Guide to the quality and safety of TISSUES AND CELLS for human application

European Committee (Partial Agreement) on Organ Transplantation (CD-P-TO)

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Guide to the quality and safety of tissues and cells for human application

5th Edition

European Directorate for the Quality of Medicines & HealthCare (EDQM)

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Foreword

Founded in 1949, the Council of Europe is the oldest and largest of all European institutions and now numbers 46 member states.* One of its founding principles is that of increasing co-operation between member states to improve the quality of life for all Europeans. Within this context of intergovernmental co-operation in the field of health, the Council of Europe has consistently selected ethical problems for study. One of the most important of these ethical issues relates to the prohibition of financial gain from the human body and its parts, including blood, organs, tissues and cells.

Transplantation-related activities at the Council of Europe are co-ordinated by the European Directorate for the Quality of Medicines & HealthCare (EDQM). This directorate is a key European organisation involved in the harmonisation, co-ordination, standardisation, regulation and quality control of medicines, blood transfusion, organ, tissue and cell transplantation, pharmaceuticals, pharmaceutical care, consumer health, cosmetics and food packaging.

Transplant and reproductive medicine have progressed in recent decades in ways that could not have been imagined previously. As with organs, the demand for some tissues and cells far outweighs the available supply. This has important consequences because human tissues and cells for human application can restore essential functions or, in some cases, save lives. However, as with all material of human origin, they carry risks of disease transmission that must be controlled by application of scrupulous donor selection and testing criteria, and also by ensuring that comprehensive quality systems are in place.

This Guide

The *Guide to the quality and safety of tissues and cells for human application*, published first in 2013 and now in its 5th edition, collates the most up-to-date information to provide healthcare professionals with a comprehensive overview of the most recent advances in the field as well as technical guidance on ensuring the quality and safety of human tissues and cells applied to patients. To increase safety for the recipients of human tissues and cells, it is essential that professionals involved in identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood and other cell and tissue type collection centres, fertility clinics, tissue establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing these establishments and Health Authorities responsible for tissues and cells for human application all have easy access to this information. This Guide aims to support professionals at a practical level and improve the rate of successful and safe clinical application of tissues and cells.

* Albania, Andorra, Armenia, Austria, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Republic of Moldova, Monaco, Montenegro, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, San Marino, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Türkiye, Ukraine, United Kingdom.

The requirements documented in this Guide represent the agreed opinions of experts in the field of tissue and cell banking as to what they consider to be the safest and most effective protocols, based on documented evidence, scientific principles and their professional knowledge and experience. Where a requirement is stated to be a "must" it is based on an EU legal requirement and/or strong documented evidence that supports the application of the requirement to ensure appropriate quality and safety profiles. Thus, "must" indicates "what must be done". However, this Guide goes further by providing additional technical advice on what "should" be done based on generally accepted good practices, expert opinion, relevant literature and the recommendations arising from results of many international projects. These additional recommendations should be taken into consideration to achieve the highest quality and safety standards. Any deviation from, or alternative protocol to these requirements (both "must" and "should"), must be demonstrated to result in an equally good or better outcome than the approach specified in this Guide. In addition, this Guide provides further explanatory and background information to help explain the underlying rationale (the "why and how") for the guidance given, and for general purposes. This information should be taken into account when considering policy decisions and educational initiatives.

Changes in the 5th edition

Whereas tissue establishments in EU member states are required to comply with EU directives, this Guide has a broader scope and is intended to facilitate ongoing improvements in the donation, procurement, testing, processing, preservation, storage, release, distribution and import/export of tissues and cells through education and the provision of non-binding recommendations for all Council of Europe member states, including those in the EU, but also beyond the Council of Europe borders. At any given time, implementation of these recommendations among member states and individual tissue establishments may vary, and alternative procedures, practices and equivalent standards of quality and safety based on a careful risk-based assessment may be in place.

In this edition, the use of the words 'must' and 'should' when providing guidance has been thoroughly revised. Throughout this Guide, use of the word 'must' indicates mandatory compliance in alignment with Council of Europe recommendations and resolutions as well as with the current EU directives,† but it also applies in those circumstances where an established good practice has been considered as essential by the working group. In the use of 'should', the aim is to indicate recommended compliance in accordance with commonly accepted good practice.

Unless otherwise stated, the guidelines apply only to human tissues and cells intended for transplantation or clinical use (including medically assisted reproduction). In this 5th edition, chapters have been updated and extended and some new ones introduced. This responds to an increased interest in the field of tissues and cells, not only from professionals with regard to their potential applications and quality standards but also from Health Authorities, since the rapid development of novel processing methods and clinical applications requires the establishment of well-defined quality and safety criteria on which to base regulatory requirements. Therefore, in this 5th edition great efforts have been made to respond to these needs. As a consequence this new edition is now divided into four parts, A to D.

Part A (Chapters 1-18) contains general requirements applicable to all tissue establishments and organisations involved in the donation, procurement, testing, processing, preservation, storage, distribution and import/export of tissues and cells, including the introduction of novel processes and clinical applications. All chapters have been extensively revised and updated incorporating all new developments in

† Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.

Commission Directive 2006/17/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.

Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

Commission Directive EU 2015/566 of April 2015 implementing Directive 2004/23/EC as regards the procedures of verifying the equivalent standards of quality and safety of imported tissues and cells.

Commission Decision 2010/453/EU of 3 August 2010 establishing guidelines concerning the conditions of inspections and control measures, and on the training and qualification of officials, in the field of human tissues and cells provided for in Directive 2004/23/EC of the European Parliament and of the Council.

the field, but some of the main changes in this part include:

- Chapter 2: The former chapter on quality management, risk management and validation has been split into two separate chapters. The new Chapter 2 – Quality management and validation has been completely revised and updated, the responsibilities of the Responsible Person and Quality manager have been defined and the differences between the various processes within the quality management systems have been clarified. Alongside this, the new Chapter 3 – Risk management provides the user with tools to evaluate risks in their tissue establishments, including fertility clinics. A new IT tool, the EDQM Microbiological Risk of Contamination Assessment tool (MiRCA), has been developed with the aim of assisting procurement organisations and tissue establishments to identify potential microbiological risks in novel, existing or changed aseptic processes, and to support management decisions to mitigate risks in aseptic processes. This tool is hosted in a dedicated IT portal and it is hoped that it will prove to be a valuable tool for tissue establishments, inspectors and Health Authorities in evaluating risks.
- Chapter 5 – Donor evaluation: In addition to general guidance when assessing tissue donors, this chapter now includes a dedicated section with guidance on undertaking risk assessments in the case of deceased tissue donors with an antecedent or tumour diagnosis (non-haematological and non-CNS tumours).
- Chapter 6 – Donor testing – markers for infectious diseases: Includes new algorithms for the interpretation of results of tests for HIV, HCV and HBV infectious markers.
- Chapter 7 – Procurement and Chapter 9 – Processing: Guidance in both chapters has been revised in full. Both chapters, used in conjunction with the MiRCA tool, would allow for an extensive assessment of individual and aggregated risk of contamination factors, which may be used by tissue establishments to decide on and prioritise risk-mitigation actions, to improve procurement and processing activities.
- Chapter 8 – Premises: Now contains guidance on environmental monitoring.
- Chapter 10 – Storage: Has become a standalone chapter, with Chapter 12 dedicated to Release, distribution and import/export.
- Chapter 11 – Principles of microbiological testing: Has been thoroughly revised to provide more guidance on testing for bacterial endotoxins and bioburden testing, and the validation of efficacy of decontamination with antibiotics. The guidance on the correct use of microbiological test methods described in the European Pharmacopoeia has been updated. In addition, the list of micro-organisms that should result in the discard of grafts (unless treated with a validated sterilisation process) has been thoroughly revised.
- Chapter 13 – Interaction between tissue establishments and organisations responsible for human application: Emphasises the need for close co-operation between tissue establishments, organisations responsible for human application and Health Authorities in responding to the introduction of novel processes and clinical applications in the field of tissues and cells.
- Chapter 17 – Biovigilance: Has been throughly revised and has real examples of the assessment of serious adverse reactions and events related to the use of reproductive and non-reproductive tissues and cells included for educational purposes.
- Chapter 18 – Introduction of novel processes and clinical applications: Has been moved to Part A of the guide and aims to outline key elements to be considered when developing and authorising novel processes and/or new clinical applications.

Part B (Chapters 19-34) contains specific guidelines and requirements for the different substances of human origin addressed in this guide. Every chapter has been revised, updated and extended. Relative contraindications for the acceptance of donors of these tissues and cells have been revised, considering case-by-case scenarios requiring careful risk assessment. A dedicated section on patient follow-up has been included in all relevant chapters. Some additional changes include:

- The inclusion of appendices related to Chapter 25, dealing with umbilical cord blood progenitors.
- Dedicated chapters expand the guidance on pancreatic islets (Chapter 26), hepatocytes (Chapter 27) and adipose tissue (Chapter 28).
- Chapter 29 – Medically assisted reproduction and Chapter 30 – Fertility preservation have been updated with new information to provide updated guidance including, among other relevant information, a dedicated section on pre-implantation genetic testing.
- Chapter 31 – Human milk, Chapter 32 –

Intestinal microbiota and Chapter 33 – Blood components for topical use or injection have been moved to this part. While the regulatory status of these substances may vary from country to country, the risks associated with their human origin and the processes applied to donor selection, procurement, processing and preservation have the same purpose and are analogous to those of the rest of the tissues and cells described in this Guide. Therefore, these chapters, without intending to pre-empt any regulatory decisions, provide a generic quality and safety framework for tissue establishments or any facility dealing with these substances.

- Chapter 34 – Tissues and cells as starting materials: This new chapter provides guidance for authorised tissue establishments on quality and safety aspects, mainly in donation, procurement, testing and distribution of the starting material for further processing for the production of novel therapies involving human tissues and cells.

Part C includes the Good Practice Guidelines (GPG) for tissue establishments, which have been extensively revised in this edition. These guidelines, which aim at promoting high levels of quality in the field of human tissues and cells, should be seen as a complementary document for tissue establishments and inspectors/auditors, describing in detail, and from a practical point of view, the key elements that should be defined and controlled for achieving comprehensive quality management systems in tissue establishments.

Finally, Part D includes tissue and cell monographs, providing information about tissue and cell preparations and clinical applications that are precisely defined and have been shown to be safe and effective when used in patients (consolidated processes for consolidated uses). Tissue and cell monographs are complementary to other sections of the Guide and aim to be useful tools for tissue establishments and Health Authorities, providing the minimum criteria and controls necessary to ensure the quality of tissues and cells processed by tissue establishments. By referring to these tissue and cell monographs, both tissue establishments and Health Authorities would know that products that do not have a matching monograph may need more attention (more risk assessment, validation, possibly clinical studies, etc.) before they can be authorised and supplied for routine use.

Expertise involved and acknowledgements

A dedicated working group, composed of well-recognised international experts nominated by member states and relevant professional associations, was convened for the preparation of this Guide. The final composition of the working group was decided taking into account the technical and scientific expertise in the field of the nominated experts, the drafting needs of the Guide and the active participation in the elaboration of previous editions of the Guide while ensuring broad and balanced geographic representation. For those areas where the working group felt additional knowledge was needed, external expertise was engaged. This group was chaired by Jacinto Sánchez-Ibáñez (Spain) and Akila Chandrasekhar (United Kingdom). This expert group made exceptional contributions by sharing their expertise, reviewing the literature in their respective areas and extracting and distilling knowledge from numerous international guidelines, collaborative projects and websites, with the aim of ensuring that all of this up-to-date information is made available and accessible to professionals and regulators. Members of the group co-ordinated preparation of the chapters and also ensured access to appropriate outside expertise, where relevant, through the engagement of a number of additional experts from European countries and beyond, who co-authored and contributed to the discussions on various parts of this Guide. All the professionals who participated in the preparation of the Guide are listed in Appendix 42.

The final draft was submitted to a stakeholder consultation where the Council of Europe European Committee on Organ Transplantation (CD-P-TO), Health Authorities, relevant professional associations and additional experts nominated by them, carefully revised the text and provided comments and suggestions. During the consultation for this 5th edition, 1 314 comments were received; all of them were carefully analysed by the working group and 55 % of the comments led to changes in the final text. In some instances (9 %), comments were deemed relevant but required extensive research and/or discussion, so their inclusion was postponed to a future edition, and in 36 % of cases the comments did not require any changes to the text. Our gratitude is extended to all those individuals who provided extremely useful feedback during this stakeholder consultation.

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entitled "Improving the quality and safety of SoHO, disseminating best practices, implementing EU and CoE Standards and tackling new challenges". Special thanks are due to Stefaan van der Spiegel and Deirdre Fehily, from DG-SANTE, who ensured that the current text remained in alignment with EU directives and who made available the results from EU-funded projects. Several professional associations – in particular, the European Association of Tissue and Cell Banks (EATCB), the European Eye Bank Association (EEBA), the European Society for Human Reproduction and Embryology (ESHRE) and the European Society for Blood and Marrow Transplantation (EBMT) – should also be thanked for sharing their experience and knowledge.

The drafting and publication of the 5th edition of the Guide was co-ordinated by Mar Lomero (Scientific Assistant) and Marta López Fraga (Scientific Officer in charge of the CD-P-TO and the transplantation activities at the EDQM/Council of Europe), with the assistance of Christine Gault, Jaime Marco, Janet Latzel, Muriel Burstin, David Crowe, Tara Hulley and Gerard M.-F. Hill. The entire project has been an exceptional combined effort of everyone involved, with extensive discussions dedicated to the common goal of improving the safety, efficacy and quality of tissues and cells for human application.

The result is this 5th edition of the Guide, which constitutes a common European standard, based on the long-standing expertise and knowledge of the EDQM.

Last, but not least, we would like to honour all healthcare professionals who have been on the front lines against the pandemic. Their efforts, dedication and tireless work against Covid-19 have made them the true heroes of this health crisis. They have not only fought for our lives and those of our loved ones but also ensured that the gift of donation continued to save and improve lives through this unimaginable period.

Marta López Fraga
European Committee on Organ Transplantation
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Part A. General requirements

Chapter 1. Introduction

1.1. Introduction

We are entering a new age of medical and biotechnological progress. Medical procedures that were unimaginable a generation ago are a reality today. One aspect of the recent and rapid advances in biological and medical research is that human tissues and cells are being used increasingly in new ways. Many of these developments, such as advances in transplantation therapy or in medically assisted reproduction (MAR), have unquestionable benefits. However, using human tissues and cells in different ways also raises questions of safety, quality and efficacy, and presents new ethical dilemmas.

Tissue from one deceased donor may be transplanted into as many as 100 individuals. Some other tissues and cells can be provided only by living donors, as long as this procedure does not risk serious harm to the donor or endanger the donor's life. Transplantation of tissues and cells can range from life-saving treatments (e.g. in the treatment of catastrophic burns) to quality-of-life improvements. In addition, donated gametes and embryos may help fulfil a person's wish to have children.

Some tissues are used practically unaltered from the condition in which they were removed from the donor. Deceased donor corneas, for example, are used to restore sight, heart valves replace damaged ones and extend life, tendons and ligaments may be used for the treatment of sporting injuries or to repair degenerative defects, and skin can be employed as temporary cover for major burns or to support the healing of ulcers. Other tissues, however, are processed into products that are almost unrecognisable as bodily material. Skin, for example, may be cut into conveniently sized dressings, incorporated into sprays or gels, or decellularised for use in various surgical procedures. Bone can be processed into hundreds of different products and distributed via a global medical market for use in orthopaedics (general and oncology), sports medicine, craniofacial/maxillofacial/dental surgery and neurosurgery. Cellular components of bone may be removed entirely and even the calcium may be removed to promote incorporation and tissue regeneration. Bone allografts may be precision-cut and sized, and bone can also be supplied in soft, pliable or injectable forms. If a deceased donor has consented to the use of any part of their body for the treatment of others (or their legal representative, e.g. a relative, has authorised this to fulfil the donor's wishes), then many tissues – including bone, heart valves, skin, corneas, ligaments, cartilage, connective and adipose tissue, glands and nerves – can be used for therapeutic purposes.

In contrast, amniotic membranes, amniotic placenta and skull bone are donated by living persons. Also, femoral heads removed during an operation to replace a hip joint and heart valves from patients receiving a heart transplant are sometimes processed and 'recycled'. In addition, many types of cell can be donated (some during life and some after death) and submitted to different degrees of manipulation before application in humans. Examples include haematopoietic progenitor cells (e.g. bone marrow, peripheral blood progenitor cells, umbilical cord blood), somatic cells (e.g. peripheral blood cells, keratinocytes, chon-

drocytes, hepatocytes), mesenchymal stromal cells and limbal stem cells. Oocytes, spermatozoa, ovarian or testicular tissue and embryos can be used in MAR procedures to achieve pregnancy.

Human tissues and cells could also be the potential starting material for much more complex products in the future.

1.2. Scope and purpose of this Guide

This is the 5th edition of the Council of Europe Guide to the quality and safety of tissues and cells for human application. This Guide has two main objectives:

1. It aims to provide sound information and guidance – for all professionals involved in donation, banking, transplantation and other clinical applications of tissues and cells – to optimise the quality and minimise the risks of these complex procedures. All material of human origin carries risks of disease transmission that must be controlled by application of scrupulous criteria of donor selection and testing, compliance with biovigilance system procedures and comprehensive systems to assess quality. The idea behind this Guide is to help professionals on a practical level by providing generic guidance and detailed good practice guidelines (GPG) that will help improve the rate of successful clinical application of tissues and cells.
2. This Guide includes ethical principles and guidelines to be considered for the donation and human application of tissues and cells.

The field of tissue and cell donation and banking is now highly regulated in many countries. In the European Union (EU), the relevant directives describe the requirements and have been transposed into the national legislation of the EU member states. This Guide refers to those requirements, where appropriate, but goes beyond them to describe generally accepted good practice at a technical level and includes some consideration of ethical issues. Therefore, it will be useful as a source of practical information for those working within the EU legislative framework and those working within national legal frameworks in all Council of Europe member states and beyond.

It is worth noting that, in the EU, activities involving cells and tissues that are subject to substantial manipulation or are used for a different essential function in the recipient than in the donor are not governed by tissues and cells directives but fall under Regulation (EC) No. 1394/2007 (the 'Advanced Therapy Medicinal Product (ATMP) Regulation'). Thus, in EU countries, this Guide would only apply to the donation, procurement and testing steps leading to the production of ATMPs. In any case, it is important to take into account that this Guide does not provide any guidance on how human tissues and cells are or should be regulated, so users of this Guide are advised to carefully consider the national legal requirements that apply to the activities they are undertaking.

According to the World Health Organization (WHO) *aide-mémoire* on the donation and transplantation of tissues and cells [1], national Health Authorities are responsible for ensuring that the donation, banking and human application of tissues and cells are promoted, regulated and monitored appropriately in the interests of patient safety and public transparency. More specifically, they are responsible for ensuring that:

a. an appropriate legislative/regulatory framework is in place;
b. national/international practice standards have been defined;
c. there is inspection/authorisation of screening, testing, procurement, processing, storage, distribution, import and export;
d. there are programmes for vigilance and surveillance of adverse outcomes;
e. there is monitoring and reporting of donation, processing, storage, distribution and import/export activity.

In this Guide, the term 'Health Authority' is used throughout to refer to a body that has been delegated the responsibility for these activities on a national or regional basis by their government. Other similar terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it. It should be noted that in some countries, the activities described in this Guide may be controlled by different Health Authorities – e.g. separate authorities may regulate MAR and the donation, banking and human application of other tissues and cells. Unless otherwise indicated, the term 'member states' signifies member states of the Council of Europe.

Human tissues and cells also play a key part in medical research. In clinical trials of new medicines, for example, vital information about the effects of the medicine on an individual can be obtained from samples of tissues or cells and other materials provided by research participants. However, tissue is also used much more widely in medical research, from

early drug 'discovery' (such as using human tumour samples to discover possible targets for treatment) to later clinical development whereby samples may be used to identify which subgroups of patient populations respond best to a new medicine. Additionally, current research aims to develop artificial tissue that could alleviate the shortage of tissue available for human application. These forms of 'basic' research using human tissue still have an ultimately therapeutic goal in mind. However, important though all these possibilities are, this Guide covers only tissues and cells used for current therapeutic purposes.

Similarly, all tissues and cells that are both procured and applied within the same medical procedure are outside the scope of this Guide.

Finally, a glossary of terms is provided in Appendix 3.

This book is the result of the collective effort and expertise gathered by experts nominated by the member states and professional associations in the field (see Appendix 42), as well as by the members and observers of the European Committee of Experts on Organ Transplantation (CD-P-TO), for which see Appendix 43.

For matters dealing with the use of organs and blood or blood products, see the *Guide to the quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality assurance of blood components* [2], both published by the Council of Europe.

1.3. Brief history of the application and banking of tissues and cells of human origin

The best documented accounts of early transplants deal with skin transplantation, though the success or failure of these procedures has not been well documented. The first reliable account is that of the Indian surgeon Sushruta in the 2nd century BCE, who used autografted skin transplantation for a nose reconstruction (rhinoplasty). Centuries later, the Italian surgeon Gasparo Tagliacozzi carried out successful skin autografts, but he consistently failed with allografts, offering the first suggestion of rejection several centuries before that mechanism could be understood. He attributed it to the 'force and power of individuality' in his 1596 work *De Curtorum Chirurgia per Insitionem*. Orthopaedic surgeons refer to the origin of their discipline as 1668 when Job van Meekeren reported on the grafting of bone from a dog's skull to correct a defect in a soldier's cranium. It was not until 1869 that the first completely documented fresh human-skin allograft was carried out by the Swiss surgeon Jacques Reverdin.

The first successful full-thickness corneal transplant, a keratoplastic operation, was carried out in 1905 by Eduard Zirm at Olomouc Eye Clinic in Moravia (now Czech Republic). Pioneering work in the surgical technique of transplantation was done in the early 1900s by the French surgeon Alexis Carrel, together with Charles Guthrie, who developed techniques for suturing arteries and veins. Their skilful anastomosis operations and new suturing techniques laid the groundwork for later transplant surgery, and Alexis Carrel won the 1912 Nobel Prize in Physiology or Medicine for his work in the field. Major steps in skin transplant occurred during the First World War, notably through the work of Harold Gillies in Aldershot, UK. Among his advances was the tubed pedicle graft, which maintained a fleshy connection from the donor site until the graft established its own blood supply.

Bone is the oldest tissue transplant on record and the most common tissue transplanted today. The first bone transplant recorded in modern times occurred in Scotland in 1878 when Sir William Macewen removed an infected humerus from a 12-year-old boy and replaced it with three allografts from an amputated tibia from another child with rickets. In 1907, Erich Lexer in Berlin developed a procedure to remove a whole knee joint from an amputee in one operating room and transport the 'warm' graft to an adjacent operating room for immediate transplant into the recipient. Five years later, Alexis Carrel's work predicted the storage of tissues for future transplantation, and surgeons began to use bones and developed their own 'bone banks'. These pioneers included Inclan in Cuba, Bush, Wilson and Hibbs in the USA, Hult working in Sweden, Judet in France, and Klen in what was then Czechoslovakia. Most of these early bone banks were simply refrigerators and, later, freezers, but greater sophistication was developed by bone banks in Leeds (UK), Berlin, Athens and Warsaw. When long-term freezer storage of long bones became feasible, limb-sparing surgery using this type of bone allograft to avoid amputations in the treatment of malignant skeletal tumours became popular. Burrwell (UK), Parrish and Mankin (USA) and Ottolenghi (Argentina) published their results.

The orthopaedic profession realised that, if very large segments of bone could be transplanted successfully, smaller segments could also be used. This resulted in a very large increase in the use of bone allografts. Tissue-storage methods were developed further during the 1950s by Hyatt at the US

Navy Tissue Bank in Bethesda, Maryland, where they adapted methods of lyophilisation from the food preservation industry and applied the process to the preservation of bone and skin, which could then be easily stored, transported and reconstituted for use when needed. This method of preservation allowed bone to be stored and transported easily without any electrical or mechanical requirements, and has had a profound effect on the availability and use of bone allografts. By the end of the 1990s, use of musculoskeletal tissue allografts (i.e. bone, cartilage, soft tissue) had become commonplace in many clinical areas. In 1944, Paton established the world's first eye bank, the Eye-Bank for Sight Restoration, in New York. Subsequently, the first deceased-donor eye bank was established in Odessa using eyes (packed in glass containers) sent by rail from a trauma centre in Moscow.

The first recorded cardiac valve transplantation was carried out in Toronto by Gordon Murray, who implanted an aortic allograft in the descending thoracic aorta to relieve aortic insufficiency in 1956. The first orthotopic transplantation of the aortic valve was undertaken by Donald Ross in London in 1962 and independently by Brian Barratt-Boyes in Auckland, New Zealand, a few weeks later. Pulmonary and mitral valves were first used as allografts in subsequent years, with the pulmonary autograft procedure being carried out first in 1967.

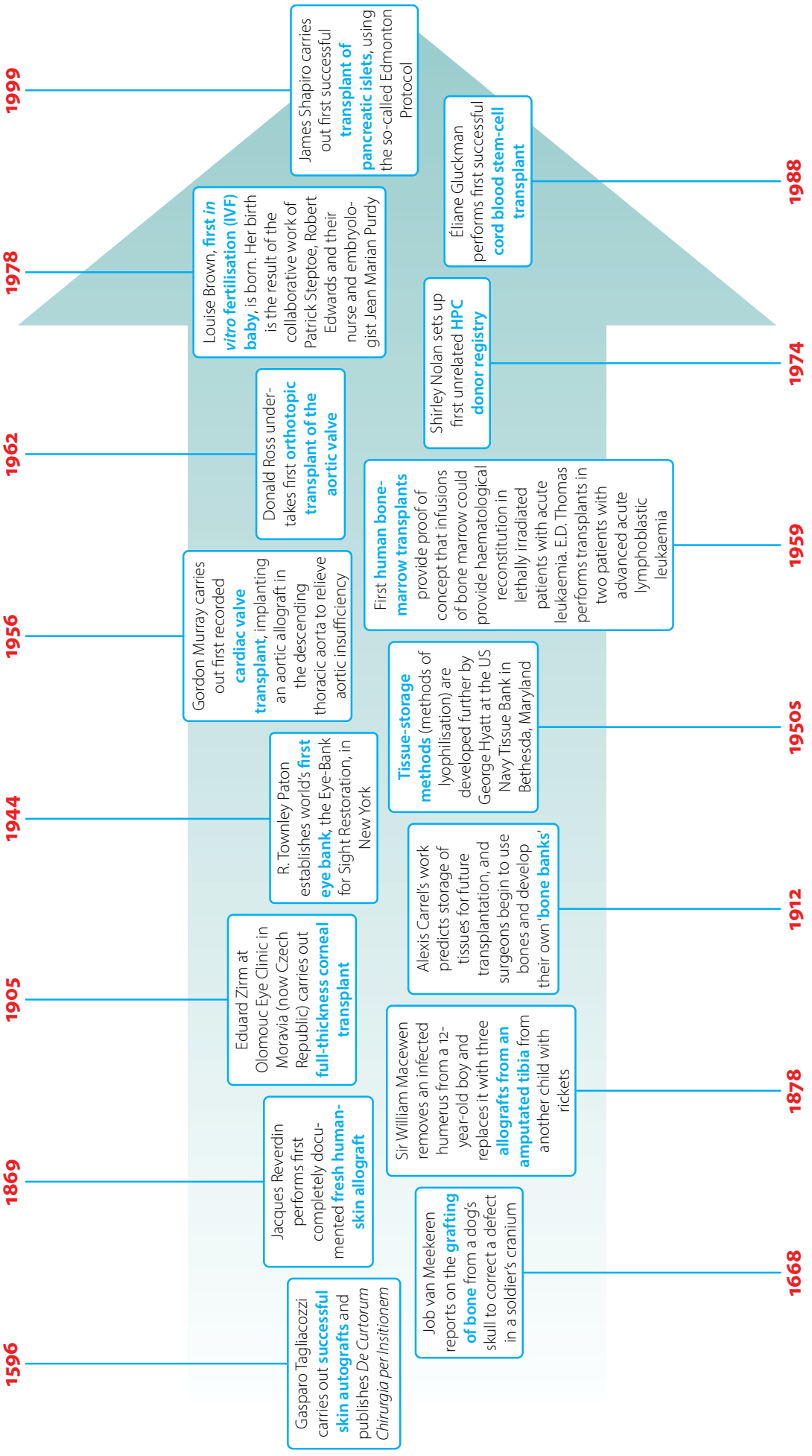
After the atomic bomb explosion in Japan that ended the Second World War, many scientists began to explore ways of protecting humans from radiation. The first experiments were done in mice and later in dogs by E.D. Thomas. As early as 1956, the idea that bone-marrow transplants might exert a therapeutic effect against malignancies was proposed by Barnes and Loutit, who observed an anti-leukaemic effect of transplanted spleen cells in experimental murine models. In 1959, the first human bone-marrow transplants gave proof of concept that infusions of bone marrow could provide haematological reconstitution in lethally irradiated patients with acute leukaemia. E.D. Thomas performed transplants in two patients with advanced acute lymphoblastic leukaemia, with a syngeneic graft after high-dose total body irradiation; the grafts were successful but the patients died a few months later of relapse. G. Mathé administered allogeneic bone marrow for the treatment of several patients who had suffered accidental exposure to irradiation, and most survived with autologous reconstitution. In 1965, Mathé was the first to describe long-term engraftment of sibling bone marrow, thereby demonstrating chimerism, tolerance and an anti-leukaemic effect. Although the transplant itself was successful, the patient eventually died of varicella with chronic graft-*versus*-host disease (GvHD). In 1970, M. Bortin reported 203 transplants carried out between 1958 and 1968, with only three patients alive at the time of the report. The major causes of death were graft failure, GvHD and relapse. After these disappointing results, few centres persisted and the number of transplants declined sharply.

Major progress came from the discovery of the human leukocyte antigen (HLA) system by J. Dausset and J.J. Van Rood. Selection of HLA-identical siblings as bone-marrow donors diminished the risk of rejection and GvHD. Using animal models, R. Storb and E.D. Thomas developed the model of total body irradiation for conditioning (in dogs) and the use of methotrexate for GvHD prevention. In mice, G. Santos showed that the use of cyclophosphamide could add immuno-suppression to the myeloablation of total body irradiation. He was also the first to use busulfan instead of total body irradiation. In 1988, the first successful cord blood stem-cell transplant was done by E. Gluckman to treat a child with Fanconi's anaemia with cells from his healthy HLA-identical sibling (related) donor. The first unrelated donor registry was established in London in 1974 by Shirley Nolan, trying to find a HLA-match donor to her son who was diagnosed with Wiskott–Aldrich syndrome. After this first donor recruitment drive, the number of bone-marrow and peripheral haematopoietic progenitor cell donors increased all over the world, with more than 40 million volunteer donors now registered, including more than 800 000 cord blood units [3].

Transplantation of pancreatic islets has been carried out in humans since 1990 [4]. However, it was not until 1999 that the first successful transplant of pancreatic islets, using the so-called Edmonton Protocol, was undertaken by James Shapiro [5]. European centres became active around the same period, but their transplant recipients had complications of type-I diabetes that could not be managed with insulin injections. The advantage of the Edmonton Protocol was that it allowed restoration of the finely tuned regulation of glucose metabolism through appropriate insulin production by transplanted islets. In 2005, the first pancreatic islet transplant from a living donor – from a 56-year-old woman to her 27-year-old diabetic daughter – resulted in transplanted cells producing insulin within minutes after transplantation.

On 25 July 1978, Louise Brown, the first *in vitro* fertilisation (IVF) baby, was born in Oldham, UK [6]. Her birth was the result of the collaborative work of Patrick Steptoe, Robert Edwards and their nurse and embryologist Jean Marian Purdy. Since then, this

Figure 1.1. **Summary of the timeline of the main relevant events in tissues and cells transplantation**



research area has seen major improvements in the laboratory – e.g. cryopreservation of gametes and embryos, intracytoplasmic sperm injection (ICSI) [7], pre-implantation genetic diagnosis [8] and improved clinical management (such as methods for ovarian stimulation and embryo culture conditions) – thereby leading to a considerable increase in the use of assisted reproductive technologies (ART) [9]. A brief summary of the main relevant events in tissues and cells transplantation is shown in Figure 1.1.

1.4. Benefits and risks of human application of tissues and cells

Progress in the medical sciences has made it possible to effectively transplant human cells and tissues from one person into another. For the application of tissues and cells of human origin, selecting the appropriate donor is essential to exclude individuals whose donation could either pose a risk to their own health or pose a risk to the recipient that exceeds the expected benefit. The expected benefit will depend on the initial pathology and the status of the recipient, using a risk assessment methodology. The use of tissues and cells for therapeutic purposes constitute well-established techniques, outnumbering organ transplants by more than tenfold. They offer major therapeutic benefits to a wide range of patients, from lifesaving (skin or arteries) to major improvement in the quality of life of the recipient (cornea, bones). Indeed, demand is rapidly increasing for bone transplantation, particularly for secondary revision of hip-replacement operations, as well as for skin treatment of severely burned patients.

There are many differences between organ and tissue transplants and these differences have an impact on the risks associated with donation and transplantation or clinical applications. For example, the time to transplantation of an organ is usually measured in hours, whereas clinical applications of tissues are measured in days or years depending on the tissue and the preservation method. Organs, however, cannot be preserved for future use. An organ donor can only supply a limited number of recipients, where a tissue donor can be used in many recipients. Organs cannot be sterilised before transplantation, whereas tissues can often be subjected to decontamination and/or sterilisation methodologies. Because of these differences, donor-selection criteria for tissue donors can often be more stringent.

Haematopoietic progenitor cells fall somewhere between organs and tissues in this comparison. They are intended to be life-saving and are usually transplanted on the basis of one donor to one recipient. However, they can be processed to some extent, though not sterilised, and they can be stored for extended periods. Where bone marrow is donated by an unrelated donor for a specific recipient and transplanted without freezing, the situation is very analogous to organ transplantation. In contrast, when cord blood is donated to a public bank, stored for years and possibly selected later for transplant to a matching recipient, the situation is more analogous to tissue banking.

In practice, the decision to use any donor-derived tissues or cells will always be based on a clinical assessment of the risk *versus* the benefit to the patient, taking any alternative potential therapies into consideration. This is because any human application of tissues and cells carries not only process-related risks, but donor-related disease-transmission risks. The factors influencing the clinical outcome are complex because there is an interaction between two different biological systems, namely, those of the donor and the recipient. Therefore, when assessing the risk of human application of tissues and cells, both donor and recipient should be considered. In both cases, the potential benefits of the procedure should outweigh the risks. Transparent communication and good collaboration between Health Authorities, tissue establishments and clinicians treating patients are vitally important in any donation process.

Some of the most widely used tissues and cells, and their benefits for recipients, are listed in Table 1.1.

With regard to the risks associated with the human application of tissues and cells, Article 6 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin clearly establishes that:

> all professionals involved in organ or tissue transplantation must take all reasonable measures to minimise the risks of transmission of any disease to the recipient and to avoid any action which might affect the suitability of an organ or tissue for implantation.

Careful evaluation of the donor's medical history, travel history, behavioural risks and history of malignancies is necessary to keep the risk of transmission of infections or malignancies to the recipient as low as possible. These risks are covered in Chapter 5. Specific criteria regarding tissues and cells are discussed in detail in the relevant chapters of Part B of this Guide.

Table 1.1. **Most widely used tissues and cells: the benefits for the transplant recipient**

Tissues and cells	Function	Benefits for the recipient
Amniotic membrane	Forms the amniotic sac, filled with amniotic fluid, which surrounds and protects the fetus; transfers oxygen and nutrients from mother to fetus	Used in burns and wound healing (to reduce surface inflammation, scarring and pain in surgical applications), in certain types of ulcers and in oral, maxillofacial and ocular surface surgery
Bones and cartilage	Support the body and protect vital organs	Used to repair or stabilise the spine and other bones and cartilage damaged by degeneration, trauma, cancer or birth defects; also used in oral surgery and in filling bone cavities or other areas where bone mass has been lost
Corneas/eyes	Cornea and sclera together form the outer coat of the eye: the cornea is transparent and lets light into the eye; the white sclera is opaque	Indicated for visual problems caused by damage or deterioration of the front part of the ocular globe; if whole eyes are donated, the corneas can be used in transplants for corneal blindness and the sclera can be used for reconstructive and glaucoma surgery
Gametes, reproductive tissues and embryos	Generate a new human being	Used primarily for the treatment of infertility in heterosexual couples and to achieve pregnancy and live birth in single women and same-sex couples; can be stored to preserve fertility or even re-establish gonadal function (in the case of reproductive tissues). Medically assisted reproduction can also be applied to avoid transmission of some genetic or infectious diseases
Fascia	Fibrous tissue that covers muscles	Used to repair tendons, muscle, ligaments and deformities
Haematopoietic progenitor cells (bone marrow, peripheral blood progenitor cells and cord blood)	Haematopoiesis	Used for the treatment of haemato-oncologic disorders, and genetic and autoimmune diseases
Heart valves	Direct the flow of blood in the heart	Used for patients with valve defects, especially in children
Pancreatic islets	Contain beta cells, which are responsible for insulin production	A transplantation method that restores an adequate mass of insulin-producing beta cells in patients with diabetes
Pericardium	Forms protective lining around the heart	Used for replacement of *dura mater* in the brain and for eye surgery
Skin	Protects the body against injury, infection and dehydration	Used for the treatment of burns patients, certain types of ulcer, abdominal wall repairs and reconstructive or plastic surgery
Tendons	Attach muscle to bone	For use in joint injuries
Veins and arteries	Provide a structure for the flow of blood through the body.	Replace blood vessels that are damaged by disease, trauma or prolonged dialysis treatment. Also used in bypass surgery to re-route blood flow.

Only tissues and cells recovered, processed, stored and distributed within well-controlled quality management systems of donation, processing, storage and distribution are likely to function satisfactorily and to reach an acceptable level of safety. The donor-selection criteria and the conditions of processing and preservation are crucial parameters that need to be tightly controlled. Therefore, any organisation involved in these processes should implement a comprehensive quality-management system. Management commitment and support are essential for the development, implementation and monitoring of a quality system to ensure continuous improvement. All staff should understand the importance of quality and their role in achieving it consistently (see Chapter 2).

In summary, human application of tissues or cells can confer great benefit for a patient, but it is not without risk. In exceptional cases, a donation of tissues or cells that does not meet all the necessary safety or quality requirements may be used for human application for a particular patient. This may occur,

for instance, where the transplant is likely to be life-saving and the alternative options for treatment of that patient carry a poor prognosis. Similarly, couples undergoing MAR treatments often use gametes that would not meet selection criteria for non-partner procedures (e.g. gametes from an infected partner, low-quality sperm, gametes with a well-known risk of transmission of a geneticdisease). Ultimately, patients contemplating use of any donated tissues or cells should discuss the risks and benefits of surgery/therapy with their surgeon/physician and make the decision that is best for them.

1.5. The process of donation of tissues and cells and their application in humans

Donation of tissues and cells and their application in humans continue to be fast-moving fields. Such rapid developments bring their own challenges. These challenges include: control of all crucial technical activities and services (procurement, transportation, processing, preservation, quality control, storage) that enable tissues and cells to be removed from one person and transferred to another body, reimbursement of expenses and service charges, safeguards from exploitation or misuse (e.g. formal requirements for consent from the potential donor before procurement of tissues or cells) and the complex chain of intermediaries (people and institutions) in the process of donation and human application.

The process of donation of tissues or cells from a deceased donor is, in many respects, quite different from the process in living donors; but, in all cases, a complex network of interactions underlies the many ways in which human material may be provided by one person for the benefit of others. Some of these complex links, using the example of a deceased donor, are summarised in Figure 1.2.

We can conceptualise the entire process in terms of organisation and workflows. In the case of donation after death, transplantation can take place only if trained professionals are available to talk to the next of kin of the deceased potential donor, if there is the necessary infrastructure to procure tissues within a given timeframe and process them, if transport services exist to transport tissues appropriately and if surgeons are available to carry out tissue transplantation into the recipient. Similarly, living donation is possible only if professionals recruit and evaluate potential donors, and adequately trained personnel carry out the processes that will generate the medical products used to treat patients.

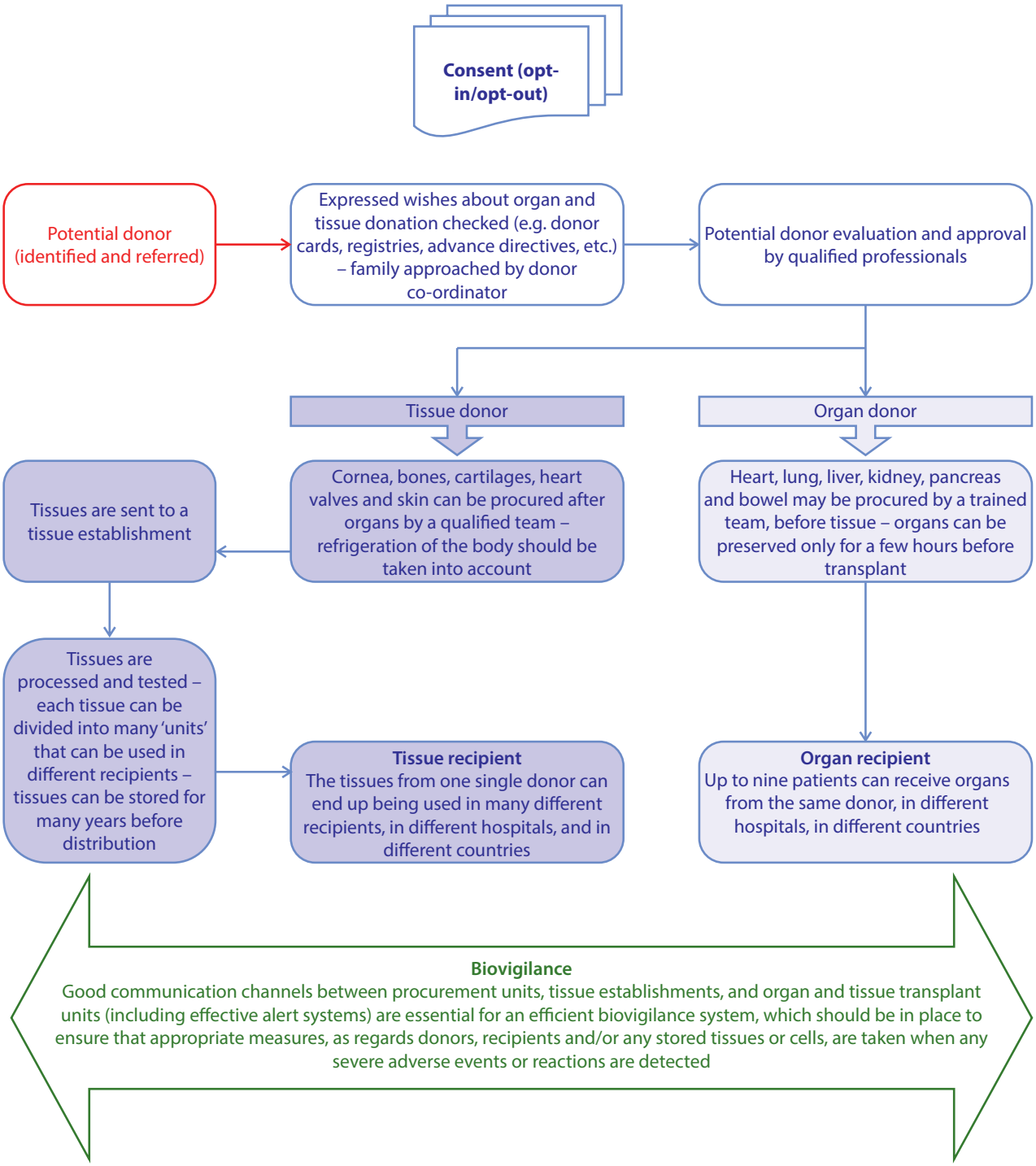
Tissue establishments play a central role in modern medicine by providing material for treatment and research. Tissue banking and cell banking are increasingly interconnected as part of the complicated networks that now connect the sources and recipients of donated bodily material, and the many intermediaries involved in processing the material to facilitate its use by clinicians.

Centralised management of tissue and cell donations could be the ideal scenario. However, tissues and cells can be provided from public organisations and private companies. Co-operation between establishments that store tissues and cells may be relatively limited. National and international efforts have focused on good practice for tissue establishments without usually providing a mechanism for comprehensive, nationwide sharing of donated material. In the meantime, an industry based on the supply of human tissue and cells has evolved worldwide, with multiple providers competing in a market driven by, among other things, biotechnology companies, pharmaceutical companies and private clinics. Thus, the flows involved between the original source or donor of the material, the amount of processing of the material involved and the commercial nature of some of those transactions are becoming ever more complex.

It is important to emphasise how consideration of policy surrounding donation must now take into account these complex flows and multiple intermediaries [10]. Awareness is needed of the central part that must be played by organisations and organisational structures in the donation and subsequent use of bodily material. Everyone involved needs to understand how the process includes, for example, the creation of professional roles such as 'donor co-ordinators', the extent to which they are expected to maximise opportunities for donation, how these professionals approach the next of kin of potential donors and form relationships with them, how well one part of the system links with another and where responsibility is seen to rest, and the way professionals in different fields interact and co-operate with one another. Awareness of this also points to added complexities in the form of legal agreements, liabilities and obligations that may arise where donated material is transformed, banked or otherwise handled as a commodity by successive intermediaries.

The increasing possibilities in using many forms of bodily material to benefit others in medical treatment has brought about increased pressure in member states to meet demand. There is a continual need to recruit new tissue and cell donors to main-

Figure 1.2. **Complex links between donors and recipients in the context of donation after death**



tain an adequate supply. Shortages of supply may affect particular subgroups of the population more than others because of the need to match material according to immunological criteria or age. 'Demand' for material is inherently variable; as scientific developments make more treatments possible, the demand for that treatment is likely to increase, whereas the development of alternatives may lead to reduced demand. Public expectations of what medical science can achieve may serve to put further pressure on demand.

Talking in terms of 'supply' and 'demand' may resonate with the experience of many professionals and patients (potential recipients), who are only too aware of the impact of any shortage in supply. This feature is exacerbated in situations in which the requirement for a high degree of matching or phenotypical similarity between donor and recipient calls for recruitment from ethnic minorities and international collaboration. However, at the same time, it may imply a lack of consideration of the human nature of the source of the material. It is important to

emphasise when using these impersonal terms that we are talking about people and people's lives.

1.6. Tissue banks, tissue establishments and biobanks

A 'tissue bank' is a term commonly used to describe an establishment that collects and stores human tissues or cells for human application and for non-therapeutic application like medical research.

Increased use of tissues and cells for human application and for research calls for terminology that will distinguish between establishments that collect and store tissues and cells for one of these purposes or the other. In Europe, the terms currently in use are 'tissue establishment' (for clinical applications) and 'biobank' (for non-therapeutic applications).

The term 'tissue establishment' became widely used in Europe following publication of the EU Tissues and Cells Directive 2004/23/EC, which defined it as:

> a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement or testing of tissues and cells.

In the field of MAR, the term 'tissue establishment' refers to the laboratories in MAR centres or clinics as well as banks of gametes. These centres or clinics often also include clinical units in which the patients are treated. In this Guide, the term 'tissue establishment' is used and refers to all these banks, units, centres and clinics. The directive does not cover research using human tissues and cells, so tissue establishments are concerned only with tissues and cells intended for human application. Similarly, the directive does not cover other clinical applications and practices undertaken in the clinical units of MAR centres, for example, standard fertility workup.

In the USA, the American Association of Tissue Banks (AATB) uses the term 'tissue bank' for:

> an entity that provides or engages in one or more services involving tissue from living or deceased individuals for transplantation purposes. These services include assessing donor suitability, recovery, processing, storage, labelling, and distribution of tissue. [11]

The term 'biobank' is widely used for repositories storing human biological samples for use in research. In its glossary, the Organisation for Economic Co-operation and Development (OECD) defines a biobank as 'a collection of biological material and the associated data and information stored in an organised system, for a population or a large subset of a population' [12]. Several other definitions, as used in EU legislation or guidelines, are available on the website of the EU-funded project PRIVILEGED (Privacy in Law, Ethics and Genetic Data) [13]. The general requirements for biobanking are described in ISO 20387.

In the USA, the term 'biorepository' is preferred to 'biobank'. For example, according to the glossary of the National Cancer Institute, a biorepository is:

> a facility that collects, catalogues, and stores samples of biological material, such as urine, blood, tissue, cells, DNA, RNA, and protein, from humans, animals, or plants for laboratory research. If the samples are from people, medical information may also be stored along with a written consent to use the samples in laboratory studies. [14]

The biobanking field is continually evolving, and tissue establishments may become interested in collecting samples for research purposes, so the terminology should also be refined to reflect these changes in the future.

In this Guide, it has been agreed to use the term 'tissue establishment' and its definition in accordance with Directive 2004/23/EC.

1.7. Quality management

High-quality, safe and efficacious procedures are essential for donors and recipients alike. The long-term outcomes of tissue and cell donation and human application should be assessed for the living donor, as well as the recipient, to document benefit and harm.

The level of safety, efficacy and quality of human tissues and cells for human application as health products of an exceptional nature must be maintained and continually optimised. This strategy requires implementation of quality systems (see Chapter 2) that include traceability (see Chapter 16) and vigilance (see Chapter 17), with adverse events and reactions reported both nationally and for imported/exported human products.

Optimising the outcome of the human application of tissues and cells entails a rules-based process that encompasses clinical interventions and *ex vivo* procedures from donor selection through to

long-term follow-up. Under the general supervision of Health Authorities, transplant and MAR programmes should monitor donors and recipients to ensure that they receive appropriate care, including information about long-term risks and benefits. Evaluation of information on long-term risks and benefits is essential to the consent process and for adequately balancing the interests of donors and recipients. The benefits to both must outweigh the risks associated with donation and human application. Donors should not be permitted to donate in clinically hopeless situations.

Locally organised donation, transplantation and MAR programmes should store details of their activity and follow-up data in national and/or international registries. All deviations from accepted procedures that could increase the risk to recipients or living donors (as well as any untoward consequences of donation or human application) should be reported to, and analysed by, the responsible Health Authorities.

Transplantation of human material that does not involve long-term medical care of the recipient may not require active, long-term follow-up, though traceability should be ensured for the anticipated lifetimes of donor and recipient. Internationally agreed means of coding to identify tissues and cells used in transplantation are essential for full traceability (see Chapter 16).

In the specific case of MAR, traceability should include the outcome of the pregnancy as well as the health of the donor, recipient and newborn. It is of the utmost importance to put the welfare of donors (especially with respect to non-partner donors) in a central position in determining what constitutes acceptable practice. This requirement might entail additional effort in the context of cross-border reproductive care. MAR registries are essential, collecting data on a systematic basis to follow up the long-term health effects of MAR activity, including the health of the donor, recipient and newborn, but also including information on non-partner donors.

1.8. Ethical issues

Human tissues and cells can be derived only from the body of a person – hence the ethical challenges associated with their use. The range of tissues and cells described in this Guide makes explicit the very different circumstances under which a person can donate. The person providing the material may be living or deceased, the material may be used almost immediately or stored for long periods of time, and the material may be used unprocessed or heavily processed. Whatever the case, handling and disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and for the human body.

Ethical standards of all aspects of tissue and cell donation and transplantation have to conform to the Oviedo Convention on Human Rights and Biomedicine (1997) [15] and the Additional Protocol on transplantation of organs and tissues of human origin (2002) [16] and also, in the EU, to the Charter of Fundamental Rights of the European Union (2012) [17]. Other important guidelines to observe from an ethical viewpoint are Committee of Ministers Resolution (1978) 29 on harmonisation of legislation of member states relating to removal, grafting and transplantation of human substances [18], the WHO Guiding Principles on human cell, tissue and organ transplantation [19], the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [20] and the Barcelona Principles on the use of human donated tissue for ocular transplantation, research and future technologies from the Global Alliance of Eye Bank Associations (GAEBA) [21].

For tissue donation from deceased individuals, the 'dead-donor rule' (which states that patients must be declared dead before removal of any vital organs or tissues for transplantation) must be strictly respected [22].

1.8.1. Consent

The Oviedo Convention states that an intervention in the health field may be carried out only after the person concerned has given free and informed consent to it. This person must make a free choice in the absence of any undue influence and must be given appropriate information beforehand as to the intended use and nature of the intervention as well as its consequences and risks. The person concerned may freely withdraw consent at any time.

Together with the Declaration of Istanbul, a joint initiative of the International Society for Nephrology (ISN) and The Transplantation Society (TTS), the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin expands these provisions further for the specific case of donation and transplantation. These provisions are explained further in detail in Chapter 4. Specific cases related to consent in MAR procedures are outlined in Chapter 29.

Tissues must not be removed from the body of a deceased person unless that person has been certified dead in accordance with the national law and consent

or authorisation has been obtained. The removal must not be carried out if the deceased person had objected to it.

Finally, it is crucial to emphasise the importance of consent in creating and maintaining the trust of the general public in health professionals and the healthcare system as a whole. 'Medical mistrust', or mistrust of the healthcare system, is one of the reasons why people are reluctant to donate bodily material. This may be associated with concerns about consent in that the terms of the consent might be misused (for example, by using the donated material in a manner which is not in accordance with consent, including its use for non-therapeutic purposes, research and/or profit) and that additional material might be taken without explicit consent. Values such as honesty and trust are central in both the professional and personal relationships when donation of bodily material takes place. Therefore, it is of vital importance that the limits of the consent are clearly established, made explicit and scrupulously respected.

The recipient – and, if appropriate, the person or official body providing authorisation for the human application – must be given suitable information beforehand on the purpose and nature of the procedure, its consequences and risks, and the alternatives to the intervention.

In summary, all donation and transplantation programmes are dependent upon the goodwill and voluntary donation of relevant material from donors to continue their activity. It is, therefore, important that public confidence is maintained by standards of good practice. By engaging donor trust and commitment when obtaining consent, healthcare professionals will reduce the risk of nefarious trading and potential physical harm from the use of transplantable tissue for human application.

1.8.2. Conflicts of interest

To avoid any potential conflict of interests, physicians determining the death of a potential donor should not be directly involved in tissue or cell procurement from the donor or subsequent transplantation procedures, and nor should they be responsible for the care of any intended recipient of such tissues or cells. Health Authorities will set out the legal standards for determining that death has occurred and specify how the criteria and process for determining death will be formulated and applied.

All living donors should receive complete information before giving their consent. Moreover, this information must be impartial and, whenever possible, given by a treating physician who is not treating the recipient of the tissues or cells, in case of allogenic donation or non-partner donation in MAR. Scarcity of tissues and cells procured from living donors should never influence the decision to accept a donor into a programme. Also, financial incentives must not be used to encourage donations because they will render living donors more likely to consider repeat donations or to continue donating despite potential risks to their health. Furthermore, financial incentives may lead donors to not disclose all the information necessary for a complete and adequate donor selection, thus compromising the quality and safety of the donated tissues and cells.

1.8.3. Financial aspects of donation and human application of tissues and cells

Discussions around how best to increase the supply of human tissues and cells often focus on questions of donor motivation: specifically, how individuals may best be encouraged to donate different forms of bodily material. Nevertheless, it is essential to recall the Oviedo Convention which, in Article 21, clearly states that the human body and its parts must not, as such, give rise to financial gain. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [23] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation. This notion is reiterated in the additional protocol to that Convention, which also clearly states in its Article 21 that the human body and its parts must not, as such, give rise to financial gain or comparable advantage. The aforementioned provision does not prevent payments that do not constitute a financial gain or a comparable advantage, in particular:

a. compensation of living donors for loss of earnings and any other justifiable expenses caused by the removal or by the related medical examinations;
b. payment of a justifiable fee for legitimate medical or related technical services rendered in connection with transplantation;
c. compensation in cases of undue damage resulting from the removal of tissues or cells from living persons.

In the donation of any tissue or cell, removal of barriers to donation must not render a decision to donate non-altruistic. Initiatives that reduce the barriers to donation should only facilitate individuals in

carrying out an action that they were already inclined to take by concern for the welfare of the recipient.

In this sense, the Nuffield Council on Bioethics suggests distinguishing between two types of intervention, both of which aim to increase donation by changing its costs and benefits [24]. The first type is 'altruist-focused interventions', which typically involve removal of various disincentives to act and, in doing so, remove countervailing concerns that may hinder potential donors from acting on their altruistic motivations. For the purpose of this Guide, we will call these interventions 'compensation'. The second type is 'non-altruist-focused interventions', which are targeted at potential donors who have no strong motivation to help others through donation of their bodily material and who, therefore, if they are to donate, need to be provided with different reasons for action, perhaps in the form of payment or 'incentive' going well beyond the reimbursement of expenses. These incentives are particularly worrisome in the case of gamete donors, where they may change the donor's perception of the relative risks and benefits of a donation that is not free of potential health hazards and psychological consequences. In addition, gamete donation for treatment purposes presents further ethical implications because it involves the potential generation of a new human being.

In summary, voluntary unpaid donation, long promoted as the only ethical basis for donation of bodily material, should continue to have a central role in the donation process of any type of tissue or cell. Compensation to donors should cover only justifiable expenses and loss of income, and should not act as a direct or indirect incentive or inducement.

Physicians and other health professionals should not engage in transplantation procedures, and health insurers and other payers should not cover such procedures, if the tissues or cells concerned have been obtained through exploitation or coercion of, or payment to, the donor or the next of kin of a deceased donor.

Promotion of altruistic unpaid donation of human tissues or cells by means of advertisement or public appeal may be undertaken in accordance with domestic regulations. However, advertising the need for availability of tissues or cells with a view to offering or seeking financial gain or comparable advantage for the donor, or their next of kin where the individual is deceased, should be prohibited. Brokering that involves payment to such individuals or to third parties should also be prohibited.

Tissue establishments storing and supplying human tissues and cells have developed largely in response to the increasing demand for supplies of human tissues and cells for therapy and research. However, professional bodies should ensure that their guidelines reflect their members' responsibilities in the acquisition and supply of human tissue. Tissue establishments should operate on a non-profit basis. Tissues and cells should be supplied on an operational cost basis and no payment should ever exceed the justifiable fee for the services rendered; in other words, tissue establishments can claim a reasonable amount for certain expenses but should not quote an unfair amount greater than the actual cost in order to make profits. When calculating the operational costs of a tissue establishment, which may have a variety of funding sources, consideration should be given to the long-term sustainability of the tissue establishment. In order to do this, it is important to analyse the clinical need (for the different types of tissue that the tissue establishment will be processing and distributing) and to efficiently manage the tissue establishment's 'value chain', which includes the costs of procurement, processing, storage, distribution, personnel, transport, infrastructure and administration, and the need to incorporate state-of-the-art processes and equipment, among others.

The allocation of tissues and cells must be guided by clinical criteria and ethical norms, not financial or other considerations. Allocation rules, defined by appropriately constituted committees, should be equitable, based on clinical need, externally justified and transparent.

1.8.4. Equitable access to transplantation or to medically assisted reproduction

Healthcare in general is a human right because it secures and protects people's access to the normal range of opportunities and because it allows people to thrive. Given the importance of health for the general well-being of a person, every person, regardless of his/her income or financial means, should have access to decent healthcare.

Requests (the demand) for human tissues and cells may often exceed what is available (the supply). Significant practical and ethical questions of efficiency and fairness arise in deciding how to distribute these limited resources. Article 3 of the Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin states that transplantation systems must exist to provide equity in access to transplantation services for patients. Except in the case of direct donations, tissues and cells must be allocated among patients only in conformity with transparent, objective and duly justified rules ac-

cording to medical criteria. The persons or official bodies responsible for the allocation decision must be designated within this framework.

With regard to access to MAR, infertility treatment covers a broad range of 'causes' (e.g. age-related decline in fertility, male factors, blocked Fallopian tubes) and applications that cannot necessarily all be fitted into the same framework. The pivotal point in this discussion seems to be whether the desire for a child should be considered a fundamental need or a personal wish. Current regulatory frameworks in MAR are heterogeneous and, in some countries, still under development. The debate on ethical and social issues (including access to MAR for social indications, anonymity of gamete donors, genetic selection of donors, compensation for donation, posthumous reproduction or surrogacy) is ongoing. There is, however, general agreement that reproductive cloning must be forbidden.

Inclusion of infertility treatment in the basic healthcare tier is dependent upon the general level of welfare in society. Cultural, educational and religious backgrounds may also affect the availability of these therapies. Nevertheless, given the rightful claims of other types of healthcare and other fundamental needs in society (as well a limited availability of non-partner gametes and embryos), access cannot be expected to be unlimited. Ultimately, access to MAR should be considered in a structured way to include efficiency, safety and equity to avoid discrimination [25]. Some countries have opted to give public access to a fixed number of cycles/treatments for everyone, even if this means that those who need more treatments have to pay for it themselves.

Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking MAR treatment cross international borders to obtain reproductive treatment outside their country of residence. The reasons for travelling vary between countries, but the most common reason is access to certain treatments or techniques not legally allowed in the country of origin (e.g. non-partner donation treatment, pre-implantation genetic testing of embryos) or if a particular group is excluded from treatment (e.g. same-sex couples, single women, women above a certain age). There may be other limitations to access at home (e.g. long waiting lists). Further reasons for travelling may be better quality of care and less expensive treatment [26, 27].

The ideal situation is fair access to fertility treatment in the home country for all patients. This ideal should be promoted at all levels [27]. However, if for some reason treatment is not possible in the country of residence or not available, cross-border reproductive care may provide a solution. Furthermore, it is in accordance with the principle of freedom of movement of patients within Europe [28]. However, crossing borders may also lead to increased shortage of scarce resources in the visited country and to the detriment of local citizens. Health Authorities may want to introduce a system for fair allocation of scarce resources that takes into account local needs, such as a maximum number (or percentage) of treatments provided to foreign patients. In addition, cross-border reproductive care should always follow the same strict quality and safety criteria that govern domestic treatments, including appropriate traceability and biovigilance measures that cover both recipients and their children born as a result of the MAR treatment.

1.8.5. Equity in donation

Individual motivation and choice is only one part of the donation picture; the central role of organisations, organisational procedures and professionals in facilitating donation should not be underestimated, nor indeed the importance of trust in these systems. An example of such organisational aspects is that, whenever a person dies in circumstances where donation is a possibility, this should be raised with their family.

The role of the state with respect to donation should be understood as one of stewardship: that is, actively promoting measures that will improve general health (thereby reducing the demand for some forms of bodily material) and facilitating donation. Such a stewardship role should extend to taking action to remove inequalities that affect disadvantaged groups or individuals with respect to donation. Equity in donation refers to the absence of systematic disparities in the burden of donation between social groups who have different levels of underlying social advantage or disadvantage (i.e. different positions in a social hierarchy). Inequities in donation would, in a systematic manner, put groups of people who are already socially disadvantaged (e.g. by virtue of being poor, female and/or members of a particular racial, ethnic or religious group) at further disadvantage with respect to their health.

As discussed above, introduction of financial incentives for donation in the field of MAR renders certain social groups particularly susceptible to disparities based on social and economic status.

With respect to cross-border care, safeguards must be in place to guarantee that all donors, regardless their origin, receive similar care and follow-up. To prevent abuse of donors coming from abroad, the

use of intermediate agencies – which may lead to violations of the rules of good clinical practice and, in the worst-case scenario, to trafficking – should be avoided. Post-donation care must be provided to the best possible standards at home or abroad.

1.8.6. Anonymity

The identity of the donor and recipient should (except in the case of donation between persons having a close personal relationship) be maintained in strict confidentiality. Such precautions will prevent abuse and protect the families of donors and recipients from feelings of anxiety associated with emotional involvement, obligation to return favours or guilt.

In the specific case of MAR, different regulations are applied in different member states with regard to the anonymity of non-partner donors. Debate has focused around the donor's right to anonymity, the welfare of the resulting offspring and his/her right to family life, and the effect of removal of donor anonymity on the supply of gametes for treatment. Presently, some countries require that donors always remain anonymous, whereas other countries require that their identity might be known only in exceptionally urgent medical situations. Some countries allow the possibility of the offspring gaining access to non-identifying information about the donor (e.g. hair colour, ethnicity). Other countries allow the offspring to contact donors after the offspring has reached a certain age. Hybrid models exist in some countries. A common standard seems to be that donors do not have the right to information about children generated from their gametes (unless the child chooses and is legally allowed to obtain information about the donor).

1.8.7. Transparency

The organisation and execution of activities based on donation and human application, as well as their clinical results, must be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are protected (if relevant).

Transparency can be achieved by maintaining public access to regularly updated comprehensive data on processes, in particular to data on donation and banking activities, allocation, distribution and import/export, demand for tissues and cells, human applications and the outcomes for recipients, living donors and children born as a result of MAR procedures, as well as data on organisation, budgets and funding. Such transparency is not inconsistent with shielding (from public access) information that could identify individual donors or recipients, while still respecting the requirement of traceability. The objective of the system should be not only to help meet the goal of self-sufficiency for countries or regions and to maximise the availability of data for scholarly study and governmental supervision to allow determination of clinical outcomes and efficacy of treatments but also to identify risks (and facilitate their mitigation) to minimise harm to donors and recipients.

1.9. Recommendations and regulations in the field

1.9.1. Council of Europe

The Council of Europe, based in Strasbourg (France), is an international organisation that promotes co-operation between all European countries in the areas of human rights, democracy, rule of law, culture and public health. After the 3rd Conference of European Health Ministers on the Ethical, Organisational and Legislative Aspects of Organ Transplantation [29], held in Paris in 1987, the Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation (SP-CTO) was created. This committee consisted of experts in different aspects of transplantation: immunologists, surgeons and physicians, as well as co-ordinators and representatives from organ-sharing and organ-procurement organisations. In 2007, the secretariat responsible for activities related to organs, tissues and cells was transferred to the European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe [30], and the newly appointed CD-P-TO took over as the steering committee [31].

Today, the CD-P-TO is composed of internationally recognised experts from Council of Europe member states, observer countries, the European Commission and the WHO, as well as representatives from the Committee on Bioethics of the Council of Europe (CD-BIO) and several professional societies and non-governmental organisations. It actively promotes the non-commercialisation of organ donation, the fight against organ trafficking, the development of ethical, quality and safety standards in the field of organs, tissues and cells, and the transfer of knowledge and expertise between member states and organisations.

Within the framework principle of sharing knowledge through international co-operation, the Council of Europe has established widely recognised recommendations and resolutions in the field of transplantation, covering the ethical, social, scientific

and training aspects of the donation and transplantation of organs, tissues and cells [32]. Whereas agreements and conventions are binding on the states that ratify them, resolutions and recommendations are policy statements to governments that propose a common course of action to be followed.

The Council of Europe Convention for the Protection of Human Rights and Fundamental Freedoms (European Treaty Series, No. 5) [33] is an international treaty to protect human rights and fundamental freedoms in Europe. It was drafted in 1950 by the then newly formed Council of Europe and came into force on 3 September 1953.

The European Agreement on the Exchange of Therapeutic Substances of Human Origin (European Treaty Series, No. 26) [34], signed in Paris on 15 December 1958, aims to provide mutual assistance with respect to the supply of therapeutic substances of human origin.

The European Agreement on the Exchange of Tissue-Typing Reagents (European Treaty Series, No. 84) [35], signed in Strasbourg on 17 September 1974, lays the groundwork for development of mutual assistance in the supply of tissue-typing reagents and the establishment of joint rules between signatory parties. The signatory parties undertake to make reagents available to other parties who are in need of them, by the most direct route, subject to the condition that no profit is made on them, that they must be used solely for medical and scientific purposes and are free of import duties. The Additional Protocol (European Treaty Series, No. 89) [36], which was opened for signature on 24 June 1976 and came into force on 23 April 1977, provides for the accession of the European Community to this agreement.

The Oviedo Convention – the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (European Treaty Series, No. 164) [15], which was opened for signature on 4 April 1997 and came into force on 1 December 1999 – is the first legally binding international text designed to preserve human dignity, fundamental rights and freedoms, through a series of principles against the misuse of biological and medical applications. The Convention is inspired by the principle of the primacy of human beings over the sole interest of science or society. It lays down a series of principles applying to medical practice as well as biomedical research, organ transplantation and genetics. The Convention includes the principle of consent, non-discrimination on the basis of genetic characteristics, and protection of private life and access to information. The Convention specifically prohibits any financial gain from the body and its parts, as such. The Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors [23] provides further guidance on how to interpret Article 21 of the Oviedo Convention in order to facilitate its implementation.

The Oviedo Convention was extended by an Additional Protocol to the Convention on Human Rights and Biomedicine concerning transplantation of organs and tissues of human origin (European Treaty Series, No. 186) [16], which was opened for signature on 24 January 2002 in Strasbourg and came into force on 1 May 2006. This additional protocol aims to protect the dignity and identity of everyone and to guarantee, without discrimination, respect for his/her integrity and other rights and fundamental freedoms with regard to the transplantation of organs and tissues of human origin, thereby establishing principles for the protection of donors and recipients. However, the additional protocol does not apply to gametes and embryos.

The Council of Europe Convention on Action against Trafficking in Human Beings (European Treaty Series, No. 197) [37], which was opened for signature in Warsaw on 16 May 2005 and came into force on 1 February 2008, alongside its Explanatory Report, addresses the trafficking of human beings for the purpose of organ removal.

The Council of Europe/United Nations joint study on Trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs [38], presented at the United Nations headquarters in New York on 13 October 2009, focuses on trafficking in organs, tissues and cells for the purpose of transplantation. The joint study made it evident that existing criminal-law instruments dealing exclusively with trafficking in human beings (including for the purpose of organ removal) left loopholes that allowed several unethical transplant-related activities to persist. This is why the Council of Europe decided to undertake the task of drafting a new international legally binding instrument against trafficking in human organs.

The Council of Europe Convention against Trafficking in Human Organs (European Treaty Series, No. 216), with its Explanatory Report [38], adopted by the Committee of Ministers on 9 July 2014, identifies distinct activities that constitute 'trafficking in human organs'. The central concept is 'the illicit removal of organs', which consists of removal without the free, informed and specific consent of a living donor; removal from a deceased donor other than as authorised under domestic law; removal when, in exchange, a living donor (or a third party)

has been offered or received a financial gain or comparable advantage; or removal from a deceased donor when a third party has been offered or received a financial gain or comparable advantage.

Other major resolutions and recommendations in the field of tissues and cells include:

- Resolution (78) 29 of the Committee of Ministers on Harmonisation of legislation of member states relating to removal, grafting and transplantation of human substances [18];
- Recommendation No. R (94) 1 of the Committee of Ministers to member states on human tissue banks [39];
- Recommendation No. R (98) 2 of the Committee of Ministers to member states on provision of haematopoietic progenitor cells [40];
- Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous cord blood banks [41];
- Recommendation Rec (2006) 4 of the Committee of Ministers to member states on research on biological materials of human origin [42];
- Recommendation CM/Rec (2020) 5 on the quality and safety of tissues and cells for human application [43];
- Recommendation CM/Rec (2020) 6 on establishing harmonised measures for the protection of haematopoietic progenitor cell donors [44].

Monitoring of practices in member states has become an evident need for the sake of transparency and international benchmarking. Keeping this goal in mind, since 1996 the EDQM/Council of Europe has published *Newsletter Transplant* [45], which is co-ordinated by the Organización Nacional de Trasplantes (ONT) in Spain. This publication summarises comprehensive data (provided by national focal points designated by governments) on donation and transplantation activities, management of waiting lists, organ-donation refusals and authorised centres for transplantation activities. *Newsletter Transplant* provides information from ≈ 70 countries, including Council of Europe member states, observer countries and observer networks (e.g. Iberoamerican Donation and Network Council on Organ Donation and Transplantation, Mediterranean Network). The *Newsletter Transplant* database is connected with other international projects on data collection (e.g. WHO Global Observatory on Organ Donation and Transplantation, Eurocet database) to avoid duplication of efforts. *Newsletter Transplant* has evolved into a unique official source of information that continues to inspire policies and strategic plans worldwide.

Achieving self-sufficiency based on voluntary unremunerated donation and security of supply, as well as timely and equitable access to safe transplantation, are important national and European goals. To ensure rational, fair, timely and equitable access to safe therapies based on the use of tissues and cells of human origin, governments need to know how many tissues and cells are available and how many are required for their populations so the transplantation requirements can be consistently met and an appropriate level of funding to support donation programmes is maintained. This information is also essential in order to avoid over-reliance on a limited number of providers or countries, as well as to prepare for risk scenarios that might affect supply. Furthermore, having accurate activity data is also essential in order to put into context the figures related to serious adverse events and reactions related to the use of tissues and cells of human origin. In an attempt to streamline and harmonise data-collection exercises in Europe, in the framework of a co-operation Grant Agreement between the European Commission and the EDQM/Council of Europe and with the support of the main professional societies and existing registries in the field, the EDQM co-ordinated the identification of a minimum dataset that, if regularly collected by member states, would serve the abovementioned objectives [46]. The exercise entailed reaching an agreement on the parameters, units and expected quality of the data to be collected, as well as making recommendations on who should be accountable for the collection and validation of this data and ensure dissemination among all relevant stakeholders.

The Council of Europe also produces other guidelines, including this *Guide to the quality and safety of tissues and cells for human application*, the *Guide to the quality and safety of organs for transplantation* and the *Guide to the preparation, use and quality assurance of blood components* [47].

1.9.2. World Health Organization

In 1987, the 40th World Health Assembly, concerned about the trade for profit in human organs, initiated preparation of the first WHO Guiding Principles on transplantation, endorsed by the Assembly in 1991 in resolution WHA 44.25 [48]. These Guiding Principles have greatly influenced professional codes and practices, as well as legislation, around the world for almost two decades. After a consultation that took several years, on 21 May 2010 the 63rd World Health

Assembly adopted resolution WHA 63.22 [49], which endorsed the updated WHO Guiding Principles on human cell, tissue and organ transplantation [19] and called on WHO member states to implement these Guiding Principles, promote voluntary and unremunerated donation, oppose trafficking and promote transparent and equitable allocation. It also urged its members to strengthen oversight, to collect and publish activity data, including adverse events and reactions, and to implement globally standardised coding. These WHO guidelines are intended to provide an orderly, ethical and acceptable framework for the acquisition and transplantation of human cells, tissues and organs for therapeutic purposes.

The World Health Assembly adopted resolution WHA 57.18 [50] in 2004, which urged WHO member states 'to take measures to protect the poorest and vulnerable groups from transplant tourism and the sale of tissues and organs, including attention to the wider problem of international trafficking in human tissues and organs'. Subsequently, the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [20] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and the International Society for Nephrology (ISN). In 2018 it was updated to incorporate revised definitions and briefer and more comprehensive principles to provide up-to-date guidance and practical advice for health professionals, policy makers and law-enforcement authorities. The declaration emphasises that organ trafficking and transplant tourism should be prohibited because they violate the principles of equity, justice and respect for human dignity, targeting impoverished and otherwise vulnerable donors and inexorably leading to inequity and injustice.

United Nations Resolution 71/322, adopted by the WHO General Assembly on 8 September 2017, aims at strengthening and promoting effective measures and international co-operation on organ donation and transplantation to prevent and combat trafficking in persons for the purpose of organ removal and trafficking in human organs [51].

Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance and surveillance worldwide. For this reason, Resolution WHA 63.22 [49] also urged WHO member states to collaborate in collecting data (including adverse events and reactions) in addition to implementation of globally consistent coding systems. The NOTIFY project was a specific follow-up action that was led by the Italian National Transplant Centre (CNT) and WHO to promote the sharing of information on adverse incidents for improving safety and efficacy [52].

As a result of resolutions WHA 57.18 and WHA 63.22 (which requested that global data on the practice, safety, quality, efficacy and epidemiology of transplantations be collected in the WHO member states that have transplantation programmes), an international watchdog on transplantation was set up as a collaborative initiative between the Spanish ONT and WHO, and was termed the Global Observatory on Donation and Transplantation [53]. The universal availability of these data is recognised as a prerequisite for global improvements in demonstrating transparency, equity and compliance, and for monitoring systems in countries. In addition, the data provided also help to give an overview of the legal and organisational aspects in very different settings and countries, which enables the regulating bodies to monitor transplantation activities.

The WHO has also published two *aide-mémoires* specifically on the donation and transplantation of tissues and cells [1, 54].

In recent years, the WHO has been promoting use of the term 'medical products of human origin' (MPHO). This category includes blood, organs, tissues, bone marrow, cord blood, reproductive cells and milk derived from humans for therapeutic use. Use of these MPHO, obtained from living and deceased donors, entails practical, scientific and ethical considerations.

1.9.3. European Union

1.9.3.1. *EU tissues and cells legislation*

Article 168 (4)(a) of the Treaty on the Functioning of the European Union [55] (previously Article 152 of the Treaty of Amsterdam) gives the EU a mandate to establish high quality and safety standards for substances of human origin, such as blood, organs, tissues and cells.

Acknowledging that the human application of tissues and cells is an expanding medical field that offers important opportunities for the treatment of disease, the EU aims for a common approach to the regulation of tissues and cells across Europe, in particular to promote cross-border exchanges where that can improve patient access.

The EU tissue and cells directives have created a benchmark for the standards that must be met if carrying out any activity involving tissues and cells for human application, including gametes, embryos and reproductive tissue. The directives also require that systems be put in place to ensure that all the tissues and cells used in human applications are traceable from donors to recipients and vice versa.

Directive 2004/23/EC [56] of the European Par-

liament and of the Council of 31 March 2004 applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human use (including reproductive cells used in ART procedures). The directive introduced obligations on EU member states' authorities, from supervision of human tissue and cell procurement and authorising and inspecting tissue establishments, to ensuring traceability and vigilance and maintaining a publicly accessible register of national tissue establishments. It also lays down rules on donor selection and evaluation (e.g. principles governing tissue and cell donation, consent, data confidentiality) and quality and safety of tissues and cells (e.g. quality management, tissue and cell reception, processing and storage conditions).

Commission Directive 2006/17/EC [57] established specific technical requirements for each step in the human tissue and cell preparation process, in particular the requirements for procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, tissue and/or cell donation, the procurement and reception procedures at tissue establishments and the requirements for direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by Commission Directive 2012/39/EU with regard to certain technical requirements for the testing of human tissues and cells [58].

Commission Directive 2006/86/EC [59] includes traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

In 2015, two new Commission directives were adopted, one an implementing directive on the procedures for verifying equivalent standards of quality and safety of imported tissues and cells (Directive 2015/566) [60] and a second one amending Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells (Directive 2015/565) [61].

Quality and safety standards for human organs intended for transplantation are laid down in Directive 2010/53/EU and Commission Implementing Directive 2012/25/EU. These detail the standards and procedures for information exchange between EU member states regarding human organs intended for transplantation [62, 63].

The EU directives oblige EU member states to encourage voluntary and unpaid donation of tissues and cells and to endeavour to ensure that the procurement of tissues and cells is carried out on a non-profit basis. Promotion and publicity activities in support of the donation of human tissues and cells with a view to offering or seeking financial gain or comparable advantage are not allowed. The EU directives also provide clear provisions on the information to be given to donors for the purposes of obtaining consent and the anonymity of all personal data collected, and instruct EU member states to adopt measures to ensure data security and prevent unauthorised modifications to files and records.

These directives do not cover laboratory research using human tissues and cells (e.g. *in vitro* research or research using animal models) and do not interfere with the decisions of EU member states on the use or non-use of any specific type of human cell, including embryonic stem cells. Similarly, these directives do not interfere with provisions of member states defining the legal term 'person' or 'individual'.

As from 25 May 2018, the new General Data Protection Regulation 2016/679 (GDPR) entered into force, repealing Directive 95/46/EC [64]. The regulation is directly applicable in all EU member states and regulates the processing by an individual, a company or an organisation of personal data relating to individuals in the EU. This directive applies to companies or organisations that process personal data as part of the activities of one of its branches established in the EU or, to a company or organisation established outside the EU, processing data of data subjects in the EU in the context of offering products or services in the EU, or monitoring behaviour of data subjects in the EU. It does not apply, however, to the processing of personal data of deceased persons, anonymised data or legal persons.

In 2019, the EU published an evaluation of the blood and tissues and cells legislation that aimed to assess whether the legislation had achieved its original objectives and whether it was still fit for purpose in light of the significant and technological developments in the sector [65]. To address the identified gaps and shortcomings identified in that evaluation, the Commission is undertaking a revision of the EU legislation in this field to ensure the framework is up-to-date, fit for purpose and future-proof [66]. The initiative aims at updating the legislation in the direction of a more flexible alignment with scientific and technological developments by removing from legislation many technical provisions, allowing for a faster update of standards. In addition, the revision aims to strengthen donor protection and oversight by authorities, to facilitate innovation in the field and to support measures in member states to achieve sufficiency and sustainability of supply. A comprehensive impact assessment of policy options has been con-

ducted to inform the revision and will be published together with the Commission proposal for revised legislation. The revision proposal is planned to be adopted by the European Commission in 2022. The adoption will be followed by debates in the European Parliament and the European Council to reach agreement on the text that will finally come into force.

In addition to this legislative work, the European Commission has, throughout the years, supported EU member states in their efforts to implement EU directives on tissues and cells by providing funding for several projects under the Programme of Community Action in the Field of Health [67]. Some of the projects supported include:

- EQSTB (European Quality System for Tissue Banking) focused on four main work packages: (i) identification of the key requirements for tissue banking; (ii) development of a registry to support exchange of tissues; (iii) provision of training programmes, both online and face-to-face, to fulfil the needs of tissue establishment professionals, and (iv) development of an audit model and audit guide for tissue establishments, with recommendations for tissue establishments and guidance for auditors.
- Eustite (European Standards and Training in the Inspection of Tissue Establishments) [68] developed guidance and training courses for EU competent authorities on the inspection of tissue establishments and on vigilance procedures for tissues and cells used in transplantation and in assisted reproduction. The guidance document served as a basis for the guidelines on implementation of inspection and control measures in the field of human tissues and cells included in Commission Decision 2010/453/EU of 3 August 2010.
- Poseidon (Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation Organisation and Networking in unrelated haematopoietic stem-cell transplantation in Europe) provided recommendations for improvements in the safety of unrelated haematopoietic progenitor cell transplantation, for the optimisation of human stem-cell donation policy, and for promoting equal access to this therapy throughout the EU.
- Eurocet [69] is a platform that was funded initially by the European Commission but is now maintained by the Italian National Transplant Centre. It collects and publishes annual activity data on donation, processing and human applications of tissues and cells. However, the Tissue Establishment Registry has been temporarily suspended in order to avoid confusion with the official EU Tissue Establishment Compendium.
- EuroGTP (European Good Tissue Practices) [70] developed a guide to good tissue practices and personnel training guidelines for tissue establishments on the recovery, processing and preservation of tissues, to ensure that all tissue establishments guarantee the highest level of quality and safety of tissues for human application. EuroGTP has provided a crucial basis for much of the technical content of this Guide. A strong collaboration between the European Association of Tissue and Cell Banks (EATCB), which will update and maintain the GTPs as their own standards, and the Council of Europe will be maintained to ensure consistency and development in the light of the most up-to-date scientific knowledge.
- The project SoHO V&S (Vigilance and Surveillance of Substances of Human Origin) [71] addressed the harmonisation of terminology and documentation relating to adverse events and reactions. It aimed to find a consensus on how information should be exchanged between EU member states, the European Commission and third countries to enhance efficient management of incidents involving cross-border distribution of tissues and cells. The project drafted important guidance documents for the EU competent authorities, on the detection and investigation of suspected illegal and/or fraudulent activity related to tissues and cells, the communication and investigation of serious adverse events and reactions associated with human tissues and cells, and vigilance and surveillance in the field of assisted reproductive technologies. The project also prepared a guidance document for healthcare professionals on vigilance and surveillance of human tissues and cells. It also provided a training model for competent authorities in the investigation and management of vigilance and surveillance of tissues and cells.
- The joint action Arthiqs (Good Practice on Donation, Collection, Testing, Processing, Storage and Distribution of Gametes for Assisted Reproductive Technologies and Haematopoietic Stem Cells for Transplantation) [72], launched in 2014, was a three-year project to build institutional and inspection guidelines for assisted reproductive technologies as well as guidelines related to the set-up and regulation of haematopoietic stem-cell donor follow-up registries and banking of cord blood.

- The joint action VISTART (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) [73], launched in 2015, aimed at promoting and facilitating the harmonisation of inspection, authorisation and vigilance systems for blood transfusion and tissues and cells for human application.
- The project EuroGTP-II (Good Tissue Practices for demonstrating safety and quality through recipient follow-up) [74], launched in 2016, aimed at developing technical guidance to assess the quality and safety of novel tissue and cell therapies and demonstrating their efficacy based on recipients' outcomes.
- The joint action European Cornea and Cell Transplant Registry (ECCTR), launched in 2016, aimed to develop a common assessment methodology, based on the three existing European corneal transplant registries in the Netherlands, Sweden and the UK, and establish a web-based European registry to assess and verify the safety, quality and efficacy of ocular tissue transplantation [75].
- The EU-funded project TRANSPOSE (TRANSfusion and transplantation: PrOtection and SElection of donors) [76] was launched in 2017 and aimed at harmonising European donor selection and protection policies, while maintaining adequate health and safety protection of the recipient.
- The joint action GAPP (Facilitating the Authorisation of Preparation Process for blood, tissues and cells) [77] was launched in 2018 and aimed at facilitating the development of a common and optimal approach to assess and authorise preparation processes in blood and tissue establishments, adapting requirements as prescribed by Article 29 of Directive 2002/98/EC and Article 28 of Directive 2004/23/EC.

These projects have strengthened collaboration among Health Authorities, and between these Health Authorities and the professional associations in the area of tissues and cells for human application, allowing continuous input from field practice into the regulatory framework.

1.9.3.2. *Other relevant EU legislation*

Cells and tissues that are substantially manipulated or used for a different essential function can be classified as a gene therapy medicinal product, a somatic cell therapy medicinal product or a tissue-engineered product.

Activities involving such cells and/or tissues have to comply with Regulation (EC) No. 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products (the 'ATMP Regulation') [78, 79] (see Appendix 41 for additional information). This Guide is not applicable to such activities, other than aspects related to donation, procurement and testing of the cells/tissues. Clinical trials of ATMPs must comply with the EU framework on clinical trials (Regulation (EU) No. 536/2014) [80]. Donation, procurement and testing of the tissues and/or cells must comply with the quality and safety standards laid down in Directive 2004/23/EC and its implementing directives.

In 2017, two new regulations on medical devices were adopted and entered into force. Regulation (EU) 2017/745 of the European Parliament and of the Council, on medical devices, amends Directive 2001/83/EC, Regulation (EC) No. 178/2002 and Regulation (EC) No. 1223/2009 and repeals Council Directives 90/385/EEC and 93/42/EEC. The revised requirements apply to medical devices combined with tissues and cells and to medical devices incorporating non-viable derivatives of human tissues or cells, in particular human collagen. Regulation (EU) 2017/746 of the European Parliament and of the Council on *in vitro* diagnostic medical devices repeals Directive 98/79/EC and Commission Decision 2010/227/EU [81].

1.10. References

1. Access to safe and effective cells and tissues for transplantation, World Health Organization, available at www.who.int/transplantation/cell_tissue/en/, accessed 18 March 2022.
2. Council of Europe blood transfusion and organ transplantation guides, https://freepub.edqm.eu/publications, accessed 18 March 2022.
3. Apperley J, Carreras E, Gluckman E, Masszi T, eds. *EBMT handbook on haematopoietic stem cell transplantation*. Barcelona, Spain: European Group for Blood and Marrow Transfusion, 2012, available at https://statistics.wmda.info/, accessed 18 March 2022.
4. Tzakis AG, Ricordi C, Alejandro R *et al.* Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 1990;336(8712): 402-5.
5. Shapiro AMJ, Lakey JR, Ryan EA *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000;343(4):230-8.
6. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978;2(8085):366.

7. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990;344(6268):768-70.
8. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;340(8810): 17-18.
9. Adamson GD, de Mouzon J, Chambers GM *et al.* International Committee for Monitoring Assisted Reproductive Technology: world report on Assisted Reproductive Technology 2011. *Fertil Steril* 2018;Nov 110(6):1067-81. https//doi.org/10.1016/j.fertnstert.2018.06.039.
10. Parry B. Entangled exchange: reconceptualising the characterisation and practice of bodily commodification. *Geoforum* 2008;39(3):1133-44.
11. American Association of Tissue Banks. *Standards of tissue banking*, 14th edn. McLean VA, USA: American Association of Tissue Banks, 2012.
12. OECD. *Glossary of statistical term*s, available at http://stats.oecd.org/glossary/, accessed 18 March 2022.
13. PRIVILEGED Project. Determining the ethical and legal interests in privacy and data protection for research involving the use of genetic databases and bio-banks, available at https://cordis.europa.eu/project/id/36775/it, accessed 18 March 2022.
14. National Cancer Institute, *Dictionary of cancer terms*, available at www.cancer.gov/dictionary?cdrid=561323, accessed 18 March 2022.
15. Council of Europe (1997) Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: [the Oviedo] Convention on Human Rights and Biomedicine, available at http://conventions.coe.int/Treaty/en/Treaties/Html/164.htm, accessed 18 March 2022.
16. Council of Europe (2002) Additional Protocol to the Convention on Human Rights and Biomedicine, on transplantation of organs and tissues of human origin, available at http://conventions.coe.int/Treaty/en/Treaties/Html/186.htm, accessed 18 March 2022.
17. Charter of Fundamental Rights of the European Union (2012), available at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:12012P/TXT&from=EN, accessed 6 July 2022.
18. Council of Europe Committee of Ministers Resolution (78) 29 on harmonisation of legislations of member states relating to removal, grafting and transplantation of human substances, available at www.coe.int/t/dg3/healthbioethic/texts_and_documents/Res (78) 29E.pdf, accessed 18 March 2022.
19. World Health Organization (2010), WHO Guiding Principles on human cell, tissue and organ transplantation, available at https://apps.who.int/iris/handle/10665/341814, accessed 29 November 2022.
20. The Declaration of Istanbul on Organ Trafficking and Transplant Tourism (2018), available at www.declarationofistanbul.org, accessed 18 March 2022.
21. Barcelona Principles on the use of human donated tissue for ocular transplantation, research and future technologies, available at www.gaeba.org/wp-content/uploads/2018/05/GAEBA-2018-The-Barcelona-Principles-FINAL.pdf, accessed 18 March 2022.
22. Truog RD, Miller FG. The dead donor rule and organ transplantation. *N Engl J Med* 2008;359(7):674-5.
23. Council of Europe (2018). Guide for the implementation of the principle of prohibition of financial gain with respect to the human body and its parts from living or deceased donors, available at https://rm.coe.int/guide-financial-gain/16807bfc9a, accessed 18 March 2022.
24. Report from the Nuffield Council on Bioethics. *Human bodies: donation for medicine and research*, available at http://nuffieldbioethics.org/project/donation, accessed 18 March 2022.
25. Pennings G, de Wert G, Shenfield F *et al.* ESHRE Task Force on Ethics and Law 14: Equity of access to assisted reproductive technology. *Hum Reprod* 2008a; 23(4):772-4, available at www.eshre.eu/Specialty-groups/Special-Interest-Groups/Ethics-and-Law/Documents-of-the-Task-Force-Ethics-Law.aspx, accessed 18 March 2022.
26. Shenfield F, de Mouzon J, Pennings G *et al.* ESHRE Taskforce on Cross Border Reproductive Care. Cross border reproductive care in six European countries. *Hum Reprod* 2010;25(6):1361-8.
27. Pennings G, de Wert G, Shenfield F *et al.* ESHRE Task Force on Ethics and Law 15: Cross-border reproductive care. *Hum Reprod* 2008;23(10):2182-4.
28. Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross-border healthcare, available at https://eur-lex.europa.eu/eli/dir/2011/24/oj, accessed 7 December 2018.
29. Conclusions of the Third Conference of European Health Ministers (1987), 16-17 November 1987, available at https://rm.coe.int/CoERMPublicCommonSearchServices/DisplayDCTMContent?documentId=0900001680 4c6d07, accessed 18 March 2022.
30. European Directorate for the Quality of Medicines & HealthCare (EDQM) [website], available at www.edqm.eu, accessed 18 March 2022.
31. European Committee on Organ Transplantation (CD-P-TO) [website], available at www.edqm.eu/en/organisation-work-programme-organs-tissues-cells, accessed 29 November 2022.

32. Council of Europe Conventions, Resolutions, Recommendations and Reports in the field of organs, tissues and cells, available at www.edqm.eu/en/organ-transplantation-recommendations-resolutions-74.html, accessed 18 March 2022.
33. Convention for the Protection of Human Rights and Fundamental Freedoms, available at http://conventions.coe.int/treaty/en/treaties/html/005.htm, accessed 18 March 2022.
34. European Agreement on the Exchange of Therapeutic Substances of Human Origin, available at http://conventions.coe.int/treaty/en/treaties/html/026.htm, accessed 18 March 2022.
35. European Agreement on the Exchange of Tissue-Typing Reagents, available at http://conventions.coe.int/treaty/en/treaties/html/084.htm, accessed 4 December 2018.
36. Additional Protocol to the European Agreement on the Exchange of Tissue-Typing Reagents, available at http://conventions.coe.int/treaty/en/treaties/html/089.htm, accessed 18 March 2022.
37. Council of Europe Convention on Action against Trafficking in Human Beings and its Explanatory Report, available at http://conventions.coe.int/treaty/en/treaties/Html/197.htm, accessed 18 March 2022.
38. Council of Europe/United Nations [joint study on] Trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs, available at www.edqm.eu/medias/fichiers/Joint_Council_of_EuropeUnited_Nations_Study_on_tra1.pdf; Executive Summary available at www.edqm.eu/medias/fichiers/Executive_summary_of_the_Joint_Council_of_EuropeUn.pdf, accessed 18 March 2022.
39. Council of Europe Committee of Ministers Recommendation No. R (94) 1 to member states on human tissue banks, available at https://rm.coe.int/0900001680 4dd7ee, accessed 18 March 2022.
40. Council of Europe Committee of Ministers Recommendation No. R (98) 2 to member states on provision of haematopoietic progenitor cells, available at https://search.coe.int/cm/Pages/result_details.aspx?ObjectID=09000016804f8a57, accessed 18 March 2022.
41. Council of Europe Committee of Ministers Recommendation Rec (2004) 8 to member states on autologous cord blood banks, available at www.edqm.eu/sites/default/files/recommendation_no_2004_8_of_the_committee_of_ministers_to_member_states_on_autologous_cord_blood_banks.pdf, accessed 18 March 2022.
42. Recommendation Rec (2006) 4 of the Committee of Ministers to member states on research on biological materials of human origin and its Explanatory Memorandum, available at www.coe.int/t/dg3/healthbioethic/Activities/10_Biobanks/Rec (2006) 4%20EM%20E.pdf, accessed 9 February 2022.
43. Council of Europe Committee of Ministers Recommendation CM/Rec (2020) 5 on the quality and safety of tissues and cells for human application, available at https://search.coe.int/cm/Pages/result_details.aspx?ObjectId=0900001680 9fdcde, accessed on 9 February 2022.
44. Council of Europe Committee of Ministers Recommendation CM/Rec (2020) 6 on establishing measures for the protection of haematopoietic progenitor cell donors, available at https://rm.coe.int/0900001680 9fdd5b, accessed on 9 February 2022.
45. European Directorate for the Quality of Medicines & HealthCare, *Newsletter Transplant*, available at https://freepub.edqm.eu/publications/PUBSD-87/detail, accessed 9 February 2022.
46. Harmonising activity data collection exercises in the field of tissues and cells in Europe, available at www.edqm.eu/en/transplantation-areas-work#Harmonisation-of-data-collection, accessed 9 February 2022.
47. Council of Europe technical guides, available at https://freepub.edqm.eu/, accessed 9 February 2022.
48. World Health Assembly (1991), Human organ transplantation: WHA44.25, available at www.transplant-observatory.org/download/resolution-wha44-25-endorsing-the-1991-guiding-principles/, accessed 18 March 2022.
49. World Health Assembly (2010), Human organ and tissue transplantation: WHA63.22, available at http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_R22-en.pdf, accessed 18 March 2022.
50. World Health Assembly (2004), Human organ and tissue transplantation: WHA57.18, available at http://apps.who.int/gb/ebwha/pdf_files/WHA57/A57_R18-en.pdf, accessed 18 March 2022.
51. United Nations Resolution 71/322: Strengthening and promoting effective measures and international cooperation on organ donation and transplantation to prevent and combat trafficking in persons for the purpose of organ removal and trafficking in human organs, available at www.edqm.eu/sites/default/files/who_res71-322_e-september2017.pdf, accessed 18 March 2022.
52. Notify: Exploring vigilance notification for organs, tissues and cells, available at www.transplant-observatory.org/download/notify-exploring-vigilance-notification-for-organs-tissues-and-cells-report-february-2011/, accessed 18 March 2022.
53. Global Observatory on Donation and Transplantation,

available at www.transplant-observatory.org, accessed 9 February 2022.

54. *Aide-mémoire* on key safety requirements for essential minimally processed human cells and tissues for transplantation, available at www.who.int/transplantation/cell_tissue/en/, accessed 18 March 2022.

55. Treaty on the Functioning of the European Union, available at http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:12012E/TXT&from=EN, accessed 18 March 2022.

56. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, available at http://data.europa.eu/eli/dir/2004/23/oj, accessed 18 March 2022.

57. Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells, available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF, accessed 18 March 2022.

58. Commission Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32012L0039, accessed 18 March 2022.

59. Directive 2006/86/EC as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32006L0086, accessed 18 March 2022.

60. Commission Directive (EU) 2015/566 of 8 April 2015 implementing Directive 2004/23/EC as regards the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32015L0566, accessed 18 March 2022.

61. Commission Directive (EU) 2015/565 of 8 April 2015 amending Directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells, available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2015.093.01.0043.01.ENG, accessed 18 March 2022.

62. Directive 2010/53/EU of the European Parliament and of the Council of 7 July 2010 on Standards of quality and safety of human organs intended for transplantation [wrongly titled as 2010/45], available at https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32010L0053, accessed 18 March 2022.

63. Commission Implementing Directive 2012/25/EU laying down information procedures for the exchange between member states of human organs intended for transplantation, available at http://ec.europa.eu/health/blood_tissues_organs/docs/organs_impl_directive_2012_en.pdf, accessed 18 March 2022.

64. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation), available at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0679&from=EN, accessed 9 February 2022.

65. Evaluation of the EU blood and tissues and cells legislation, available at https://ec.europa.eu/health/blood-tissues-cells-and-organs/overview/evaluation-eu-blood-and-tissues-and-cells-legislation_en, accessed 9 February 2022.

66. Revision of the EU legislation on blood, tissues and cells, available at https://ec.europa.eu/health/blood-tissues-cells-and-organs/overview/revision-eu-legislation-blood-tissues-and-cells_en, accessed 9 February 2022.

67. The EU's Second Programme of Community Action in the Field of Health, 2008-2013, available at www.eubusiness.com/topics/health/programme, accessed 18 March 2022.

68. Eustite Project, available at www.notifylibrary.org/background-documents#European-Union-Standards-and-Training-for-the-Inspection-of-Tissue-Establishments-Project-(Eustite), accessed 18 March 2022.

69. EUROCET Project [website], available at https://joinup.ec.europa.eu/collection/ehealth/document/european-registry-organs-cells-and-tissues-eurocet, accessed 18 March 2022.

70. EuroGTP Project, available at http://eurogtps.com, accessed 18 March 2022. For guidance, visit http://eurogtps.com/Portals/0/pdf/Euro%20GTP%20Final%20Delivery.pdf, accessed 18 March 2022.

71. SoHO V&S Project, available at www.notifylibrary.org/background-documents#Vigilance-and-Surveillance-of-Substances-of-Human-Origin-Project-(SOHOV&S), accessed 18 March 2022.

72. ARTHIQS Joint action [website], available at www.arthiqs.eu, accessed 18 March 2022.

73. VISTART Project [website], available at https://vistart-ja.eu/home, accessed 18 March 2022.
74. EuroGTP-II Project, available at www.goodtissuepractices.eu/, accessed 18 March 2022.
75. European Cornea and Cell Transplant Registry, available at www.ecctr.org, accessed 18 March 2022.
76. EU-funded project TRANSPOSE [website], available at www.transposeproject.eu, accessed 18 March 2022.
77. GAPP Joint Action [website], available at www.gapp-ja.eu, accessed 18 March 2022.
78. Eudralex [collection of rules and regulations governing medicinal products in the European Union], available at http://ec.europa.eu/health/documents/eudralex/index_en.htm, accessed 18 March 2022.
79. Regulation (EC) No. 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No. 726/2004, available at https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32007R1394, accessed 18 March 2022.
80. EU legislation on clinical trials, available at www.ema.europa.eu/en/human-regulatory/research-development/clinical-trials/clinical-trials-regulation, accessed 18 March 2022.
81. Regulations (EU) Nos. 2017/745 and 2017/746 on medical devices, available at https://eur-lex.europa.eu/eli/reg/2017/745/2017-05-05 and https://eur-lex.europa.eu/eli/reg/2017/746/oj, accessed 18 March 2022.

Chapter 2. Quality management and validation

2.1. Quality management

This chapter outlines the general principles of a quality management system (QMS) that should be applied at all stages, from identification of a potential donor through processing and storage of the tissues or cells to the final preparation for application to the patient. Quality of tissues and cells is achieved through compliance with requirements at four levels:

a. The legal framework that provides the overall context in which the donation, procurement, testing, processing, storage, distribution until human application and import/export activities for tissues and cells are performed;

b. The QMS, which is a management method to ensure that tissues and cells consistently comply with technical and legal requirements;

c. The technical requirements specific to each type of tissue or cell, which ensure quality, safety and efficacy, as detailed in Part B and Part C of this Guide;

d. The authorisations in place for the specific activities, from the specific Health Authority.

A tissue establishment must implement a QMS that covers the scope of all of its activities. The following non-exhaustive list of relevant standards and legal instruments includes tools to support a tissue establishment in the construction of a robust and efficient programme:

- Directive 2004/23/EC [1] – which sets the standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells – and its associated technical directives provide key elements to be included in a tissue establishment QMS; these requirements are legally binding in EU member states.
- Good Practice Guidelines for Tissue Establishments (see Part C) that follow the EU directives. The guidelines are based on a QMS approach. They form the basis of good practice in all tissue establishments and should be used in preparation for inspection and in continuous improvement.
- Good Tissue Practices for European tissue banks were developed by the EU-funded project EuroGTP, which aimed to agree harmonised practices and techniques across Europe and to increase the know-how and level of competence of tissue establishment personnel. Much of the guidance developed in that project has been incorporated in the chapters of this Guide.
- FACT–JACIE International Standards for cellular therapy product collection, processing and administration, published by the Foundation for the Accreditation of Cellular Therapy and the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation.
- NetCord-FACT International Standards for cord blood collection, processing and release for administration.
- European Society of Human Reproduction

and Embryology (ESHRE) Guidelines for good practice in IVF laboratories [2].
- World Marrow Donor Association International Standards for Unrelated Haematopoietic Stem Cell Donor Registries.
- The International Organization for Standardization (ISO) requirements, as addressed in the ISO 9000 QMS family of standards. ISO standards have been developed to assist organisations of all types and sizes to implement and operate effective QMS. ISO 9001 on QMS requirements is particularly relevant to tissue and cell processes [3].
- The EU Guidelines for Good Manufacturing Practices (GMP) [4] provide specific guidance on the preparation of medicinal products. However, much of their content is also relevant to the procurement, processing, storage and distribution of tissues and cells. Wherever (in the EU) products containing tissues or cells are classified as advanced therapy medicinal products (ATMPs), then the EU guidelines on good manufacturing practice specific to advanced therapy medicinal products must be applied [5].

2.2. Applying a quality management system in donation and banking of tissues and cells

Quality is the responsibility of all personnel involved in the process of providing tissues and cells for clinical application. A systematic approach to quality management must be implemented and maintained throughout all processes that influence the quality of tissues and cells. A good system addresses quality management under the following headings:

a. Personnel and organisation;
b. Premises;
c. Equipment and materials;
d. Outsourced activities management (contractual agreements);
e. Documentation;
f. Quality control;
g. Quarantine and release;
h. Change control;
i. Traceability;
j. Complaints;
k. Investigation and reporting of deviations, adverse events and reactions;
l. Recall;
m. Self-assessment, internal and external audit;
n. Validation, verification and qualification;
o. Quality risk management;
p. Fiscal and continuity planning;
q. Tools for continuous quality improvement.

2.3. Personnel and organisation

There must be sufficient, suitably qualified personnel to carry out all tasks in compliance with quality and safety requirements. Tasks and responsibilities must be clearly defined, understood and documented. All personnel should have clear, documented and up-to-date job descriptions. There should be an organisational chart that describes the structure of the organisation with clear delineation of responsibilities and lines of reporting.

2.3.1. Key personnel

Key personnel in each organisation involved in the process (from the initial donor-selection stage to the final delivery of tissues and cells) should include an identified person who is responsible for all activities carried out in their organisation, along with a designated person who takes over this responsibility in their absence. In principle, processing and quality control activities should be independent of each other to ensure effective and reliable evaluation of processes, and be performed by different individuals. However, it is recognised that in practice this separation of responsibilities may be difficult to accomplish in smaller TEs, where due to staffing constraints individuals may have to perform both processing and quality control activities. It is however strongly recommended that individuals do not take responsibility for quality control checking of activities that they themselves have performed.

2.3.1.1. *Responsible person*

For those countries that are members of the EU, the Responsible Person (RP) in a tissue establishment must meet qualification criteria defined in Directive 2004/23/EC [1]. Each relevant organisation must also have an identified medical specialist/adviser who may or may not be the RP.

The responsibilities of the RP should include (but are not limited to):

- ensuring that human tissues and cells in the establishment for which that person is responsible, and intended for human applications, are procured, tested, processed, stored and distributed (including import and export) in accord-

ance with this directive and with the laws in force in the member state;
- providing information to the Health Authority on any substantial changes to its activities;
- implementing appropriate control measures in order to ensure compliance with the requirements of this directive;
- keeping records of their activities, including the types and quantities of tissues and/or cells procured, tested, preserved, processed, stored and distributed, or otherwise disposed of, and on the origin and destination of the tissues and cells intended for human applications;
- ensuring that the Health Authority(ies) is (are) notified of any serious adverse events and reactions and is (or are) provided with a report analysing the cause and the ensuing outcome;
- putting in place and updating a quality system based on the principles of good practice;
- providing training and qualifying personnel directly involved in activities relating to the procurement, processing, preservation, storage and distribution of tissues and cells.

2.3.1.2. *Quality manager*

The quality manager who manages the quality system should ensure (but their responsibilities are not limited to) implementation of the following:
- qualification of personnel,
- verification or validation of processes,
- qualification, maintenance, cleaning, disinfection and monitoring of premises and equipment,
- verification or validation of test methods,
- qualification of materials,
- qualification and monitoring of suppliers and contractors,
- document control,
- retention of records,
- compliance of all other personnel with establishment requirements,
- scheduling and follow-up of audits (internal or external ones),
- follow-up of non-compliances and corrective measures,
- implementation of risk mapping as part of the quality management system, notably for critical activities.

2.3.2. Training and qualification of staff

Tissue and cell procurement and processing should be carried out by appropriately qualified personnel. Personnel must receive initial and continued training appropriate to the tasks assigned to them. Criteria should be defined and satisfied before declaring personnel qualified for a specific task. Training methods must be documented and must ensure that each individual:
- has demonstrated necessary competence in, and is authorised to perform, their designated tasks;
- has an adequate knowledge and understanding of the scientific/technical processes and principles relevant to their designated tasks;
- understands the organisational framework, the quality principles relevant to their tasks and the safety rules of the TE in which they work;
- is adequately informed of the broader ethical, legal and regulatory context of their work;
- has knowledge of the general data protection regulation, including information technology (IT) security and data management rules.

When applicable, personnel should have relevant knowledge of microbiology and hygiene, and should be constantly aware that microbial contamination of themselves, donors and recipients, as well as tissues and cells, should be avoided. The training programme should include mid- to long-term training plans, be adequately resourced and target all the personnel that might be involved in any activities within the scope of this Guide, irrespective of whether the activity is routine or occasional.

Training programmes should be periodically re-evaluated, and the competence of personnel must be monitored regularly. This can be done, for example, by the monitoring of quality indicators, proficiency testing, external quality assurance, and internal or external audits.

2.3.3. Safety issues for healthcare personnel working with tissues and cells for human application

Personnel carrying out tissue and cell procurement and processing activities are exposed to a risk of infection to a similar degree as operating theatre personnel. In some cases, donors will not have been fully tested at the time of procurement or initial processing and, even where they have been tested, a residual risk of infection by untested agents remains. There may also be occasions when a donation is still required to be processed following receipt of positive test results, increasing the risk to healthcare personnel, for example when an autologous donation is assessed as being the most appropriate treatment

methodology. Standard universal precautions and appropriate procedures should be in place to avoid transmission of infections and suitable personal protective equipment (PPE) must be applied to protect personnel from these risks. Documented procedures should be in place describing the actions to be taken if an individual is directly exposed to the blood or tissues of a donor or their donation (e.g. needle-stick injury). These procedures might include: accelerated and extended testing of the donor, rapid testing of the staff member, and prophylaxis for the transmissible agent(s) where appropriate.

Procedures should define rules for personnel hygiene and safety and the behaviour of the personnel in the TE, as follows.

In the procurement and processing rooms of the TE:

a. eating, drinking and smoking are prohibited, and using private cellphones (mobiles) is strongly discouraged;
b. personnel should be educated as to the potential impact of their health status on their suitability for working with substances of human origin (SoHOs) because compromised immunity, their vaccination status or infectious disease may increase the risk to themselves, their co-workers or the SoHO they are manipulating;
c. the usual rules for dressing should be detailed – in particular, personnel need to be reminded of basic hygiene conditions regarding hair, beard and hands – and the use of gloves, glasses and specific protection for SoHO preparations should be mentioned; and
d. behaviour in case of a work accident occurring with biologic fluid or SoHO that carries potential risks of disease transmission, with toxic chemistry reagents, etc.

In areas with controlled atmosphere or with specific risks:

e. for each situation (e.g. gas manipulation, risk of anoxia, risk of burns), the dressing, hygiene and protective rules should be defined and evaluated by trained professionals (e.g. prevention and biosafety officers) according to the kind of risks;
f. these rules should be accepted and understood by the personnel concerned;
g. attention should be paid to occasional workers in the TE, as for example cleaning staff, who need to be informed of the risks (e.g. regarding microbiological hazards and liquid nitrogen) and should be regularly trained to the specific rules on hygiene and safety mentioned above; and
h. regular training should be delivered to the TE personnel and additional training envisaged if necessary.

2.3.4. Safety issues for tissues or cells handled by personnel with bacterial or viral infections

Personnel involved in procurement and processing of tissues and cells might also pose a risk to the quality and safety of the tissues and cells if they themselves are infected with a transmissible agent. Organisations should have documented policies describing the requirements for health screening of personnel and for individuals to inform the organisation, in a confidential manner, if they have accidentally exposed tissues or cells to risk of contamination.

2.4. Premises

Premises must be designed, located, constructed, adapted and maintained to suit the operations to be undertaken. Their layout and design must aim to minimise the risk of errors and permit operations to proceed in an orderly sequence. Their layout must also allow effective cleaning and disinfection to avoid contamination and cross-contamination.

Suitable premises should be available for confidential interviewing of living donors or the families or friends of deceased donors.

Facilities in which tissues or cells are procured must meet appropriate grades of air quality and cleanliness. The appropriate standard of cleanliness will depend on the type of tissues or cells being procured, the degree of exposure of the tissues or cells during the procurement process, and the decontamination or sterilisation processes that will subsequently be applied to the tissues or cells during processing. Most operating theatres are now environmentally monitored and have controlled air systems that make them suitable for the procurement of tissues that are not subsequently sterilised. Other types of facilities, such as mortuaries, may also be adequate for the procurement of certain types of tissues, but they should be assessed for fitness of purpose. Further guidance on facilities for tissue and cell procurement is given in Chapter 7, Chapter 8 and Chapter 9, and in Part B of this Guide.

Processing facilities should be dedicated to this activity and should be designed, qualified and monitored to ensure that air quality is appropriate for the

process being carried out. Further guidance is given in Chapter 8 and in Part B of this Guide.

Processing and storage facilities should be cleaned, and if applicable disinfected, according to a schedule and procedure that has been validated to achieve the required level of cleanliness, and all cleaning and disinfection procedures should be documented. They must be spacious enough to facilitate the work and provide good ventilation. Premises for storing tissues, cells, critical consumables and reagents must also be qualified, maintained and monitored. If certain storage conditions are critical for preserving the required properties of tissues, cells, consumables and reagents, the appropriate monitoring and alarm systems must be in place to indicate if conditions are approaching, or fall outside, predefined limits. Standard operating procedures (SOPs) should define the actions to be taken and the organisation in place in response to alarms. Storage requirements apply equally to interim storage of tissues and cells before transport to a processing facility. Further guidance on requirements for storage is given in Chapter 8 and Chapter 10, and in Part B of this Guide.

Premises should include adequate dedicated areas that allow the 'first in, first out' – or, when applicable, the 'first expired, first out' – principle for critical consumables and reagents to be respected. These areas should allow for adequate (physical or electronic) segregation of those materials in quarantine from those released for use.

2.5. Equipment and materials

2.5.1. Equipment

A list or register of critical equipment should be maintained. In this context, 'critical' means those items of equipment that come in contact with the tissues, cells or critical consumables and reagents, or influence the quality/safety attributes of the tissues, cells, consumables and reagents either directly (e.g. a freezer) or indirectly (e.g. donor testing equipment). All equipment on this list must be designed, identified, qualified, maintained and, if applicable, monitored to suit its intended purpose and it must minimise any hazard to donors, recipients, operators or the quality and safety of the tissues and cells. Equipment should conform to international standards and EU and national licensing arrangements, where these exist.

In the EU, critical equipment must meet the requirements of Regulation 2017/745 of the European Parliament and of the Council of 5 April 2017 concerning medical devices [7] and Regulation 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices [8], and national licensing arrangements where these exist. All critical equipment should be qualified before being taken into service, to assure proper functioning, and re-qualified periodically in service (see §2.12.6) [6], for example, temperature mapping of a freezer to identify temperature deviations that might affect the quality of stored tissues or cells. Requalification intervals should be defined through risk assessment, taking into account reported deviations, monitoring results and the lifespan of the equipment. All qualification activities should be scheduled and recorded.

Critical measuring equipment, for example micropipettes or temperature sensors, must be calibrated at defined intervals to assure the accuracy of measurements. The calibration intervals should be defined through risk assessment, taking into account reported deviations, monitoring results and the lifespan of the measuring device. Calibration results must be maintained and recorded (see §2.12.8).

Apparent deviations in the quality and/or performance of equipment must be investigated and registered promptly. Outcomes of these investigations should be reported in a timely manner to the RP, who should consider and approve the corrective and preventive actions to be implemented. Critical deviations from the design or the use of the device that could endanger the quality and/or safety of tissues and cells must be reported to the manufacturer and, where appropriate, must be reported to the Health Authority.

Defective equipment should, if possible, be removed from production and quality control areas, or at least be clearly labelled as defective. Repair operations should not present any hazard to the quality of the tissue and cells and should be recorded. The requalification of repaired equipment before being returned to service could be risk-based.

Equipment should be designed so that it permits effective maintenance, cleaning and if applicable disinfection. Maintenance, cleaning and if applicable disinfection must be performed regularly and recorded. Preventive maintenance should be scheduled as defined by the manufacturer. The cleaning and disinfection intervals should be defined through risk assessment, taking into account reported deviations, monitoring results and the validation of the cleaning and disinfection procedure.

2.5.2. Materials, consumables and reagents

A controlled list must be constructed of all materials, consumables and reagents that come into contact with the tissues or cells or that influence the

quality or safety of the tissues or cells. Detailed specifications for these critical materials, reagents and consumables must be documented and, when applicable, must meet the requirements of Regulation 2017/745 of the European Parliament and of the Council of 5 April 2017 concerning medical devices [7] and Regulation 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices [8].

Only materials from qualified suppliers that meet the documented specifications should be used. Manufacturers should provide a certificate of compliance for every lot/batch of materials supplied. Batch acceptance testing or checking of each delivery of materials should be carried out and documented before release for use in tissue or cell procurement or processing.

Inventory records must be kept for traceability and to prevent use of materials after their expiry date. Each batch of critical materials, reagents or consumables must be traceable to the session of procurement or processing of tissues and cells in which they were used.

Apparent deviations in the quality and/or performance of materials must be investigated and recorded promptly. Outcomes of these investigations should be reported in a timely manner to the RP, who should consider and approve the corrective and preventive actions to be implemented. For relevant deviations, a notice should be sent to the manufacturer and, where appropriate, must be reported to the Health Authority.

Further guidance on reagents and materials used in tissue and cell processing is provided in Chapter 9.

2.6. Outsourced activities management (contractual arrangements)

Where steps influencing the quality or safety of tissues or cells (i.e. critical steps) are carried out by a third party, there must be a contract or service-level agreement in place that must describe the roles and responsibilities of all parties for maintaining the quality chain and the quality requirements for the service provided. Agreements should allow for audits of contracted third parties to confirm their compliance with expectations. An example of an expectation is that if a supplier changes specifications for equipment or reagents provided to a tissue establishment, or they provide a substitute for an ordered item, they must inform the TE in in advance in a timely manner with regard to these changes.

In EU member states, tissue establishments must establish written agreements with a third party each time an external activity takes place that influences the quality and safety of tissues and cells procured or processed in co-operation with a third party. They should keep a complete list of these agreements and make them available at the request of the Health Authorities.

Agreements should be dated, reviewed and renewed on a regular basis. Written agreements should be in place for at least the following service suppliers:

a. testing laboratories (including donor, tissue and environmental testing);
b. procurement teams that are independent from a tissue establishment;
c. transport companies;
d. suppliers of critical equipment, consumables and reagents;
e. suppliers of services such as tissue and cell storage, processing or sterilisation;
f. suppliers of IT services.

Once tissues and cells have been distributed for clinical application, they usually leave the QMS of the tissue or cell facility. However, appropriate control of transport and transport conditions, appropriate handling and preparation before use and full traceability should all be maintained. Maintenance of quality and traceability is usually achieved by providing users with clear and detailed written instructions. The tissue establishment should implement appropriate measures, such as instructions for use and traceability record labels, which are provided to the clinical user in order to ensure product quality and traceability down to the recipient. Some national standards require the organisation responsible for human application of tissues and cells (ORHA) to provide the supplying tissue establishment with details of the patient to whom the tissues or cells were clinically applied (see Chapter 13).

Distribution to the patient for use without direct supervision of a health professional requires particular attention to instructions for storage and use, and should be carried out only if it is the only available option.

2.7. Documentation and records

There must be a process in place that results in clearly defined and effective documentation, that controls modification of records and that registers

approval of SOPs for all activities for which accreditation/designation/authorisation/licensing is sought. Documentation must enable all recorded data relating to the quality and safety of the tissues and cells to be checked and traced, from the donor to the recipient and vice versa (see Chapter 16 – Traceability). In MAR, traceability also involves follow-up of the outcome from these treatments, including the children (see Chapter 29). Written documentation ensures that work is standardised and prevents errors that may result from oral communication. Where oral communication is necessary for critical information exchange, audio recordings may be useful. Donor information, in general, and donor-referral records in particular, must meet the same criteria.

2.7.1. Documentation

Quality-assurance (QA) document control is the process used in the management, co-ordination, control, delivery or support of an item required for QA purposes. QA document control is an essential part of the QA system of good tissue and cell practice. It allows authorised persons to approve, review and update documents; make changes and identify revision status; control document distribution; and prevent use of obsolete documents, facilitating proper archiving. National regulations concerning document control could apply.

Procedures must be adopted to ensure that:

1. all documents issued as part of the quality management system are periodically reviewed, revised when necessary, dated and approved by authorised personnel prior to issue;
2. only the current version of the document is in use, and its distribution is maintained;
3. all personnel have access to documents needed for the execution of their tasks;
4. invalid or obsolete documents are promptly removed from all locations;
5. retained or archived documents are appropriately identified to prevent their inadvertent use.

All documents relevant to the quality management system should be uniquely identified to include:

1. title;
2. edition or current revision date or revision number;
3. number of pages;
4. owner, author and authoriser.

For every critical activity, the materials, equipment and personnel involved must be identified and documented. Documentation should include at least the following items:

a. a quality manual, if required by national legislation or other applicable quality standards;
b. policies and SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;
c. specifications for materials, consumables and reagents;
d. qualitative and quantitative specifications for tissues and cells;
e. specifications for equipment;
f. technical guidelines;
g. contract and service-level agreements;
h. job descriptions;
i. instruction manuals;
j. reliability of the traceability system;
k. implementation and traceability of corrective measures;
l. implementation of internal or external audits;
m. implementation of a risk mapping as part of the quality management system, notably for critical activities.

Documentation must be retained according to national requirements.

2.7.2. Records

Records must be maintained for all critical steps, including the rejection of non-conforming tissues and cells. Procedures must be adopted to ensure that:

- records are reliable and a true representation of the results;
- records are legible and indelible;
- records meet the confidentiality requirements laid down in Article 14 of Directive 2004/23/EC [1];
- access to registers and data is restricted to persons authorised by the responsible person, and to the Health Authority for the purpose of inspection and control measures (see also Chapter 14);
- any alteration made to a record is dated and signed or, in the case of digital records, an audit trail of alterations is maintained;
- records contain the identification of the operators and date of completion of the activity and, where appropriate, the identification of any person who checked these activities.

Procedures should be adopted to ensure that

the use of handwritten records, including transcriptions of records, is minimised. Records should preferably be registered in qualified information systems.

Records should include at least the following items:

a. records of the performance of operations critical to the safety and quality of tissues and cells,
b. records of critical equipment, information systems, materials, consumables and reagents,
c. records of complaints and deviations,
d. records of corrective and preventive actions,
e. records of internal audits, external audits and inspections,
f. training and competency records of personnel,
g. records of internal and external quality control,
h. identification and analysis of risks and a risk-mitigation plan,
i. donor records,
j. information on the final destination of tissues and cells,
k. records of quality indicators.

The QMS should define the period of time for which records will be retained. In the EU, records that are critical for the safety and quality of tissues and cells, including quality-system documentation and raw data, should be retained for 10 years and traceability records for 30 years after clinical use, expiry date or disposal of the tissues and cells. Records can be stored on paper or any electronic support.

An establishment responsible for using personal data has to follow strict rules on data protection. They should make sure the information is:

a. used fairly, lawfully and transparently,
b. used for specified, explicit purposes,
c. used in a way that is adequate, relevant and limited to only what is necessary,
d. accurate and, where necessary, kept up to date,
e. kept for no longer than is necessary,
f. handled in a way that ensures appropriate security, including protection against unlawful or unauthorised processing, access, loss, destruction or damage.

Information which is deemed more sensitive may require additional protection. This includes data on:

- race,
- genetics,
- biometrics (where used for identification),
- health.

2.8. Change control

Change-control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a product of the desired quality consistent with the approved specifications. Change control should be carried out prior to the implementation of a revised/new process. Written procedures should be in place to describe the actions to be taken if a change is proposed to any starting material, final product specification, equipment, environment (or site), method of production or testing, or any other change that may affect the quality of tissues or cells or the reproducibility of the process. All such changes should be requested, investigated, documented, traced and authorised. The likely impact of the change in facilities, systems and equipment on the final product should be evaluated (including a risk analysis). The need for, and the extent of, any re-validation or re-qualification should be determined.

The training programme should be re-assessed for any critical change in environment, equipment or processes. Training records (including plans and training plans) should ensure that training needs are identified, planned, delivered and documented appropriately by taking into account any changes to systems and equipment.

Some changes may require notification to, or licence amendment from, a national health authority.

For further information, see Chapter 9.

2.9. Complaints

All complaints must be documented, carefully investigated and managed in a timely manner. The complaints procedure should take into consideration complaints from:

a. living donors or the families of deceased donors,
b. personnel,
c. third-party health professionals,
d. clinical users, including those in another jurisdiction,
e. patients.

A mechanism for categorising, tracking and trending complaints should be in place and should be readily available for audit. Categorisation of complaints should in any case lead to an assessment of whether the complaint is justified and is related to a potential deviation. If the latter is the case, then based on the potential seriousness and frequency of appearance, a root cause analysis and possible identi-

fication of corrective measures should be performed (see §2.12 and Chapter 4 for details).

2.10. Investigation and reporting of deviations, adverse events and adverse reactions

All deviations, including (serious) adverse events and (serious) adverse reactions, from the required standards of quality and safety must lead to a documented investigation. There should be an SOP in place that defines how the organisation manages deviations. This SOP should ensure that:

- the personnel responsible for managing and investigating deviations are designated;
- any deviations are categorised depending on how critical they are to the quality and safety of tissues and cells, and how frequently they occur;
- the medical significance of any deviation is considered and, where appropriate, the RP and/or clinician responsible for the procurement or clinical application is informed;
- the released tissues and cells are recalled as necessary (see §2.11 and Chapter 12);
- there is a clear allocation of responsibility to discontinue or resume processes;
- reporting of deviations in a non-punitive context is encouraged to help achieve improvements in practice.

However, any deviation that, depending on its impact, might result in a serious adverse event or serious adverse reaction in a living donor or recipient, must be linked to the vigilance reporting system (see Chapter 17).

There should be a process in place for taking immediate remedial actions. These actions should be clearly documented. Based on the estimation of occurrence and severity (e.g. risk matrix) of the deviation, a root cause analysis should be performed to identify the underlying cause or causes of the deviation. These should, where appropriate, lead to corrective action. At regular specified intervals, the records of deviations should be reviewed to detect trends and initiate preventive actions.

Corrective actions must be documented, initiated and completed in a timely and effective manner. Preventive and corrective actions should be assessed for effectiveness after implementation.

Serious adverse events and serious adverse reactions should be reported through a vigilance system. For detailed guidance on vigilance of tissues and cells, see Chapter 17. If products containing tissues or cells are classified in the EU as ATMPs, adverse occurrences should be reported either through pharmacovigilance systems for process events or through biovigilance systems for donor reactions, or other events and reactions detected before delivery of the tissues and cells to the pharmaceutical facilities.

2.11. Recall

An effective written procedure must be in place for recalling defective tissues or cells or those suspected of not meeting required quality or safety requirements. This written procedure must encompass the need to agree and document any corrective and preventive actions that might be necessary, remembering that other tissues or cells procured from the same donor might be affected. Therefore, a recall procedure could affect more recipients than initially presumed. If other organs, tissues or cells from the same donor were used, transplant teams should be promptly informed. The actions should be communicated to the end user, where appropriate. Further guidance on recall is provided in Chapter 17.

2.12. Qualification, validation and verification

2.12.1. General principles

Validation, verification and qualification together form the part of the QMS concerned with proving that all critical aspects of the establishment's operations are sufficiently under control to provide continual assurance that tissues and cells will remain safe for patients and fit for purpose; see Table 2.1. The critical aspects subject to validation, verification and qualification include:

a. the facilities and equipment used in procurement, processing, storage, testing and distribution, and any equipment and software used to manage their operation and data,
b. materials and reagents that come into contact with cells and tissues,
c. labelling and tracking materials, equipment and software,
d. operational staff and the written procedures that instruct their work,
e. process stages from procurement to distribution where there is a risk of a detrimental effect on the quality and safety characteristics of tissues and cells if not performed correctly, based notably on the risk mapping tool,
f. analytical test methods used to assess and

confirm the safety and quality of donors, donations, tissues and cells,

g. other auxiliary processes such as the transport and cleaning processes.

Validation, verification and qualification combine to form a highly technical activity requiring a good understanding of the risks associated with critical processes conducted by the tissue establishment and the potential risks and impact of materials and equipment used in these processes. Risk mapping is a useful tool for this exercise. Establishments performing very simple, minimal manipulation of a limited range of tissues and cells in accordance with published methods, or following long-established practices using the same materials and equipment, may rely on ongoing quality control and periodic reviews. Such establishments should still document their validation policy, explaining their approach on the basis of risk assessment and should perform a retrospective verification of their critical processes to confirm that the method has the intended (clinical) outcome. The qualification of premises, equipment, materials, consumables, reagents and personnel should be ensured.

The risks increase significantly with the introduction of more complex processes, a wider range of tissue and cells handled, computerised systems, expansion of facilities and significant growth in workforce. In these circumstances it becomes more important to take the formal approach to validation as described in this section to ensure that the establishment's processes remain safe for donors and patients.

Validation is usually split into two components: qualification and validation (or verification). The term 'qualification' is applied to each part of the process and to individual items, including cleanroom facilities, equipment, computer systems, materials and operators. Such items should be qualified before they are first used in a process and then re-qualified at predetermined intervals, or when significant changes are made. Each individual item should be qualified separately to demonstrate consistent performance. Validation provides objective evidence that the method or process is fit for purpose, meaning that that the predefined requirements for a specific intended use are fulfilled. If a TE wishes to apply a previously validated method, or a standard method, that has been extensively validated via another TE or published by professional organisations or international guidelines, consideration should be given to verification of the requirements of these standard methods. Verification tests of standard methods are typically less extensive than those required for validation.

Process (or test-method) validation or verification should only be performed once all the items used have been qualified; it should be performed before a new process or method is used routinely and, where required by local legislation, before any associated tissue or cell product is released for human application (prospective validation). It is possible to conduct process validation during the processing of tissues and cells intended for subsequent release for human application (concurrent validation). Where establishments have not validated any of their processes or methods because they have been in routine use without change for many years, they may use existing data and information as a basis for retrospective verification of their critical processes to confirm that the method has the intended (clinical) outcome. Any process or method changes should be assessed for impact and risk in accordance with quality risk-management principles (see Chapter 4), and verification should be considered where there is unacceptable risk.

The objective of validation is to challenge the critical aspects of items through a series of controlled tests representative of the conditions under which they are expected to operate, to demonstrate that they achieve predefined acceptance criteria for quality and safety. The challenge should include the normal variation of possible conditions expected, but also more extreme conditions where there are high risks, to provide a safety margin, for example with sterilisation processes. The objective of verification is to demonstrate the ability to achieve the published performance characteristics of the standard or validated method under their own test conditions.

The test methods to be used and the acceptance criteria should be documented and approved by the establishment management before qualification or validation or verification commences. This document is commonly called the validation plan. The validation and verification should be performed by trained and competent persons. The results of the validation and verification should be compared with the acceptance criteria and any deviation from the plan should be recorded during the validation and documented in summary form with a conclusion. This document is commonly called the validation report. Following validation and verification, the acceptance or rejection of the item or process by designated establishment management should be documented.

It should be clear through documentation and/or status labelling which processes and items have been validated, qualified or verified and which are in the process of validation, verification or qualification. Where anything is not in a fully validated state, there must be controls to prevent its use.

Table 2.1. **Processes or resources that should be qualified, validated, verified or calibrated**

	Qualification	Validation	Verification	Calibration
Premises	×			
Personnel	×			
Equipment	×			×
Software	×			
Measuring equipment	×			×
Critical materials	×			
Suppliers	×			
Analytical tests (IVDR, *Ph. Eur.* monographs)			×	
In-house analytical tests		×		
In-house process methods		×		
Established process methods (e.g. guidelines, monographs, other tissue establishments)			×	
Cleaning and disinfection methods		×		

2.12.2. Validation planning

The validation, verification and qualification policy should consider a process design phase where deep knowledge of the process is achieved. In the following paragraphs, the term 'validation' is also used to cover method validation, verifications and qualification.

In this stage, the critical quality attributes of the tissues/cells are identified and the subsequent critical process parameters are identified. According to the critical process parameters affecting the critical quality attributes, a process control strategy should be developed.

During the validation phase itself, the process control strategy is implemented, and all the elements involved, such as equipment, utilities, suppliers and transport, are qualified before proceeding with the process validation.

All validation must be carefully planned in advance. Validation planning requires technical expertise in the processes involved and items used in the processes as well as expertise in any applicable regulations and technical and quality standards. It should therefore involve a validation team of relevant operational, quality, regulatory and medical experts in the establishment. Where necessary, for example the qualification of new cleanroom facilities, external experts may be employed to advise.

Effective validation is not possible unless the establishment management is completely clear what its specific technical and quality requirements are. Establishments should use quality risk-management procedures to help determine their expectations for the processes and items used, which must address any significant risks to donors, recipients or quality of products, and any risks of regulatory non-compliance. Consideration should be given to possible modes of failure and the need to detect failure. Each establishment should document their expectations in the form of specifications. For processing stages there should be product specifications, for test methods there should be test specifications and for items used in processes, there should be user-requirement specifications (URS). For bespoke items of equipment and facilities, the manufacturer or supplier will need to interpret the URS and write a design specification to instruct their engineers, who will then write associated detailed technical specifications for the construction. For off-the-shelf items, the URS is used to assess suitability and to inform purchase decisions.

The validation team will need to develop validation plans that prove that the relevant specifications will be met under all likely conditions and worst case scenarios, with expected margins of safety where necessary. Appropriate tests and associated acceptance criteria should be established. This requires knowledge of the critical operational parameters and the expected variation in those parameters. Such parameters may include operating temperatures, exposure times, air flows, bioburden, location and product characteristics. Statistical methods will often be needed to demonstrate consistent performance with the necessary level of confidence. Means for detecting failures and alarms will need to be tested. Where relevant standards such as ISO and the *European Pharmacopoeia* provide detailed validation methods, these should be included in the validation plan.

If it is not possible to complete any procedures strictly in compliance with the plan, then there should be a review by the validation team, who should decide whether to repeat all or part of the validation or to allow a deviation for the modified procedure. Such decisions must be recorded in the validation records and report. Where establishments

employ an independent quality manager or a designated RP in accordance with EU legislation, then that person should supervise all validation activity and be responsible for approving or rejecting the outcome of validation.

2.12.3. Documentation

As with all elements of the quality system, the policy and process for planning, executing and recording validation must be documented in written procedures. This documentation may be assembled into a validation master plan (VMP). The VMP will typically include the following:

- validation policy,
- organisational structure of validation activities,
- summary of facilities, systems, equipment and processes to be validated,
- documentation format,
- planning and scheduling,
- change control,
- reference to existing documents.

2.12.4. Qualification of operators

Operators are qualified as part of the training programme (see §2.3.2). There should be a documented training specification and plan for each operator, identifying how they are to be trained and listing the desired outcomes (acceptance criteria) from the training. The capability of individual operators to meet the desired outcomes should be assessed through observation and tests before they are approved as qualified to carry out procedures unsupervised. Particular attention is given to qualification of operators' aseptic techniques working in Grade A environments with Grade B background. The usual approach under such circumstances is to conduct simulated processes using culture medium or broth in place of, or added to, tissues or cells.

Before written procedures (SOPs and Work Instructions) are approved as part of the document-control system (see §2.7) they should be read and approved by an authorised operator to confirm that they are clear, understandable, accurate and practical.

2.12.5. Qualification of materials and suppliers

A detailed URS should be available for materials (see §2.5.2). Before introducing a new material into a process it must be qualified. This will involve confirmation that it meets the URS through examination of the material and of test data either from the manufacturer (Certificate of Analysis), a third party or in-house testing. Where the URS requires, or where there may be significant variation that might affect the outcome from the process in which the material is to be used, there may be a need for qualification of the material. Qualification may be carried out on a scaled-down version of the process or at full scale and may be run in parallel with the existing material to demonstrate a comparable outcome.

Suppliers and manufacturers of materials, equipment and contract services should be qualified before any purchase is made. The purpose is to ensure that they can be relied upon to continue supplying the goods and services that meet the URS. This relies upon compliance with quality specifications and operation of an acceptable quality management system. This should be confirmed through audit (see §2.13) of their operations and quality management system or internal quality control.

2.12.6. Qualification of premises and equipment

Facilities and equipment must be qualified before use and when any significant change is implemented.

Facilities and equipment should be qualified following the four steps shown in Figure 2.1. and the extension of the qualification should be determined through a risk assessment exercise. Some steps might be executed together.

2.12.6.1. *Design qualification*

The first element of the qualification of new facilities, systems or equipment can be considered 'design qualification' (DQ). This qualification involves demonstration and documentation of the compliance of the design with good practice (i.e. the design is suitable for the intended purpose). DQ should be used to confirm that proposed solutions meet the URS. If the proposed solution is an off-the-

Figure 2.1. **Steps in qualification of facilities and equipment**

shelf item DQ is still required by the Tissue Establishment to confirm it meets their requirements prior to purchase.

2.12.6.2. *Installation qualification*

Installation qualification (IQ) should be carried out on new or modified facilities, systems and equipment once installed on site. IQ should include (but is not limited to) the following:

a. installations of equipment, piping, services and instrumentation, which are checked to current engineering drawings and specifications,
b. collection and collation of the operating and working instructions as well as the maintenance requirements of the supplier,
c. calibration requirements, including verification of the uncertainty of measurement for any measuring equipment,
d. Qualification of construction materials.

IQ for new facilities or modified facilities and more complex equipment may be performed by the supplier, but the establishment should verify that agreed acceptance criteria have been met. An example of a cleanroom qualification document is available in Appendix 4 and an example of an incubator is available in Appendix 5.

2.12.6.3. *Operational qualification*

OQ should include (but is not limited to) the following:

a. tests that have been developed from knowledge of processes, systems and equipment to ensure that the system is operating as designed,
b. tests to include a condition or a set of conditions encompassing upper and lower operating limits (sometimes referred to as 'worst-case' conditions).
c. Completion of a successful OQ should allow calibration, operating and cleaning procedures, operator training and preventive maintenance requirements to be finalised. It should permit a formal 'release' of the facilities, systems and equipment.

2.12.6.4. *Performance qualification*

PQ should include (but is not limited to) the following:

a. tests, using production materials, qualified substitutes or simulated tissues and cells, which have been developed from knowledge of the process and the facilities, systems or equipment,
b. tests to include a condition or set of conditions encompassing upper and lower operating limits.

The number of tests carried out should achieve reproducibility of the process, to the grade of warranty that the process is required to demonstrate. The more knowledge there is of the process, the less testing during PQ may be needed.

2.12.7. Qualification of software

Computer and automated systems controlled by bespoke and off-the-shelf software are extensively used by tissue and cell establishments to help manage procurement, processing, testing and distribution activities and data. The design of, and changes to, software can have a significant impact on the quality and safety of tissues and cells and the integrity of critical data. It is therefore essential to maintain effective version control over software in use and to qualify new software and requalify existing software when changes are made. Establishments should be aware of the current version of software operating their relevant computer and automated systems at all times and should not allow upgrades of existing software by system suppliers without their approval.

Software requirements should be included in the appropriate URS for the system they control. For bespoke software, the URS will be used by software system engineers to produce a detailed technical specification to be used by a programmer to write new or revised code for the system. For an off-the-shelf system the URS will be used to assess candidate software and to inform the local IT staff how to configure the software. New and revised versions of software should be qualified in operation by users (user acceptance testing or UAT) before being put into service. At the very least, this testing should include verification of critical settings encoded in the software, for example when there is a version update to software for running an apheresis machine or testing system. However, usually the software will be tested in its operational state through process-simulation tests or parallel running as part of the system (equipment) qualification. It is important during user acceptance testing to verify that existing functionality continues to operate as expected (regression testing) as well as testing new functionality.

For additional information, see Chapter 14.

2.12.8. Calibration

All equipment or systems with a critical measurement function must be calibrated according to

a planned schedule. Calibration is a qualification method that confirms, under defined conditions, the relationship between values obtained from the measuring equipment or system and those obtained using an appropriate certified standard. Calibration addresses measurement uncertainty (U). 'Measurement uncertainty' refers to the closeness of agreement between a measured quantity value and the true quantity value of what is being measured. Hence, if the measured value is close to the true value, the measuring system has high accuracy. The acceptable tolerance should be set according to the critical quality attributes of tissues/cells, and these tolerance limits of the same equipment may have different needs depending on the tissues/cells subject to the process.

In practice, the measurement result of each piece of critical measuring equipment or system should be traceable through a documented and unbroken chain of calibrations back to the International System of Units or to a recognised standard. The uncertainty of measurement should be established [9, 10] and quoted with subsequent results. Hence, the critical measuring equipment or system is compared against a standard; the standard is compared against a higher standard; and the chain is documented through calibration certificates. If calibration is carried out by a third party, a copy of the calibration certificate for the specific measuring equipment or system used should be provided together with the calibration report.

If the calibration result ± the measurement uncertainty falls outside the tolerance limits of the measuring equipment or system, there should be provisions for remedial action to re-establish conformity with these limits. These remedial activities must be documented. If calibration activities provide evidence that tissues or cells were processed and released for use when critical measurement equipment was not measuring accurately, risk assessment should be applied to decide on appropriate corrective or preventive actions regarding the fate of the tissues and cells.

2.12.9. Verification or validation of analytical tests

The approach to verify or validate analytical tests will depend on whether test systems and kits, certified as compliant with the EU *In vitro* Diagnostic Medical Device Regulation [8], are used or in-house developed analytical tests. In all cases the validation plan should take into account the variety of sample types and analytes to be tested, as there may be substances present that interfere.

Verification studies should be done to demonstrate that the performance of the IVD kit or test system, as used in the establishment, meets the expected specification. If using *Pharmacopoeia* methods, e.g. for sterility testing, the methods should be verified in accordance with the method monograph. For quantitative assays, the acceptance criteria should consider trueness (bias), precision, interferences, linearity, limits of detection, stability and verification of the reference values of the own population. The uncertainty of measurement should be established [9][10] and quoted with subsequent results. For qualitative tests, then specificity and sensitivity are the key criteria.

In-house developed analytical tests should be validated. The acceptance criteria should consider trueness (bias), precision, analytical sensitivity, analytical specificity, linearity, stability, diagnostic specificity, diagnostic sensitivity and verification of the reference values of the own population.

2.12.10. Process validation

More specific guidance on approaches to process validation is given in Chapter 9 of this Guide. Some examples of validation are in Appendix 6 and Appendix 7.

2.13. Quality review

2.13.1. Quality control

'Quality control' refers to those activities, such as verification steps, sampling and testing, which are used to ensure that materials, processes and the final product meet the required specifications.

Internal quality control in a testing laboratory includes use of positive, weakly positive or negative control samples as appropriate. External quality assessment (sometimes called 'proficiency testing') involves analysis of unknown samples and evaluation of the results by a third party. Quality control of critical functions can be undertaken using audit techniques that include a sampling plan.

Detailed guidance on microbiological testing is provided in Chapter 11. Guidance on specific quality-control tests for specific types of tissues and cells is provided in Part B of this Guide.

2.13.2. Self-assessment, internal audit and external audit

Auditing is an essential tool for ensuring compliance with the quality system and for supporting continuous quality improvement.

Internal audits should be scheduled and conducted in an independent way by designated, trained and competent persons. Internal audits are normally carried out by the organisation's quality assurance personnel, or can be done by a mandated person from outside (quality assurance personnel from another TE) with the help of one internal person.

External audits are undertaken by independent bodies (often designated as approved ISO certifying bodies) and are required for certification and accreditation purposes. External audits provide an opportunity for critical review by experts unfamiliar with the systems in place locally. They can provide an excellent opportunity for systems improvement.

All audits should be documented and recorded. Clear procedures are required to ensure that the agreed corrective and preventive actions are undertaken appropriately. These actions and the evaluation of their effectiveness should be recorded.

2.13.3. Monitoring

To ensure appropriate performance of premises, systems or equipment, a monitoring plan must be developed and implemented. The plan should take into account the criticality of the premises, system or equipment, and should outline monitoring, user notification and mechanisms for problem resolution. If an unusual event is observed, personnel should follow the standard response described in the monitoring plan. The standard response should involve notifying affected personnel and, if possible, initiation of a resolution response to the problem and risk assessment of the affected tissues or cells. Depending on the severity of the problem and the criticality of the system or piece of equipment, a back-up plan may need to be implemented to keep the process or system operating.

2.14. Continuity planning

General quality-management responsibilities include budgetary/fiscal oversight and contingency planning to ensure that essential services for patients are not interrupted. Each organisation in the chain – from donation to distribution and biovigilance of tissues and cells – must have a continuity plan in place that details how procurement services, donated tissues and cells and all associated documents will be maintained in the event that activities must temporarily be suspended or permanently ceased. Usually this plan will include a mutual agreement (a service-level agreement or contract) with another organisation for the transfer of tissues or cells, documentation and services in these circumstances.

2.15. References

1. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32004L0023, accessed 19 March 2022.
2. De los Santos MJ, Apter S, Coticchio G *et al.* Revised guidelines for good practice in IVF laboratories (2015). *Hum Reprod* 2016;31(4):685-6.
3. EN ISO9000:2015. Quality management systems – Fundamentals and vocabulary. CEN, 2015.
4. EudraLex [collection of rules and regulations governing medicinal products in the European Union]. Volume 4, EU guidelines for good manufacturing practices – medicinal products for human and veterinary use (GMP), available at http://ec.europa.eu/health/documents/eudralex/vol-4/, accessed 3 December 2021.
5. EudraLex [collection of rules and regulations governing medicinal products in the European Union]. Volume 4, EU guidelines on good manufacturing practice specific to advanced therapy medicinal products, available at http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf, accessed 19 March 2022.
6. EudraLex [collection of rules and regulations governing medicinal products in the European Union]. Volume 4, EU guidelines for good manufacturing practices – medicinal products for human and veterinary use (GMP), Annex 15, available at http://ec.europa.eu/health/documents/eudralex/vol-4_en, accessed 19 March 2022.
7. Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R0745, accessed 29 November 2022.
8. Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices, available at https://eur-lex.europa.eu/eli/reg/2017/746/oj, accessed 29 November 2022.
9. EA-4/16. EA guidelines on the expression of

uncertainty in quantitative tests. European Accreditation, 2003, available at https://european-accreditation.org/wp-content/uploads/2018/10/ea-4-16-g-rev00-december-2003-rev.pdf, accessed 7 July 2022.

10. EN ISO 15189: 2012. Medical laboratories – Requirements for quality and competence, ISO, 2012 available at www.iso.org/obp/ui/#iso:std:iso:15189:ed-3:v2:en, accessed 7 July 2022.

Related material

Appendix 4 – Example of cleanroom qualification
Appendix 5 – Example of incubator qualification
Appendix 6 – Example of process validation – tissue transportation
Appendix 7 – Example of method validation – oocyte vitrification

Chapter 3. **Risk management**

3.1. Introduction

The procurement, testing, processing, storage and distribution of tissues and cells should be subjected to comprehensive risk assessment to allow identification of those steps where most of the quality-system controls are required and where validation of procedures is necessary. A 'process flow' diagram listing all relevant steps, processes, reagents, tests and equipment can form the basis for the risk assessment exercise. Risk-mapping methodology is one of the risk-management tools. This methodology offers a visualised, comprehensive view of the likelihood and impact of an organisation's risks.

Risk assessment should include an estimation of the severity of any identified hazard (potential source of harm) and an estimation of the probability that the hazard will result in harm. Probability should be based on evidence and experience (e.g. events collected in the framework of the QMS or biovigilance) whenever possible.

Risk-mitigation strategies should be developed to identify and manage risks to ensure the safety of the tissues and cells, the donor and recipient, the personnel and the process itself, as well as other processes being undertaken in proximity to it. The degree of control within the quality system should be related to the degree of risk associated with each step in the process.

Risk assessment should refer to current scientific knowledge, should involve appropriate technical expertise and should be related to the protection of the patient and the donor. The level of effort, standardisation and documentation of the risk-control process should be aligned with the estimated risk level.

A periodic review mechanism should be implemented proportionally to the level of risk and considering new knowledge and potential inputs from inspection, internal audits or unexpected events. Risk assessment should be repeated and documented whenever a critical process is changed as part of a change-control process. Actions to mitigate any significant new risks, including validation, should be completed before any change is implemented.

Risk assessment is also an essential tool for making important decisions and for tracing them, particularly when deviations from standard procedures or their standards and specifications are under consideration. Examples include:

a. selection of a donor where full compliance with the normal criteria has not been met, but where the donation has a particular clinical value and the potential risk can be mitigated sufficiently to justify the deviation from standard procedures;

b. exceptional release of non-complying tissues or cells on the basis that the potential benefits for the recipient and the lack of availability of alternatives outweigh the potential risks;

c. retention or removal of tissues and cells in storage that had been historically released according to former criteria, when new, more sensitive procedures or tests have been implemented that imply an additional level of safety

or quality and new, more stringent criteria for release;

d. eligibility determination where certain test results are reactive, for example, where EU Directive 2006/17/EC Annex 2 requires further investigations with a risk assessment when antibody to hepatitis B core antigen (anti-HBc) is positive and hepatitis B surface antigen (HBsAg) is negative, or where a donor is reactive for a *Treponema*-specific test (see Chapter 6 for further guidance on donor testing);

e. prioritisation of a list of corrective actions following an audit or inspection, or prioritisation of quality improvements in general.

The approach to risk assessment should be systematic and should be documented. The most commonly applied risk-assessment methods are Hazard analysis and critical control points (HACCP), Failure mode and effects analysis (FMEA), Failure mode, effects and criticality analysis (FMECA), risk mapping and EuroGTP II tool.

3.2. Hazard analysis and critical control points

HACCP was developed in the 1950s in the food industry, but is now widely used for many manufacturing processes, including biological control. HACCP is also recognised by ISO 14644 as a formal system for risk assessment. HACCP is a system that requires that potential hazards are identified and controlled at specific points in a process. HACCP has seven principles:

1. Conduct a hazard analysis (HA).
2. Identify the critical control points (CCP).
3. Establish the critical limits.
4. Monitor CCP.
5. Establish corrective actions.
6. Verification.
7. Record keeping.

The World Health Organization published a paper in 2003 providing more details on using HACCP as a tool within a pharmaceutical environment, details which can be further adapted and interpreted for use within a tissue establishment [1].

The *Quality Assurance Journal* has also published papers on the use of HACCP as a tool within a QMS [2].

3.3. Failure mode, effects and criticality analysis

FMECA is an extension of Failure mode and effects analysis (FMEA) that includes a factor for detectability, taking into consideration those hazards that are more easily detected and represent a lower overall risk. FMECA allows the estimation of a risk priority number (RPN) for the ranking of identified risks [3]. The estimated level of risk should take into account the benefits, possible alternatives and costs associated with reducing risk further. An organisation should have a clear policy regarding risk acceptance (see Figure 3.1).

Undertaking risk assessment at various stages helps to define requirements and alternatives, aids the process of supplier selection and helps to determine the scope and extent of validation. The methodology has been used for tissue and cell banking for specific process steps [4] and for reviews of an entire process [5].

Risk assessment is not a once-only process but a cyclical one (Figure 3.2). Risk assessment should be followed by risk avoidance and reduction (if possible) and continual re-evaluation of residual risk.

Guidance on quality risk management is provided in Part III Q9 of the Rules governing medic-

Figure 3.1. **Failure mode, effects and criticality analysis (FMECA)**

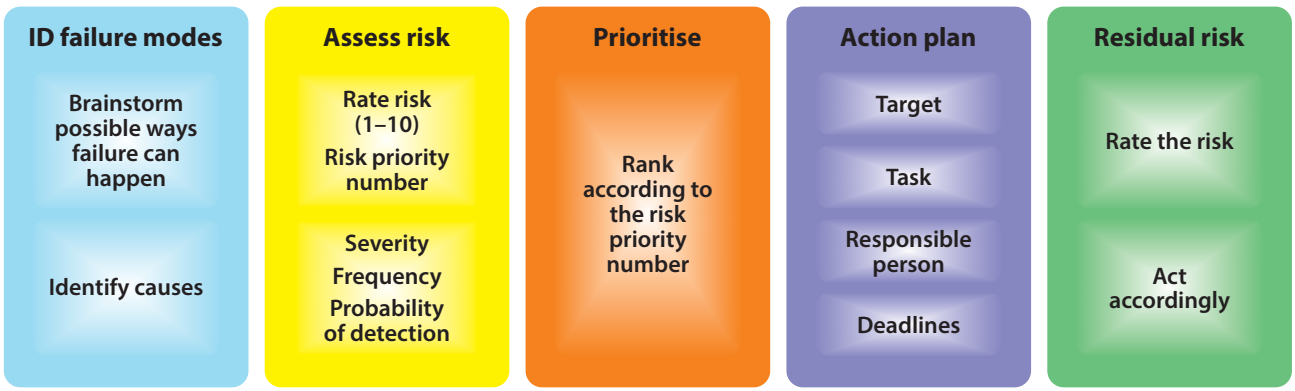
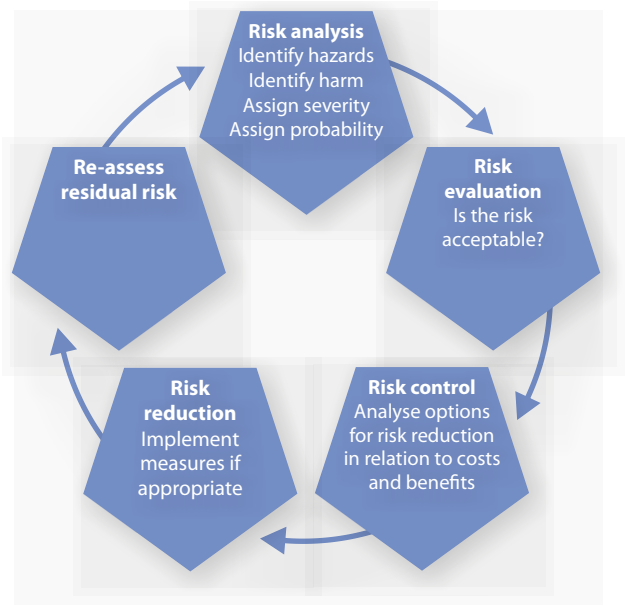


Figure 3.2. **Cycle of risk assessment**



inal products in the EU, Volume 4: EU Guidelines for good manufacturing practice for medicinal products for human and veterinary use [6], in which several well-established risk-assessment methodologies are listed. Inclusion of this new section in GMP guidance reflects the current thinking that risk management should be an integral part of quality management.

3.4. Root cause analysis

Root cause analysis (RCA) is a tool used to understand the true cause of why an adverse event has occurred. Through an investigation of the adverse event and getting to the root cause, RCA is able to identify corrective and preventive actions (CAPA) that will prevent any occurrence of the event in the future.

RCA has five purposes:

a. Establish the facts and events that led to the adverse event;
b. Identify what went well;
c. Determine what went wrong;
d. Establish the root cause of what went wrong;
e. Identify CAPA.

When carrying out RCA there are five main factors which need to be considered:

1. Plant – what was used, was it calibrated, was it maintained etc?
2. People – who was involved, were they trained, competent and capable?
3. Procedure – is there an SOP, was it followed, is it correct, has it been validated?
4. Premises – location, department, environment etc?
5. Product – how many times has this occurred, number of products affected, damage, defects?

When performing RCA, there are additional tools that can be used, such as fishbone diagrams, 5 whys, flowcharts and timelines. Examples of these have been included as Appendices 8, 9 and 10.

3.5. EuroGTP II process

3.5.1. Background

Whenever a novel process is implemented, or an existing process revised, it is incumbent on a tissue establishment (TE) to assess the risk resulting from this change. The Euro Good Tissue Practices II (EuroGTP II) project was funded by the European Union to develop a process to enable TEs to do this in a systematic and consistent manner [7]. The project brought together TEs, Competent Authorities, professional societies and independent experts, and produced a number of key deliverables, including an interactive assessment tool (IAT) to perform risk assessment and a detailed guide for application of the IAT. Both of these deliverables can be accessed online [8].

3.5.2. Principles

There are three steps to the EuroGTP II methodology (see Figure 3.3):

a. *Identification of novelty*
With any new process, or change to an existing process, there is a likelihood that significant changes, which could impact on the safety and/or clinical efficacy of the product, will need to be implemented. The first step of the process requires that the user considers each step of the supply chain, from donation to transplantation, to determine if any such significant change has been made. If any is identified, the user proceeds to step 2.

b. *Identification and evaluation of specific risks*
If significant change (such as a new process, or significant change to an existing process) is identified, the user must then determine the specific risks and risk consequences that the change raises, and the likelihood, severity, detectability and evidence that can be used to mitigate the risk, for each risk. Based on the scores allocated, the IAT calculates an overall

risk score for the change, ranging from negligible to high.

c. *Risk mitigation and extent of clinical evaluation*
Depending on the outcome of step 2, the user may need to take steps to reduce the risk resulting from the change. The EuroGTP II guide provides guidance as to how this can be done, listing *in vitro* and *in vivo* assays that can be used to further investigate specific risks. The Guide also suggests clinical follow-up methodologies that are appropriate to designing clinical evaluations or registries for the products in question, either to collate additional data to inform the risk analysis or to implement routine follow-up methods and registries following implementation of lower risk products.

3.5.3. Rationale

The EuroGTP II process provides a structured and systematic methodology to enable TEs to identify, quantify and mitigate risks associated with introducing novel procedures, and changes to existing ones.

The online IAT can be used to document and record the decisions made and rationales used in the different stages of the risk-assessment process, and the outcome can be printed and shown to Health Authorities as evidence that an appropriate risk-assessment process has been undertaken with respect to novel and changed processes. Furthermore, it is a continual process that can be repeated as and when new data become available. A key requirement of the process is that sufficient resource and expertise be committed to the risk assessment to ensure an accurate outcome. The process is already being applied in a number of TEs [9, 10].

3.6. EDQM Microbiological Risk of Contamination Assessment tool

3.6.1. Background

The aseptic procurement and processing of tissues and cells (TC) are some of the more difficult processes conducted by tissue establishments (TEs) and entail some degree of risk. Failure in some of these process steps may lead to microbiological contamination and loss of TC, or may even pose a potential health risk if the contamination is not detected before implantation or use. Microbial safety cannot be assured only by finished-product testing because the available microbiological test methods are not always fully conclusive, with the possibility of false negative results, and sometimes test results are not yet available at the time of implantation or use. In addition, the environmental monitoring that is often performed during aseptic processing of TC can hardly be considered as a quantitative measurement of the level of cleanliness of the aseptic environment because of the limitations of classic microbiological air-sampling methods.

The EDQM *Guide to the quality and safety of tissues and cells for human application* collates the most up-to-date technical guidance to designing and maintaining an aseptic procurement and processing. Since the beginning of the 21st century it has been recognised that a risk-based approach provides justification for choices made throughout the design and implementation of processes, and it is becoming evident that risk mitigation is a valuable component of an effective quality management system.

Numerous risk analysis methods, such as Fault tree analysis (FTA), Failure mode effect analysis (FMEA), Hazard analysis and critical control points (HACCP) or Failure mode effect and criticality analysis (FMECA), have been proven successful in a variety of applications.

However, none of these methods was specifically developed for aseptic procurement and processing of TC, which rely on a large number of variables: cleanliness of the local infrastructure, garment, TC type, number and competence of personnel, degree of qualification of infrastructure, equipment and materials used, procurement and processing methods, decontamination processes, environmental monitoring processes and local expertise, among others. Therefore, experts from the EDQM have developed a methodology specifically to assess aseptic risks in procurement and processing of tissues and cells.

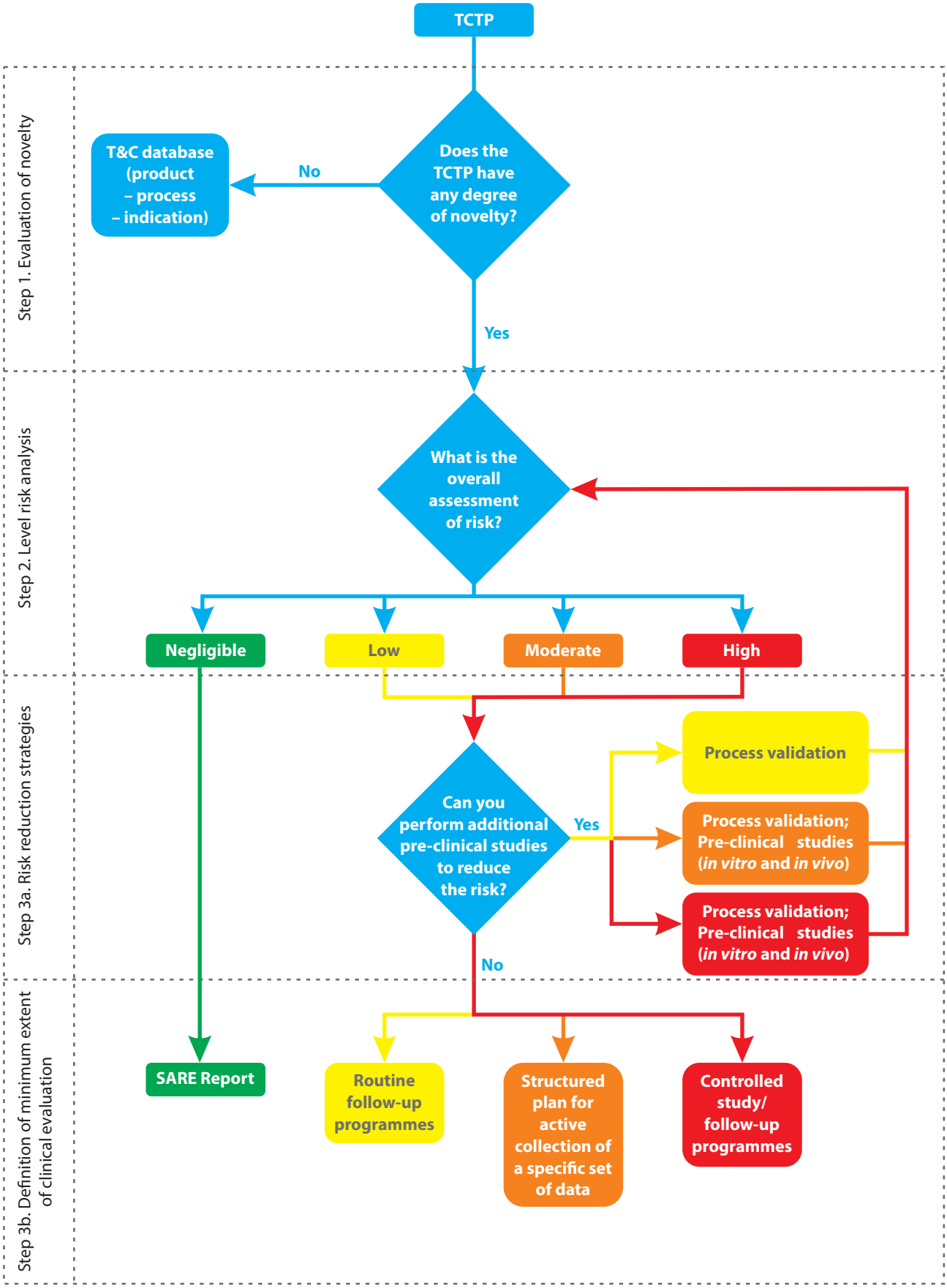
3.6.2. Approach

The EDQM Microbiological Risk of Contamination Assessment tool for tissues and cells (MiRCA) [11] has been developed to:

a. identify potential risks in novel, existing or modified aseptic processes;
b. alert management to the degree of risk of introducing microbiological contamination during the procurement or processing of TC;
c. support management decisions to mitigate risks in aseptic processes.

The objective of MiRCA is to prevent the loss

Figure 3.3. **Overview of the EuroGTP II process: assessing tissue and cellular therapies/products (TCTPs)**



of TC due to microbial contamination during aseptic procurement and processing and to reduce the risk of infection being transmitted to recipients. MiRCA starts from the assumption that the primary source of microbial contamination during aseptic processing is human, more specifically personnel and patients. This assumption is based on the knowledge that a gowned operator may release continuously as many as 10 000 colony-forming units per hour [12, 13].

However, MiRCA has developed a much broader approach focusing on all sources of contamination affecting the deposition of micro-organisms during aseptic processing. In many aseptic processes, operators are using inanimate objects, such as equipment, materials and reagents, that could disperse their load of microbial contamination to the environment or directly to TC. Also, the training of personnel and the qualification and monitoring of environments and equipment has an impact on the deposition of micro-organisms. Special interest is also given to the presence and effectiveness of decontamination processes, quality control and monitoring processes. The implementation or non-implementation of these processes is included in MiRCA and each process is assessed as an individual risk factor.

MiRCA identified in total 55 individual risk factors: 22 individual risk factors that affect the deposition of micro-organisms during aseptic procurement of TC and 33 individual risk factors that affect the deposition of micro-organisms during aseptic processing and distribution of TC. Among international standards and guidelines there is broad consensus that risk has two dimensions: probability of occurrence and impact of occurrence on the objective. Normally the duration of occurrence is part of the probability of occurrence. In our approach it was not always possible to incorporate the duration of the exposure into the probability of the occurrence. For that reason, a third dimension was created: temporal distribution.

The semi-quantification of risk, expressed as an individual risk value (IRV), is calculated by multiplying the probability of occurrence (P), the impact of occurrence on the objective (I) and the temporal distribution (T) of each risk factor:

$$IRV = P \times I \times T$$

3.6.2.1. *Probability of occurrence*

The probability of occurrence (P) estimates how likely is the deposition of micro-organisms to occur due to individual risk factors. The probability spectrum is expressed on a scale of 0 to 5.

0 = negligible or not applicable
1 = very low
2 = low
3 = moderate
4 = high
5 = very high

For example, for the risk factor "air filtration system of the procurement environment":

0. Not applicable or closed system. The probability of deposition of micro-organisms is negligible.
1. Temperature controlled HEPA-filtered air flow. The probability of deposition of micro-organisms is very low due to this air filtration technology.
2. Vertical or horizontal laminar HEPA-filtered air flow. The probability of deposition of micro-organisms is low due to this air filtration technology.
3. Turbulent mixed HEPA-filtered air flow. The probability of deposition of micro-organisms is moderate due to this air filtration technology.
4. Turbulent mixed non-HEPA-filtered air flow. The probability of deposition of micro-organisms is high due to this air filtration technology.
5. No ventilation. The probability of deposition of micro-organisms is very high because there is no air filtration technology available.

3.6.2.2. *Impact of occurrence on the objective*

The objective of MiRCA is to prevent the loss of TC due to microbial contamination during aseptic procurement and processing. But not every risk factor has the same impact on the loss of TC due to microbial contamination. In order to classify the risk factors according to their impact (I), MiRCA applies an impact factor of 1 to 5, based on the assumption that the main mechanism for microbial dispersion during aseptic processing is airborne deposition:

1 = The risk factor indirectly affects the deposition of micro-organisms in the background of the environment that TC are exposed to. For example, periodical qualification of the background at rest has no direct influence on the deposition of micro-organisms in the background, but it tells us whether the microbiological load of the background is within the specifications.

2 = The risk factor directly affects the deposition of micro-organisms in the background of the environment that TC are exposed to. For example, the number of personnel present in the background of the processing environment has a direct influence because these operators

continuously release micro-organisms in the background of the environment that TC are exposed to.

3 = The risk factor indirectly affects the deposition of micro-organisms in the environment that TC are exposed to. For example, the frequency of sanitisation of the procurement environment affects the microbiological load of furniture, equipment, floors and walls, which has in turn an impact on the microbiological load of the procurement environment that TC are exposed to.

4 = The risk factor directly affects the deposition of micro-organisms in the environment that TC are exposed to. For example, the number of personnel present during procurement because they continuously release micro-organisms into the procurement environment that TC are exposed to.

5 = The risk factor directly affects the deposition of micro-organisms to TC, for example, reagents used for the storage of TC. Non-sterile reagents directly contaminate TC.

3.6.2.3. *Temporal distribution*

The temporal distribution (T) of risk factors that have an impact on the airborne deposition of microbial contamination is estimated by the time during which the TC are exposed to their direct environment, expressed in hours – for example, the time that an open container containing TC is exposed to the process environment, or the time that musculoskeletal tissues are exposed to the air of the procurement environment during procurement. Other risk factors, mostly intervention-dependent, are assessed as present/applicable or not present/not applicable – for example, the use of reagents, personnel competence.

At the end, all IRVs are added up to calculate a final risk score for the procurement of TC. The outcome of the procurement of TC is added as an individual risk factor in the assessment of the processing and distribution of TC. The outcome of the global process is calculated as the sum of all individual risk values of processing, multiplied by the individual risk value of the terminal sterilisation or decontamination method, expressed as a percentage, with the sterility assurance level (SAL) shown in brackets.

- Validated sterilisation process (SAL $\leq 10^{-6}$), e.g. gamma irradiation
- Any validated method that achieves (SAL $\leq 10^{-5}$ or $\leq 10^{-4}$), for example a chemical treatment.
- Any validated method that achieves (SAL $\leq 10^{-3}$), for example a validated antibiotic cocktail at 22 to 37 °C.
- Any validation method that achieves (SAL $\leq 10^{-3}$ and $\leq 10^{-2}$) for example, a validated antibiotic cocktail at 2 to 8 °C
- No decontamination or not validated decontamination method

3.6.3. Interpretation of outcomes

The final risk scores, expressed as a percentage of the maximal achievable risk score, are then translated to a risk profile by an exponential algorithm, which is proportional to the overall risk of introducing microbial contamination during procurement or processing and distribution of TC:

0-3.12 %	Extremely rare
3.13-6.24 %	Rare
6.25-12.4 %	Unlikely
12.5-24.99 %	Possible
25.00-49.99 %	Likely
50.00-100 %	Almost certain

MiRCA provides a structured and systematic methodology to enable TEs to identify, quantify and mitigate aseptic risks of established and novel aseptic processes. Furthermore, it is a continual process, which can be repeated in preparation of management decisions, or as a step within the CAPA process or the change-control process. The aseptic risk outcome can be used during the EuroGTP II exercise when assessing the probability of a specific aseptic risk consequence.

MiRCA can help TEs to define the minimal requirement of their procurement and processing facilities with respect to all applicable aseptic risk factors and any subsequent decontamination step taken after procurement or processing (see also Chapter 7 and Chapter 9). MiRCA cannot be used as proof of deviation from the minimal air quality requirements set by the directive and/or national laws.

The online tool [11] can be used to document and record the decisions made and rationales used in different stages of the risk-assessment process, and the outcome can be printed and shown to Health Authorities as evidence that an appropriate risk-assessment process has been undertaken with respect to novel and changed aseptic processes.

A key requirement of the process is that sufficient resource and expertise be committed to the risk assessment to ensure an accurate outcome.

3.7. References

1. World Health Organization. Application of hazard analysis and critical control point (HACCP) methodology to pharmaceuticals. Technical Report Series, No. 908, Annex 7, 2003.
2. Dahiya S, Khar R, Chhikara A. Opportunities, challenges and benefits of using HACCP as a quality risk management tool in the pharmaceutical industry. *Qual Assur J* 2009:12(2):95-104.
3. Stamatis DH. *Failure mode and effect analysis: FMEA from theory to execution*. Milwaukee WI, USA: ASQC Quality Press, 1996. ISBN 0-87389-300-X.
4. Wijk MJV, Geyt CV, Laven ABH *et al.* Physical examination of potential tissue donors: results of a risk management procedure to identify the critical elements of the physical examination. *Cell Tissue Bank* 2012:13(4):547-63.
5. Bambi F, Spitaleri I, Verdolini G *et al.* Analysis and management of the risks related to the collection, processing and distribution of peripheral blood haematopoietic stem cells. *Blood Transfus* 2009;7(1):3-17.
6. EudraLex [collection of rules and regulations governing medicinal products in the European Union]. Volume 4: EU guidelines for good manufacturing practices – medicinal products for human and veterinary use (GMP), Annex 15, available at http://ec.europa.eu/health/documents/eudralex/vol-4_en, accessed 26 March 2022.
7. EuroGTP II: a tool to assess risk, safety and efficacy of substances of human origin. Trias E, Lomas R, Tabera J *et al*; EuroGTP II Study Group. *Int J Qual Health Care*. 2020 April 21;32(1):80-84. https//doi.org/10.1093/intqhc/mzz048.
8. EuroGTPII. *Good tissue & cell practices*, available at www.goodtissuepractices.eu/, accessed 26 March 2022.
9. Banking of corneal stromal lenticules: a risk-analysis assessment with the EuroGTP II interactive tool. Trias E, Gallon P, Ferrari S *et al. Cell Tissue Bank*. 2020 Jun;21(2):189-204. https://doi.org/10.1007/s10561-020-09813-8.
10. Lierman S, Bus A, Andries S *et al. Cryobiology* 2021 Jun;100:164-72. https://doi.org/10.1016/j.cryobiol.2021.01.013. Epub 2021 Jan 21. PMID: 33485899.
11. EDQM Microbiological Risk of Contamination Assessment tool (MiRCA), available at https://soho-guides.edqm.eu/.
12. Reinmüller B. "People as a contamination source – clothing systems" in: Dispersion and risk assessment of airborne contaminants in pharmaceutical cleanrooms. *Royal Institute of Technology, Building Services Engineering, Bulletin No. 56*, Stockholm (2001), pp. 54-77.
13. Agence de la Biomédecine. *Guide d'aide à l'identification et à la réduction des risques appliqué aux erreurs d'attribution et à la gestion d'une salle de cryoconservation*. Agence de la Biomédecine, France (2019), available at www.agence-biomedecine.fr/IMG/pdf/190917_guide_d_aide_a_l_e_blaboration_d_une_cartographie_des_risques.pdf, accessed 14 December 2022.

Related material

Appendix 8. Example of root cause analysis: 5 whys – receipt of tissue
Appendix 9. Example of root cause analysis: fishbone diagram – receipt of tissue
Appendix 10. Example of root cause analysis: fishbone diagram – medically assisted reproduction

Chapter 4. Recruitment of potential donors, identification and consent

4.1. Introduction

Human tissues and cells may be used for transplantation therapy or for medically assisted reproduction (MAR). They can be obtained from living or deceased donors, as long as the retrieval procedure does not endanger the life or health of a living donor or compromise the respect due to a deceased person. Transplantation of tissues and cells ranges from life-saving treatments (e.g. serious burns victims, general sepsis due to prosthesis infection, haematological malignancies) to quality-of-life improvements (improvement of vision or restoration of joint movement). The donation of donated gametes and embryos may help fulfil a person's wish to have children. Human tissues and cells are also starting material for advanced therapy medicinal products (ATMPs).

In order to ensure availability of tissues and cells, and the safety and success of any transplantation or human application programme, potential living and deceased donors need to be identified and recruited for potential tissue donation. In all cases, screening must be performed to ensure the safety of the tissues and cells donated, to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donor themselves.

Donation programmes should at least include [1]:

a. adequate public-awareness strategies, promoting organ donation and tissue and cell donation;

b. effective systems to facilitate the recruitment of living donors in an ethical manner, ensuring their safety and well-being, and the identification and referral of potential deceased tissue donors to the appropriate organisation/tissue establishment (e.g. routine medical chart reviews in every case of in-hospital death);

c. adequate training of professionals involved in the recruitment, identification and referral of potential donors.

Once potential donors are recruited, or identified and referred, informed consent is an essential requirement before donation can take place. Consent is obtained from the donors themselves in the case of living donors or from their legal representatives if the donor is juvenile or incapable. In the case of deceased donors, consent is obtained either from the donor before death (e.g. donor registries, donor card, advanced directives) or from their relatives; see Appendix 11 and following. The way in which consent is obtained depends on the type of donor, the specific circumstances and the specific legal system for consent in each particular country. Although the term 'consent' will be used throughout this chapter, the Guide recognises that in some countries the term 'authorisation' rather than 'consent' is used to enable lawful recovery of tissues and cells.

This chapter describes the process, requirements and key elements at the beginning of the donation pathway for both living and deceased donors.

4.2. Living donors

Some tissues and cells can only be obtained from living donors. This is true for haematopoietic progenitor cells (HPC); for oocytes, spermatozoa, ovarian or testicular tissue and embryos, used in MAR procedures; and for mesenchymal stromal cells and some somatic cells, such as keratinocytes and chondrocytes.

Some tissues can be collected as surgical residues (e.g. umbilical cord blood and placenta following child birth, femoral heads removed during surgery to replace a hip joint, heart valves from patients receiving a heart transplant). Depending on how the tissues or cells will be used, their clinical application can be described as:

a. autologous: when the tissues or cells procured from a patient are used for the patient's own treatment (e.g. skull bone obtained from a decompression craniotomy, ovarian tissue as part of specific programmes to preserve fertility in oncological female patients, HPCs in therapies for haematological malignancies); or

b. allogeneic: when the tissues or cells donated by one person are used for the treatment of another person. In this case, the material can be donated for:

i. an intended recipient, who can be related or unrelated to the donor (e.g. HPC) – a directed donation; or

ii. a recipient who is unrelated to the donor (e.g. amnion/bone).

In the case of MAR, depending on the origin of the gametes and embryos, the donation can be classified as:

i. partner donation (donation between a couple who declare that they have an intimate physical relationship);

ii. non-partner donation (donation of reproductive cells between individuals who do not have an intimate physical relationship; also called 'third-party donation')

The selection of a living donor must be based on a risk–benefit analysis for both the donor and the recipient. In the case of surgical residues, there is no risk to the donor derived from the donation itself.

In some instances, donation may occur years after the initial selection and registration (e.g. in HPC donation, when potential donors are included in a registry and the donation only takes place if a matching recipient needs it).

4.2.1. Donor recruitment

Donor recruitment is a continuous process. It begins with increasing the awareness of the general public, by education at local and national levels about the benefits of the clinical applications for different tissues and cells. Recruitment drives should focus on altruism, solidarity and social engagement, based on the principles of voluntary unpaid donation.

Success stories describing donor and patients' experiences and testimonials of family members may drive people to consider whether tissue or cells donation is right for them. Public campaigns should aim to diversify and increase the pool of available donors, increase the number of donations in general and augment registration in living donor registries. Publicising and/or advertising the need of donated tissues or cells for a given patient should be discouraged (and in some countries such activities are forbidden/regulated by law).

The most common instances of donation from living donors where donor recruitment plays an essential role are the donation of HPC and of gametes and embryos for MAR procedures.

4.2.1.1. *Recruitment of haematopoietic progenitor cell donors*

Donation of HPC must be voluntary and unpaid, and informed consent must be obtained in the preliminary steps. Successful HPC transplantation depends on matching the donor and recipient for human leukocyte antigens (HLA). If a patient needs an HPC transplant, their siblings and close relatives should be screened to determine if their HLA types are compatible. If the donor is under the age of legal consent, specific consideration is needed (see Chapter 24).

For patients without a matching family donor, there is a possibility that an HLA-compatible voluntary donor can be found. Therefore, it is of crucial importance that volunteer donors are recruited and registered on donor registries around the world, particularly from diverse ethnic communities. Donor registries and cord blood banks are linked internationally, in a global database operated by the World Marrow Donor Association (WMDA), using tissue-typing to establish rapid identification of potential donors.

When a person volunteers to be an HPC donor, an initial evaluation is performed. Suitable donors are HLA-typed and their details are placed on a donor registry. Potential donors are requested to inform the registry in case of any change to their health status that could contraindicate donation or the possible use of the cells.

Potential HPC donors will only be contacted if they are identified as the best possible match for a patient. In this case they will be requested to attend a donation centre for a full explanation of the procedures by the clinical staff and a thorough medical examination with blood tests to detect any medical and/or infectious contraindication to donation. Further information on donor registries and recruitment of HPC donors can be found in Chapter 5 and Chapter 24.

4.2.1.2. *Recruitment of cord blood donors*

Umbilical cord blood (UCB) donor recruitment usually starts during pregnancy, but it may also occur as late as at admission into the maternity unit, provided the mother is fit to provide an informed consent. The UCB donation can be made available for public or private uses. Public banks provide UCB units stored for unrelated transplantation, in order to increase access to UCBs through worldwide donor registries, whereas in private banks, which are usually for-profit organisations, the donor keeps ownership of the product. These organisations offer the processing and storage services for future potential medical applications for donors and their family. Information leaflets or brochures about UCB banking are an important part of the recruitment plan, and any claims made in recruitment materials must be supported by scientific evidence [2] (see Chapter 25).

4.2.1.3. *Recruitment of donors in medically assisted reproduction*

With the development of MAR and changes in lifestyles, a growing demand for gamete and embryo donors has emerged. These developments in most countries also reflect changing social values and government financial support. This growth has not always been accompanied by similarly swift development of laws and regulations in the field. Gradually, countries have set legal provisions and/or guidelines based on their own historical, cultural, religious and social traditions and their political and economic situations [3]. Consequently, there are wide variations in the techniques available and permitted by law in each country, and the types of reproductive cell that can be donated. Whatever the situation, national laws must be respected and donors recruited accordingly.

As mentioned previously, gamete and embryo donation can be classified as partner or non-partner donation, depending on the link with the donor. This chapter will focus on the recruitment of non-partner donors.

The practice of gamete and embryo donation is complex, and multiple ethical rules, legal restrictions, medical facts and social and psychological consequences intermingle. Things are further complicated by such factors as:

a. the reasons why a person donates;
b. what kind of reproductive cells are donated (donation of oocytes or sperm or embryos commits the donor to a different investment in the medical interventions required and the risk of harm);
c. the level of anonymity, whether a known donor, identifiable or anonymous;
d. the level/type of compensation schemes for donors;
e. how often a donor can donate and how many offspring may be derived from a single donor.

The scarcity of donor gametes has stimulated discussion about acceptable systems of recruitment, especially since it has become evident that different clinics and countries are using different approaches to recruit donors, with various modes of compensation for donors (e.g. compensation of proven expenses, compensation through lump sums, oocyte-sharing schemes). However, it is essential to emphasise that the donation of reproductive material should strictly follow the same principles of being voluntary and unpaid as for other donations of tissues or cells. Extra care is required since any type of reward, benefit or incentive may be a threat to voluntariness and compromise the degree of confidence that can be placed in the donation process (see Chapter 29).

The donor-recruitment activity, whether performed by a public health system or by private clinics (where allowed by national legislation), must be authorised by the Health Authority.

As with any living donors, it is essential that gamete donors are entered into registries, not as a database of potential donors, such as for HPC, but to guarantee traceability and transparency of practice (see Chapter 16). In addition, registries allow adequate follow-up of the donors, including the collection of data on serious adverse events or adverse reactions in donors, in recipients and in the resulting offspring (see Chapter 17).

Further information on recruitment of gamete and embryo donors can be found in Chapter 29.

4.2.2. Consent for living donation

The donation process differs depending on whether a person donates tissues and cells for the benefit of others, and accepts risks and inconvenience that they would not otherwise face, or donates while undergoing a medical intervention for their

own benefit. Donation implies an altruistic act and, to some extent, a trade-off between individual well-being and societal utility. As a consequence, there is potential for the abuse and exploitation of individual donors. Obtaining individual consent, in any situation of donation, either collection of surgical residues or tissues and cells procurement, is crucial to assure that donation process conforms to professional ethical standards and the individual's own goals and values.

Donation of tissues or cells must only be carried out after the person concerned has given free, informed and specific consent, either in written form or orally by a trained professional.

Consent should be recorded and/or documented in the donor/patient's record. Informed consent must be discussed with the donor or their legal representative in a language and with terms that they can understand. If translation is needed, it is recommended that neither family nor friends serve as interpreters or translators. The record should mention that the prospective donor has understood – and, where appropriate, their legal representatives or their relatives have understood – the information given, had the opportunity to ask questions, received satisfactory answers and confirmed their position on donation. Some examples of forms to obtain consent for MAR may be found in Appendix 11, Appendix 12 and Appendix 13.

Recruitment of persons not able to consent (e.g. donors who are too young or who are mentally incapacitated) must never be done through public registries. In addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some countries do not allow procurement of peripheral blood progenitor cells from the very young and/or administration of growth factors to donors).

In accordance with Article 14 of the Additional Protocol to the Oviedo Convention, no tissue or cell procurement may be carried out on a person who does not have the capacity to consent. Exceptionally, and under the protective conditions prescribed by law, the removal of HPC for allogeneic transplantation from a person who does not have the capacity to consent may be authorised provided the following conditions are met:

a. there is no compatible donor available who has the capacity to consent;
b. the recipient is a brother or sister of the donor;
c. the donation has the potential to be life-saving for the recipient;
d. the authorisation of their representative or an authority or a person or body provided for by law has been given specifically and in writing and with the approval of the competent body;
e. the potential donor concerned does not object.

Potential donors must beforehand be given appropriate information as to the type(s) of tissues or cells to be procured, the purpose and nature of the intervention, its consequences, possible side-effects and risks, whether the tissues or cells will be processed and stored, and the purpose or use to which the tissues or cells will be put. All relevant information should be given because consent must be specific and cannot be assumed for uses of tissues or cells about which the donor has not been informed. Donors must be given all the information needed to ensure that they understand all potential purposes and benefits for the recipient before they give consent. Full understanding of the consent is particularly important when practices may be controversial or sensitive. Similarly, some donors may not wish to donate tissues or cells to a commercial organisation where, for example, their donated tissues or cells may be used as starting material for developing therapies in a commercial setting.

Potential donors should have an advocate, who must not be involved in the treatment of the recipient. Their main role is to look after the interests of the donor, ensuring at all times that the donor's safety and the consent processes are appropriately handled. Information about potential risks for the donor, and risks and benefits for the recipient, must be clearly understood before consent is given. Where relevant, the donor must also be informed that the results of the qualification of tissues and cells for transplantation and the transplant itself are uncertain because they depend on many factors, including the recipient's condition. Where relevant the donor should also be provided with information about psychological support in case the transplant does not lead to survival or cure of the recipient.

The scope and duration of the consent should be stated explicitly. When the tissues and cells are to be stored, the consent should include information about the storage time and the policy once the period has expired. The interview for consent should be conducted in a suitable environment. The interviewer should have received specific training for this purpose to be able to consider the donor's needs and to answer questions about donation, risks and transplantation processes.

Potential donors, or their legal representatives, or their relatives, should be informed of the tests that will be performed to evaluate the possible existence of any transmissible diseases that would be

a risk to the recipient, and of the action to be taken in the case of a positive result. In such a case they should be informed and receive adequate counselling. All results of the medical evaluation should be kept available to the donor. If a potential donor is found to be unsuitable, the reasons and the results of the medical assessment should be discussed with them and advice should be given on recommended actions regarding their health. If the findings do not exclude the potential donor but may have implications for the recipient (e.g. an HPC potential donor carrier of a haemoglobinopathy), the transplant centre must also be informed.

In the case of HPC donors, consent must be obtained at several stages: before HLA typing, before donor testing and before the conditioning regimen on the recipient has begun. The interview must include information about donation procedures, their risks and side-effects, the procurement method and the need to administer growth factors when peripheral HPC is being procured. In the case of cord blood donation, consent is usually obtained months before the delivery and should at least be obtained before the mother goes into labour to avoid interfering during the delivery.

Documentation must be made available to the cell or tissue establishments that receive the procured material for processing and storage to confirm that detailed consent has been duly given (see Chapter 2).

Donors must be informed that they may withdraw consent at any time. In the case of HPC donors, they have to be informed of the possible consequences for the recipient if they withdraw their consent once the conditioning regimen on the recipient has already begun. Ultimately, this situation cannot be used to coerce the donor and their final decision must be respected. In that case every effort needs to be made to find alternative donors e.g. haplo-identical related donors or HPC donation should take place and cells cryopreserved prior to conditioning of the patient.

In the case of autologous donation, the patient must be informed about options and the balance of risks and benefits of the procedure. The consent must specify what would happen if the clinical application could not be performed for any reason (e.g. contamination of the transplant or if the patient's condition contraindicated application). In such instances, the consent should indicate whether the tissues or cells could be used for the treatment of others or for research, or must be disposed of.

Consent should be obtained from the patient before procurement. In some circumstances, this is not possible (e.g. emergency craniotomies) and the decision to procure and, if necessary, to process the tissues or cells should be taken by the medical team.

In the case of planned surgical procedures where it may be possible to donate surgical tissues, consent should be obtained before procurement. As is the case for all tissue or cell donors, the donor should be informed beforehand about tests to determine the suitability of the transplant, and consent should include information about the processing, storage and intended use of the donated material. The consent must also specify the fates of tissues or cells if their clinical application cannot be performed for any reason.

Donors, or their legal representatives, or their relatives, should be aware of the importance to recipient safety of providing the medical staff with information about any medical condition of the donor that may occur after donation.

They should be informed about, and give consent for, the use of personal identifiable data in computerised data processing.

4.3. Deceased donors

Tissues donated by a single donor can be used to treat as many as 100 recipients. Tissues donated by deceased donors typically include the following tissue types:

a. musculoskeletal: bones, tendons, ligaments of the upper and lower extremities, menisci, fascia, cartilage;
b. cardiovascular: heart valves, blood vessels, pericardium;
c. skin: split thickness and full thickness (epidermis and dermis);
d. ocular: corneas, sclera;
e. specific cell types from certain tissues (e.g. limbal stem cells) or from certain organs (pancreatic islets, hepatocytes).

In order to guarantee the success of any donation programme from deceased tissue donors it is essential that hospitals (and other centres where potential tissue donors may be found) have a system to

a. identify potential donors, to
b. check consent, shown by
  i. the presence of donor's consent or
  ii. the lack of donor's objection expressed by donor before their death or
  iii. consent obtained from family members or relatives of the potential donor, and
c. refer donors to the appropriate procurement organisations and tissue establishments.

Communication with bereaved family members or relatives will require clear and sensitive procedures or protocols, with consent obtained by appropriately trained specialists (see Appendix 14).

4.3.1. Identification and referral of deceased tissue donors

Tissue donation depends on commitment and the development of strong working relationships between tissue establishments, procurement organisations and hospitals and other centres where potential tissue donors may be found, such as medical examiners' offices, mortuaries, coroners' offices, forensic institutes, funeral homes, emergency medical services, and nursing or retirement homes [7].

It is recommended that any potential donors considered for organ donation are also referred for potential tissue donation [8]. Furthermore, it is recommended that all deaths (typically hospital but also community deaths) should be routinely referred to a donor co-ordinator, procurement organisation or tissue establishment, regardless of the age of the patient or the cause of death. Routine referral of all potential donors with no known medical contraindication gives every individual the opportunity to donate and allows for the standardisation of donor-selection criteria.

Similarly to the WHO Critical Pathway for deceased donation [9], which was conceived as a useful clinical tool applicable in every country, region or hospital for assessing the potential of deceased organ donation, evaluating performance in the deceased donation process and identifying areas for improvement, a Clinical Pathway for deceased tissue donation has been designed by the CD-P-TO with the same goal [10]. The particular value of this tool is that creates uniformity in the description and assessment of the deceased donation process, and defines types of donor based on the different phases of the donation process: possible, potential, elegible, actual and utilised tissue donors (see Figure 4.1).

In every deceased donation programme, it is essential to identify all of the parties who may be involved, in order to co-ordinate and facilitate the process and maximise the opportunities for successful procurement. The key parties are summarised in Table 4.1.

Before procurement, checking the donor's identity is an essential prerequisite.

A proper investigation needs to gather as much information as possible about the donor, using medical records, interviews with medical staff who treated the donor (attending physician, general practitioner, nurse) and relevant information provided by the donor's relatives or legal representatives, family physician or other persons who have information about the donor's behavioural and medical history. The donor's medical suitability is assessed in accordance with the selection criteria, and after the evaluation of risk factors such as sexual behaviour, travel and exposure to sources of infection. A *post mortem* examination may also provide useful information. These criteria may vary, depending on the type of tissue to be procured for human application (see Chapter 5 for general criteria and Part B for tissue-specific criteria).

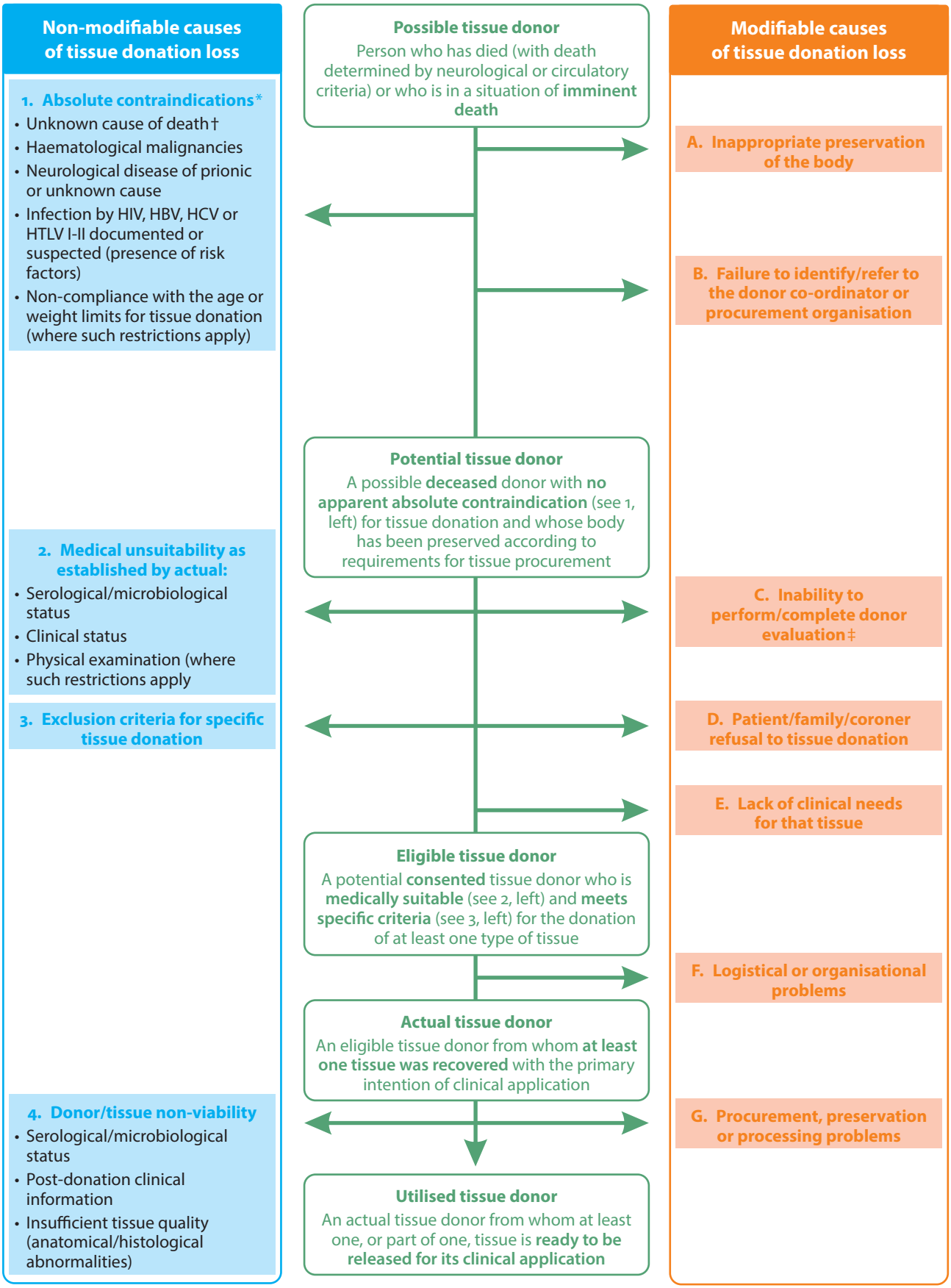
In the event that a health facility does not have the means to manage a potential tissue donor or is not licensed/authorised for tissue procurement by their Health Authority, arrangements should be made, where possible, for transfer of the potential donor to a suitable hospital or procurement centre.

4.3.2. Consent for deceased donation

Before procurement of tissues from a deceased person can take place, consent to donation must be obtained and recorded in order to ensure that their wishes are fulfilled. In some cases, the deceased person may have expressed their wishes while alive through a donor registry, donor card or advanced directive. In other cases, it is members of the family of the deceased person who decide whether donation was in accordance with the person's wishes, values and beliefs, or whether the deceased had expressed an objection to donation during their lifetime, and who give consent accordingly.

It is important to emphasise that consent needs to be specific. Therefore, donors' relatives or legal representatives must be given all the information needed to ensure that they understand all potential uses, including processing and storage, before they give consent. The specificity of consent is particularly important when the donated tissues and cells may not be used for transplantation/human application. Tissues and cells may be donated specifically for other purposes, or be used for other purposes should they prove unsuitable for transplantation/human application. These alternative uses may include (for example) use for teaching and training, validation and development, research or the development/preparation of commercial products. Specific consent must be obtained for any potential use (see Chapter 1). Consent needs to be given also for the final fate of the donation if the tissue/cells cannot be used for clinical purposes.

Figure 4.1. **CD-P-TO Critical Pathway for deceased tissue donation [10]**



* See European Guidelines.
† Unless an autopsy is performed to clarify the cause of death after tissue procurement.
‡ Including feasibility to perform validated serological/microbiological blood testing. See corresponding non-modifiable causes of tissue loss.

Table 4.1. **Key parties in deceased tissue-donation programmes and the challenges and opportunities they may pose**

	Opportunities	Weaknesses/Obstacles
Public	• Learn about donation and the benefit of transplantation to recipients • Promote solidarity and altruism • Express wishes to family and friends in relation to donation • Increase awareness of tissue donation at every opportunity – media, schools etc.	• Lack of knowledge about donation and tissue needs • Unwillingness to face death • Fear that an expressed desire to become a donor may interfere with medical care • Lack of trust in the fairness of the donation and transplantation system
Donor family	• Honour the wishes of the loved one • Make some sense out of the death of a loved one • Find comfort in knowing that donated tissues of the loved one helped save or improve lives [4] • Gain some control after the death of a loved one • Know that a part of the family member 'lives on' • Recognition and gratitude from society and recipients	• Grief • Lack of understanding or knowledge about donation • Fear of disfigurement • Not knowing the wishes of the loved one • Lack of trust in the medical profession or fairness of the donation system
Hospital staff	• Support wishes of the donor and their family • Increase availability of tissues for patients in need • Contribute to public awareness of donation and transplantation • Take part in the chain of donation, contribute to saving lives	• Discomfort or lack of knowledge about donation [5] • Lack of trust in the donation and transplantation system • Not acknowledging the value of tissues • Increased workload • Lack of acknowledgement from those involved in procurement • Lack of resources
Transplant co-ordinator	• Support wishes of donor and their family • Maximise donation opportunities – tissue donation may benefit as many as 100 patients per donor • Increase availability of tissue for patients in need • Contribute to public and professional awareness of donation and transplantation	• Concern that tissue consent will have an impact on consent for some organ donation [5, 6] • Lack of knowledge about tissue donation and its benefits • Increased workload
Coroner	• Mutually beneficial for coroner and procurement team/tissue establishment, who may share findings, tissue/blood samples and test results to help investigations • Support wishes of the donor and their family • Increase availability of tissues for patients in need	• Lack of knowledge about donation • Concern over potential impact on death investigation
Funeral homes	• Extend support to bereaved families • Raise awareness by including donation information in funeral homes and obituaries • Allow procurement of certain tissues to occur in funeral homes • Contribute to the conditioning of the body • Increase availability of tissues for patients in need	• Lack of knowledge about donation • Fear of difficulty in preparing body for funeral services • Increased time and costs • Delay in funeral services
Retirement homes, nursing homes and hospices	• Support wishes of the donor and their family • Raise awareness by including donation information • Allow procurement of certain tissues to occur on their premises • Increase availability of tissues for patients in need	• Lack of knowledge about donation • Lack of trust in the medical profession and in the donation and transplantation system • Many of the residents may be affected with conditions that contraindicate donation

4.3.2.1. *Legal consent systems*

Consent for the donation of organs and tissues from deceased donors is subject to national legislation and regulation in each country.

There are two legal consent systems for expressing consent to donation. Opting-in is a system in which consent to donation has to be obtained explicitly from the donor during their lifetime or from an authorised individual (usually the next of kin). Opting-out is a system in which consent to donation is presumed, no objection to donation has been registered by an individual during their lifetime or is known to have existed, or consent is inferred by the donor's family who could testify to the donor's position. In practice, variations exist within both systems, and the relatives may play a prominent role in the decision.

According to opt-in legislation, consent for removal of tissue from deceased donors must be given by the person when they were alive or, after their death, by their legally authorised representative or a 'person in a qualifying relationship to the deceased'. A hierarchy of qualifying relationships may exist (e.g., ranked from highest to lowest: spouse or partner, parent or child, brother or sister, grandparent or grandchild, niece or nephew, etc.) and consent should be obtained, where possible, from the person ranked highest in the hierarchy.

The opt-out system was introduced to help meet the shortfall in organs and tissues available for transplantation. However, in most programmes, if the family is against the idea of the donation proceeding, tissue procurement will not proceed; this is called a 'soft' opt-out system.

Even if the legislation is based on a presumed consent or opt-out system, the family and/or other persons who knew the donor well must be engaged in the donation process to provide information on medical and behavioural risks about the potential donor to ensure the safety of donated substances of human origin (tissues or cells).

Table 4.2 gives an overview of national consent systems in Europe. The information is up to date as of June 2022. Of the 42 answering countries, it appears that the majority (24 countries) have an 'opt-out' system, and 14 countries have an 'opt-in' system. Four countries have a mixed system, either combining elements of both 'opt-in' and 'opt-out' or, as in the United Kingdom, with an 'opt-in' system in one of the four UK administrations (Northern Ireland), and an 'opt-out' system in Scotland, Wales and England.

Different countries have different procedures to help people express their wishes regarding organ and tissue donation [2]. In each country, national legislation (or, if this is lacking, operational policies) should make clear what evidence (i.e. written or oral) is valid in their country to confirm consent or objection to organ and tissue donation.

Among the means to express wishes regarding organ and tissue donation after death are donor cards and donor registries. Individuals who have donor cards are also often simultaneously recorded in the national donor registry. Consent to donation recorded on a donor card or in a registry may contain detailed information, e.g. consent or not to various types of donation (donation of specific organs or tissues). In some countries, those who apply for a passport or driving licence have to state whether or not they are willing to donate organs, tissues and cells after death. Advance directives ('living wills') may also enable individuals to state prospectively under which medical conditions they do not want to receive life-sustaining therapy and allow documentation of people's wishes related to donating organs and tissues after death.

All national systems should enable individuals to modify or withdraw their consent or objection at any time. This ensures that the most recent information about an individual's wishes is recorded in some way and is available at all times should an enquiry be received from a physician or a donor co-ordinator involved in the donation process.

4.3.2.2. *Establishing consent in other circumstances*

In countries with no legal framework for consent to donation, or where a potential donor is not able to express their donation preference, for example a minor, the decision is, as a rule, left to the family of the potential donor, based on the assumption that the family would respect and represent the potential donor's wishes. Alternatively, consent may pass to those who are the nominated legal representatives of the potential donor, according to the laws of the country.

In some circumstances (e.g. when death occurs in suspicious circumstances or as a result of an illicit act), authorisation to proceed must be given by a coroner, a judge or a family court to avoid the procurement interfering with an investigation, even if the family has consented to donation.

In other circumstances, when a person was to become a donor (expressed wish or absence of refusal/objection), if no relatives can be found or contacted, national procedures and regulations should enable organ and tissue procurement where possible, but only if sufficient medical, social and behavioural information to support safe donation and transplantation can be obtained by other means (e.g. from family physician or friends). If this level of information cannot be reached, donation should not be considered, as it does not guarantee the safety of the recipient.

4.3.3. Approaching the families of potential tissue donors

Grief and mourning are crucial processes to help cope with the death of a loved one. When approaching a bereaved family to seek consent or information on the position of the deceased regarding donation, the many aspects of acute reactions to grief following a death should be understood in order to deal with the circumstances sensitively and in an understanding way. Donation professionals should learn to navigate the environment of acute grief to obtain a decision about donation that is best for the family. By applying their skills and experience, donation professionals can support the family in their mourning and provide appropriate help. The physician or donor co-ordinator who is in charge of approaching the family for donation should have accurate knowledge of the purpose and needs in tissue transplants.

4.3.3.1. *Conversations with the family*

Conversations with a family about organ donation do not generally differ from conversations related to tissue donation. Therefore, where appli-

Table 4.2. **Legal provisions in European countries for consent to/authorisation of organ donation from deceased persons**

Country	National consent system	Donor registry	Non-donor/refusal registry
1 Armenia	opting-out		
2 Austria	opting-out		x
3 Belgium	opting-out	x	
4 Bosnia Herzegovina	opting-out		
5 Bulgaria	opting-out		x
6 Croatia	opting-out		x
7 Cyprus	opting-in	×	
8 Czech Republic	opting-out		x
9 Denmark	opting-in	×	x
10 Estonia	opting-out	×	x
11 Finland	opting-out		
12 France	opting-out		x
13 Georgia	opting-in	×	x
14 Germany	opting-in		
15 Greece	opting-in	×	x
16 Hungary	opting-out		x
17 Iceland	opting-out		x
18 Ireland	opting-in		
19 Israel	opting-in	×	
20 Italy	opting-out	×	x
21 Latvia	opting-out	×	x
22 Lithuania	opting-in	×	x
23 Luxembourg	opting-out		
24 Malta	opting-in		x
25 Moldova	mixed system		
26 Montenegro	opting-in		
27 Netherlands	opting-out	×	
28 North Macedonia	opting-in	NA	NA
29 Norway	opting-out		
30 Poland	opting-out		×
31 Portugal	opting-out		×
32 Romania	opting-in	×	
33 Russian Federation	opting-out	×	×
34 San Marino	opting-out	NA	NA
35 Serbia	opting-out		×
36 Slovak Republic	opting-out		×
37 Slovenia	mixed system	×	×
38 Spain	opting-out	*	*
39 Sweden	mixed system	×	×
40 Switzerland	opting-in		
41 Türkiye	opting-in	×	
42 United Kingdom		×	×
a. England	opting-out		
b. Northern Ireland	opting-in		
c. Scotland	opting-out		
d. Wales	opting-out		

×: system in use; NA: data not available; *: some countries do not have opting-in or opting-out registries, but fulfil this requirement by advance will directives and/or registries that allow people to record their wishes about donation.

Source: Adapted from European Commission's implementation survey regarding Directive 2010/53/EU.

cable, it is best to perform interviews about the donation of organs and tissues in a single interview session with the family, allowing them time to reach a decision with which they are comfortable. The physician or donor co-ordinator who will conduct the conversation should prepare carefully for the interview. It is important that he or she is familiar with the context, the history of the hospitalisation and the situation of the relatives, and that he or she gathers as much information as possible before interviewing them.

It is frequently impractical to discuss donation with a large number of family members, and it is recommended that participating family members should be limited to the lawful next of kin and/or those who are key to making the decision, taking into account the legal framework in place and cultural or religious practices. This should be explained to the other family members so that they do not feel excluded.

When there are social, cultural or language barriers or difficulties, the support (if there are no other possible helpers) of interpreters or friends of the potential donor who have a greater level of integration or similar religious sympathies may be beneficial for the family. These persons should be previously informed about the donation and about the processes involved so that they can support the family and champion a favourable attitude towards donation; they should not be limited to making a simple translation. Ideally, it is best practice to work with authorised interpreters who are familiar with the necessary terminology and who can explain and translate the relevant medical terms.

The conversation should be planned and carried out at the right time, in the right place and by trained people. Proper preparation for the conversation reduces the likelihood of errors and the need for improvisation [11, 12, 13, 14]. The discussion should take place in an environment that helps facilitate the conversation, perhaps located close to the place where their loved one died, to give family members the opportunity to say goodbye. It is important to provide the family with a quiet room, where they can speak freely. It is also advisable to have resources that meet the minimum needs (e.g. telephone, handkerchiefs, water, food).

In certain circumstances, relatives of potential tissue donors may be interviewed over the telephone [15]. Before the interview, the co-ordination staff should be very careful and verify whether the interviewee has been informed of the death of their relative. It may happen that the hospital staff did not reach them before the interview. Such interviews need to ensure that the conversation takes place when the relative is in a private space and preferably not in an unfamiliar environment, such as a hospital. Telephone conversations can make it more difficult to offer reassurance and support to a family since there is little opportunity to demonstrate a personal touch, a lack of which can increase the emotional distance. Trained personnel should be able to find appropriate words, and respect silent moments, to provide support under these circumstances to enable a standardised interview to take place [16].

The physician or donor co-ordinator who is conducting the conversation with the relatives should respect their grief. This type of conversation requires interpersonal skills, sensitivity and empathy. In situations when there is a huge pressure on medical staff, conversation with families can become difficult, rushed or insensitive. These telephone conversations should be documented or recorded, if possible.

4.3.3.2. *Informing the family*

There is often debate about the amount of information a donor family should receive, how much information is enough and when does it become too much. One opinion is that the family should be given only the information they request; another view is that the family must be told absolutely everything: which tissues will be donated, a description of the procurement process including reconstruction, potential uses of the tissue in both clinical practice and research, the method of discard, the potential need for follow-up if some test results are found positive, and so on. The first approach has the limitation of not taking into consideration the fact that the family may not know much about donation and will therefore not know what to ask. The latter could cause harm to the family and could risk converting an interest in donation into a refusal. The solution to this dilemma should be found by the donation professionals on a case-by-case basis. Professionals should be able to adapt the information given to the family, taking into account their knowledge, circumstances and requests.

In cases of donation for cells donated as starting material for ATMPs, some special considerations apply and should be specifically discussed (e.g. the implications for the donor in the event of a novel genetic disease marker being identified many years after the donation; the commercial potential of the ATMPs produced; and the fact that, in view of their scaleability, such products derived from one biological system may reach a significant number of patients).

While providing the information, they may observe agitation, frustration or irritability in

a family member; this may signal unwanted or stressful information and suggest reconsideration of how much information is needed or wanted. Ultimately, the amount of information made available to donor families in order to obtain consent should vary and should be increased if the potential application of the donated material is potentially sensitive, e.g. research or commercial applications. The interview in any case should be prepared in advance with systematic content, built with the interviewer's own words. Global and generic sentences may be helpful to raise the main subjects, which the family can investigate further with questions if needed.

It is helpful to ensure that, during the whole donation, the family receives the appropriate care and support they need. In many countries, hospitals have dedicated bereavement teams to provide psychological support, access to social services, administrative support or religious counselling. The clinical team should establish whether there are any specific religious or spiritual requirements of the family and whether the family wishes to retain keepsakes such as locks of hair or handprints.

4.3.3.3. *Family objections*

Conversations about tissue donation aim to fulfil the will of the deceased donor and to obtain family consent or support for donation. Regardless of the legal position, conversations must aim to achieve a decision that is acceptable and accepted by the relatives. Agreement to donation must not be coerced or conditional, nor should it be achieved under pressure or by offering any financial inducements or other material benefit.

It is difficult to proceed with donation when a family is strongly against it, even if there is evidence that their deceased family member wished to be a donor. The family has the right to express their opinion about donation, and clinicians need to make a balanced decision to continue with the procurement without the support of the family and risk damaging the emotional health of the relatives, incurring possible bad publicity and a loss of public confidence in the donation programme, or accept that it is not feasible to follow the wishes of the deceased and abandon the donation process. Family co-operation is essential to obtain a good medical and behavioural history of the donor.

It might be helpful to use the following when discussing refusal with the family:

a. If the family claims that the deceased patient did not agree to donation or had changed their mind, explore the basis on which the family gives such a statement.

b. When the family does not know anything about the attitude of the deceased to donation, discuss whether the deceased helped people generally, e.g. as a blood donor or by donating to charity, and how donation could help many people to benefit from a transplant.

c. The experience of interviews with families suggests that some difficulties and possible opposition may occur in procurement of tissues from 'visible places' like skin, bone and, in particular, eyes when family members fear disfigurement of the body. In these situations, reassure them that the deceased's body will be fully respected. If necessary, some technical aspects of procurement should be explained, for example the use of specific surgical incisions and sutures or suitable prostheses or artificial eyes or bones. Reassurance should be given that they should not notice anything if they see the deceased person after procurement, albeit there can be rare problems such as bruising or bleeding, and they should be made aware of such possibilities.

d. In the case of religious concerns, offer a consultation with a religious leader or representative.

e. Give special attention to cases of dissatisfaction with the healthcare provided and record the complaints, but explain that the issue of donation should be kept separate.

f. Identify the persons involved in the refusal to donate and their role within the family, and attempt to communicate with them separately to understand and try to address their concerns.

g. Identify whether a disagreement to donation by individual family members is based on conflicts between family members, which come to light when a person has died. In this case, try to separate the conflict from the issue of tissue donation.

4.4. Special considerations

Some challenging aspects of consent for donation of cells are related to informed consent, follow-up of the recipients and traceability (see Chapter 16 for traceability). A number of complex issues exist, mostly relating to the potential length of storage of these cells and to tests and manipulations that may be performed. These issues include:

a. the implications for the living donor in the event of identification of known or novel infectious or genetic disease markers, either at the time of donation or at any time in the future;

b. the implications for potential recipients if there

is development of post-donation disease in the donor which may have an infectious or genetic basis;

c. the extent to which the development of clinical problems in recipients may have implications for the living donor and/or their family;

d. any findings that cause concern during any stage when the tissues and cell are in the TE or in process to become ATMPs;

e. the possibility of multiple cells or tissue products (ATMPs) being offered to multiple recipients over an extended period.

Where each product sits in relation to the others is usually with respect to two key areas. How consent is affected by these issues will mostly depend on the complexity and the potential risks of the tissue or cell procured.

The subject of cell-based advanced therapies should be discussed openly and transparently, in order to build growing and informed public awareness. Consent should always be considered as a process, not an event. The scope of the consent needs to be explicit, although a balance needs to be struck. A blanket, generic consent means that the person giving consent does not know or understand the implications of what they are consenting to; however, if the initial consent form is too detailed, it may be too restrictive.

The scope of the consent should cover not only the tests available today, but also tests that may be available in the future. To aid decision-making, donors should be provided with examples of the type of tests that may come on line and the implications of them.

Whoever is giving consent should be told the circumstances in which they would receive feedback on any new findings. They should be prepared to receive such information, and the institution arranging the donation should be prepared to provide or arrange appropriate support when such information is communicated. Where there is no public health need for a donor to be told of such a result, they might choose to be given feedback only if the condition concerned has consequences for the donor's health and can be treated.

4.5. Conclusions

The continuing development of transplant medicine gives hope to many patients in need. However, the need for tissues and cells for clinical application cannot be met only by autografts or surgically discarded tissues. The ability of a tissue establishment to meet patients' needs requires multiple efforts to increase tissue and cells donation activity. These efforts involve organisational measures, the development of proactive donor recruitment and identification programmes, and the engagement of many parties, including the general public, hospital staff, coroners, procurement organisations and tissue establishments. By establishing strong links with and co-ordination between all these parties, and by adequately training personnel to acquire the necessary medical expertise and key social and emotional skills, tissue establishments can ensure the success of tissue and cell donation programmes.

Because tissues and cells come from a human being, either living or deceased, it is essential to ensure that donors have the autonomy to decide freely about matters that are entirely their own choice. Obtaining informed consent is relatively straightforward when donors are alive. It is essential, however, that they fully understand the risks and consequences of the donation procedure and the final use that will be given to their donated material. In the case of deceased donors, it is less clear how respect for autonomy applies but, ultimately, it is crucial that the wishes and best interest of the potential donor are scrupulously respected. This chapter has aimed to offer practical guidance for obtaining consent in all possible contexts.

4.6. References

1. Rodríguez A, Sandiumenge A, Masnou N *et al.* Medical students for tissue procurement, a 10-year experience in a large university hospital: an exportable model? *Transplant Proc* 2015 Oct;47(8):2314-17. https://doi.org/10.1016/j.transproceed.2015.08.027.
2. Warwick R, Fehily D, Brubaker S, Eastlund T. *Tissue and cell donation – an essential guide*. Chichester UK/ Hoboken NJ: Wiley-Blackwell, 2009.
3. Rosenblum AM, Li AH, Roels L *et al.* Worldwide variability in deceased organ donation registries. *Transpl Int* 2012;25(8):801-11. https://doi.org/10.1111/j.1432-2277.2012.01472.x.
4. Netcord–FACT. Foundation for the accreditation of cellular therapy at the University of Nebraska medical center, available at www.factwebsite.org/cbstandards/, accessed 26 July 2022.
5. Melo J, Batista A, Teixeira A *et al.* Knowledge and behavior among health professionals in relation to cadaveric organ donation and transplantation: a questionnaire-based analysis in Portuguese hospitals. *Transplant Proc* 2011 Jun;43(5):1429-33. https://doi.org/10.1016/j.transproceed.2010.11.028.
6. Quintana S, Navarro A, Navas E *et al.* Consent to

donate some but not all organs or tissues. *Organs Tiss Cells* 2013;(16):37-9.

7. Bofill-Ródenas AM, Genís X, Brillas P *et al.* Out-of-hospital tissue donation: multidisciplinary donor circuit in a forensic institute. *Transplant Proc* 2019 Dec;51(10):3219-21. https://doi.org/10.1016/j.transproceed.2019.09.007. Epub 2019 Nov 19.
8. Rodríguez-Villar C, Paredes D, Ruiz Á *et al.* Optimising tissue donation process in a university hospital: 10 years of experience. *Med Clin (Barc)* 2014 Apr 22;142(8):343-7. https://doi.org/10.1016/j.medcli.2013.01.030.
9. Domínguez-Gil B, Delmonico FL, Shaheen FAM *et al.* The critical pathway for deceased donation: reportable uniformity in the approach to deceased donation. *Transpl Int* 2011;24(4):373-8. https://doi.org/10.1111/j.1432-2277.2011.01243.x.
10. Sandiumenge A, Domínguez-Gil B, Pont T *et al.* on behalf of the CD-P-TO. Critical Pathway for deceased tissue donation: a novel adaptative European systematic approach. *Transpl Int* 2021 May;34(5):865-71. https://doi.org/10.1111/tri.13841.
11. Haddow G. Donor and non-donor families' accounts of communication and relations with healthcare professionals. *Prog Transplant* 2004 Mar;14(1):41-8. https://doi.org/10.7182/prtr.14.1.r3510160u371qj20.
12. Jacoby LH, Breitkopf CR, Pease EA. A qualitative examination of the needs of families faced with the option of organ donation. *Dimens Crit Care Nurs* 2005;24(4):183-9. https://doi.org/10.1097/00003465-200507000-00009.
13. Sanner MA. Two perspectives on organ donation: experiences of potential donor families and intensive care physicians of the same event. *J Crit Care* 2007;22(4):296-304. https://doi.org/10.1016/j.jcrc.2007.03.002.
14. Potter JE, Herkes GH, Perry L *et al.* and the COMFORT study investigators. COMmunication with Families regarding ORgan and Tissue donation after death in intensive care (COMFORT): protocol for an intervention study. *BMC Health Serv Res* 2017;17:42.
15. Rodríguez-Villar C, Ruiz-Jaramillo MC, Paredes D *et al.* Telephone consent in tissue donation: effectiveness and efficiency in postmortem tissue generation. *Transplant Proc* 2007 Sep;39(7):2072-5. https://doi.org/10.1016/j.transproceed.2007.07.004.
16. Dincer PC, Birtan D, Arslantas MK *et al.* The effect of standardized interviews on organ donation. *Exp Clin Transplant* 2018 Mar;16 Suppl 1(Suppl 1):51-4. https://doi.org/10.6002/ect.TOND-TDTD2017.O21.

Related material

Appendix 11. Example of consent form: female (NHS, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (NHS, UK)
Appendix 14. Medical and social history questionnaire (NHS, UK)

Chapter 5. **Donor evaluation**

5.1. Introduction

Donor evaluation is critical to ensuring the safety and quality of the donation/product, as well as the safety of living donors [1, 2]. This chapter considers the principles of evaluation of (a) haematopoietic progenitor cells (HPC) from autologous and allogeneic bone marrow and peripheral blood donors, (b) living allogeneic tissue donors, (c) deceased allogeneic tissue donors, and (d) medically assisted reproduction (MAR) non-partner donors.

The main objectives of donor evaluation are:

1. to identify absolute contraindications to donation, to exclude such donors and thereby to minimise the risk of transmitting a disease from a donor to recipient,
2. to identify relative contraindications to donation by careful assessment of information in the medical history and thus to identify conditions that might adversely affect the quality of tissues and cells and influence efficacy and clinical outcome, and
3. to ensure that the donation will not cause harm to a healthy living donor.

When dealing with living donors, an important part of the evaluation is to assess whether the procurement process itself could harm the donor, and whether it is necessary to implement long-term follow-up of living donors (HPC and oocytes donors) after procurement. The World Marrow Donor Association and similar organisations are active in many countries to help protect the health and safety of unrelated HPC donors [1, 2]. The European Committee on Organ Transplantation (CD-P-TO) has recommended the same level of protection for related and unrelated HPC donors [3]. Special consideration is required for paediatric living donors. These aspects of living donation are detailed in Chapter 4 – Recruitment of potential donors, identification and consent; Chapter 24 – Haematopoietic progenitor cells from bone marrow and peripheral blood; Chapter 29 – Medically assisted reproduction; and Chapter 30 – Fertility preservation.

5.1.1. Donor evaluation

To meet the above objectives, the following information must be evaluated:

i. medical history (including genetic disease, family history of disease and transfusion history);
ii. social history (personal and behavioural information, including travel history);
iii. physical examination;
iv. psychological examination (living HPC and non-partner MAR donors) – see Chapter 24 and Chapter 29;
v. tests for markers of transmissible disease, as detailed in Chapter 6 and Chapter 11.

During donor evaluation, confirmation of the validity of the consent and of the donor identity are essential (see Chapter 4). For European Union (EU) member states, the selection criteria for deceased donors (including additional exclusion criteria for de-

ceased paediatric donors) and living donors of tissues and cells are specified in Annex I/III of Directive 2006/17/EC. These criteria are the legal minimum, and individual member states can set additional criteria as necessary.

5.2. Assessment of potential autologous donors

5.2.1. General evaluation

The donor eligibility criteria for autologous donors can be very different from the criteria for allogeneic donors [1] because the direct benefit of transplant may outweigh potential risks associated with donating cells and tissues. The clinician caring for the donor should make the decision on autologous donation/application according to clinical guidelines and relevant scientific data. Eligibility for donation should be evaluated on an individual basis, taking into consideration the possible risks and benefits, and should be based on all available information, including the relevant medical history, results of laboratory tests and physical examination results.

5.2.2. General contraindications

There are no absolute contraindications for autologous donors. The potential benefits and risks should be analysed on an individual basis and a decision made by the clinician in charge, subject to full informed consent from the patient. This process should be clearly documented.

If the procured tissues or cells will be processed and/or stored, the same screening with biological testing for mandatory markers must apply as for an allogeneic living donor (see Chapter 6), although the results are not necessarily a contraindication for autologous donation. Potential or proven infectious (e.g. HIV/HBV/HCV-positive) materials collected from autologous donors should be handled in such a way that the risk of cross-contamination with tissues and cells from other donors within the tissue establishment is minimised. The risk of transmission of infection to personnel, during procurement, processing and storage of these cells, should also be considered. Written standard operating procedures (SOPs) should be present for these situations (see Chapter 8, Chapter 9 and Chapter 10).

5.3. Assessment of potential allogeneic donors

5.3.1. General evaluation

Allogeneic donors can be living donors related to the intended recipient, unrelated voluntary living donors or deceased donors. This section includes assessment of:

a. living tissue donors – for example, femoral heads surgically removed during primary hip replacement, amniotic membrane donated by mothers at the time of delivery and heart-valve donation from the discarded heart of a heart transplant recipient (known as a 'domino donor');

b. HPC donors: related or unrelated, and cord blood – for example, bone marrow collected in

Table 5.1. **Deceased donors: sources of information and types of record for donor evaluation**

Sources of information	Types of record
1. interview with family and friends/close acquaintances 2. interview with attending clinician and nurse, as well as the healthcare provider 3. detailed review of the medical notes (see Types of record, to the right) 4. general practitioner notes 5. physical examination findings 6. autopsy findings (for deceased donors), which must be communicated as soon as possible after procurement 7. tests for infectious markers (see Chapter 5 and Chapter 10) and other relevant test results (see *Types of record*, to the right)	1. emergency room and emergency medical transport (ambulance) records 2. admission records, progress notes, clinician's orders/notes, nursing observations 3. surgical records 4. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling) 5. discharge summary or death certificate (for deceased donors to confirm cause of death or to determine whether an autopsy is planned) 6. results of laboratory tests (microbiology, chemistry, haematology, virology, toxicology, genetic screening, pathology) 7. physical evaluation form 8. information relating to transfusions and infusions (to be used for evaluation of haemodilution) 9. radiography/magnetic resonance imaging/computed tomography
For deceased donors whose death occurred outside a healthcare facility, the records listed to the right may also be available and, if so, they should be reviewed	10. police records 11. records from the medical examiner or coroner 12. records from the extended-care facility

theatre under general anaesthesia or peripheral blood stem cells collected by apheresis techniques;

c. deceased tissue donors – for example, musculoskeletal, cardiovascular, ophthalmologic or skin tissue donated after death;

d. MAR donors – in the case of non-partner donation (sperm/oocyte donors).

5.3.1.1. *Sources of information*

In the setting of living donation, the information on medical and social history required for donor evaluation should be obtained directly from the donor. For HPC donors, the health risks for the donor must be considered by a clinician not involved in the treatment of the potential recipient, to avoid conflicts of interest. Additional information, where applicable, should be sought from other sources, similar to that obtained for deceased donors as given below. The types and extent of records to be reviewed will vary depending on the type of donation.

Several sources of information should be used to gather medical and social history about deceased donors (see Table 5.1). Information obtained must be included in the donor's medical evaluation record. Procurement of tissues from deceased donors takes place after circulatory arrest. Thus, the time available for full donor evaluation is normally limited. An interview with relatives of deceased donors should be undertaken, bearing in mind that, under emotional stress, some details might be forgotten. Even when donor relatives trust the interviewer, they may be reluctant to disclose sensitive information, or they may not know the entire truth.

Contact with the general practitioner of the donor and a review of hospital records (for historical data or other sources of information, e.g. tumour registry/pathology reports if available) are both important in supplementing and/or confirming information provided by the family. The donor medical evaluation record should include the details of hospital admission (if the donor died in a healthcare facility); cause of death; medical and behavioural history; general data such as age, gender, body weight (if necessary, e.g. to calculate haemodilution), date and time of death; and signs of obvious medical interventions, i.e. scars, skin or mucosal lesions.

Standardised questionnaires should be used for interviews to ensure that all the relevant information is obtained (see Appendix 14). The interviews should be performed, documented and signed by a suitably trained and competent authorised person and comply with national regulations. They should be held in private and be carried out before donation (see Chapter 4). The donation record, whether paper or electronic (see Chapter 2), must fully and accurately reflect the relevant information gained from reviewing these records and from discussions with medical or other personnel. Transferring information from records to a new document carries the risk of transcription or interpretation errors. These steps must be carried out by well-trained, competent staff.

Careful review of all the collected donor information will help ensure an accurate donor evaluation and assessment of the risks, including the identification of any potential contraindications for donation, either absolute or tissue-specific (see Part B for specific chapters). This analysis should preferably be performed before procurement; but, if this is not possible, the procured tissues and cells should be quarantined until a final decision is made by the Responsible Person (RP) of the tissue establishment. In addition, it is the responsibility of the person/team performing the procurement to document any suspicious anatomical findings observed during the procurement procedure and to obtain samples for histological examination if relevant.

An HPC donor should be pre-screened for factors to assess if the donation process would place them at increased risk of adverse incidents or harm to their health. Pre-screening may include general health status, medical history questions, physical exam, blood tests and other relevant medical evaluation.

5.3.1.2. *Donor medical and social history*

5.3.1.2.1. Medical history

a. Current clinical information, e.g. diseases/diagnoses, transfusions/infusions and medication/vaccinations, should be reviewed. If large volumes of blood are to be collected in HPC donations, the donor's haemoglobin should be measured before the donation, and potential donors with low levels should be adequately prepared (e.g. iron supplementation in the case of iron deficiency) or excluded from donating. If marrow is to be aspirated to obtain bone-marrow stromal cells, or a skin biopsy, the donor's platelet count and coagulation measures should meet pre-defined criteria to be sure that they are not at increased risk from bleeding [1, 2, 4].

b. In deceased donors, cause of death must be available. Haemodilution should be assessed in donors with trauma, intra-operative blood loss or ruptured aneurysms, including bleeding from oesophageal varices, spleen rupture etc.,

as the donors may also have received blood products. If haemodilution is > 50 %, serology testing on blood samples drawn at the time of procurement may not be reliable (see Chapter 6 and Appendix 18). When haemodilution is suspected or confirmed, blood samples taken before transfusion must be used for virology and serology testing, and serology testing has to comply with sample testing requirements mentioned in Chapter 6. If pre-transfusion/infusion samples are not available, haemodiluted samples can only be accepted if the testing procedures used have been validated for such samples.

c. Previous diagnoses of disease, surgeries, vaccinations, genetic disease, chronic diseases and family history should be evaluated. For living allogeneic donation, where applicable (e.g. umbilical cord blood or gamete donation), attention should be given to cases of family adoption or conception by donated gametes/embryos, as it may not be possible to trace the genetic family history.

d. The evaluation of current and past medical history should include

i. chronic/previous disease, e.g. chronic persistent infection, malignancy, autoimmune disease, neurological disease, genetic disease;
ii. medication;
iii. information on recent vaccinations [5]:
- to identify recent vaccinations that indicate travel risks or other types of exposure to potential infection e.g. hepatitis B or tetanus,
- to identify vaccinations with live attenuated virus,
- to help with interpretation of test results (a recent HBV vaccination can be expressed as reactive/positive HBsAg);

iv. family history – for instance, if individuals are at familial risk of prion-associated diseases (have had two or more blood relatives develop a prion-associated disease, or have been informed following genetic counselling that they are at risk for public health purposes) [6], or for malignancies or connective tissue disease.

5.3.1.2.2. Social history, evaluation of behavioural and personal risk

Behavioural and personal risk (including travel history) must be evaluated because they may completely exclude a donor, or can indicate that certain tissues/cells may be compromised or may suggest an increased risk of infectious diseases [7]. It is necessary to ask lifestyle questions, including sexual history and habitual drug use, to determine behavioural risk as detailed in section 5.3.3.1.

Recent or pertinent travel history or residence abroad/overseas must be evaluated to rule out the risk of tropical or endemic infections, e.g. malaria, trypanosomiasis or Zika, as well as the subsequent risk of vertical transmissions. Travel history helps to identify risks related to places/countries with less rigorous regulatory standards or with a high prevalence of certain infections. Emerging, non-tropical infections also exist in some European regions, e.g. West Nile virus, chikungunya virus. See 5.3.3.3.

Information about hobbies (e.g. home, garden, animals, woodlands) should also be obtained with the same intention. Seeking information about contact with fauna, especially bites from pets, domestic or wild animals, bats and birds, is necessary to evaluate the risk of infections.

5.3.1.3. *Physical evaluation of donors*

Physical evaluation of the donor (see Table 5.2) must be carried out and documented in the donor evaluation record. Each donor must be thoroughly examined following established protocols (see Chapter 7) to detect any sign that may be sufficient in itself to exclude the donor or that must be assessed in the light of the donor's medical and personal history. The information obtained through physical examination is supplementary to the comprehensive summary of clinical data.

For living donors, a complete physical examination should be undertaken to ensure the safety of donors and recipients according to the specific requirements of the particular type of tissue or cell donated. This examination should be done in the context of a clinical evaluation that includes an interview and a comprehensive physical examination, together with psychological evaluation of the potential donor where relevant. More detailed information about HPC donors, including paediatric donors and non-partner MAR donors, is given in Chapter 24 and Chapter 29.

For deceased donors, the physical examination should look for evidence of high-risk behaviour, or external signs of underlying medical conditions (see Table 5.2). Visual examination of the body is advisable during early, initial screening if adequate information on the condition of the body cannot reliably be obtained orally. The physical examination may include taking a photograph of suspicious lesions that may indicate a risk and/or taking a sample for histology. Any findings that may indicate the risk of transmissible disease or unsatisfactory quality of the tissues should result in exclusion of the donor [8]. Any

Table 5.2. **Deceased donors: physical examination prior to donation**

Look for signs of	
Possible systemic disease	• malignancy (suspicious skin or subcutaneous lesions; see Appendix 16) • malnutrition, multiple deformities
Bacterial or viral infection	• recent receipt of a live vaccination (vaccination site infection, scabs, vaccinia) • recent receipt of a tattoo, body piercing or acupuncture (shaved area, redness, swelling or scabbing may require further investigation to assess risk) where there is a risk that non-sterile instruments may have been used • skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles, scabs • oral lesions such as ulcers or thrush (not always possible to examine due to rigor mortis) • visible/palpable enlarged lymph node(s) • icterus
High-risk behaviour	• injected drug abuse (non-medical injection sites) • inspection of tattoos for hidden injection sites or for any additional information (e.g. some tattoos may suggest imprisonment or high-risk sexual behaviours) • genital or peri-anal skin lesions indicative of a sexually-transmitted disease
Trauma	• fractures, avulsions, lacerations or abrasions that may affect (contaminate or compromise integrity of) the tissue to be procured • suspected internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries) • cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination) • scars (surgical or other); if findings do not match the donor's history, further investigation may be required

new information related to lesions (tumours, skin lesions, scars), diseases or treatments that becomes apparent during the physical examination must be investigated further by the professionals responsible for donor selection. Excessive weight of the donor should not compromise the requirement to carry out a thorough assessment.

In all cases of abnormal findings, the tissue establishment should undertake further investigations, if required, according to specific SOPs. The limited sensitivity and specificity of physical examination for discovering pathologies should be considered in the donor risk assessment. An example of a tissue-donor physical assessment form can be found in Appendix 15.

Paediatric donors should be screened with as much diligence as adult donors. Physical assessment should not be overlooked or shortened simply because the donor is a child.

5.3.2. Generic contraindications for tissue and cell donation

Donors should be considered as high-risk if one (or more) of the following conditions is present.

5.3.2.1. *Unknown cause of death (deceased donors)*

If the cause of death (COD) is not known, the donation cannot be accepted, as death may have been due to a transmissible disease. The only exception to this rule would be in those cases when an autopsy report will be available after procurement, to provide information on the COD. The circumstances of death and medical history for differential diagnosis contributing to death may help to exclude contraindications to donation until the certified cause of death is available.

5.3.2.2. *Infectious diseases*

Infectious agents transmissible by tissues and cells belong to one of five groups of pathogens:

- viruses, which can be transmitted either during active or in latent infections; DNA viruses may persist latently in the tissues without detectable viraemia and evaluate the risk of transmission and weigh the benefit for the recipient; RNA viruses usually cause direct infection and disease;
- bacteria by bacteraemia or colonisation/infection of tissues or cells;
- fungi by fungaemia or colonisation/infection of tissues or cells;
- parasites by latent or acute infection;
- prions (see §5.3.2.2.5 and §5.3.2.2.6).

5.3.2.2.1. Active systemic infection

Donors with systemic infection that is not controlled at the time of donation (including bacterial diseases, viral, fungal, protozoan or parasitic infections, or significant local infection in the tissues and cells to be donated) must be excluded. Donors with

bacterial septicaemia may be evaluated and considered for cornea donation, provided that the corneas will be stored by organ culture (see Chapter 19).

If the aetiology of an active infection cannot be established, the donor is not a suitable candidate for donation. Communication with the physician or medical staff caring for the potential donor is necessary if there is any doubt. These healthcare providers may know if there was a suspicion of sepsis or another infectious disease at the time of death, which may not have been well documented in the records.

5.3.2.2.2. Chronic persistent infection

Consideration should be given to the history of bacterial and protozoic diseases that can lead to chronic persistent infections, including tuberculosis, brucellosis, leprosy, Q-fever, chlamydiosis and salmonellosis. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis. The risk of transmitting these infectious agents with specific tissues must be assessed.

5.3.2.2.3. Confirmed transmissible viral infection

Donations must be screened for evidence of transmissible viral infections (see Chapter 6). Persons with clinical or laboratory evidence of active HIV, HCV, HBV or HTLV-I/II infection must be excluded from donation. Behavioural risks that could increase the risk of acquiring transmissible infections are discussed in section 5.3.3.1. These have to be evaluated to determine the suitability of the donor.

Newborns, and infants aged under 18 months, or children who have been breastfed by their mothers during the previous 12 months, who were born to a mother with confirmed HIV, HBV, HCV or HTLV infection, cannot be considered as donors, regardless of the results of analytical tests of their sample (see Chapter 6). This is due to the fact that vertical transmission cannot be excluded because a newborn's immune systems are not fully developed to mount an immune challenge. Similarly children who have been breastfed by infected mothers within the 12 months before death should not be accepted. This is a requirement of the EU Tissue and Cell Directive.

5.3.2.2.4. Recent history of vaccination with a live attenuated virus/bacterium

Vaccinations with live vaccines [5] may result in transmission of a vaccine-derived pathogen to the recipient. Therefore, it is imperative to determine if the donor has received live vaccines during the previous 4 weeks. Live vaccines include:

a. viral: inhaled attenuated influenza, varicella–zoster, rotavirus, measles-mumps-rubella (MMR), oral polio and yellow fever. Vaccinia for smallpox should be deferred for 8 weeks;
b. bacterial: bacillus Calmette-Guérin (BCG), oral *Salmonella typhi*.

Note that injectable live attenuated non-replicating vaccines are not contraindications to donation. If the vaccine has been administered following potential exposure, consideration needs to be given to the residual risk of infection transmission.

5.3.2.2.5. History of prion disease

Transmissible spongiform encephalopathies (TSE), which include Creutzfeldt–Jakob disease (CJD), Gerstmann-Stäussler-Scheinker (GSS), Kuru and fatal familial insomnia (FFI), are rare neurological degenerative diseases that are progressive and inevitably fatal. They are associated with transformation of the normal form of prion protein (PrPC) into an abnormally-folded form (PrPSc).

There are four clinical forms of CJD: sporadic (sCJD), which is the most common; variant (vCJD); genetic (gCJD); and iatrogenic (iCJD). While Western blot and ELISA assays have been investigated for testing blood, retinal tissue, optic nerve, spleen and tonsillar tissue, diagnosis can currently be confirmed only by autopsy. Adherence to European Centre for Disease Prevention and Control (ECDC) recommendations is suggested and the risk of transmission should be considered as detailed in 5.3.2.2.6.

Prion related diseases (any form of CJD, GSS or FFI) are contraindications to tissue donation.

5.3.2.2.6. Risk of transmission of prion diseases

In the following circumstances, possible TSE and other CNS infections must be ruled out before accepting tissue donation:

a. any suspicion of prion-associated disease, such as rapid progressive dementia;
b. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination); but, if dementia has a primary cause (e.g. dementia of vascular origin), donation can be accepted;
c. degenerative or demyelinising disease or a disorder of unknown aetiology involving the central nervous system.

In addition, TSE transmission risk should be evaluated in the following cases:

d. persons treated with hormones derived

from human pituitary gland, such as growth hormone;

e. recipients of cornea, sclera and *dura mater* as well as persons who have undergone neurosurgery in which the *dura mater* may have been used;

f. when following the precautionary advice given by some countries (other than UK) that prion risk should be considered in persons who lived in the UK between January 1980 and December 1996 for longer than 6 months;

g. individuals who have been told that they may be at increased risk because a recipient of blood or tissues that they have donated has developed a prion-related disorder or they have received blood transfusion from a donor who had subsequently developed prion disease [6].

5.3.2.3. *Malignancies*

5.3.2.3.1. Haematological malignancies

Myeloid and lymphoid neoplasia and leukaemia are malignant diseases caused by dysregulated multipotent haematopoietic stem cells and should be considered as absolute contraindications to donation. Other myeloproliferative diseases (e.g. *Polycythaemia vera*, essential *thrombocythaemia* and mastocytosis, myelodysplastic syndromes) may also affect the stem cells; thus, these donors require special attention, and donation of living cells must be avoided. The World Health Organization (WHO) has published classifications of myeloid neoplasms and acute leukaemia [9], and of lymphoid neoplasms [10]. Any information indicating haematological alterations that would be suggestive of any of the malignancies must be evaluated further. A recent blood test carried out before death, if available, may offer valuable information indicative of these alterations. Although an experienced haematologist will be able to provide a differential diagnosis, certain results should be individually evaluated [11], such as the examples shown in Table 5.3.

5.3.2.3.2. Non-haematological malignancies

A history of malignancy should be evaluated carefully to determine its effects on the quality and safety of tissue, due to the risk of either the presence of a tumour or the treatment given to the donor for the malignancy.

Results of donor evaluation may imply a donor risk, a recipient risk or both. An increased risk of harm to a living donor through the donation process is not acceptable, even if the benefit of transplantation for the recipient is considered to outweigh the risk of transmission (for example in the case of a donor's past malignancies).

Detailed history of type, duration, course/recurrence and treatment history must be considered. Availability of screening programmes has improved early detection, which increases the treatment options and can lead to cure. For donors who had been diagnosed with a pre-malignant condition (e.g. Bowen's disease, polyposis coli or Barrett's oesophagus), further information should be sought to exclude malignancy because these patients are likely to be monitored regularly (see Appendix 17).

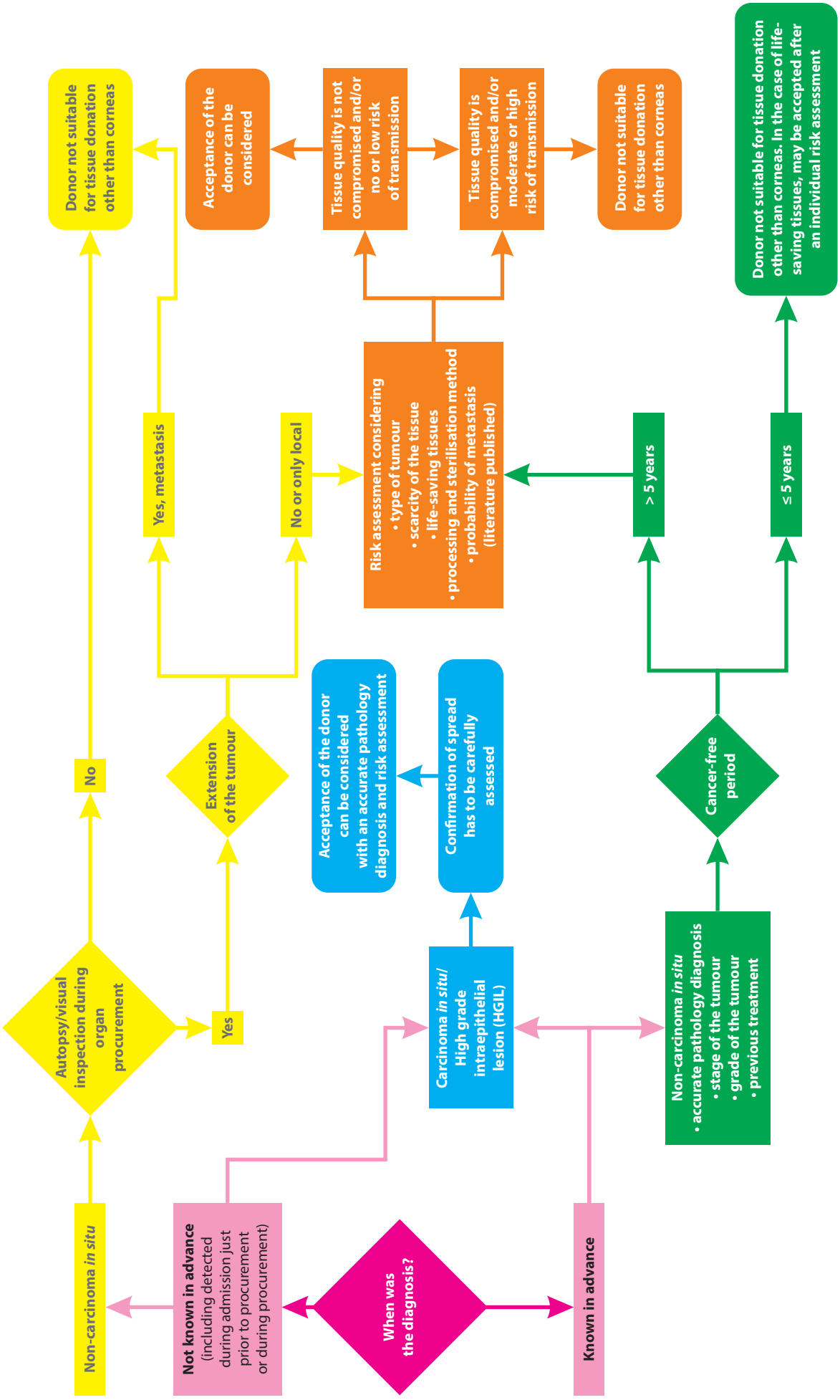
Some international bodies provide assessments on risk of transmission of malignancies through organ transplant for CNS (central nervous system) and non-CNS tumours [12], which can be used as a basis for determination of the risks of transmission through tissue transplant. The role of processing steps applied to tissues and cells in reducing the risk of transmission of malignancy for tissues and cells following transplantation should be considered. The effect of high-dose terminal sterilisation (25-40 KGy) and the decellularisation process removing viable cells are examples of tissue-processing steps that reduce the potential for transmission of malignant cells. It should be also noted that tissue recipients usually do not require immuno-suppression.

The *Guide to the quality and safety of organs for transplantation* (Chapter 9: Risk of transmission of cancer) [13] provides useful guidance on assessing

Table 5.3. **Haematological parameters that should be individually evaluated**

Altered haemoglobin	men > 18.5 g/dL women > 16.5 g/dL	should be carefully assessed for potential contraindications, such as *Polycythaemia vera*
Altered haematocrit	men > 55.5 % women > 49.5 %	
Platelet count	< 50 × 10^9/L	is highly indicative of a haematological disorder
	50-100 × 10^9/L	should be carefully assessed for contraindications due to a possible haematological problem
	> 450 × 10^9/L	should be carefully assessed for contraindications such as essential thrombocytosis
Altered white blood cells	> 50 × 10^9/L	should be carefully assessed for contraindications such as chronic myeloid leukaemia

Figure 5.1. **Decision tree algorithm for tumour approach**



the risk of malignancy transmission through organ transplantation, based on published evidence in the literature and national transplant registries. Other than absolute contraindications (unacceptable risk), the risk of transmission is classed as minimal (< 0.1 %), low (0.1 to 1 or < 2 %), intermediate (1 or 2 % to 10 %) and high (> 10 %) for CNS and non-CNS tumours. A similar approach could be applied for tissue and cell transplantation based on literature review.

5.3.2.3.2.1. CNS tumours

Malignancy gradation in the CNS should be thoroughly evaluated, including a complete histological exam rather than a simple biopsy, due to possible heterogeneity of the mass. In 2021 the WHO updated their classification of 2016 [14] of selected CNS tumours is shown in Appendix 17 (Table A17.1 compiles a combination of 2016 and 2021 classifications). In addition, following SaBTO guidelines, WHO Grade 1 and 2 CNS tumours have a minimal or low risk of metastasis and would not contraindicate tissue donation. The Grade 3 and 4 primary CNS tumours have either low (WHO grade 3: < 2 % transmission risk) or intermediate (WHO grade 4: 2.2 % with an upper 95 % CI of 6.4 % transmission risk) risk of transmission through organ transplantation [14] (Appendix 17, Table A17.2). There are no described cardiac metastases with any CNS tumours in the literature. There are published reports of extracranial bone metastasis of glioblastoma multiforme (Grade 4) [14-20]. Individual risk assessment is required for decisions whether to accept donors with malignancies that have low or intermediate transmission risk for tissue donation. Appendix 17 provides a generic risk assessment for accepting a donor with CNS tumours (Grade 3 & 4) for heart valves.

In CNS tumours, cerebral lymphoma and secondary intracranial lymphomas are considered absolute contraindications.

5.3.2.3.2.2. Non-CNS tumours

The assessment of transmission risk for non-CNS tumours in an organ transplant setting [12] is summarised in Appendix 17, Table A17.3. Malignant neoplasms have been transmitted to immunosuppressed recipients through transplantation organs from donors with known or unknown malignancies. In an organ transplant setting, this risk needs to be considered against the perspective of the important, life-enhancing and life-saving benefits afforded by organ transplant. The increasing number of patients on waiting lists, along with the shortage of organs available for transplant, has encouraged reconsideration of the criteria for acceptance of organs from donors with a past or current history of malignancy [13].

The risk–benefit analysis is very different in a tissue-transplant setting. Tissue grafts are mostly used in elective settings and are life-enhancing (rather than life-saving). However, there is a scarcity of certain types of tissue to meet clinical requirements. This should be taken into consideration where the risk of transmission is negligible or very low, and when there are no metastases described in the relevant tissue (see Appendix 17). The processing steps and terminal sterilisation may vastly reduce the potential for transmission of tumour cells, and tissue recipients, unlike organ recipients, usually do not require immuno-suppression. Thus, the risk of transmission is generally much lower compared with organ transplant. Guidance for undertaking these assessments is provided in Figure 5.1 (decision tree algorithm). Transmission of malignancy via tissue allografts is rare; there have been two recent case reports (in 2017) of donor-derived malignancy in viable keratolimbal allograft recipients [22, 23].

5.3.2.3.2.3 Carcinoma *in situ*

Carcinoma *in situ* (CIS) is an early form of cancer that is defined by the absence of invasion of tumour cells into the surrounding tissue, usually before penetration through the basement membrane. There may be differences in terminology used to describe CIS depending on the anatomical location where it occurs, but all share the same characteristics. A list of all types of malignancy considered as CIS is provided in Appendix 17. CIS is, by definition, a localised phenomenon, with no potential for metastasis unless it progresses into an invasive carcinoma. Therefore, its removal eliminates the risk of subsequent progression into a life-threatening condition. As most forms of CIS have a high probability of progression into invasive carcinoma [13], it is usually recommended that the lesion is completely removed. Therefore, CIS is usually treated in much the same way as a malignant tumour. If a donor had been successfully treated and cured after a CIS (e.g. uterine cervical or vulval carcinoma *in situ*, some intra-ductile carcinoma of the breast, intraepithelial cancer of the prostate etc.) the donation could be suitable because CIS do not adversely affect the safety or quality of other types of tissues.

5.3.2.3.2.4. Specific considerations for EU member states

It must be noted that, for EU member states, the EU directives for tissues and cells general donor exclusion criteria require that donors with malignancy

must be excluded from donation unless justified on the basis of a documented risk assessment approved by the responsible person (as specified below). Commission Directive 2006/17/EC states that the presence, or previous history, of malignant disease, except for primary basal cell carcinoma, carcinoma *in situ* of the uterine cervix and some primary tumours of the central nervous system that have to be evaluated according to scientific evidence, is a criterion for exclusion of donors for tissue or cells. This regulatory requirement must be considered as part of the risk assessment in decision-making.

Donors with malignant diseases can be evaluated and considered for cornea donation (see Chapter 19), except for retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Malignant melanoma with known metastatic disease also excludes use of ocular tissue, including avascular cornea. Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should be evaluated as discussed above.

5.3.2.4. *Exposure to toxic substances*

In case of ingestion or exposure to a toxic substance (e.g. cyanide, lead, mercury, gold, arsenic, pesticides), the quality and safety (due to the presence of a high level of a toxic substance) of some types of tissues and cells may be affected and, as a result, can cause harm to recipients, although if the potential donor remained well with no evidence of toxicity in life then the amount of toxin present in donated tissue products is unlikely to be at a level that will cause harm in recipients; however, consideration of the timing of exposure to the toxin in relation to when donation will take place needs to be assessed. Exposure to asbestos in the past is a risk for developing a mesothelioma. In this case a thorough risk assessment should be performed.

5.3.2.5. *Tissue-specific contraindications*

Certain medical conditions can adversely affect specific tissues and cells which, if procured, processed and made available for human application, may result in unfavourable outcomes for the recipients of tissue and cells. This risk is evaluated on a case-by-case basis and for specific tissue types. For guidance on the specific contraindications for each tissue and cell type, please refer to the relevant chapters in Part B of this Guide.

5.3.3. Evaluation of personal and social history

All substances of human origin (SoHO) have the potential to transmit infections to a recipient. Personal and social history should be carefully evaluated to inform donor suitability. Evidence-based donor selection is the first safeguard in minimising the risk of transmission while not compromising sufficiency of valuable grafts for clinical use.

The incidence and prevalence of these SoHO-related infections varies, depending on different risk factors [24, 25, 26], and the causes of *de novo* infection vary between European regions [24, 27]. The tissue establishment must consider available evidence from the epidemiological data on transmissible bloodborne infections such as HIV, HCV and HBV in the population, the performance (sensitivity and specificity) of screening tests used for detecting these infections and the residual risk of undetected infection that could be potentially transmitted to the recipient. This residual risk may be the result of one or all of a number of factors: error in the process, poor assay sensitivity or a donation collected from a donor in the infection window period. It is recommended to adopt an interval of at least twice the window period since the last 'at-risk behaviour' for the length of deferral before donation [24].

It is recommended that a risk-assessment framework, such as the Alliance of Blood Operators model [28], is used to systematically analyse the information and document the decision, based on the acceptable level of risk tolerance. The outcome of this systematic approach would provide the basis for evidence-based donor deferral and acceptance policies for donors with high-risk behaviours, or who have sexual partners with high-risk behaviours [29].

5.3.3.1. *Behavioural risk factors*

Potential donors should be considered at high risk if they have participated in any of the following behaviours or if they have had sexual contact with persons who have participated in any of the following behaviours:

a. People who have injected drugs by an intravenous, intramuscular or subcutaneous route for non-medical reasons;

b. Tattoos, ear piercings, body piercings and/or acupuncture, which are very popular in some European countries; usually they are applied by sterile methods and, in many countries, there are specific approvals for those establishments. If tattoos, piercings or acupuncture treatments were done in approved settings, the donor can be accepted without temporary deferral, but in case of doubt the associated risk should be con-

sidered to be the same as that of non-medical injections;

c. Persons who have been newly diagnosed with, or have been treated for, sexually transmitted diseases (e.g. syphilis, gonorrhoea, *chlamydia* or genital ulcers);

d. Persons who have had sex in exchange for money or drugs;

e. Persons whose sexual behaviour (such as frequent changes of sexual partner; sex under the influence of drugs, except for cannabis and sildenafil citrate; or anal sex with a new partner) puts them at risk of acquiring sexually transmitted infectious diseases.

In countries where tissue establishments do not have access to data to perform risk assessment, a deferral period of 12 months is a safer option after cessation of the high-risk behaviour or sexual contact. This may be reduced to 3 months if supported by risk assessment, considering risks and benefits of the transplant, together with individual NAT testing and bacterial screening.

5.3.3.2. *Personal risks, exposure events [6]*

Exposure events that increase the risk of acquiring a transmissible disease can occur at any time during life. They include accidents, certain medical therapies, occupations and travel to, or residence in, an area endemic for certain diseases. Examples of exposure events include:

a. persons from a high-risk region for endemic disease, as well as the risk of vertical transmission, e.g. HIV1 group O, human T-cell lymphotropic virus (HTLV1); the Caribbean is, for example, high-risk for HTLV1;

b. exposure to someone else's blood or other body fluids (such as needlestick injury, human bite) when that person was known to be infected with HIV, HBV or HCV (or of unknown status);

c. sharing a residence with someone who has HBV or clinically-active HCV;

d. patients on regular haemodialysis;

e. people who have been in a lockup, jail, prison or juvenile correctional facility for more than 72 consecutive hours should be carefully evaluated for the risk of high-risk behaviours (see §5.3.3.1);

f. history of travel to, origin in or visiting relatives in malaria-endemic areas; the minimum deferral period recommended for blood donors in EDQM guidance [27] for all groups of potential donors (visitor, origin/previous resident, travel-related illness, history of malaria) is 4 months if the result of a validated malaria antibody assay, performed at least 4 months after last exposure incident or resolution of symptoms, is negative;

g. history of animal bites should be carefully evaluated to rule out the possibility of transmissible diseases such as rabies.

5.3.3.3. *New and emerging diseases*

New and emerging diseases, including those that have spread to a new geographical area, can pose a significant challenge when screening donors for risks of communicable disease due to travel history. Professionals responsible for donor selection should be vigilant regarding surveillance of changes to the global movement of infectious-disease risks. Diseases that should be considered include: Middle East respiratory syndrome (MERS), dengue fever, yellow fever, malaria, trypanosomiasis, tuberculosis, plague, chikungunya virus, West Nile virus (WNV), severe acute respiratory syndrome-associated coronavirus (SARS-CoV), Q fever, antibiotic-resistant diseases, and HIV1 group O, rabies, Ebola virus and Zika virus. The most recent pandemic in 2020 due to SARS-CoV-2 impacted in an extraordinary way the donor selection procedure and provision of tissues and cells internationally. In Europe, regular monitoring of the Rapid Communication Reports originating from the Eurosurveillance website [30] is recommended, as well as actively seeking information to assess the epidemiological status of diseases in the areas where a donor has lived or travelled [31]. Specific information about geographic distribution of infectious diseases can be obtained from the websites of the European Centre for Disease Prevention and Control (www.ecdc.eu), the World Health Organization (www.who.int/ith/en) and the Centers for Disease Control and Prevention in Atlanta (the Yellow Book at wwwnc.cdc.gov/travel/page/yellowbook-home/).

The risk of transmission of an infectious agent through procurement of tissues or cells from a donor who may have visited an affected area should be balanced against the likelihood of transmission occurring.

The *Guide to the quality and safety of organs for transplantation* (Chapter 8: Risk of transmission of infectious diseases) [13] provides useful guidance on assessing the risk of infection transmission through organ transplantation, based on published evidence in the literature and national transplant registries.

Regional risks within an affected country can vary. In cases of recent travel, if the donor remains well after return or after known contact with someone

infected, the donor should be deferred for at least twice the length of the incubation period [25]. If the donor was infected, they can only be accepted after full recovery and when the donation is no longer infectious. In cases of community transmission, further risk assessment is essential, taking into the account all available scientific and clinical information, mitigation factors and any other relevant external factors. A multidisciplinary approach with involvement of different stakeholders is required to respond to threat posed by new and emerging infections.

5.3.4. Relative contraindications

Below are listed the potential risks that have to be analysed on an individual basis, considering the potential harm and benefit.

a. *Additional contraindications/risks to donation of tissues and cells for living donors*
   i. pregnancy (except for donors of umbilical cord blood cells and amniotic membrane, and sibling donors of haematopoietic progenitor cells);
   ii. breastfeeding;
   iii. health risks for donors related to specific procedures.

b. *Organ-transplant recipients*
   Organ recipients receive immunosuppressive drugs to prevent rejection, but this could make the serology testing unreliable; moreover, organ donor-selection criteria are less stringent than for tissues and cells. This risk should be assessed on a case-by-case basis, taking into account the feasibility of performing NAT testing and the level of immunosuppression in combination with the possibility of tracing the medical details of the organ donor.

c. *Impact of immuno-suppressive agents in the donor*
   Treatment with immuno-suppressive agents can weaken the immune system and thus influence the reliability of serological tests (Chapter 6). Steroid therapy in high doses causes immunosuppression and may mask infective and inflammatory conditions that would otherwise prevent donation (e.g. donation of umbilical cord blood). Evaluating the effect of the immuno-suppressive agents on the haematological parameters (erythrocytes, leukocytes and thrombocytes) can be indicative for immuno-suppression. NAT testing may be helpful in such circumstances. All other medication of the donor should be always interpreted by a risk assessment for impact on the tissue, e.g. chronic use of corticosteroids can affect the quality of skin and musculoskeletal tissue (see Part B. Specific requirements for substances of human origin) and also increase the possibility of a secondary malignancy.

d. *History of genetic disease*
   A family history of inherited genetic disease should be assessed. Where the occurrence of genetic disease in the family history cannot be traced/assured, it presents a risk of transmission of inherited genetic diseases, especially in non-partner MAR (see Chapter 29). This risk should also be evaluated in the case of HPC (Chapter 24) and umbilical blood (Chapter 25) donors.

e. *Deferred for blood donation for known reason*
   If it is known that the potential donor was excluded or deferred from donating blood by a blood-collection establishment, the specific reason for the deferral must be discovered, and the eligibility of the donor is then evaluated on an individual basis. If the reason is not known, it may be safer to exclude the donor for tissue donation.

f. *Xenotransplantation*
   Xenotransplantation is any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues or organs [32, 33, 34, 35].
   Biological products, drugs, or medical devices sourced from non-human animals that do not contain living cells, tissues or organs, including (but not limited to) porcine insulin, porcine heart valves, porcine skin and acellular porcine corneal stroma, and collagen matrices derived from acellular porcine, bovine or any other xenogeneic source [36, 37] are not considered as xenotransplantation.
   The main concern with the use of xenotransplantation is the potential infection of recipients with both recognised and unrecognised infectious agents and the possible subsequent transmission to their close contacts and into the general human population. Moreover, new

infectious agents may not be readily identifiable with current methods/tests used for screening. Therefore, "transplantation with xenografts" is included as an exclusion criterion for donors (at §1.1.13 of Annex I) in Directive 2006/17/EC.

Exposure to non-human live animal material has the potential for cross-species infection by retroviruses, which may be latent and may lead to disease years after infection. Infections that would not normally affect humans such as porcine endogenous retroviral viruses (PERVs) that are integrated into the pig genome could potentially integrate into the host-genome [38, 39, 40] and be transmitted through donated material. *In vitro* studies demonstrated transmission of a PERV to a human cell line lacking the intracellular machinery that protects against retroviral infection [39, 41]. However, available data from clinical studies, with a limited number of patients, did not report any transmission of pathogens in the recipients who received porcine cell-based bioartificial liver transplant or porcine islets [42, 43, 44, 45]. In total, in over 200 individuals who were exposed to pig cells or tissues or *ex vivo* perfusion of pig organs or pig cell-based bioreactors, no transmission has been reported [40, 41]. The use of animal products with insufficient testing can result in the transmission of other microbial infections. For example, transmission of *Coxiella burnetti*, causing severe Q fever in recipients of intramuscular xenotransplantation of injected fetal lambs cell extracts, has been reported [46].

Although the published studies have not demonstrated a transmission of retroviruses via clinical xenotransplantation, the risk is difficult to quantify given the small number of recipients to date [37, 39], and long-term monitoring is necessary to demonstrate safety. It is recommended that precautionary donor exclusion is applied if a potential donor had previous xenotransplantation with living cells, tissues and organs. The medical device and medicinal sectors are regulated by high standards to achieve stringent quality and safety measures to mitigate the risk of infectious agents transmission by biological grafts that contain non-living cells through selective sourcing of pre-screened animals, effective collection and handling, and measures applied for elimination/inactivation or removal of agents. The donors who received these biological grafts can therefore be accepted as tissue and cell donors.

As a precautionary measure, a few countries have applied a broader interpretation of the exclusionary term, to also include non-viable cells or tissues of animal origin utilised in therapeutic products. Tissue establishments should apply documented systems to justify their local practices in relation to xenotransplantation products by the evaluation of scientific evidence, professional standards and national guidance.

5.4. References

1. Stroncek D, England L. Protecting the health and safety of cell and tissue donors. *ISBT Sci Series* 2015 April 1;10(Suppl1):108-14. https://doi.org/10.1111/voxs.12150.
2. Shaw B, Ball L, Beksac M *et al.* Donor safety: the role of the WMDA in ensuring the safety of volunteer unrelated donors: clinical and ethical considerations. *Bone Marrow Transplant* 2010;45:832-8.
3. Council of Europe. Recommendation CM/Rec (2020) 6 of the Committee of Ministers to member states on establishing harmonised measures for the protection of haematopoietic progenitor cell donors, available at https://rm.coe.int/0900001680 9fdd5b, accessed 7 July 2022.
4. O'Donnell P, Pedersen T, Confer D *et al.* Practice patterns for evaluation, consent, and care of related donors and recipients at haematopoietic cell transplantation centres in the United States. *Blood* 2010; 115(24):5097-101.
5. Centers for Disease Control and Prevention. The Pink Book: Immunology and vaccine-preventable diseases, available at www.cdc.gov/vaccines/pubs/pinkbook/prinvac.html, accessed 5 April 2022.
6. JPAC (Joint United Kingdom Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee). Guidelines for the blood transfusion services, available at www.transfusionguidelines.org/, accessed 5 April 2022.
7. Brubaker SA. Tissue donor screening. McLean VA, USA: American Association of Tissue Banks; available at https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.737.9153&rep=rep1&type=pdf, accessed 5 April 2022.
8. Van Wijk MJ, van Geyt C, Laven AB *et al.* Physical examination of potential tissue donors: results of a risk management procedure to identify the critical elements of the physical examination. *Cell Tissue Bank* 2012;13(4):547-63.
9. Arber DA, Orazi A, Hasserjian R *et al.* The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;

127(20):2391-405 and *Blood* 2016;128(3):462-3. https://doi.org/10.1182/blood-2016-03-643544.

10. Swerdlow SH, Campo E, Pileri SA *et al.* The updated WHO classification of hematological malignancies: the 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 2016;127(20): 2375-90. https://doi.org/10.1182/blood-2016-01-643569.
11. Tefferi A, Varian JW. Classification and diagnosis of myeloproliferative neoplasm: the 2008 World Health Organization criteria and point-of-care diagnosis algorithms. *Leukemia* 2008;22:14-22.
12. SaBTO (Advisory Committee on the Safety of Blood, Tissues and Organs). Transplantation of organs from deceased donors with cancer or a history of cancer, independent report (December 2020); available at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/948068/transplantation_of_organs_from_deceased_donors_with_cancer_or_a_history_of_cancer-revised_FINAL44266_JNcw_cw.pdf, accessed 5 April 2022.
13. European Directorate for the Quality of Medicines & HealthCare (EDQM). *Guide to the quality and safety of organs for transplantation*, 8th edition. Strasbourg: Council of Europe, 2022, available at https://freepub.edqm.eu/publications/PUBSD-88/detail, accessed 18 November 2022.
14. Louis DN, Perry A, Wesseling P *et al.* The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro-Oncology*;2021:23(8) 1231-51, available at https://doi.org/10.1093/neuonc/noab106, accessed 13 June 2022.
15. Montagne K, Marie B, Cahn V *et al.* Glioblastome avec métastases systémiques d'emblée. *Ann Pathol.* 2004;24(3):268-70.
16. Takanen S, Bangrazi C, Caiazzo R *et al.* Multiple bone metastases from glioblastoma multiforme without local brain relapse: a case report and review of the literature. *Tumori* Sep-Oct 2013;99(5):e237-40.
17. Birbilis TA, Matis GK, Eleftheriadis SG *et al.* Spinal metastasis of glioblastoma multiforme: an uncommon suspect? *Spine (Phila Pa 1976)* 2010;35(7):43-5.
18. Didelot A, Taillandier L, Grignon Y *et al.* Concomitant bone marrow metastasis of a glioblastoma multiforme revealed at the diagnosis. *Acta Neurochir (Wien).* 2006; 148(9):997-1000.
19. Bugalho P, Chorão M, Fontoura P. Miliary brain metastases from primary gastric small cell carcinoma: illustrating the seed and soil hypothesis. *J Neurooncol* 2005;73(1):53-6.
20. Elena A, Melina C, Raffaele N *et al.* Extraneural metastases in glioblastoma patients: two cases with YKL-40-positive glioblastomas and a meta-analysis of the literature. *Neurosurg Rev* 2016;39(1):37-46.
21. Goodwin CR, Liang L, Abu-Bonsrah N *et al.* Extraneural glioblastoma multiforme vertebral metastasis. *World Neurosurg* 2016;89:578-82.
22. Miller AK, Young JW, Wilson DJ *et al.* Transmission of donor-derived breast carcinoma as a recurrent mass in a keratolimbal allograft. *Cornea* 2017 June; 36(6):736-9.
23. Sepsakos L, Cheung AY, Nerad JA *et al.* Donor-derived conjunctival-limbal melanoma after a keratolimbal allograft. *Cornea* 2017 Nov;36(11):1415-18.
24. European Centre for Disease Prevention and Control. Publications and data, available at www.ecdc.europa.eu/en/publications-data, accessed 5 April 2022.
25. Kucirka LM. Risk of window period HIV infection in high infectious risk donors: systematic review and meta-analysis. *Am J Transplant* 2011 June;11(6):1176-87. https://doi.org/10.1111/j.1600-6143.2010.03329.x.
26. Kucirka LM. Risk of window period hepatitis-C infection in high infectious risk donors: systematic review and meta-analysis. *Am J Transplant* 2011 June;11(6):1188-1200. https://doi.org/10.1111/j.1600-6143.2011.03460.x.
27. European Directorate for the Quality of Medicine & HealthCare (EDQM). *Guide to the preparation, use and quality assurance of blood components*, 20th edition. Strasbourg: Council of Europe 2020; available at www.edqm.eu/en/blood-guide, accessed 5 April 2022.
28. Alliance of Blood Operators. Risk-based decision-making framework, available at https://allianceofbloodoperators.org/abo-resources/risk-based-decision-making/rbdm-framework.aspx, accessed 5 April 2022.
29. SaBTO (Advisory Committee on the Safety of Blood, Tissues and Organs). *Blood, tissue and cell donor selection criteria report*. Independent report 2017, available at www.gov.uk/government/publications/blood-tissue-and-cell-donor-selection-criteria-report-2017, accessed 5 April 2022.
30. Eurosurveillance. New and emerging diseases, available at www.eurosurveillance.org, accessed 5 April 2022.
31. European Centre for Disease Prevention and Control. Rapid risk assessment: Zika virus disease epidemic, 7th update, available at http://ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1525#sthash.ypWPenLz.dpuf, accessed 5 April 2022.
32. Cox MA, Brubaker SA. Interpretive conundrum on the exclusion criterion of 'transplantation with xenografts' for tissue and cell donation. *Cell Tissue Bank* 2012;13(2):225-9.
33. CHMP (Committee for Medicinal Products for Human Use of the European Medicines Agency). Guideline on xenogeneic cell-based medicinal

products, London, 22 October 2009, available at www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/12/WC500016936.pdf, accessed 5 April 2022.

34. Nuffield Council on Bioethics. *Animal-to-human transplants: the ethics of xenotransplantation*, 1996, available at https://www.nuffieldbioethics.org/publications/xenotransplantation, accessed 29 November 2022.
35. European Commission: Scientific Committee on Medicinal Products and Medical Devices. Opinion on the state of the art concerning xenotransplantation, adopted 1 October 2001, available at https://ec.europa.eu/health/archive/ph_risk/committees/scmp/documents/out38_en.pdf, accessed 29 November 2022.
36. Scobie L, Padler-Karavani V, Le Bas-Bernardet S *et al.* Long-term IgG response to porcine Neu5Gc antigens without transmission of PERV in burn patients treated with porcine skin xenografts. *J Immunol*. 2013; 191(6):2907-15.
37. Zhang MC, Liu X, Jin Y *et al.* Lamellar keratoplasty treatment of fungal corneal ulcers with acellular porcine corneal stroma. *Am J Transplant* 2015;15(4):1068-75.
38. Weiss RA. Infection hazards of xenotransplantation: retrospect and prospect. *Xenotransplantation* 2018; 25(4):1-5.
39. Fishman JA. Infectious disease risks in xenotransplantation. *Am J Transplant* 2018;18(8):1857-64.
40. Denner J, Tönjes RR. Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. *Clin Microbiol Rev* 2012;25(2):318-43.
41. Denner J, Schuurman HJ, Patience C. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes – Chapter 5: Strategies to prevent transmission of porcine endogenous retroviruses. *Xenotransplantation* 2009;16(4): 239-48.
42. Di Nicuolo G, D'Alessandro A, Andria B *et al.* Long-term absence of porcine endogenous retrovirus infection in chronically immunosuppressed patients after treatment with the porcine cell-based Academic Medical Center bioartificial liver. *Xenotransplantation* 2010;17(6):431-9.
43. Valdes-Gonzalez R, Dorantes LM, Bracho-Blanchet E *et al.* No evidence of porcine endogenous retrovirus in patients with Type 1 Diabetes after long-term porcine islet xenotransplantation. *J Med Virol* 2010;82:331-4.
44. Morozov VA, Wynyard S, Matsumoto S *et al.* No PERV transmission during a clinical trial of pig islet cell transplantation. *Virus Res* 2017;227:34-40.
45. Irgang M, Sauer IM, Karlas A *et al.* Porcine endogenous retroviruses: no infection in patients treated with a bioreactor based on porcine liver cells. *J Clin Virol* 2003;28(2):141-54.
46. CBER FDA. Source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans; guidance for industry. *Xenotransplantation* 2016;23(6):1-64.

Related material

Appendix 14 – Medical and social history questionnaire (NHS, UK)
Appendix 15 – Physical assessment form (Dutch Transplant Foundation)
Appendix 16 – Evaluation of pigmented skin lesions
Appendix 17 – Evaluation of malignancies for risk assessment in tissue and cell donors

Chapter 6. Donor testing – markers for infectious diseases

6.1. Introduction

Use of tissues and cells for human application can result in unintentional transmission of infection or disease from a donor to the recipients. However, such events can be minimised by scrupulous evaluation of donors, including laboratory testing of each donor close to the time of donation in accordance with established good practice. The risk can be reduced substantially by appropriate donor sample testing, but adequate controls must be in place to ensure that test results are accurate and reliable. Adequate controls include:

a. ensuring that the screening programme includes relevant infectious diseases (related to behaviours and travels, if any) and their appropriate individual screening target(s);
b. selecting a suitable testing laboratory, which should be qualified to perform screening tests for cell and tissue donors;
c. ensuring that donor blood samples are labelled, handled and stored appropriately and that the time interval between collection and testing meets the sample requirements of the test kit;
d. use of Conformité Européenne (CE)-marked tests or, when CE-marked tests are not available, appropriately validated in-house tests for infectious diseases;
e. providing well-written standard operating procedures (SOPs) and training for all personnel involved in collection and labelling of donor samples, for sample storage and transport, and for technical staff carrying out testing and reporting results, as well as for those receiving and interpreting them.

These are fundamental elements of a tissue establishment's quality system, and any laboratory undertaking tissue-donor testing must ensure that the most appropriate technology for this activity is used, including selecting test kits that demonstrate high clinical and analytical sensitivity and specificity. If the tests are performed by an external laboratory a service-level agreement between this laboratory and the tissue establishment is mandatory.

6.2. General concepts

Tissue establishments must ensure that donor blood samples (or other analytes) from all donations of human tissues and cells are subjected to biological tests mandated by national or other applicable legislation, or by a specific situation such as travel. In EU member states, Annex II of Directive 2006/17/EC, amended by Directive 2012/39/EU, specifies mandatory laboratory tests and general testing requirements for living and deceased donors of tissues and cells, and requires that any such laboratory and its tests must be accredited, designated, licensed and/or authorised by the competent authority.

SOPs that define the criteria for acceptance or rejection of tissues and cells based on those test results must be in place. The Responsible Person (RP) who will interpret test results should be knowledgeable about the accuracy of the infectious-disease test and the interpretation of the test results, and deci-

sions must meet the expectations in regulations or, if regulations are not prescriptive, follow professional standards of practice [1] or the European Centre for Disease Prevention and Control (ECDC) SoHO risk assessments when they are available. In EU member states the RP is defined by Article 17 of Directive 2004/23/EC.

Documented measures must be taken by tissue establishments that receive tissues or cells from another country or distribute tissue or cells to another country to ensure that the donor-testing requirements of the destination country are met. Evidence should also be available to show that any laboratory involved in testing of donor samples has been accredited, designated, licensed and/or authorised by the appropriate authority to carry out such testing.

6.3. Quality of donor samples

Manufacturers of assays for infectious-disease testing provide specific sample requirements for which their assays have been validated. Personnel of procurement organisations and tissue establishments involved in collecting, transporting (having particular regard to packaging, temperature, duration), storing or testing donor samples must be aware of these requirements to ensure optimal assay performance. If inadequate or otherwise compromised samples are provided to the laboratory and tested, the results may not be valid, and unreliable test results increase the risk of donor-related transmission of infectious pathogens.

Shipment conditions should be validated to avoid degradation of the quality of the test sample. Donor-related conditions that could affect the quality of a test specimen must also be taken into account. Infectious-disease test results may be invalidated by haemodilution if the extent of any dilution is such that it may dilute any screening target present to a level below that which is detectable by the assay(s) used. Therefore, in cases where haemodilution is known to have occurred, ideally pre-transfusion/infusion samples should be obtained for testing purposes (see §6.3.2).

Haemolysis may also affect test results. Haemolysis is the destruction of red blood cells in whole blood that discolours the plasma or serum, and it is noticeable after centrifuging the tube. Depending on the degree (severity) of haemolysis, the colour of the serum or plasma may be orange to red. This darker colour can promote a higher density reading by the optical component of test equipment, leading to a false positive test result. Haemolysis may be caused by rapid collection of blood through a small-bore needle, or by improper sample storage or transport, such as allowing the tube of whole blood to freeze prior to testing. Other causes, besides the haemolysis that occurs regularly after death, can be donor-derived and include an infection, a toxin, medication or autoimmune haemolytic anaemia, and haemolysis can occur after haemodialysis or after a haemolytic transfusion reaction.

Although serology tests must be used, detection of antibodies against pathogens can be impaired if the donor has received immunosuppressive treatment prior to sample collection or when the donor has received blood products containing antibodies against certain microbiological agents (e.g. Epstein–Barr virus, *Cytomegalovirus*, *Toxoplasma* or hepatitis B (HBs/HBc) antibodies). Immunosuppressive treatment may lead to false-negative results, and passive transfer of antibodies present in validated and authorised labile blood products may lead to false-positive results. Adding molecular screening tests (i.e. nucleic acid amplification technique/NAT) can be valuable because detection of viral nucleic acid in blood samples is generally not affected by immunosuppressive therapy [2]. The underlying condition requiring immunosuppression will demand further assessment because the disease/condition in itself may constitute an independent reason for determining that the donor is not eligible. If any of these donor-related conditions exist, they must be documented in the donor record and evaluated by an RP before release of tissues or cells for clinical application. In the second situation, pre-transfusion/infusion samples should be used.

Additionally, false-negative results can occur in other scenarios, including haemodilution, incorrect sampling or inappropriate test quality.

6.3.1. Sample collection (sample type, tubes, labelling, time limits and handling)

All personnel involved in any stage of the testing process must be fully trained. Testing is often carried out on plasma or serum of the donor according to the specification laid out by the manufacturer of the test kit. However, sometimes testing can be performed on other fluids or secretions, e.g. urine, seminal fluid, respiratory swabs or sperm, when the assays have been specifically validated for use with that sample type. In the case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using a blood sample from the donor's birth mother. Another important consideration is that, during the first 18 months of life, a child's immune system is only in development and protective anti-

bodies may not yet have been produced against an infection, thereby increasing the risk of hidden infections in child donors (see §6.5.1).

The test manufacturer's instructions on donor sample collection and testing must be followed with regard to:

a. the type of sample collection tube (no anti-coagulant or a specific anti-coagulant) required for the test being carried out;
b. sample storage and transport conditions post-collection, which can include centrifugation and/or separation within time limits or specimen refrigeration/freezing; and
c. testing required to be carried out within a specified timeframe post-collection.

To ensure traceability at each stage of the testing process, all donor samples must be identified with a permanently affixed label that contains information or references that link the sample and the laboratory test results to the donor (see Chapter 15 and Chapter 16). The date and time when the sample was drawn must be accurately documented. Unless a unique codification system is used, at least two donor identifiers, such as the donor's full name, date of birth, medical record number or other unique identifier, should be used. In the case of a sample from a donor (deceased or living), the label or associated documentation should also include some identification of the person who collected it and a description of the site on the donor's body the sample was taken from (e.g. cephalic vein, femoral artery, subclavian artery, superior vena cava) and, in the case of deceased donation, if the sample was extracted *pre* or *post mortem*. If any donor blood samples were drawn before death, they can be assessed for use (see §6.3.1.1), but there must be assurance that the patient identifier (i.e., appropriate labelling) used for any such specimen is confirmed as coming from the donor so mix-ups do not occur (i.e. to avoid carrying out testing for critical communicable diseases on the wrong person) [3]. Other donor-identification methods can be used, if validated, to ensure traceability [4].

Specimens of blood, serum or plasma from the same donor must not be mixed together for testing, whether collected at the same time or at a different time.

An adequate volume of whole blood must be collected, because otherwise the quantity of serum or plasma after centrifugation may not be sufficient to undertake all of the required tests for infectious diseases, or for any further investigations that may be required. The volume of blood required will be dependent upon the minimum requirements of the testing laboratories as well as the sample requirements of each test kit, and these parameters should be known and verified before blood collection. Moreover, in some cases more than one sample might be needed, notably when samples have to be managed by different laboratories. Indeed, laboratories responsible for routine virus testing may be different from the ones responsible for parasite testing, e.g. plasmodium, or from the ones responsible for non-routine viruses testing, e.g. arboviruses. In such cases the primary samples could be aliquoted, unambiguously labelled (ideally with a fully automated aliquoting device) and divided between the laboratories. Other considerations could include a donor with a high haematocrit (which could necessitate collection of extra tubes) and a donor who took (or was given) anti-coagulant medication (fibrin clots may appear in the serum after centrifugation and cause a reduction in the volume of testable serum). However, in the case of a living donor, care should be taken not to collect an unnecessarily large volume of blood because an adverse clinical event could result.

Proper handling of any donor blood sample after it is collected is necessary to ensure that testing protocols can meet the required specifications. For example, when a blood sample is collected in a tube containing an anti-coagulant, this liquid or powder requires that a completely filled tube be gently mixed by slowly inverting the tube 5 to 10 times immediately after collection [5].

After collection, specimen handling by personnel can include centrifugation and/or separation of the serum or plasma from red cells within specific time limits. In addition, specimen storage and/or transport conditions can involve refrigerating or freezing the plasma or serum aliquot. Specific instructions from the test kit manufacturer should be followed and can differ between tests [3]. In all cases, qualified transport containers and validated shipping conditions should be used when sending donor samples to a testing laboratory.

The facility receiving any donor sample for testing should have an SOP in place to define the criteria for acceptance or rejection of the sample, based on collection, storage and transport conditions. The testing facility should document acceptance or rejection of the sample and should share this sample status in a timely manner with an RP at the procurement organisation or tissue establishment.

6.3.1.1. *Deceased donor*

In the case of a deceased donor, blood samples must have been obtained just before cardiocirculatory arrest or, if this was not possible, the time of

sampling must be as soon as possible after death, and in any case within 24 h after death.

It is important to collect blood samples without untoward delay after death to avoid sample characteristics that could cause a non-specific test result (e.g. partial haemolysis) or that could lead to its rejection for testing (e.g. complete haemolysis). Delays in donor sampling have been shown to increase the incidence of red cell haemolysis, and other substances can appear in non-circulating blood due to growth of micro-organisms and release of enzymes (including by-products of tissue and cell death) [3].

There is some evidence that blood samples collected more than 24 hours after cardiocirculatory arrest can yield valid results; however, it is essential that all infectious disease testing kits used to analyse these samples are validated for use under these circumstances [5, 6, 7, 8, 9]; see also Appendix 19. These practices must be controlled by national regulations.

6.3.1.2. *Living donor*

In the case of living donors, blood sampling must be obtained at the time of donation or, if this is not possible, within 7 days before or 7 days after donation. However, for practical reasons, collection of a sample from an allogeneic bone-marrow stem cell or peripheral blood stem-cell donor must occur within 30 days before donation (taking into account that re-testing at the time of donation will be informative), but before reaching a point-of-no-return when irreversible measures for preconditioning of the recipient have been initiated. If tissues and cells of allogeneic living donors can be stored for long periods before use, repeat sampling and testing is required after 180 days, unless specific exemption criteria are met. (See §6.5.2; see also Chapter 20; Chapter 28).

6.3.2. Haemodilution assessment

When possible, a donor blood sample collected before administration of any transfusions and infusions should be used for testing purposes.

If a donor has recently received transfusions of blood or blood components, or infusions of colloids or crystalloids, and has lost blood, any testing of donor blood collected post-transfusion or post-infusion may not be valid due to haemodilution or plasma dilution of the donor's blood and, thus, of any samples taken from the donor. Assessment of the extent of any haemodilution includes the use of a formula to calculate dilution of the donor's original circulating blood volume (and circulating levels of antigen and/or antibody, if present) and should be done by the physician in charge or the transplant co-ordinator. Current practice in a number of countries is to consider 50 % calculated haemodilution to be the maximum allowable with minimal risk of a false-negative test result arising because of the haemodilution. Further possibilities for calculating haemadilution have been developed taking into account different physiological conditions [10]. These calculations should only be used after extensive studies and in compliance with the specifications of the algorithm presented here.

Examples of when a haemodilution calculation may need to be carried out include:

- *ante mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding blood sampling, or if crystalloids were infused in the hour preceding blood sampling;
- *post mortem* blood sample collection: if blood, blood components and/or colloids were administered in the 48 h preceding death (circulatory arrest), or if crystalloids were infused in the hour preceding death (circulatory arrest).

Refer to Appendix 18 for an example of a commonly used formula to assess the donor's potential haemodilution or plasma dilution that can be applied when the donor received any fluids that may lead to haemodilution. Adaptations of the algorithms may be needed for body sizes outside the normal adult range. Allowances may need to be made for a very large or a very small adult donor, or a paediatric donor. In brief, a donor's total plasma volume (TPV) and total blood volume (TBV) are estimated by calculations based on the donor's body weight, then direct comparisons are made to amounts of recent transfusions and/or infusions that were administered before circulatory arrest or before collection of the blood sample, whichever occurs first [3]. In summary:

a. estimate TPV of donor (weight in kg × 40 mL/kg; or, weight in kg ÷ 0.025);
b. estimate TBV of donor (weight in kg × 70 mL/kg; or, weight in kg ÷ 0.015);
c. calculate total blood (mL) received in the last 48 h (A);
d. calculate colloids (mL) received in last 48 h (B);
e. calculate crystalloids (mL) received in the last 1 h (C);
f. add b + c and compare to TPV (fluid volumes are compared);
g. add a + b + c and compare to TBV (mass/fluid volumes are compared);
h. does either comparison show > 50 % dilution? If not, the blood sample qualifies and can be used for testing for infectious diseases.

Although not normal practice, a tissue establishment may accept tissues and cells from a donor with plasma dilution of > 50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases, to help reduce risk, additional testing should also be performed using molecular tests (i.e. NAT) for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), and possibly for other viruses, depending on the donor's travel history, underlying disease or other factors.

The blood collected can also be diluted if the specimen is drawn in close proximity to an infusion or transfusion intravenous line, even if the donor is not actually haemodiluted or plasma-diluted. Samples should be drawn from the opposite side of the body in relation to the site of any infusion/transfusion.

Furthermore, in theory, a transfusion shortly before the donation could result in transmission of infectious agents to the donor.

6.4. Testing laboratories

To meet quality and safety requirements, all testing of infectious diseases for deceased and living donors must be carried out by laboratories that are accredited, designated, authorised and/or licensed for these activities according to the regulations set by the relevant Health Authority. Such laboratories will have the knowledge, skills, resources and competence required for testing blood samples from tissue donors, and should use appropriate algorithms to ensure that their testing procedures have maximum sensitivity without loss of specificity. They should also participate in relevant external quality assessment schemes (proficiency testing) and be subject to regular internal and external audits.

If additional biological assays are carried out, the laboratory used should be accredited, designated, authorised and/or licensed and should participate in an appropriate external quality-assessment programme [11]. (See §6.5.1 and Chapter 3.)

Tissue establishments can undertake these testing protocols themselves or must have a written agreement with any laboratory that carries out these tests [12]. Tissue establishments should evaluate and select a testing laboratory on the basis of its ability to generate reliable and appropriate results, and to keep relevant records. In addition the testing laboratory should comply with regulatory requirements and any other specific expectations of the tissue establishment (e.g. time-sensitive availability of test results, record retention). The tissue establishment should ensure by means of a service-level agreement that the laboratory is competent to perform this work and is using appropriate assays and procedures. There should be evidence that good practice is being followed and that personnel are appropriately trained and experienced in relevant testing procedures. The obligation of the laboratory to notify the tissue establishment when deviations occur is mandatory. To ensure a consistent level of competence and performance, audits of the testing laboratory(ies) should be undertaken periodically by the tissue establishment or by qualified external experts as part of the tissue establishment's quality system.

In addition, test records at the laboratory should be retained for 10 years at least and should include sample data, laboratory results, reference values and acceptance criteria. See Chapter 2 and Chapter 16.

6.5. Tests to be carried out

The donor-screening assays selected must be validated or verified and used in accordance with current scientific knowledge. The use of the latest generation of tests leads in general to a shortened serological window period and the latest tests should be used, if available [13, 14, 15].

Where appropriate, all assays used for donor testing within the EU should be CE-marked (see Appendix 19). Most of the major international manufacturers of donor-screening assays provide CE-marked assays and systems, and in some cases the manufacturers have undertaken validation work with samples from deceased persons. Where such data are not provided, laboratories performing this work will be expected either to have validated the assays for this purpose themselves or to verify the assay in accordance with other available peer-reviewed data [16, 17].

6.5.1. Mandatory tests

Mandatory serological tests for HBV, HCV, HIV and *Treponema pallidum* are listed below; additional molecular assays can be carried out to confirm a putative infection. All assays must be carried out in serum and/or plasma samples of the donor according to the manufacturer's instructions [3]. Though this may depend on the laws of the relevant country, it is better to test individual samples, not pooled samples. However, for some tissue and cells it is more accurate to test on other test samples (e.g. sperm after washing) which could provide additional relevant information.

a. *Human immunodeficiency virus type 1 and 2 (see Appendix 21) [18]*
A 4th or 5th generation assay including detection of anti-HIV-1/2 antibodies plus HIV-1 p24 antigen is strongly recommended. If a 3rd generation test is used, an HIV-1 RNA test (qualitative or quantitative) has to be performed additionally to exclude an HIV-1 infection. It is recommended that the HIV-1 RNA assay should be able to detect 50 IU/mL or lower. Samples with initially reactive (i.e. weak reactive or borderline) serological screening results can be re-tested in duplicate using the same assay. If the results are negative the donated tissues can be released. Samples that are repeatedly reactive in a screening assay can be re-tested using an alternative certified serological assay of equal or greater sensitivity. Donations that are non-reactive in the alternative assay and negative for HIV-1 RNA can be considered suitable for clinical use. If an HIV-1 RNA test is performed and the result is reactive, independently of the serology result, the donations cannot be considered suitable for clinical use.

b. *Hepatitis B virus (see Appendix 23) [18]*
HBV surface antigen (HBsAg) and total antibodies to HBV core antigen (anti-HBc). HBsAg must be negative. If anti-HBc is 'reactive', an additional determination of a highly sensitive HBV-DNA method must be performed (≤ 30 IU/mL detection limit; it is recommended to use the most sensitive test); but haemodilution may influence the limit of detection. If anti-HBc is positive and HBsAg and HBV-NAT is negative, the donated tissues can be released. HBV-DNA positivity reflects potential infectivity, also in cases of occult HBV infections, and leads to a discard of the donated tissues.

c. *Hepatitis C virus (see Appendix 22) [18]*
The screening for an HCV infection is based on detection of anti-HCV antibodies. But combined assays like the assays for HIV are not commercially available at present, and the performance of HCV-antigen assays is not yet sufficient to exclude an early infection. Presence of anti-HCV may indicate an acute, chronic or past infection. Furthermore, the pre-seroconversion window phase takes several weeks; thus, an HCV-RNA assay is strongly recommended to exclude active HCV infection. It is recommended that the sensitivity limit for the HCV-RNA assay should be ≤ 50 IU/mL. An anti-HCV-positive and RNA-negative result, which is indicative of a non-specific reaction or a past infection (confirmed from the donor's medical history, i.e. type and duration of HCV treatment and serology), needs to be confirmed by immunoblot analysis. If the result of the HCV immunoblot is negative or indeterminate (anti-HCV false-positive result) the donated tissues can be released.
In the case of an HCV immunoblot positive result (confirmed positive anti-HCV result), it is only with a record of two negative HCV RNA results six months apart as evidence for a finished successful HCV treatment under medical supervision, together with a negative HCV-RNA test on the donor sample, that the donated tissues can be released for clinical use.

d. *Treponema pallidum*
In the serological diagnosis of syphilis, a treponemal screening test should be used, e.g. *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test, treponemal enzyme immuno-assays (EIA) or chemiluminescence immuno-assays (CLIA). If the screening test is reactive, the results should be confirmed by means of a second treponemal test based on a different analytical method (see Appendix 20). Alternatively, an approved non-treponemal screening assay may be used to screen donors. Reactive samples can be re-tested using a treponemal-specific assay. Donations that are non-reactive in that assay can be considered suitable for clinical use.

Testing for HTLV-I antibodies must be performed for donors living in high-prevalence areas. Likewise, testing is necessary if the donor themselves, the donor's parents or the sexual partners originate from such areas [19]. Reactive screening results need to be confirmed by immunoblot analysis and/or specific NAT.

If a child donor is 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should be evaluated for risks associated with HIV, HBV, HCV, HTLV and *Treponema pallidum*. In the case of a neonatal donor (i.e. age ≤ 1 month), the required tests should be carried out using both, a blood sample from the donor's birth mother and one from the newborn. IgG antibodies in the newborn blood sample are likely to be maternal; therefore, testing for anti-HIV, anti-HCV and anti-HBc is not suggested in the newborn. However, if a maternal infection or another exposure event is sus-

pected, additional testing for HBs antigen and HIV-1 RNA, HBV-DNA and HCV-RNA in the newborn sample should be performed, to exclude a congenital or postnatal infection. In the very rare case of a treated HIV infection in the mother, testing for presence of active HIV-1 infection in the newborn must be carried out (HIV-1 cDNA).

In addition, special screening considerations are applicable to other paediatric donors and additional testing for communicable diseases (e.g. Covid-19, Zika virus infection, malaria or Chagas disease) may be indicated. Special attention needs to be paid to putative infections while maternal antibodies may still be detectable (up to the first 18 months of life).

6.5.2. Additional tests

It is well recognised that NAT assays for HIV, HBV and HCV reduce the risk of inadvertent disease transmission due to the substantial decrease in window period when compared with routine serological tests [14, 15, 20, 21].

Because NAT assays are more sensitive, serious consideration should be given to carrying out NAT tests for HIV, HBV and HCV in addition to serological tests. Considerations that support the use of NAT assays for each donor screening include the following:

a. The medical and behavioural history obtained from a third party such as a partner, parent, relative, friend or a legal guardian for a deceased donor can be less reliable than collecting this information from a living donor.

b. There is a risk that recent exposure to HIV, HBV and HCV (several days prior to death) might not be detected by serological (antibody) assays as they have not developed sufficient antibody response against the specific virus.

c. If the donation includes multiple tissue types and it results in a large number of tissue grafts to be made available for many recipients, the potential risk is increased if the viral tests selected cannot detect early infection in a donor.

d. The molecular methods used for the screening have to meet the requirements of each individual pathogen. Haemodilution (see §6.3.2) especially has to be scoped. In cases of acute or untreated chronic infections, viral loads are usually in a range where haemodilution may not cause false-negative results. But, if low-level viraemia is expected, for instance in occult HBV infections, the NAT method must be as sensitive as possible (currently ≤ 30 IU/mL). Tissue from a donor with suspected occult HBV infection [20, 21] and haemodilution might not be considered to be safe.

e. Molecular assays from deceased donors should be performed in individual samples (see current legislation of each country), not in pooled samples. Some of these NAT assays are combination tests that can detect HIV, HCV and HBV from a single blood specimen in one run (triplex assays), thus improving the feasibility of routine NAT for donor screening. (In the case of samples from living donors, the pooling could be accepted if the national requirements for the comparable NAT testing of blood donors are fulfilled.)

Any risk factors in donors associated with high-prevalence areas for specific infections/diseases must be considered carefully. Scientific evidence for risk factors for certain diseases is provided by ECDC [17], which regularly publishes risk assessments and maps that can be helpful, notably for emerging diseases.

Additional testing may be considered (depending on the donor's history and/or the characteristics of the tissues or cells donated):

- antibodies to *Cytomegalovirus*, Epstein–Barr virus, *Coxiella burnetii* and *Toxoplasma gondii* might be relevant for donor-recipient risk stratification;
- hepatitis E virus RNA (i.e. NAT).

Depending on factors like individual travel history and specific current or past clinical abnormalities of the donor as well as the epidemiological situation, the decision can be made to carry out other optional tests, which can include screening for tropical infections such as malaria, trypanosomiasis, strongyloidiasis, schistosomiasis and viral infections such as West Nile virus and Zika virus. The need to perform such assays, or others, must be examined on a case-by-case basis. In the case of paediatric donors, such infections must be reviewed for their impact in mother-to-child transmission.

Results of blood culture tests can be very useful tools to aid in the determination of bacteraemia in a donor of tissues and/or cells (see Chapter 11).

6.5.3. Re-tests of samples from living donors (allogeneic use)

Repeat sampling and serology testing is required after 180 days, unless any of the following specific exemption criteria are met:

a. if samples from a living donor undergo se-

rology testing and are also tested by molecular tests – (i.e. NAT) for HIV, HBV and HCV. NAT is recommended because it can increase sensitivity in the detection of recently acquired infections. Molecular testing of all donors using this technology is highly recommended as standard practice;

b. if the tissue/cells come from a living donor and have been processed using an inactivation step that has been validated for the pathogens concerned;

c. if the tissue/cells come from a living donor and will not be stored longer than 180 days prior to use.

Test-kit assays for infectious-disease markers are typically optimised for testing a sample from a living donor. For living donors, initial infectious-disease testing is carried out at the time of donation or, when this is not possible, within 7 days of the donation. In the case of bone marrow and peripheral blood stem-cell collection, blood samples must be drawn for testing < 30 days before donation. Minimum testing requirements are the same as for deceased donors. More tests could be indicated because there may be unique risks of infectious disease pertinent to a profoundly immunosuppressed recipient of bone marrow or of similar types of haematopoietic allograft [18, 22].

For testing individuals involved in medically assisted reproduction (MAR), see Chapter 29.

6.5.4. Testing of autologous samples

For autologous donors, if the removed tissues or cells are stored or cultured, they must undergo the same serological tests as for allogeneic donors before they can be transplanted back into the donor. If an autologous donor's blood sample has not been appropriately tested or if a test is indicative for a relevant infectious disease, this will not necessarily prevent the tissues or cells, or any product derived from them, from being stored, processed and re-implanted in the autologous donor; but this is only true if appropriate storage can provide isolation/segregation to ensure there is:

- no risk of cross-contamination with stored allografts;
- no risk of contamination with adventitious agents;
- avoidance of mix-ups due to misidentification (see Chapter 15 and Chapter 16).

Additionally, testing of autologous donors is useful to reduce the risk of transmission of potential infectious agents to staff and into facilities.

SOPs based on risk analyses should be in place to define the criteria for acceptance and rejection for contaminated autologous tissues and cells, or if the autologous donor has not been tested for infectious diseases (see Chapter 2).

6.6. Reporting and documentation of test results

Tissues and cells must be held in 'quarantine' until such time as requirements relating to donor testing have been completed. With this in mind, donor infectious-disease testing should be carried out and reported without delay. Reporting methods must be used that link the donor's unique identifier to the test results, while also keeping the donor anonymous to third parties. Data-security measures are required, as well as safeguards against any unauthorised additions, deletions or modifications to donor test results. There must be no disclosure of infectious-disease test results to unauthorised persons.

Arrangements between the testing laboratory and the tissue establishment, or the clinical team responsible for use of the donated tissues or cells, should include agreed methods for the reporting of test results to ensure that mix-ups are avoided and to prevent misinformation. Laboratories and tissue establishments should have policies relating to the management of test results from a donor that may be pertinent to family members and other contacts of the donor or that have implications for them and public health.

Reporting procedures should ensure that accurate, rapid and verifiable results are provided. In addition, there should be a system in place to ensure prompt alerts using an immediate notification system when an indicative test result for an infectious disease occurs. Other precautionary measures in reporting may include [23] the following:

a. where manual systems are still used (although they are not recommended), analysis reports should be cross-checked to ensure that the transcription of test results has been confirmed by two independent assessors (the 'four eyes principle');

b. using computerised procedures for the transfer of test results from laboratory equipment to the laboratory data-processing management system (e.g. medical records) to eliminate the need for manual transcription of data or oral information;

c. using clearly interpretable, computerised graphic symbols to highlight pathologic results;
d. recording (semi)quantitative values (e.g. titre, IU/mL) of antibodies and/or the related positivity threshold next to the viral negative/positive result;
e. recording the number of copies/mL, if applicable (or, preferably, IU/mL) of nucleic acid measurement as well as (for qPCR systems) the linear range of the assay (if qualitative PCRs are used, then semiquantitative values should be recorded, e.g. Ct-Values, Cp-Values);
f. using formal laboratory reporting structures and accreditation or certification pathways to improve quality standards;
g. using widely recognised international units of measurement;
h. mentioning systematically the name of the kit used (or making available on demand the data of the diagnostic assays, e.g. name, manufacturer).

6.7. Archived samples

If there are no national requirements, a risk-analysis-based decision based on the type of tissue/cell and the type of donor (autologous, allogeneic, living, deceased, multiorgan) should be performed to decide if, and for how long, archived samples must be retained. A storage time of at least 1 year after the distribution of the last tissue from the donor is recommended. In some countries, archiving donor samples may be required.

Archived samples may be used for several purposes: look-back testing involving a new infectious agent, development of more accurate or new tests, or if investigating a report of a serious adverse reaction in a recipient of tissues or cells. If these samples are to be used for research and development purposes, this must be covered by the donor's consent.

A documented risk assessment, approved by the tissue establishment's RP, should be carried out to determine the fate of all stored tissues and cells following the introduction of any new donor test that could reasonably be considered to affect safety or quality (see Chapter 3).

6.8. References

1. SaBTO (Advisory Committee for the Safety of Blood, Tissues and Organs) [UK], Microbiological Safety Guidelines, 26 March 2020, available at https://www.gov.uk/government/publications/guidance-on-the-microbiological-safety-of-human-organs-tissues-and-cells-used-in-transplantation, accessed 6 July 2022.
2. Watzinger F, Suda M, Preuner S *et al.* Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. *J Clin Microbiol* 2004 Nov;42(11): 5189-98.
3. Gillan H, Pamphilon D, Brubaker S. Principles of cell collection and tissue recovery. In: Fehily D, Brubaker S, Kearney JN, Wolfinbarger L, eds, *Tissue and cell processing: an essential guide*. London, UK: Wiley-Blackwell 2012.
4. Warwick RM, Rushambuza FG, Brown J *et al.* Confirmation of cadaveric blood sample identity by DNA profiling using STR (short tandem repeat) analysis. *Cell Tissue Bank* 2008;9(4):323-8.
5. Edler C, Wulff B, Schroeder AS *et al.* A prospective time course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 hours after death. *J Med Microbiol* 2011;60(7):920-6. https://doi.org/10.1099/jmm.0.027763-0. Epub 2011 Mar 24.
6. Kalus U, Wilkemeyer I, Caspari G *et al.* Validation of the serological testing for anti-HIV-1/2, anti-HCV, HBsAg, and anti-HBc from post-mortem blood on the Siemens-BEP-III automatic system. *Transfus Med Hemother* 2011;38(6):365-72.
7. Meyer T, Polywka S, Wulff B *et al.* Virus NAT for HIV, HBV, and HCV in post-mortal blood specimens over 48 h after death of infected patients – first results. *Transfus Med Hemother* 2012 Dec;39(6):376-80.
8. Kalus U, Wilkemeyer I, Pruss A, Caspari G. Validation of serological testing for anti-Treponema pallidum from postmortem blood on the Siemens-BEP®-III automatic system. *Transfus Med Hemother* 2013 Dec; 40(6):403-8.
9. Wilkemeyer I, Pruss A, Kalus U, Schroeter J. Comparative infectious serology testing of pre- and post-mortem blood samples from cornea donors. *Cell Tissue Bank* 2012;13(3):447-52.
10. Ross SW, Britton Christmas A, Fischer PE *et al.* Control trial with blood donation as a model for hemorrhage. *J Am Coll Surg* 2018 Sep;227(3):321-31.
11. Clinical and Laboratory Standards Institute (CLSI). *Procedures for the handling and processing of blood specimens: approved guideline*, 3rd edition, vol. 24, no. 38.
12. Outline scope of testing agreement, available at http://plasma-test.com/agreement-and-contracts/, accessed 6 April 2022.
13. Superior Health Council. Federal Public Service Health, Food Chain Safety and Environment of Belgium. Reporting and interpreting biological tests

carried out on samples from donors of human body material. CSS no. 9314, 2016.

14. Humar A, Morris M, Blumberg E *et al.* Nucleic acid testing (NAT) of organ donors: is the 'best' test the right test? A consensus conference report. *Am J Transplant* 2010;10(4):889-99.
15. Zou S, Dodd RY, Stramer SL *et al.* Probability of viremia with HBV, HCV, HIV, and HTLV among tissue donors in the United States. *N Engl J Med* 2004; 351(8):751-9.
16. Kitchen AD, Newham JA. Qualification of serological infectious disease assays for the screening of samples from deceased tissue donors. *Cell Tissue Bank* 2011;12: 117-24.
17. European Centre for Disease Prevention and Control. Geographical distribution of areas with a high prevalence of HTLV-1 infection. Stockholm: ECDC, 2015 (ISBN 978-92-9193-625-0), available at https://ecdc.europa.eu/en/publications-data/geographical-distribution-areas-high-prevalence-htlv-1-infection#no-link, accessed 6 April 2022.
18. Pruß A, Chandrasekar A, Sánchez-Ibáñez J *et al.* Algorithms for the testing of tissue donors for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV). *Transfus Med Hemother* 2021;48:12-21.
19. Gessain A, Cassar O. Epidemiological aspects and world distribution of HTLV-1 infection. *Front Microbiol* 2012;3:388.
20. Sánchez Ibáñez J, Vilarrodona Serrat A, Seoane Pillado T *et al.* Evaluation of occult hepatitis B infection in tissue donors: a multicenter analysis in Spain. *Cell Tissue Bank* 2019 Dec;20(4):513-26.
21. Mirabet V, Álvarez M, Luis-Hidalgo M *et al.* Detection of hepatitis B virus in bone allografts from donors with occult hepatitis B infection. *Cell Tissue Bank* 2017 Sep;18(3):335-41.
22. Punzel M, Patrick M, Hunter H *et al.* Detection of hepatitis B virus DNA in the blood of a stem cell donor after granulocyte colony-stimulating factor treatment. *Hepatology* 2016;64(5):1803-5.
23. Villa E, Nanni Costa A. HIV-positive organs used for transplant in Italy due to human error. *Eurosurveillance* 2007;12(3):E070308.1, available at www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3150, accessed 5 April 2022. https://doi.org/10.2807/esw.12.10.03150-en.

Related material

Appendix 18. Sample haemodilution algorithm
Appendix 19. Example of validation of screening: infectious disease assays of blood from deceased donors
Appendix 20. Treponema pallidum testing
Appendix 21. Algorithm for HIV testing
Appendix 22. Algorithm for HCV testing
Appendix 23. Algorithm for HBV testing

Chapter 7. Procurement

7.1. Introduction

To ensure high standards of quality and safety during the procurement process for tissues and cells, it is recommended that a quality system be in place in the procurement organisation or the tissue establishment (TE) undertaking the process. This quality system must guarantee adequate training of all personnel involved, as well as documented standard operating procedures (SOPs) covering all stages of the process. Procurement professionals should take measures to ensure that appropriate safety and quality conditions are in place.

Procurement of human tissues or cells must take place only in authorised institutions, after donor consent/authorisation requirements have been satisfied, as described in Chapter 4.

Chapter 2 sets out the requirements about the personnel, facilities, equipment, materials, procedures and documentation that should be applied when considering the quality and safety of tissues and cells for human application. After procurement, tissues and cells must be identified, packaged and labelled correctly (see Chapter 15) and then transported to the TE for processing, or to the clinical team for direct use, in accordance with established requirements.

Procurement protocols must be appropriate to the type of donor (deceased or living) and tissue to be procured [1]. They should consider relevant risk factors that may impact the quality of procured tissues and, for living donors, also the safety of the donor. Deceased donation of tissues or cells can also occur after organ donation, and in those cases aseptic technique needs to be ensured throughout the whole procedure, including during organ procurement. If more than one tissue is to be procured from a single deceased donor, procurement may be performed by a multi-tissue team or by different tissue-specific teams.

7.2. Personnel

Procurement activities must be undertaken by personnel with appropriate qualifications, training, expertise and experience [2-7]. This includes successful completion of a comprehensive technical and/or clinical training programme, including the broader ethical, legal and regulatory context of procurement and, for deceased donors, how to appropriately reconstruct the body following procurement. The training programme must be tailored to the specific tissues or cell types to be procured and will also depend on whether the procurement is from a living or deceased donor.

Persons undertaking procurement must be made aware of the risks and potential consequences if policies and procedures on procurement are not followed as directed in written SOPs and according to relevant legislation.

To promote compliance with donor-selection criteria and procurement procedures, the TE must have written agreements with each person, clinical team or third-party procurement organisation involved in carrying out procurement, as well as those collecting critical information used in donor selection. The written agreements must include detailed

descriptions of expectations and responsibilities related to quality and safety measures, as well as any additional regulatory requirements. A written agreement is not necessary for individuals directly employed by the same TE or organisation responsible for these steps but expectations and responsibilities pertaining to procurement must appear in their job description (see Chapter 2).

7.3. Facilities, equipment and materials

7.3.1. Facilities

Procurement of tissues and cells may take place in various facilities, ranging from a hospital operating room, TE, hospital clinic, mortuary, funeral home or care home, to a donor's own home [8, 9]. These facilities can be broadly categorised as:

- operating theatre or equivalent;
- dedicated procurement area with air-quality monitoring and controlled cleaning (e.g. TE procurement room);
- dedicated clean area (controlled cleaning);
- non-dedicated area, with local cleaning of the recovery space, and suitable to prevent cross-contamination.

Procurement activities must be authorised by the Health Authority. Each procurement event should take place in an appropriate facility and follow the required clinical/technical procedures (see §7.4). The procedure must include steps aimed at minimising the risk of contamination of procured tissues and cells.

Minimising the risks of contamination during procurement (see below) and processing (see Chapter 9) is crucial to ensure the safety of grafts. Risks are often multi-factorial so, to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as a tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, how risk factors are distributed along the tissue/cell supply chain from procurement to distribution, and can implement the most efficient measures to mitigate and reduce risk.

For reasons of privacy and control of contamination, access to the area where procurement takes place must be restricted to essential personnel during the actual recovery of tissues or cells. In addition, the donation of tissues or cells by living donors should take place in an environment that ensures their health, safety and privacy. The procurement sites must be fit for purpose and a risk assessment should be carried out to determine the suitability of the procurement site, depending on the types of tissues or cells to be procured.

The room where procurement takes place should meet the following requirements:

a. adequate floor area, and ancillary work areas (e.g. work-tops and benches);
b. appropriately located and access controlled to ensure privacy;
c. furnished with sufficient and suitable lighting;
d. in a good state of maintenance;
e. able to provide a sufficiently clean and cleanable environment that will not increase the risk of contamination of the cells or tissues;
f. comfortable in temperature and humidity for donor and procuring staff.

Before procurement, steps taken to minimise the risk of contamination should include the cleaning of working surfaces with an appropriate and effective disinfectant. Procurement of tissue should follow aseptic procedures. The procurement area should also be cleaned and disinfected after recovery. Appropriate and safe disposal of single-use instruments, consumables and any other waste, including clinical waste that poses a biohazard, must be carried out. Any re-usable instruments must be cleaned immediately or as soon as possible following use, and sterilised before being used again. If a TE (or third party carrying out the recovery) uses the general services of the host facility to clean the procurement area and/or sterilise any re-usable instruments, the TE must have a written agreement with the host facility, and the procedures in place must be validated and inspected.

7.3.2. Equipment and materials

Materials (i.e. consumables and reagents) and equipment (i.e. surgical instruments, packaging and containers) used during procurement must comply with the relevant national and international regulations, standards and guidelines for the intended use of the donated tissues and cells (see Chapter 2). All equipment and materials that may have direct contact with tissue and cells should be sterile, single-use (where possible) and CE-marked for their intended purpose (where available). Instruments or devices must be suitable for their intended use, and must be maintained in good working order. This must include visual inspection and scheduled calibration of measurement devices, where appropriate, against relevant defined standards at specified intervals. Routine maintenance inspections, at least annually, of equipment used for procurement are

encouraged and if relevant a re-qualification assessment is required whenever repairs or modifications have occurred. Procurement personnel must receive appropriate training, supported by records, on the proper use of equipment.

Where possible, use of single-use instruments for procurement is recommended. When re-usable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents should be used and documented. A traceability and tracking system should be in place for operators and critical equipment/materials, for each tissue- or cell-procurement event, and for the donor.

Personnel conducting procurement activities must be provided with protective clothing appropriate for the type of procurement. Usually, this will involve wearing a sterile gown, sterile gloves, glasses and a face shield or protective mask. Approved materials necessary for reconstruction of a deceased donor's body must be provided to allow this step to be completed effectively.

7.3.3. Identification, packaging, containers and labelling

At the time of procurement, tissues and cells must be uniquely identified. They must be packaged so as to minimise the risk of environmental contamination. At least two layers of packaging should be used. The labels must be appropriate to ensure identification and traceability of tissues and cells and should be designed to adhere firmly to the container. Labels must be resistant to storage conditions to avoid the loss of identification of tissues and cells.

Storage containers must be appropriate for the type of tissue or cells, the temperature and method of storage, and the intended application. They must withstand sterilisation (where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport. Each tissue or cell container must be examined visually for damage or evidence of contamination before processing, or distribution for clinical use, and by the end user.

Guidance on coding, packaging and labelling is provided in Chapter 15.

7.4. Procedures

Written SOPs for procurement must be in place, based on the requirements of the relevant Health Authority, the recommendations laid out in this Guide and the expectations of the TE or end-user needs. These SOPs must outline the correct steps to be taken for each stage of procurement [10]. Procedures that ensure contamination control must be applied, including use of aseptic techniques, sterile materials and equipment and appropriate clothing for the personnel conducting the procurement (see §7.2 and §7.3.2). Procurement SOPs must be reviewed by an authorised person periodically (or as required) and updated as necessary. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured, and must be validated [11].

The SOPs must be readily accessible so that procurement personnel can follow the required steps, including:

a. verification of the donor's identity and what constitutes evidence of donor (or the donor family's) consent or authorisation (see Chapter 4);
b. where necessary, ensure that permission from any relevant legal authorities (for example, judges or coroners) has been obtained before tissues or cells are procured;
c. assignment and appropriate use of a unique identifier/code (see Chapter 15);
d. knowledge of selection (risk) criteria required for donor assessment, including physical examination of the donor (see Chapter 5) and mandatory infectious disease testing required;
e. knowledge of the types of blood and other samples required for mandatory laboratory tests to ensure that they are of appropriate quality (see Chapter 6);
f. steps that minimise the risk of microbiological contamination during procurement (see this chapter, as well as Chapter 3, Chapter 11 and Chapter 19 and following);
g. procurement steps that protect the properties of the tissue and cells required for clinical use (see this chapter and Chapter 19 and following);
h. for deceased donation, how to reconstruct the donor's body so it is as similar as possible to its original anatomical appearance;
i. considerations for packaging, labelling and transportation of procured tissues or cells to the TE or, in the case of direct distribution, to the clinical team responsible for their human application or direct use (see this chapter, Chapter 13 and Chapter 15);
j. considerations for collecting, packaging, labelling and transporting samples of donor blood or other samples to the laboratory for testing (see this chapter, Chapter 6 and Chapter 15);
k. procedures that protect the health and safety

of the living donor (see Chapter 20, Chapter 26 and following);

l. how to register deviations from the approved procurement procedure.

In addition, the TE is expected to have procedures in place to notify, without delay (see Chapter 17), other TEs or the relevant Health Authority of all available information about:

a. knowledge of deviations from approved procedures that have occurred or that are suspected to have occurred and which could have affected the quality and/or safety of procured tissues and cells and that could have resulted in a serious adverse reaction or serious adverse event;

b. any serious adverse reaction in a living donor that may influence the quality and safety of the tissues or cells procured;

c. any serious adverse event during procurement that may influence the quality and/or safety of human tissues and cells.

To minimise the risk of tissue or cell contamination by procurement personnel who may be infected with a transmissible disease, policies and procedures must be established and followed to address this risk [2-6, 12].

Additional procedures and policies that minimise the risk of microbiological contamination during procurement must be considered (see also Part B), including those listed here:

a. the personnel allowed to be present during procurement should be defined and such restrictions respected;

b. preparation of the donor's recovery site must follow the recommended standards of practice used for surgical patients and must occur at the beginning of procurement using an appropriate antimicrobial agent designed for this purpose;

c. the procedure for the disinfection of the recovery site should account for the reduction or elimination of bacterial spores as well as vegetative micro-organisms, and should include the type of suitable disinfectants, their concentrations and durations of exposure;

d. before use, all materials and equipment must be visually inspected by procurement personnel to ensure that they meet specifications (e.g. sterile, seals not broken, equipment functioning as expected, expiration date);

e. for deceased donation, it is advisable to procure tissue before the autopsy takes place but, if this is not possible, detailed procedures must be written to address the increased potential for contamination when procurement takes place after autopsy.

Procurement must include procedures that protect those properties of tissues and cells required for their ultimate clinical use. These are described more fully in Part B of this Guide (the tissue-specific chapters), but generally include:

a. *post mortem* time limits [8, 13, 14, 15] – it is recommended that tissue should be recovered within 24 h after cardiocirculatory arrest, provided that the body has been cooled or refrigerated within 6 h of cardiocirculatory arrest (to reduce the risk of tissue degeneration and microbiological growth). If the body was not cooled or refrigerated within 6 h of cardiocirculatory arrest, tissue should be recovered within 12 h of cardiocirculatory arrest. Alternative time limits should be validated by quality assessments and tests for microbiological contamination. It may be possible to extend the *post mortem* interval up to 48 h after death if processing has been validated to guarantee quality and microbiological safety (in which case the blood samples for serological testing must be still taken within 24 h after death, as specified in EU Tissues and Cells directives, to minimise the risk of blood deterioration; see Chapter 6 for details on sample collection);

b. preservation of important anatomical structures and other tissue or cell characteristics;

c. temperature requirements during storage and transport to the next destination;

d. avoidance of delays in transport due to time limits in place for processing after procurement.

Instead of specialised procurement teams recovering different tissues from a deceased donor, a multi-tissue procurement team may be used, consisting of a group of individuals who are trained to procure all tissues for which there is consent. The roles of the individual multi-tissue team members must be defined by SOPs.

The main advantages of a multi-tissue procurement model are:

a. better co-ordination, because all tissues are procured by the same team;

b. less time taken to procure all tissues, thereby decreasing the risk of microbial contamination because of long warm ischaemia times [2-6, 9, 12], and avoiding delays in funeral rites;

c. fewer equipment and consumables resources needed (e.g. same draping may be used to procure cardiovascular and musculoskeletal tissues).

In every deceased-donor procurement team, an appropriately trained senior person must take overall responsibility to ensure that SOPs are adhered to and that the following tasks are carried out to the required standards:

a. identification of the donor (Appendix 25);
b. review of donor documentation, including medical history, laboratory tests (if completed), lawful consent/authorisation;
c. physical examination of the donor (Appendix 15);
d. organisation and co-ordination of the procurement;
e. evaluation of abnormal procurement findings;
f. review of tissue packaging and labelling;
g. review of donor reconstruction;
h. completion of all required procurement documentation.

The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue. The recommended procurement sequence, whether carried out by separate teams or by a multi-tissue team is: skin (first back and lower limbs), skin, eyes/corneas, cardiovascular and musculoskeletal. Justification for this recommended procurement flow includes the following reasons:

- Skin is the first procured tissue because the donor is placed in a prone position to obtain skin from back and lower limbs, and the support provided by the presence of musculoskeletal tissues (in particular, bones) facilitates the procedure. Reconstruction after skin procurement is recommended to be performed at the end of donor procurement to avoid any interference in subsequently procured tissues (e.g. musculoskeletal).
- Eyes are recommended to be procured after skin to avoid eye bleeding from the sockets if the donor should be placed in a prone position following enucleation of the eyes (see Chapter 19).
- Cardiovascular and musculoskeletal tissues are recommended to be procured last because the same donor draping may be used. Some cardiovascular tissues (e.g. femoral arteries) may be procured simultaneously with the musculoskeletal tissues.

Where a tissue donor has already donated organs, all surgical approaches to obtain the organs should have been sutured to reduce as much as possible the risk of contamination of thoracic and abdominal tissues before their procurement. If the procurement is performed simultaneously with organ procurement, the sequence varies: starting with the tissues from the cavities open for organ recovery, thorax and abdomen (arteries, heart for heart valves or vertebral bodies), then the recommended sequence of skin, eyes, cardiovascular and musculoskeletal should be followed. It is important that all procurement teams involved know that tissues will be procured after organs, first to prepare the body before starting surgery, and second to guarantee sterile conditions during the whole procedure and to minimise the risk of cross-contamination.

Efforts should be made to ensure that procurement procedures do not unnecessarily interfere with funeral arrangements or other formalities such as religious or cultural rituals. If this is not possible, the donor's family must be informed at the time of consent. Timely and effective communication with all parties involved can help to meet expectations in regard to delays, as well as aesthetic considerations when tissues are procured from areas of the body that may be visible (e.g. if the body is to be viewed subsequently by the family and those attending the funeral).

7.4.1. Processing at the procurement site

Microbiological safety during the procurement of tissues or cells must always be considered; but control of contamination and cross-contamination at the procurement site is typically less stringent than the controls applied in a TE (see Chapter 3, Chapter 11 and Part B). Therefore, simultaneous undertaking of processing steps during the procurement phase, or in the procurement area, is not recommended, if avoidable. However, if processing (excluding close systems), including decontamination, shaping, cleaning, sizing and final packaging (for direct distribution) at the procurement site is unavoidable, the procurement area must fulfil the criteria of a processing area (see Chapter 9).

Processing duration and extent must be limited to the minimum, and a Grade A air-quality environment (surrounded by, at least, Grade D air quality) is required for the open processing steps. Records supporting the qualification and monitoring of the processing site must be available for inspection. Selection of the use of suboptimal conditions must be

supported by written justification and be authorised by the relevant Health Authority.

7.4.2. Temporary storage and transportation to the tissue establishment

Once the tissue is procured and until it arrives at the TE, critical variables related to maintaining the quality of the tissues or cells (e.g. temperature, sterile packaging) must be controlled (see Chapter 15). Records to demonstrate compliance with specified storage conditions must be completed and maintained.

7.5. Documentation

TEs must have procedures in place that address the retention of procurement records, which must include descriptive documentation of the steps taken, the materials and equipment used, and identification of the personnel involved. Such records must be clear and legible, protected from unauthorised amendments, retained and readily retrievable throughout a specified retention period, and must comply with data-protection legislation. Procurement records must be sufficiently detailed to facilitate robust and reliable traceability, to provide a complete history of the work undertaken and to be capable of linking the records to the particular donor of the procured tissues and cells (see Chapter 16). When tissues and cells are to be sent across national borders, potential language barriers should be addressed and a common language agreed for all documentation related to donors, tissues and cells.

A unique identifier (e.g. a donation number for a donation event and/or a donor identification number) must be allocated to the donor as well as the procured tissues and cells (see Chapter 15). This coding must be in place to ensure an effective and accurate system capable of tracking tissues throughout all stages, including an identifiable link to the procurement steps. For each donor, there must be a record containing the donor's identity with at least two forms of identifiable information, e.g. given name, family name, date of birth, sex. If a mother and child (both living) are involved in the donation, records must indicate not only the name and date of birth of the mother, but also the name (if determined) and date of birth of the child. These coded data should be entered in a registry maintained for this purpose.

To ensure that all steps are traceable and verifiable, the TE (or procurement organisation) must produce a report, recorded at the time of procurement, which must be forwarded without delay to the location where processing takes place. This procurement report, depending on the type of donor, could contain the following:

a. donor identification data (donor code, donor given name, family name, date of birth and sex, as well as how and by whom the donor was identified) or donor identification. In the case of an unrelated haematopoietic progenitor cell (HPC) donor, care should be taken to maintain donor confidentiality (Global Registration Identified for Donors or GRID) if the procurement report (including donor health status) is forwarded to the recipient's clinical team;
b. unique coding number, which will be either the donation identification sequence of the Single European Code (SEC) for EU countries, or a code generated by a Health Authority or by use of an internationally recognised coding system such as 128 Eurocode, or the WMDA GRID code for unrelated HPC donors (Chapter 15); in the case of partner donation in MAR there is no need for the SEC (see Chapter 29);
c. the environmental conditions of the procurement facility, i.e. location or description of the physical area where procurement took place (see Appendix 24);
d. a list of observations made during the physical examination of the donor's body – but, for a living donor, only when such an examination is justified (see Appendix 15);
e. a description and identification of procured tissues and cells, including samples for testing of infectious diseases;
f. the identification of the person who has overall responsibility for the procurement session (including his/her signature);
g. date, time (where relevant, start and end times) and location of procurement;
h. the type, volume, manufacturer and lot/batch/serial number of reagents, additives and the tissue and cell transport solution(s) used;
i. name and address of the TE;
j. name and destination of the tissues and cells.

In addition, for procurement of tissues or cells from a deceased donor, this report must contain:

a. a sufficiently detailed summary of the events surrounding death;
b. the date and time of donor death and tissue procurement (and, where relevant, start and end times) to facilitate determination of the time interval from death to procurement;
c. the date and time of, and quantity of, blood collection for donor testing (when applicable);

d. the conditions under which the donor body was kept before procurement (whether or not the donor body was cooled or refrigerated and, where appropriate, the time when cooling or refrigeration began and ceased);
e. if possible, whether procurement took place before or after autopsy and whether or not an autopsy is planned;
f. when applicable, a description of other tissues and cells from the same donor sent to different TEs, including their identification;
g. if applicable, information regarding reconstruction of the donor's body.

If procurement from a living donor involves a directed donation, the recipient's identification must be documented to ensure traceability (see Chapter 2 and Chapter 15).

7.6. References

1. Shiroff A, Gale S, Merlin M *et al.* Enhancing the tissue donor pool through donation after death in the field. *Prehospital and Disaster Medicine* 2013;28(2):187-90.
2. Bohatyrewicz A, Bohatyrewicz R, Klek A *et al.* Factors determining the contamination of bone tissue procured from cadaveric and multiorgan donors. *Transplant Proc* 2006;38:301-4.
3. Segur JM, Suso S, García S *et al.* The procurement team as a factor of bone allograft contamination. *Cell Tissue Bank* 2000;1(2):117-19.
4. Paoli A, Romualdi C, Romagnoli L, Troja D. Analysis of potential factors affecting allografts contamination at retrieval. *Cell Tissue Bank* 2017;18:539-45.
5. Terzaghi C, Longo A, Leganani C *et al.* Incidence of bacterial contamination and predisposing factors during bone and tendon allograft procurement. *Cell Tissue Bank* 2015;16:151-7.
6. Lannau B, Van Geyt, Van Maele G, Beele H. Analysis of potential factors affecting microbiological cultures in tissue donors during procurement. *Cell Tissue Bank* 2015;16:65-71.
7. Gaucher S, Khaznadar Z, Gourevitch JC, Jarraya M. Skin donors and human skin allografts: evaluation of an 11-year practice and discard in a referral tissue bank. *Cell Tissue Bank* 2016 Mar;17(1):11-19. https://doi.org/10.1007/s10561-015-9528-3.
8. Louart B, Charles C, Nguyen *et al.* Microbial contamination and tissue procurement location: a conventional operating room is not mandatory. An observational study. *PloS ONE* 14(1):e0210140.
9. Schubert T, Bigaré E, Van Isacker T *et al.* Analysis of predisposing factors for contamination of bone and tendon allografts. *Cell Tissue Bank* 2012;13:421-9.
10. Vignola R, Giurgola L, Colabelli Gisoldi RAM *et al.* Monitoring the microbial contamination of donor cornea during all preservation phases: a prospective study in the Eye Bank of Rome. *Transpl Infect Dis* 2019; 21(2): e13041.
11. Fehily D, Brubaker SA, Kearney JN *et al. Tissue and cell processing: an essential guide.* Chichester: Wiley-Blackwell, 2012.
12. Pianigiani E, Ierardi F, Cuciti C *et al.* Processing efficacy in relation to microbial contamination of skin allografts from 723 donors. *Burns* 2010 May;36(3): 347-51.
13. Sawa B, Ribeiro VST, Kraft L *et al.* Risk factors associated with contamination of allograft valves in a tissue bank. *Cell Tissue Bank* 2019; 20: 87-94.
14. Linke SJ, Fricke OH, Eddy MT *et al.* Risk factors for donor cornea contamination: retrospective analysis of 4546 procured corneas in a single eye bank. *Cornea* 2013;32:141-8.
15. Mathur M, De A, Gore M. Microbiological assessment of cadaver skin grafts received in a skin bank. *Burns* 2008;35:104-6.

Related material
Appendix 15. Physical assessment form (Dutch Transplant Foundation)
Appendix 24. Sample form to assess working environment (NHS, UK)
Appendix 25. Sample donor-identification form (NHS, UK)

Chapter 8. **Premises**

8.1. Introduction

Tissue establishments must have suitable facilities to carry out the activities for which accreditation, designation, authorisation or licensing is sought. This chapter provides generic guidance on the facilities used for processing, testing and storing of tissues and cells. Processing of tissue and cells, while exposed to the environment, must take place in an environment with controlled air quality and cleanliness in order to minimise the risk of contamination, including cross-contamination between cells and tissues of different donors. This chapter gives guidance on creating, implementing and maintaining a validation master plan (classification and qualification) and a monitoring plan in order to gain assurance that the cleanrooms are performing adequately and that the aseptic processing of tissue and cells is monitored (see also Chapter 2). Tissue- and cell-specific guidance on selecting the appropriate air quality for processing is given in Part B of this guide.

8.2. Requirements of storage facilities

Tissue establishments should have specific storage facilities/areas for the storage of tissues and cells. Such storage areas should be:

a. designated;
b. located in a secure area, and access must be limited to authorised personnel;
c. of sufficient capacity to allow orderly storage of the various status of tissues/cells, according to national requirements:
  i. in quarantine;
  ii. released for processing;
  iii. rejected;
  iv. returned;
  v. recalled;
  vi. for research use;
d. covered by an adequate management system, ensuring clear identification of each status of tissues and cells;
e. clean and dry, and maintained within an acceptable temperature range; and, where special storage conditions are required (e.g. specific temperature and/or humidity) these should be specified, maintained and monitored;
f. cleaned, with a schedule based on the frequency of access by personnel and goods, by properly trained personnel and with approved products in order to keep the environmental bioburden under control.

The design of a cryostorage room (e.g. storage rooms equipped with liquid nitrogen tanks or equipment using liquid nitrogen) must comply with applicable regulations and safety requirements of the relevant country. Items related to safety should include at least:

a. good ventilation (capable of supporting emergency conditions in terms of operator and product safety);

b. oxygen-level monitoring with a local audio and visual alarm;
c. visual surveillance from outside;
d. adequate space to contain the necessary freezers and tanks, including back-up systems;
e. easy access to all the storage devices, with a smooth pathway to and from the facility for liquid nitrogen supply and for prompt removal and transfer of tissues and cells in case of emergency;
f. personal protective equipment available for use, which may include items such as cryo-gloves, safety goggles, cryo-aprons and respirators;
g. a specific SOP already in place to support the safety issues.

A system to monitor all the alarms, including oxygen level in the room, the level of liquid nitrogen in the tanks, and the temperature of the tanks, is essential. When necessary, the ambient temperature of the storage area should be monitored. Personnel must be trained to react to different alarms. Personnel need to be trained to use personal protective equipment.

Further details on storage can be found in Chapter 10.

8.3. Requirements of processing facilities

Processing facilities must be designed, qualified and monitored to ensure that the air quality is appropriate for the processes carried out. International standards, such as the EU Guidelines to good manufacturing practices for medicinal products for human and veterinary use (known as GMP) [1] and the ISO 14644 guidelines for Cleanrooms and associated controlled environments [2], provide information to help achieve the appropriate air quality.

Processing of tissues and cells should be carried out in cleanrooms. In these cleanrooms, the concentration of airborne particles (viable and non-viable) must be controlled to specified levels. Each processing operation requires an appropriate level of environmental cleanliness in the operational state to minimise the risks of particulate or microbial contamination. According to GMP, four grades can be distinguished.

1. Grade A: The local zone for high-risk operations provided by localised airflow protection, such as laminar airflow workstations, isolators or restricted access barrier systems (RABS). Unidirectional airflow systems should provide a homogeneous air speed in the range 0.36-0.54 m/s (guidance value) across the whole of the Grade A area (GMP, Annex 1). Maintenance of the unidirectional airflow should be demonstrated and qualified.
2. Grade B: For aseptic operations, this is the background environment for the Grade A zone. Lower grades can be considered as defined in the tissues- and cells-specific chapters of this guide (Part B). The risk-assessment tool for defining the air quality can be used to select the background environment for the Grade A zone (see §8.4).
3. Grades C and D: Clean areas for carrying out less critical stages in the processing and storage of tissues and cells. These cleanliness grades can be considered where isolator technology is used.

Different cleanroom standards are compared in Table 8.1 [1, 2].

Whichever classification is applied, the premises and their facilities should have:

a. floors, walls and ceilings of a non-porous material with smooth surfaces to minimise the shedding or accumulation of viable and non-viable particles and to permit the repeated application of cleaning agents and disinfectants;
b. temperature control and (based on risk assessment) humidity control;

Table 8.1. **Air cleanliness classifications in Europe**

Classification		**Maximal number of particles/m^3**					
ISO 14644-1	***EU GMP***	***ISO 14644-1***		***EU GMP***			
				at rest		in operation	
		≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
ISO 5	A	3 520	not applicable	3 520	20	3 520	20
	B			3 520	29	352 000	2 900
ISO 6		35 200	293				
ISO 7	C	352 000	2 930	352 000	2 900	3 520 000	29 000
ISO 8	D	3 520 000	29 300	3 520 000	29 000	not defined	not defined

Source: [1, 2].

c. a filtered air supply that maintains a pressure differential and airflow to adjacent cleanrooms of different cleanliness levels to prevent reversal of airflow direction between the segregated cleanrooms; a combination of negative and positive pressure can also be used to achieve specific biosafety requirements;
d. a documented system for manual or continuous monitoring of temperature, humidity, air-supply conditions, pressure differentials, and viable and non-viable particle numbers (for environmental monitoring, see below at §8.5.2 and §8.5.3);
e. a documented system for cleaning and disinfecting cleanrooms and equipment;
f. a documented system for gowning and laundry;
g. adequate space for personnel to carry out their operations;
h. adequate space for storage of sterile garments (if applicable);
i. access limited to authorised personnel.

Characteristics such as temperature and relative humidity are dependent on several factors (air changes in the room, number of personnel, heat load of the equipment, processing methods and external influences such as weather changes). Parameter settings should not interfere with the defined cleanliness levels. The environmental temperature and relative humidity should be set to guarantee the safety and quality of the tissue and cells, staff comfort, electrostatic charging and discharge. Energy consumption can also be taken into account. For relative humidity, the generally accepted guidance range is 30 % to 65 % (ISO 14644-4) [3].

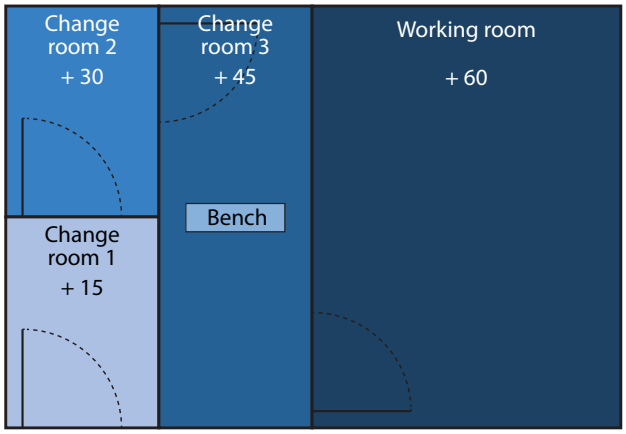
To minimise the risk of contamination, a positive pressure should be created relative to adjacent cleanrooms of a lower grade. The pressure differential between adjacent cleanrooms of different grades should be at least 10 Pa (guidance values in GMP, Annex 1) with the maximal air pressure in the background environment for the Grade A zone (the working room in Figure 8.1) [1]. This forms a 'pressure cascade' to prevent reversal of airflow direction between the segregated cleanrooms and limits the entry of contamination into the cleanrooms of a higher cleanliness level.

Stringent biosafety requirements should be followed if processing tissues or cells from patients having known viral infections, e.g. hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV). These should be determined by a documented risk analysis, considering the pathogenicity of the virus involved, and should consider:

a. The potential requirement for a containment laboratory, having reduced air pressure with respect to adjacent rooms. It may be possible to temporarily generate a containment laboratory in a standard cleanroom facility by increasing the air pressure in the change rooms to result in the working room having reduced air pressure with respect to the last change room (see Figure 8.1);
b. The use of class II biosafety cabinets or isolators to handle contaminated material;
c. The risk of cross-contamination of other tissues and cells processed at the tissue establishment;
d. Personnel safety, according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [4];
e. Handling and treatment of contaminated waste materials; any waste material generated during the process that could potentially be contaminated with viruses must be carefully handled and treated to inactivate any contami-

Figure 8.1. **Schematic plans indicating air pressure differentials between adjacent cleanrooms**

Typical processing facility design



Biohazard processing facility design

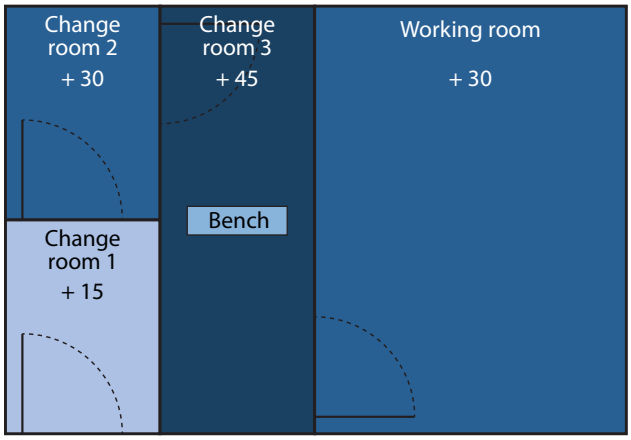


Table 8.2. **Risks of contamination that should be considered when determining air-quality specifications of processing facilities**

Risk	Explanation
Tissue or cell contamination during open *versus* closed processing.	Processes that are functionally 'closed' need a less stringent processing environment than processes where tissue and cells are exposed to the environment.
Effectiveness of the processing method to remove contaminants.	Some tissues, even though not terminally sterilised, can be treated with various antimicrobial agents; this reduces the risks of transferring any contaminants.
Suboptimal detection of contaminants due to the sampling method.	If the only option for final microbiological sampling is swabbing or testing of unrepresentative samples, the risk that contaminants will be undetected is higher than in processes where 5-10 % destructive testing of final tissue and cell grafts can be performed [6].
Transfer of contaminants at transplantation.	Tissues that are minimally processed or cellularised, or that contain blood, blood vessels and lipids, are more likely to support microbial contaminants than those that are blood- and cell-depleted. Method of application (i.e. permanent *versus* temporary) and site of transplantation both affect the risk of transfer of contaminants.

Source: Euro GTP Hot Topics guidance [16]

nation. Ideally, the inactivation process should take place before the waste material leaves the processing laboratory. Where this is not possible, it should be placed in hermetically sealed containers before being removed from the laboratory for inactivation elsewhere.

Figure 8.1 shows schematic plans indicating the air-pressure differentials between adjacent cleanrooms of a processing facility for conventional or biohazard use. Conventional processing facilities are designed to protect the sample from any contamination and therefore there is an air-pressure increase between adjacent rooms, with the maximum air pressure in the processing room. However, biohazard-processing facilities must be designed to protect both the tissue and cells and the environment. Therefore, the installation must increase the air pressure in the change rooms, resulting in maximum air pressure in one of the change rooms, and the air pressure of the working room being less than in this change room. It is recommended that pressure differentials between different rooms be set to at least 15 Pa, in order to reliably maintain a minimum pressure differential of 10 Pa.

8.4. Selecting the appropriate air quality for processing

For tissue and cell establishments in the EU, there must be the equivalent of Grade A with a surrounding environment of at least Grade D (GMP classification). A less stringent processing environment may be acceptable if one of the following applies:

a. a validated microbial inactivation or validated terminal sterilisation process is applied; or,
b. if it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissues or cells concerned; or,
c. if it is demonstrated that the mode and route of application of the tissues or cells to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with transplantation of tissues and cells; or,
d. if it is not technically possible to carry out the required process in a Grade A environment – e.g. due to the requirements for specific equipment in the processing area that is not fully compatible with Grade A (Directive 2006/86/EC) [5].

Some national requirements are more stringent, requiring Grade A with a surrounding environment B or C for certain processes. If GMP conditions are required, aseptic processing must be done in a Grade A zone within a Grade B background environment.

As outlined in Table 8.2 the specification of the air quality of the processing environment should be decided on the basis of the particular types of tissue or cell and the processing method that is being applied. Based on a risk assessment, several factors (such as limitations of sampling methods, contamination during manipulation, use of antimicrobials, transfer of contaminants at transplantation) should be taken into consideration when determining the air-quality specifications, especially when less stringent conditions are applied. Where the risk of tissue or cell contamination during processing is high, and the chances of any contaminants being transferred to the recipient are high, a more stringent air-quality specification should be adopted. See also Chapter 3 (the MiRCA tool), Chapter 9 and the tissue-specific recommendations provided in Part B of this guide.

The utilisation of isolator technology to min-

Table 8.3. **Qualification tests for cleanrooms, clean zones and laminar flow hoods**

Tests	Specification	Recommended time interval
Airborne particle count (classification test)	The total count of airborne particles (viable and non-viable) performed at rest and in operation, to determine cleanliness class	12 months
Airflow test	Average airflow velocity and air changes per hour	12 months
Air pressure difference	Differential pressure between different rooms	12 months
Installed filter system leakage test	Detection of leaks in the absolute filter and integrity testing of seals between filter and mounting arrangements	24 months or if resistance across the filter changes abnormally
Temperature and relative humidity		12 months
Recovery test	The time required for a cleanroom to recover after a particle-generation event – normally tested for cleanrooms classified as Grade A or B. Maximum delay given by GMP Annex 1 is 15-20 min	24 months
Airflow direction test and visualisation	Airflow pattern type, i.e. unidirectional, non-unidirectional or mixed	24 months
Containment leak test	Detection of leaks on structure	24 months
Laminar airflow velocity (laminar flow hoods)	The average velocity must meet the specified acceptance criteria	12 months
Microbial contamination	The total count of viable particles performed in operation	12 months

imise human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured tissue and cells. The air classification required for the background environment depends on the design of the isolator and its application. It should be controlled, and for aseptic processing it should be at least Grade D. Isolators should be used only after appropriate qualification. Qualification should take into account all critical factors of isolator technology, for example the quality of the air inside and outside (background) the isolator, sanitisation of the isolator, the transfer process and isolator integrity (checking for defective seals and pinhole leaks in the isolator gloves).

8.5. Qualification and monitoring

Processing environments should be qualified and monitored in accordance with EN ISO 14644 [2, 3, 8, 9], EN 17141 [10] and EU GMP Annex 1 [1]. The validation master plan should be built following a risk-assessment exercise aiming to define the extent and frequency of the qualification tests in a proportional manner to the risks identified. The validation plan should consider the initial and consequent qualification, as well as the at-rest and in-operation classification (see also Chapter 2). An example of a validation master plan is provided in Appendix 4 of this guide.

The qualification strategy should consider the monitoring strategy displayed over the process. Whenever a particular process is submitted to a continuous air-quality monitoring programme, the frequency of qualification of cleanrooms, laminar airflow workstations, isolators and RABS might be adapted accordingly to a less stringent programme.

8.5.1. Qualification

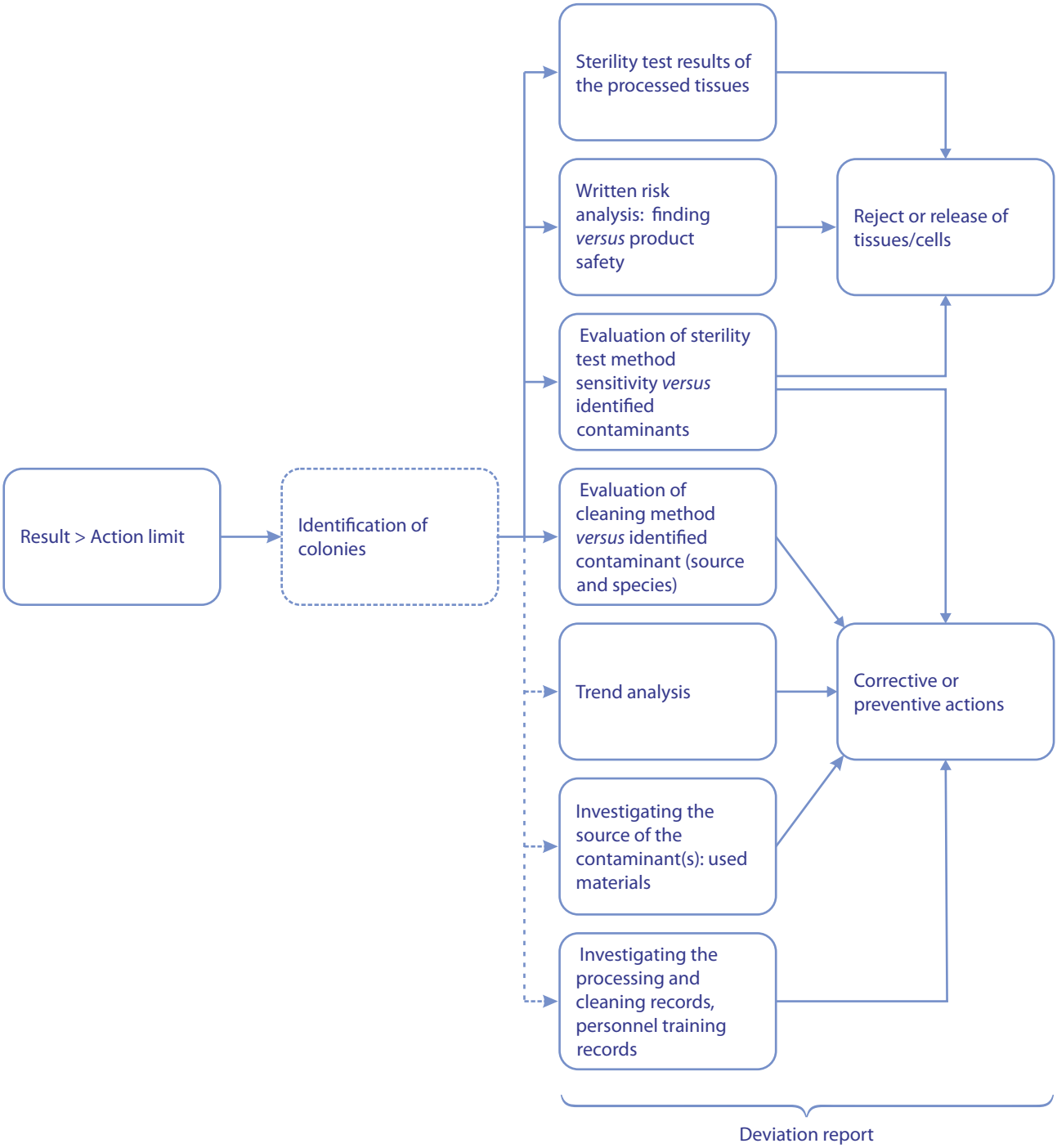
Qualification of cleanrooms and clean zones is required to support and verify the operating parameters and the limits for critical parameters. The specified acceptance criteria set should be verified, and therefore testing of certain parameters and specifications should be performed.

Qualification studies enable cleanrooms to be classified into their appropriate grade. Classification is a method of assessing the level of air cleanliness against a specification for a cleanroom or clean-zone device by measuring the airborne particle concentration. For classification, the required tests and acceptance criteria should be defined in the approved classification protocol. Classification should be performed at rest and in the operational state. The classification is part of the qualification of cleanrooms and clean zones and should be clearly differentiated from monitoring operational processes.

For particle count, the minimum number of sampling locations related to the area of each cleanroom or clean zone to be classified is provided in EN ISO 14644-1. It divides the whole cleanroom or clean zone into sections of equal area and selects in each section a sampling location considered to be representative of the characteristics of the section. The position of the particle probe should be located at the same height as, and in the plane of, the work activity.

The particle counter must have a valid calibration certificate. The frequency and method of

Figure 8.2. **Decision tree: topics and actions to be considered if microbiological-monitoring results (number of colonies) exceed the action limit**



Note: In Grade A and B areas, detected colonies must be identified by the genus and species, and for other cleanliness according to the microbial-monitoring programme. Solid lines indicate minimal actions to be considered, and dashed lines indicate topics of broader investigations

calibration should comply with the requirement of ISO 21501-4 [11].

For classification, the airborne particles equal or greater than 0.5 µm should be measured. Classification in operation may be carried out during routine or simulated operations, with a specified number of personnel present.

The minimum air-sample volume per sampling location should be determined in accordance with EN ISO 14644-1 Annex A. Sequential sample techniques could be useful to classify a cleanroom or clean zone with a very low particle concentration at the class limit (EN ISO 14644-1 Annex D).

The cleanroom or clean zone has met the specified classification if the particle concentration measured at each of the sampling locations does not exceed the concentration limits as defined in the classification protocol.

Periodic classification testing should be performed annually in accordance with ISO 14644-1. This frequency can be extended, based on risk assessment, the extent of the monitoring system and data that are consistently in compliance with acceptance limits or levels defined in the monitoring plan.

8.5.1.1. *Other qualification tests*

Table 8.3 specifies optional test methods that characterise the performance of cleanrooms and clean zones. The choice of tests should be based on factors such as the design of the installation, its operational states and the required level of air cleanliness. The selected tests should be repeated as specified in Table 8.3 as a part of validation master plan. Deviations from the pre-set frequencies should be based on a formal risk assessment. All these tests should be undertaken by qualified professionals, at least in an at-rest situation in accordance with EN ISO 14644-3 [9], which specifies ancillary tests related to other aspects of cleanroom performance such as pressure difference and airflow. The microbial load of the cleanroom should be determined in operational state as part of the cleanroom qualification. The recommended maximum limits for microbial contamination during qualification for each grade are given in Table 8.6.

Biohazard laminar-airflow hoods should also be certified to national or international performance standards at the time of installation and recertified annually.

8.5.2. Monitoring particle concentration

Monitoring particle concentration provides evidence of continuous compliance with the specified air-cleanliness class. ISO 14644-2 provides information about a monitoring plan for a cleanroom, related to air cleanliness by particle concentration.

Cleanrooms and clean zones should be monitored while in operation. Measuring locations should be determined on the basis of a formal risk analysis

Table 8.4. **Environmental microbiological monitoring methods**

Method	Air or surface qualitative or quantitative	Notes
Volumetric sampling	Air quantitative	Can be used to quantify bacteria and fungi suspended in the air surrounding the open product. Active sampling can be used to detect homogeneous suspensions of micro-organisms in the air, but it is not a reliable measure of the sporadic contamination that occurs during operations. The chosen device should be shown to correspond to current standards of sensitivity and detection. In general, the manufacturer of the sampling instrument will recommend sample sizes, and this recommendation should be taken into account in the design of sampling strategies. Sample sizes ≥ 1 m^3 should be taken for each measurement. If this sample size results in an unreadable number of colonies, reduced volumes may be employed to monitor Class C and D areas if justified. The effect of capture-plate drying during sampling and transport to the microbiological laboratory should be determined by a validation study. Time limits should be set to ensure that micro-organisms remain viable up to the point of transfer to an environment for detection of growth promotion.
Settle plates	Air qualitative	This is a method that can provide continuous monitoring of micro-organisms. Can be used to detect bacteria and fungi that descend in a column of air over the plate. Need to determine how long the plates can be open (usually 2-4 h). Exposed plates may be replaced by unused ones so that total time of exposure is reached.
Contact plates	Surface qualitative	Plates can be used. Contact plates can be used to detect contamination by micro-organisms in the immediate vicinity of the work area. The pressure and duration of contact have a significant influence on microbial yield (recommendation: uniform pressure for 10 s). Tested surfaces must be cleaned after sampling.
Swabs	Surface qualitative	Used for wiping of surfaces that cannot be sampled with plates or strips. A swab dampened with sterile physiological (0.9 %) NaCl solution is used to test dry surfaces. A dry swab is used to test damp surfaces. The sample material collected is then wiped onto an agar plate. The sample area should be ≈ 25 cm^2, if possible. Tested surfaces should be cleaned after sampling.
Glove prints	Glove or fingertips qualitative	Fingertips are the most likely area to come into contact with microbial contamination on work surfaces, on materials or arising from the operator and then transferred onto products. Glove prints (all five fingers) should be taken of both hands to assess this possibility. Usually placed on contact plates after processing or before changing gloves. Gloves must not be disinfected immediately before samples are taken. A firm and even pressure should be applied for ≈ 5-10 s taking care not to damage the agar surface.

and the results obtained during qualification of cleanrooms. A monitoring plan, taking into consideration the level of air cleanliness specified, critical care points and performance attributes of the cleanroom, should be created and maintained.

Adequate alert and action limits should be set, based on the intent and purpose of monitoring, taking into account the nature of the process. For example, bone cutting may generate numerous particles, and corneal lamellar cutting may generate numerous aerosols. If the alert limits are exceeded, further investigation or increased observation is required. If the action limits are exceeded, appropriate corrective actions must be taken. Frequent and continuous high particle counts should raise concerns because they may indicate the possibility of pollution, problems with a heating, ventilating and air-conditioning (HVAC) system, or incorrect practices during routine operations. The performance of the monitoring systems and related trends should be periodically reviewed.

Monitoring may be continuous, sequential or periodic (but indicating specified frequency).

The system selected must be adequate for the monitoring operations required. If using sequential systems, particle losses because of the length of the tubes and kinks in the tubing should be considered. For airborne-particle counters, the frequency and method of calibration should be based upon current accepted practice as specified in ISO 21501-4 [11].

Selection of the particle monitoring system should also involve consideration of the risks generated by sampling during processing. The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of cleanrooms and clean-air devices.

The Grade A zone should be monitored with a frequency that allows detection of sporadic increases in particle counts which may exceed acceptable limits. It is recommended that a similar system be used for Grade B zones, though the sample frequency may be decreased. The importance of the particle-monitoring system should be determined by the effectiveness of the segregation between adjacent Grade A and B zones.

The particle limits given in Table 8.1 for the at-rest state should be achieved after a short recovery time period of 15-20 min in an unmanned state after completion of operations.

Monitoring of Grade C and D areas should be performed in operation and in accordance with the principles of quality risk management.

Temperature, relative humidity and differential pressure of clean areas should be monitored every day.

8.5.3. Microbiological monitoring

Microbiological monitoring provides evidence of continuous compliance with the specified air-cleanliness class as well as evidence of contamination control of aseptic process operations and cleaning and sanitation methods. Microbiological monitoring is mandatory and should be done in accordance with:

a. EN 17141 *Cleanrooms and associated controlled environments – Biocontamination control* [10]; or
b. EU GMP Annex 1 [1].

To define and control microbiological hazards it is necessary to identify the potential risks relating to each processing step and the potential risks of the tissues or cells themselves, as well as the probability of these risks and the mitigation actions intended to minimise the risks. Tissue establishments must have a monitoring plan that specifies:

a. acceptance limits of microbial contamination (action level, alert level);
b. sampling plan and frequency;
c. sampling methods and equipment;
d. sampling culture media and incubation of samples;
e. analyses and evaluation of results (including trend analyses);
f. handling of out-of-specification results.

Selection of the microbial monitoring method should involve consideration of the risks generated by sampling during processing. The sample sizes taken for monitoring purposes will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal qualification of cleanrooms and clean-air devices. Microbial samples can be taken using four sampling methods: volumetric air sampling, settle plates, contact plates and glove prints – or fingerprints. A non-selective culture medium, permitting growth of the expected micro-organisms and containing additives to overcome the residual effect of biocides and cleaning agents, should be selected. Additives inhibiting residual biocides and cleaning agents are an essential component of the culture medium.

At present no commercial neutraliser is able to inactivate all biocides. The choice for a sanitation and disinfection programme with a specific cleaning agent and biocide should be well considered. The

Table 8.5. **Incubation conditions for environmental microbiological monitoring**

	Culture medium	Incubation temperature	Incubation period
Aerobic	Trypticase soy agar irradiated	20-25 °C + 30-35 °C	3-5 days + 2-3 days
Fungi			
Alternative incubation conditions			
Aerobic	Trypticase soy agar irradiated	30-35 °C	2-3 days
Fungi	Sabouraud agar	20-25 °C	5-7 days

Note: If applicable, consider also anaerobic testing in the same culture conditions as aerobic testing but in anaerobic atmosphere.

concentration of residue left on the surfaces after cleaning depends on the type of biocide and the sanitation programme. The culture medium used for environmental monitoring has to be appropriately qualified for the growth of diverse bacteria and fungi, and it should be possible to demonstrate that the residues generated by the sanitation programme do not interfere with micro-organism recovery. Table 8.4 summarises the characteristics of these sampling methods.

The frequency of sampling should take into account the processes and activities of the staff. Aseptic process operations performed in a Grade A or B environment must be monitored routinely, during every process. Background and surrounding areas should be monitored periodically, at a pre-defined frequency.

8.5.4. Incubation of samples

Environmental monitoring samples should be incubated at a minimum of two different temperatures to detect bacteria and fungi. Incubation conditions for environmental microbiological testing are detailed in Table 8.5 Incubation for 3-5 days at 20-25 °C followed by incubation at 30-35 °C for an additional 2-3 days has been shown to be sufficient to detect most bacteria and fungi. The method chosen should be validated and standardised very carefully. Alternative methods are acceptable if high recoveries (> 50 %) of micro-organisms of interest can be demonstrated consistently [12].

If micro-organisms are expected in the environment and cannot be detected using standard media for environmental monitoring with the temperatures recommended above, the procedure should be adapted accordingly.

Results of monitoring should be considered when making the decision whether tissues or cells can be released (Figure 8.2).

Recommended limits for microbiological monitoring (EU GMP Annex 1) of clean areas during operation are shown in Table 8.6. Alert and action levels for microbial contamination should be determined and the actions to be taken in the event that these levels are exceeded should be documented. The level should be specific to the area, determined on the basis of historical data and based on, for example, data from a single year. Levels should be reviewed periodically and, if necessary, updated if there are changes to processes.

The alert level emphasises an acceptable number for microbial contamination, but acts as a warning. Exceeding the alert level does not require corrective measures, but should trigger an investigation aimed at early detection of errors or deviations. The alert level is set at a lower level than the action limit. The action level emphasises a certain level of microbial contamination that necessitates immediate corrective action and corrective measures.

In Grade A and B areas, any detected colonies should be identified by the genus and species, and for other cleanliness grades according to the microbial monitoring programme of the tissue establishment.

Table 8.6. **Recommended limits for microbial monitoring (EU GMP Annex 1)**

	Recommended limits for microbial contamination			
Grade	**Air sample (CFU/m^3)**	**Settle plates, diam. 90 mm (CFU/4 hours)***	**Contact plates, diam. 55 mm (CFU/plate)**	**Glove print, 5 fingers (CFU/glove)**
A†	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	not applicable
D	200	100	50	not applicable

* Individual settle plates may be exposed for less than 4 h. Where settle plates are exposed for less than 4 h, the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 h.

† Note that for Grade A, the expected result should be 0 CFU recovered; recovery of 1 CFU or more should result in an investigation.

It is considered good practice to systematically identify colonies by the genus and species found in other cleanliness grades because this will assist with understanding trends and may prevent action levels being reached, if corrective and preventative actions can be identified and implemented. Figure 8.2 describes topics and actions to be considered if microbiological-monitoring results exceed the action limit. Any presence of fungi must be considered to denote deviation and should be identified.

After such results are obtained, tissue establishments should evaluate whether the finding will affect the risk that tissues or cells could have been contaminated during processing. Tissue establishments should also evaluate whether corrective or preventive actions should be initiated. All investigations that are carried out should be reported in a deviation report.

8.6. Avoiding contamination and cross-contamination

Entry of personnel and materials to the processing facilities, transit and exit of personnel and material through the processing area and the rules of use and clothing to be worn in them should be established to:

a. minimise the risk of contamination of tissues and cells;
b. reduce the environmental bioburden;
c. protect staff from biohazards.

A written procedure designed to avoid potential contamination and/or cross-contamination from personnel and materials to tissues and cells should be in place.

Entry of personnel, tissues and cells and materials should be done through airlocks by following specified procedures to avoid the direct flow of non-treated air into cleanrooms. Both airlock doors should not be open simultaneously. An interlocking system or a visual and/or audible warning system should be operated to prevent the opening of more than one door at a time.

Only the minimum number of personnel required for efficient processing should enter the processing areas. The need for additional persons to be present in processing areas should be taken into account during risk assessment when the procedure is being designed.

High standards of personal hygiene and cleanliness are essential. Changing and washing should follow a written procedure designed to minimise contamination of clean area clothing or transfer of contaminants to the cleanrooms. Wristwatches, make-up and jewellery must not be worn in clean areas. Outdoor clothing must not be brought into changing rooms that lead to Grade B and C rooms.

Required clothing should be chosen, based on the process and grade of the processing area. Minimum requirements modified from EU GMP Annex 1 are listed in Table 8.7. Clothing should be pocket-less, made of lint-free material, with tightly

Table 8.7. **Minimum clothing requirements (adapted from EU GMP Annex 1)**

Classification	Clothing	Description
Grade D	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A general protective suit
	Shoes	disinfected or sterilised shoes or overshoes
	Gloves	Dependent upon the process
Grade C*	Facemask	Depending on the process, at least beards and moustaches should be covered
	Cap	Hair should be covered
	Suit	A single or two-piece trouser suit gathered at the wrists and with high neck
	Shoes	disinfected or sterilised shoes or overshoes
	Gloves	If directly handling tissues and cells, sterile non-powdered rubber or plastic. Otherwise, non-sterile, non-powdered rubber or plastic are acceptable
Grade A/B*	Facemask	Sterile, single-use. Headgear should totally enclose facial hair. Sterile eye protection/ coverage is dependent upon the process
	Cap	Sterile headgear should totally cover hair, beards and moustaches; it should be tucked into the neck of the suit
	Suit	Sterile coverall
	Shoes	Sterilised footwear, boot-like structure to enable the trouser-legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered rubber or plastic gloves

* In general, the protective clothing material should shed no fibres, and clothing should retain the particles shed by the body.

fitting fasteners at the neck, wrist and ankles. For each worker in a Grade A/B area, clean sterile (sterilised or sanitised adequately) protective garments should normally be provided at each work session (or slightly less often if monitoring results justify it) but in any case at least once a day. Masks and gloves should be changed at least after each working session. Gloves should be changed regularly during operations. Disinfection of gloves is acceptable in Grade C and D environments as long as direct contact with tissues and cells is excluded. When operators are trained in the use of good aseptic practices, documented with a successful process simulation, the operator glove sites can be considered as a non-critical surface [13]. The efficacy of disinfection depends on the disinfectant/type of gloves combination. Disinfection of some gloves has an influence on the tightness of the gloves [14]. A complete investigation should be performed and documented to evaluate the impact of glove disinfection.

Gowning procedures for personnel should be validated at least in Grade A/B areas to ensure that gowning materials and protocols are adequate. Samples should be taken from the surface in several fixed sites on clothing:

a. fingers of gloves;
b. facemask;
c. sleeve (forearm) of a suit;
d. front of the suit at chest height;
e. hood.

8.7. Cleaning

Appropriate sanitation of clean areas is of the utmost importance to satisfy environmental requirements. The cleaning process (both schedule and procedure) should be validated, and the validated cleaning process should be followed to achieve the required level of cleanliness. The cleaning validation should consider the influence of the time between processing and cleaning and the time between cleaning and processing to define how long the cleaning process can be delayed after processing and how long the cleaning process remains effective.

All cleaning procedures should be documented. Cleaning should be done by personnel trained for the procedure, cleanroom environment, workflows and gowning. Appropriate disinfectants should be selected, based on the types of micro-organism found in the environment. Rotation of different types of disinfectant should be included in the disinfection programme to avoid any antibiotic resistance effect due to biocides [15] and to cover a wider range of micro-organisms. Cleaning products are made up of broad-spectrum disinfectants containing quaternary ammonium compounds, stabilised chlorine dioxide, hydrogen peroxide and sodium hypochlorite.

Disinfectants and detergents used in Grade A and B areas should be sterile before use, and be replaced within a defined period after opening, to be determined by risk assessment. Cleaning products should therefore not only disinfect the premises but also be safe for the human tissues and cells. Especially in ART centres, certain biocides might be detrimental for gametes and embryos, and therefore care should be taken in choosing the appropriate cleaning products and disinfectants [16]. Disinfectants and detergents used in Grade A and B areas should be sterile before use. Microbiological monitoring of the cleanroom should be undertaken regularly to detect development of resistant strains. Fumigation may be useful for reducing microbiological contamination on inaccessible surfaces.

Some tissue banks and cell banks accept material for autologous use from donors infected with HIV, HBV or HCV. In such cases, separate processing should be done and validated cleaning procedures applied. After processing, the surface should be decontaminated using disinfectant with specific label claims for blood-borne pathogens (e.g. HIV, HBV, HCV) or a freshly diluted bleach-based product in accordance with manufacturer's instructions, and the surface should be allowed to dry.

Inactivation of prions should be considered if risk of prion contamination has occurred, e.g. if tissues or cells from a Creutzfeldt–Jakob disease-positive donor have been processed or stored. Prions are very resistant to inactivation. Published methods for prion inactivation include physical and chemical methods. Concentrated solutions of sodium hypochlorite achieve inactivation but other chlorine-releasing compounds are less effective. Sodium hydroxide (2 M) leads to substantial (but incomplete) inactivation. Other chemical procedures, such as the use of proprietary phenolic disinfectants, are much less effective. Infectivity can survive autoclaving at 132-138 °C and, under certain conditions, the effectiveness of autoclaving declines as the temperature is increased. The small resistant subpopulations that survive autoclaving are not inactivated simply by re-autoclaving, and they acquire biological characteristics that differentiate them from the main population. Despite the limitations of autoclaving, combining autoclaving (even at 121 °C) with treatment using sodium hydroxide is extremely effective [17].

Storage facilities should be cleaned according to a schedule. Also, handling and disposal of wastes

should include appropriate collection, storage and transportation procedures according to applicable European, national and local regulation.

8.8. References

1. EudraLex [collection of rules and regulations governing medicinal products in the European Union]. Volume 4, EU guidelines for good manufacturing practices for medicinal products for human and veterinary use (GMP). Annex 1: Manufacturing of sterile medicinal products. Brussels: European Commission, November 2008, available at https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en, accessed 7 April 2022.
2. ISO Technical Committee 209 (ISO/TC 209) EN ISO 14644-1:2015, Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration.
3. ISO Technical Committee 209 (ISO/TC 209) EN ISO 14644-4:2001, Cleanrooms and associated controlled environments – Part 4: Design, construction and start-up.
4. Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work, available at http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0054&from=EN, accessed 7 April 2022.
5. Directive 2006/86/EC as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1544568681860&uri=CELEX:32006L0086, accessed 7 April 2022.
6. Sterility, general chapter 2.6.1. *European Pharmacopoeia (Ph. Eur.)*, 10th edition. Strasbourg, France: Council of Europe 2022.
7. Euro GTP hot topics. *European Union Project in the framework of the Public Health Program*. Barcelona: Transplant Services Foundation, 2007.
8. ISO Technical Committee 209 (ISO/TC 209) EN ISO 14644-2:2015, Cleanrooms and associated controlled environments – Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration.
9. ISO Technical Committee 209 (ISO/TC 209) ISO 14644-3:2005, Cleanrooms and associated controlled environments – Part 3: Test methods.
10. EN 17141:2020 Cleanrooms and associated controlled environments – Biocontamination control, available at https://standards.iteh.ai/catalog/standards/cen/786bdc13-6821-4f3d-800a-96e24631df62/en-17141-2020, accessed 25 July 2022.
11. EN ISO 21501-4:2007 Determination of particle size distribution – Single particle light interaction methods – Part 4: Light scattering airborne particle counter for clean spaces, available at www.iso.org/standard/40274.html, accessed 25 July 2022.
12. WHO. Environmental monitoring of clean rooms in vaccine manufacturing facilities: Points to consider for manufacturers of human vaccines, November 2012, World Health Organization, available at www.dcvmn.org/IMG/pdf/who_env_monitoring_cleanrooms_final_2_.pdf, accessed 21 April 2022.
13. PDA Task Force. *Points to consider for aseptic processing: part 1*. Bethesda MD, USA: Parenteral Drug Association, 2015.
14. Scheithauer S, Häfner H, Seef R *et al.* Disinfection of gloves: feasible but pay attention to the disinfectant/glove combination. *J Hosp Infect* 2016;94(3):268-72.
15. Scientific Committee on emerging and newly identified health risks (SCENIHR). Assessment of the antibiotic resistance effects of biocides. Brussels: European Commission, 19 January 2009, available at http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_021.pdf, accessed 7 April 2022.
16. Verheyen G, Sas ST, Souffreau R *et al.* Toxicity testing of decontaminating agents and cleaning products used in human IVF laboratories. *Hum Reprod* 2014;(29)_supp 1. Munich: ESHRE, July 2014.
17. Taylor DM. Inactivation of prions by physical and chemical means. *J Hosp Infect* 1999;43(Suppl):S69-S76.

Chapter 9. **Processing**

9.1. Introduction

The term 'processing' means all operations involved in the preparation, manipulation, preservation and packaging of tissues or cells intended for human application as well as inactivation of micro-organisms in such tissues and cells. Storage is necessary at various stages from procurement to clinical use and must be controlled and documented to ensure that the required properties of the tissues or cells are maintained and that cross-contamination or loss of traceability is avoided (see Chapter 10 – Storage, Chapter 12 – Release, distribution and import/export, and Chapter 16 – Traceability). Packaging and labelling are described in more detail in Chapter 15.

The aims of processing tissues and cells include:

a. improving the clinical outcome of a tissue graft or cells by removing those elements that may contribute to decreasing the performance of the tissues or cells before application, *ex vivo* – for example, decellularisation of a tissue graft, or removing seminal plasma by density gradient centrifugation before intra-uterine insemination;

b. reducing the risk of disease transmission by the inactivation of micro-organisms or even sterilisation, in circumstances where cell viability is not required;

c. preservation of the required properties of the biological material, making extended storage for future use possible;

d. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells.

Processing includes a range of activities including (but not limited to) washing, cutting, grinding, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, separation, decellularisation, concentration or purification of cells, freeze drying, freezing, cryopreservation and thawing.

Although it can deliver great benefits, processing can also introduce risks. The potential risks include:

- microbial contamination from the environment or the operator, or cross-contamination from other tissues or cells,
- errors in identification or labelling,
- damage to the tissues or cells that reduces their clinical efficacy.

For these reasons, processing of tissues and cells must be carried out within a comprehensive quality-management system, must be documented using standard operating procedures (SOPs) and must be thoroughly validated, to demonstrate that the quality and efficacy of the final product have not been unacceptably compromised and that contamination or cross-contamination has not been introduced during processing.

The process of procurement ends with the collection of cells and tissues from a donor, and usually takes place in facilities outside the TE, such as hospital mortuaries, operating theatres or apheresis units. Following procurement, the cells and tissue

are transferred to the TE, where they can be processed and stored under controlled conditions. There are occasions when the initial steps of processing are performed at the procurement facility for operational reasons, but this is not recommended and should be avoided unless absolutely necessary, due to elevated risks of microbial contamination and cross-contamination in these environments. (see §7.4.1).

This chapter provides generic guidance on the processing of tissues and cells carried out by tissue establishments (TEs). Further, more specific, guidance is provided in Part B of this Guide. It is also important that TEs follow the Good Practice Guidelines for tissue establishments (GPG) to provide the key elements of their procedures, which should be defined and controlled within the TE's quality system. Where relevant, the GPG sections pertinent to the topic are referenced in each subsection of this chapter.

9.2. Receipt at the tissue establishment

Each TE must have a documented policy and specifications against which each consignment of tissues and cells (including blood samples from donors) is verified. Verification is the confirmation through the provision of objective evidence that specified requirements have been fulfilled. These specifications must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. When the procured tissues or cells arrive at the TE, there must be documented verification of the consignment. Documents must be completed covering the transport – including the transport conditions, packaging, labelling and associated documentation – and samples (including blood) to ensure that they meet the requirements and specifications of the receiving establishment (and, in EU countries, the requirements of Annex IV of Commission Directive 2006/17/EC).

Upon receipt of the documentation, the procurement report and shipping record (if the donation was transported by a third party) must be cross-checked with the contents of the package. The packaging, the tissues and cells received, and any accompanying samples must all be examined to ensure that they have not been damaged or tampered with during transit.

The following must be checked and recorded (where appropriate):

a. (no) evidence of unauthorised opening or manipulation;
b. (no) signs of damage that might result in the deterioration of tissues and cells and (no) signs of incidents relating to storage or transport;
c. transport conditions and storage temperature and time in transit (unless a validated transport method has been used, or a validated surrogate method of confirmation, such as the presence of solid refrigerant in the container, can be employed);
d. unique identification of the donor (donation number);
e. description of the tissues or cells (including number of units per device or ampoule);
f. procurement report including procurement date and time;
g. purpose of tissues and cells (i.e. for transplant/research);
h. associated samples (including blood).

The TE must ensure that the tissues and cells received are quarantined and stored in a defined, separated and adequate location under appropriate conditions until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The acceptance or rejection of received tissues or cells must be documented.

The data that must be registered at the TE include:

a. consent/authorisation, including the purpose(s) for which the tissues and cells may be used and any specific instructions for disposal if the tissues or cells are not used for the purpose for which consent was obtained;
b. all required records relating to the procurement and donor medical/behavioural history (see §7.5);
c. for allogeneic donors, eligibility, i.e. a properly documented review of donor evaluation against the appropriate selection criteria by an authorised and trained person;
d. in the case of tissues and cells intended for autologous use, if relevant, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

Review of the relevant donor/procurement information, and thus acceptance of the donation, needs to be carried out by specified/authorised persons.

The TE must have documented procedures for the management and segregation of non-conforming tissues or cells, or those with incomplete test results for infectious diseases, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

If the material is not being transported by their own personnel, the TE should prepare an agreement to be signed by third parties that defines the responsibilities of each party in the transport of tissues and cells to the TE. Such transport should ensure the safety of the tissues and cells and maintenance of their temperature conditions, and prevent unauthorised access.

Quality-control checks of procurement and transportation methods should be reviewed regularly by TEs to ensure that the integrity of tissues or cells and the storage temperatures are maintained during procurement and transit.

9.3. Coding

TEs must ensure that human tissues and cells are correctly identified at all times. Upon receipt of the tissues and cells, the TE must assign a unique identification code to the material if this has not already been done at procurement. This code can then be extended to identify the different products and batches of tissues or cells obtained during processing.

Tissues and cells must be labelled at all stages of processing (see Chapter 15 for further guidance on labelling and Chapter 16 for further guidance on traceability). The label must include a unique identifier and, depending on the product, may also contain the following information:

a. identification of the TE;
b. type and characteristic of the product;
c. batch or lot number (if applicable);
d. recipient name (if applicable);
e. expiry date.

The coded data must be entered in a register maintained for the purpose.

9.4. Processing methods

Tissues and cells should be appropriately processed, preserved and decontaminated for clinical use. TEs must address all processes that affect quality and safety through their quality system and associated SOPs.

TEs must ensure that the equipment being used, the working environment, process design, and validation, verification and control conditions are in compliance with established quality and safety requirements (see Chapter 2). Each step of processing must be carried out under defined conditions to guarantee the quality and safety of tissues and cells, as well as the safety of TE personnel.

If a TE entrusts one of the stages of processing to a third party, a written agreement is needed between the TE and the third party. The TE must evaluate and select third parties on the basis of their ability to meet the established standards.

The recommended time limits between procurement, processing and storage are described in the tissue- and cell-specific sections of this Guide (see Part B). When appropriate, these maximum times from procurement (or circulatory arrest) until processing and storage must be defined. Procurement, processing and storage times must be documented in the records for tissues and cells.

9.4.1. Processing reagents

The reagents used in processing should be of an appropriate grade for their intended use, be sterile (if applicable) and comply with existing national regulations. Use of antibiotics during procurement, processing and preservation should be minimised, and, if used, information regarding the possibility of allergic reactions in the recipient must be included in the information provided to the end users. Whenever possible, reagents used for procurement, processing, decontamination and preservation should be CE (Conformité Européenne) marked and/or of pharmacopoeial grade, if available. Reagents that are not of appropriate grade must undergo risk assessment and validation to confirm that they are suitable for their intended purpose. Reagents not approved for human use may be used if an equivalent reagent of appropriate grade is not available, if the use has been authorised by national authorities and if the use is supported by risk assessment. The origin, characteristic conditions for storage (physical, chemical, microbiological) and expiry dates of reagents should be monitored and recorded. Reagents should be used in a manner consistent with the instructions provided by the manufacturer. Critical reagents and consumables should have written specifications describing, if applicable:

a. materials, including:
  i. the designated name and the internal code reference;
  ii. the reference (if any) to a pharmacopoeia;
  iii. the approved suppliers and, if possible, the original manufacturer of the products;
  iv. a specimen of printed materials;
  v. certificate of compliance from the manufacturer.

b. directions for sampling and testing, or reference to procedures;

c. critical quality attributes, with acceptance limits;
d. shelf life, storage conditions and precautions;
e. the maximum period of storage permitted before re-examination.

9.4.2. Processing techniques

9.4.2.1. *General principles*

Processing methods must not render the tissues or cells clinically ineffective or harmful to the recipient. They should be designed to ensure the safety and biological functionality of prepared tissues and cells. Processing methods should be validated to ensure they achieve their objectives (see the general text on validation in Chapter 2 and §9.10 below).

Processing procedures must undergo regular re-validation to ensure that they continue to achieve the intended results.

Pooling of different tissues and cells from two or more donors during processing is not recommended. The only exception is where it is supported by a comprehensive risk–benefit assessment and it has been demonstrated to be the only way of providing sufficient clinically effective tissues or cells. If performed, traceability must be fully ensured. In the case of MAR, in each MAR cycle an oocyte or oocytes from one patient/donor can only be mixed with sperm from one donor.

9.4.2.2. *Procedures*

The main types of processing procedure that can be applied to tissues and cells include, but are not limited to:

- Cleansing of procured material by removal of extraneous tissues and bodily fluids is a common initial processing step. Commonly, scalpels, scissors and gauze wipes are used in this process.
- Separation is used to partition the specific type of tissue or cell to be processed from another type, for example to divide dermis and epidermis, or amniotic membrane and chorion. It may involve the use of enzymes to separate and isolate tissues or cells.
- Cutting and shaping allows initial preparation of procured tissues into the shapes and forms required for transplantation. Different types of cutting device can be used, depending on type of tissue. For cutting bone, different types of saw may be used, such as oscillating saws, bandsaws or rotary saws, whereas for soft tissues such as skin and tendon, scissors or scalpels may be used.
- Grinding, by different types of mill, is used to pulverise bone tissue into smaller pieces. Depending on the type of bone being ground, actively cooled grinding mills that dissipate heat may be used, to prevent the bone being damaged by excessive temperatures caused by grinding friction.
- Washing may be performed for one or more of the following purposes:

– as an initial step in processing, e.g. to remove surface blood and lipids,
– as an integral part of a process, e.g. to remove bone-marrow components from musculoskeletal allografts,
– to remove traces of chemical compounds used during processing.

- Washing may also be used to decrease the bioburden of tissues. Several types of washing solution may be used, e.g. distilled water, 0.9 % NaCl, balanced salt solution, phosphate-buffered saline, or alcohols. The washing protocol utilised should be validated to demonstrate that it does not detrimentally affect the clinical efficacy or safety of the tissue [1, 2].
- Centrifugation may be used to concentrate and separate cells from a suspension or different fractions of suspensions, or to remove marrow, blood and lipid components from musculoskeletal allografts.
- Supercritical CO_2 processing involves the use of carbon dioxide under high pressure. Under these conditions, this substance behaves as a supercritical fluid solvent, which is very effective at removing fluids, lipids and cells from solid tissues.
- Disinfection by soaking or rinsing in antibiotic or antimicrobial solutions is commonly used for decontamination of viable tissues that cannot be terminally sterilised, and as a stage in the processing of subsequently sterilised tissues to reduce the bioburden.
- Cell concentration and selection is used as an initial step for HPC processing or for *in vitro* cell cultures. This may also include the isolation of particular cell types, e.g. mononuclear cells from peripheral blood.
- Filtering procedure is used after bone-marrow collection.
- Decellularisation is a technique that aims to remove most of the cellular content of the tissue, leaving behind just the extracellular matrix (ECM). These extracellular matrices may be implanted directly or used as a scaffold for the

manufacture of tissue engineered products. See Chapter 21, Chapter 22 and Appendix 34 for a more detailed discussion of decellularisation.

- Devitalisation is a term used to describe a process which will intentionally kill donor cells in a tissue, but not necessarily remove the dead cells from the tissue, such as freeze thawing or glycerolisation.
- Demineralisation is a process of chemical removal of the bone mineral, resulting in exposure of biologically active bone morphogenetic proteins present in bone tissue. Demineralisation is usually performed using a dilute (0.5 M or 0.6 M) HCl solution.
- Freeze-thawing of tissues can be used as a processing step, for lysis of cells prior to washing procedures.

TEs should satisfy themselves that any processing that they apply to tissues and cells is not classified as 'substantial manipulation' according to Regulation (EC) No. 1394/2007, the Advanced Therapy Medicinal Products (ATMP) regulation. If so, there is a possibility that the tissue or cell product could be classified as an ATMP. Further information can be found in Annex 1 of the Regulation, and guidance can be obtained from the Committee for Advanced Therapeutics (CAT) of the European Medicines Agency. In case of doubt, TEs should contact their Health Authority for advice.

9.5. Preservation methods

Preservation of tissues and cells for long-term banking is central to the operation of a TE. It is essential that a preservation technique appropriate for the graft in question is selected. The selected technique must be capable of retaining the essential properties of the graft (e.g. viability, structural integrity) for the duration of the maximum possible storage period.

9.5.1. Types of preservation

Tissues and cells can be preserved by different techniques including, but not limited to, refrigeration, freezing, lyophilisation, dehydration, cryopreservation, organ culture, vitrification or glycerolisation.

Refrigeration, or hypothermic storage, is used for short-term storage of tissues and cells following procurement, or during processing. It may also be used for the short-term preservation of tissues and cells between processing and transplantation, where other types of preservation are not feasible, for example the storage of corneas, or fresh osteochondral grafts where donor cell viability is essential. Refrigeration involves storing tissues and cells above freezing point, normally at temperatures between 2 and 8 °C or a tighter range within this overall range.

Freezing is used for pre-processing storage of procured tissues, for in-processing storage between different processing steps of non-viable tissues and for storage of processed tissue awaiting release for transplantation. Freezing can disrupt tissues and cells [3]. Hence, the method of freezing used must take into account the eventual use of the tissues and cells.

Lyophilisation (or freeze-drying) involves dehydration of tissues by freezing and then reducing the surrounding pressure to allow the frozen water in tissue to sublimate directly from the solid phase to the gas phase. Lyophilisation prevents tissue autolysis and allows storage at room temperature.

Dehydration accomplishes the removal of water from tissues by passive evaporation, without the use of freezing and negative pressure as required for lyophilisation. It is generally used for the preservation of thin tissues such as amniotic membrane which can dry quickly at room temperature. Dehydration can be accelerated by placing the tissue under a continuous air flow, and by the use of hygroscopic materials in contact with or adjacent to the tissue.

Cryopreservation is a process where the biological and structural functions of tissues or cells are preserved by cooling to sub-zero temperatures in a cryoprotectant. This is used where cell viability must be maintained. The rate of cooling must also be controlled to prevent formation of ice crystals within cells, which can result in damage and loss of viability and integrity. Once cryoprotectants are added, tissues/cells may be placed in a freezing device (such as a controlled-rate freezer) that gradually reduces the temperature of the grafts.

Organ culture involves storing tissues at normothermic temperatures, for a short period between completion of processing and transplantation. It is generally used where preservation of donor cell viability and phenotype is essential and requires the use of nutrient-rich storage media.

Vitrification is ice-free cryopreservation. The crystallisation of ice is avoided by a combination of high cryoprotectant concentrations and rapid cooling.

Glycerolisation is a procedure for soaking tissues, mainly skin, in a concentrated glycerol solution. The glycerol binds water in the tissue and prevents degradative processes occurring.

9.6. Decontamination methods

Microbiological and viral safety are critical for cell and tissue transplants. Validated sterilisation and virus-inactivation processes are mandatory in many jurisdictions. In some countries a validated disinfection process may be accepted as an alternative to sterilisation.

It is important to clearly define terms when discussing the removal of micro-organisms and the inactivation of viruses. Typically, more than one process contributes to the overall effect. Initial steps generally serve to reduce and/or control the bioburden for the main process.

9.6.1. Types of decontamination

9.6.1.1. *Disinfection*

Disinfection is a term used for non-sterilising processes that kill bacteria, and/or fungi and/or spores, and/or inactivate viruses via a known, direct and quantifiable physical or chemical mode of action. In the preparation of tissue grafts, disinfection processes are either precursors to sterilisation processes or in some cases, where sterilisation is either not required or not possible, are themselves the primary mechanism for ensuring microbiological graft safety.

Generally, when used as a precursor to sterilisation, a disinfection process is intended to reduce the bioburden on the tissue prior to sterilisation such that it does not exceed a level that can be reliably and completely eliminated by the sterilisation process.

When used as the main step for the removal of bacteria, spores and fungi and the inactivation of viruses, the process should be validated according to national requirements. In some instances, sterility tests on individual transplants may be required.

Some establishments rely upon a validated thermodisinfection process for the attainment of microbiological transplant safety [4]. Tissues that cannot be subjected to high temperatures or other disinfection procedures may be treated with antibiotic-based disinfection steps. The risk that antibiotic residues could remain in the tissue post-disinfection, and compromise post-disinfection sterility, must be considered [5]. Such processes should therefore be validated and verified for both efficacy and residual antibiotic levels. Information provided to users must include information about such residues, as some patients may be allergic to certain antibiotics and other decontaminants, such as povidone iodine.

9.6.1.2. *Sterilisation*

Sterilisation is defined as a process that results in the complete absence of all cell-based micro-organisms capable of replication. In the preparation of tissue grafts a sterilisation process will usually also have to meet the requirements for virus inactivation.

Sterilisation processes can be based on moist or dry heat, chemical agents, irradiation or high pressure. Sterilisation processes used in tissue-graft preparation are held to exacting standards and must be validated and verified according to national requirements.

Individual validation methods for processes used to sterilise tissue transplants are feasible, but the following established, standardised approaches are available:

a. Sterility Assurance Level (SAL). For sterilisation processes with a well-defined dose/kill relationship, a very high degree of sterility assurance can be achieved and quantified with an SAL. The SAL is expressed as an experimentally-derived number defining the likelihood of a contaminant surviving the process. The smaller the number, the higher the likelihood of sterility. For some classes of medical device and for some medicinal products, there is a European requirement that a sterilisation process must be validated to give a value of SAL $\leq 10^{-6}$ for the product to be labelled 'sterile'. This SAL means that the likelihood of non-sterility is 1 in 1 million. (This is often interpreted as meaning that in a theoretical batch with 1 million 'units', at least 999 999 units must be sterile. More relevant for tissue transplants is the interpretation that the process should result in all units being sterile in 999 999 from 1 million cycles.) At the time of writing there are no defined national requirements within Europe for sterilisation processes used to treat tissue transplants to be validated for a specific SAL. At the same time, when an SAL approach is used, a manufacturer may have difficulty convincing national authorities that a process with a 'lower' SAL should be approved. There is a very important limitation of the SAL approach for sterilisation processes used for the treatment of tissue grafts: the SAL method cannot be used to quantify the efficacy of virus inactivation. For this reason, in countries with the most stringent quality standards for tissue grafts, an alternative approach is considered appropriate.

b. Potency against a panel of bacteria, spores and

viruses. Validation of potency can be achieved by application of the EN 1040 standard and/or Committee for Proprietary Medicinal Products (CPMP) guidelines [6, 7]. This type of validation requires that the maximum anticipated level of bacterial, fungal and viral contamination can be eliminated, by establishing the elimination capacity in terms of the number of log-scale reductions in the concentration of samples spiked with a panel of bacteria, fungi and viruses. The panel should cover Gram-positive and Gram-negative bacteria, spores and fungi, and should include known relevant 'resistant' species. In the case of viruses, relevant species of enveloped and non-enveloped viruses covering the range of virus particle sizes should be included [7].

9.6.1.3. *Virus-inactivation process*

The virus-inactivation process is based on the capability of the process to inactivate and/or remove virus. Validation and verification of the virus-inactivation process will provide the evidence that the process will effectively inactivate or remove viruses that are known to contaminate the starting material or that could conceivably do so. The validation is based on a choice of virus similar to the virus that may contaminate a tissue. Such types include enveloped, non-enveloped, DNA and RNA viruses [7].

9.6.2. Sterilisation methodologies applicable to tissue grafts

9.6.2.1. *Irradiation*

Irradiation with gamma particles or accelerated electron beams can be used for the sterilisation and viral inactivation of tissue transplants. For the sterilisation component, such processes are well suited to validation according to SAL. Gamma radiation is effective in killing bacteria, fungi, spores and, to a more variable degree, viruses. However, depending on the dose and irradiation conditions, gamma radiation can have a negative effect on the mechanical properties of the grafts. Applying appropriate, validated and verified irradiation conditions can substantially reduce these negative effects and protect the graft integrity. There are data that suggest that treatment of allografts with less than 25 kGy does not affect the integrity of the allograft [8]. The dose of irradiation required for sterilisation is determined by the number and identity of potential contaminants. TEs should undertake studies to evaluate the bioburden present on allografts prior to irradiation, and based on these calculate an appropriate dose of irradiation to reliably accomplish sterilisation [9, 10].

High doses of irradiation and concomitant transient high temperatures (≥ 60 °C) are likely to have negative effects on transplant properties. It is, however, not possible to make generally applicable statements about the extent to which such effects will influence the clinical performance of transplants. The adverse effects of irradiation can be ameliorated by reducing the temperature and inclusion of radioprotectant chemicals [10, 11]. Irradiating musculoskeletal tissues in the frozen state retains the primary effects of gamma irradiation sterilisation (the breaking of covalent bonds by high-energy gamma rays) while minimising the secondary effects of the process (generation of free radicals). Overall, this can reduce the damage done to allografts by the irradiation process but may also provide some protection to micro-organisms.

9.6.2.2. *Peracetic acid-ethanol treatment*

This method is typical of the 'panel' approach in 9.6.1.2*b* above. In one example of a peracetic acid (1 %) ethanol sterilisation process, the method was tested in allogenic avital bone transplants with a thickness of 15 mm. The process led to a reduction of virus titres of more than 4 log10 TCID50/mL. For viable bacteria, fungi and spores, a titre reduction below the detection level (5 log10 CFU/mL) was achieved after an incubation time of 2 hours [12]. In the case of chemical treatment of tissues the question of potential residues could be a concern and the risk, if applicable, should be included in the 'instructions for use'.

9.7. Requirements for processing facilities

Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be designed, qualified and monitored to ensure that the air quality is appropriate for the process being carried out (see Chapter 8).

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure graft safety. Risks are often multi-factorial and, to help quantify and map them, a dedicated chapter (Chapter 3) has been developed, as well as a tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and how to implement the most efficient mitigation or risk-reducing measures.

9.7.1. Avoiding contamination and cross-contamination

In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled. A separate set of clean, sterile instruments should be used for each donor. Where possible, these should be single-use and disposable. In some cases, e.g. for ocular tissue, single-use surgical instruments for procurement and processing are available and recommended. Each tissue or cell product should have a batch number that is also recorded in the processing records.

Pooled tissues or cells should be treated as a single batch, ensuring that full traceability to all the donations included in the pool is maintained. Due to the high probability of cross-contamination resulting from pooling, it must be supported by a thorough risk–benefit analysis.

9.8. Quality control

9.8.1. General considerations

Tests and procedures should be carried out to measure, assay or monitor processing, preservation and storage methods, equipment and reagents to ensure compliance with established tolerance limits. Written procedures must be in place that govern quality control at key stages during processing. The written procedures should include as a minimum the test method, the sample size and the acceptance criteria. The minimum requirements for evaluation of each type of tissue and cell are described in tissue- and cell-specific chapters (see Part B). The results of all tests or procedures should become part of the permanent processing record.

If in-process quality controls are undertaken in the processing area, they should be carried out so that there is no risk to the processing steps being followed.

9.8.2. Microbiological testing

In many cases, it is not possible to exclude contaminated material during processing because the tissue originates from parts of the body which contain natural microbial flora, and pre-processing disinfection is not 100 % effective. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, with the use of protocols to control and monitor contamination during the entire procurement process. Chapter 11 describes methods of microbiological control. Sampling and testing methods must be validated to demonstrate that the sampling method accurately represents the tissue and that the testing methods are suitable and fit for purpose.

Various procedures exist for securing microbiological control, such as decontamination by antibiotics, or physicochemical methods. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated. The effectiveness of a decontamination or inactivation procedure must be shown for relevant micro-organisms in the tissue or cell preparation itself and not only in an aqueous solution. The risk that some micro-organisms may survive decontamination with antibiotics, but not be detected by post-decontamination microbiological testing, must be considered. This factor has been implicated in the death of, and serious injury to, patients [13, 14].

TEs should also consider the potential risk that use of antibiotics to decontaminate tissue and cell allografts may cause endotoxins to be released, due to the destruction of the cell walls of Gram-negative bacteria. This phenomenon is recognised clinically [15] when antibiotics are used to treat sepsis, but has not been reported following decontamination of tissue allografts (see Chapter 11 for further details).

Non-conforming products must be identified and separated from conforming products. The fate of non-conforming products will be decided by the Responsible Person (RP) in charge of the TE.

9.9. Significant changes in processing protocols

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor selection or testing criterion, or any significantly modified processing step, that enhances safety or quality. Guidance on risk assessment is provided in Chapter 3.

A documented change-control procedure must be followed before any significant change is implemented in processing. This must be supported by a comprehensive risk assessment. The purpose of this is to ensure that the change is justified, is documented and will not affect the quality of the product (see Chapter 2). All relevant persons should be involved in evaluation of the change.

As a minimum, the following aspects of any change in processing should be evaluated:

a. significance;
b. effect on quality;
c. need to update SOPs;
d. need to re-validate the process;
e. effects on quality-control (QC) analyses;

f. need to inform, and be authorised by, regulatory authorities;
g. need to train personnel;
h. effect on risk analyses.

9.10. Process validation and verification

If processing is carried out according to GMP, the processing validation and subsequent verification activities must also be done according to GMP guidelines. In the EU, Commission Directive 2006/86/EC allows for validation studies to be based on any of the following:

a. studies undertaken by the establishment itself;
b. data from published studies;
c. for well-established processing procedures, retrospective evaluation of the clinical results for tissues and cells supplied by the establishment.

Where validation is based on studies carried out by the establishment itself, reports should include at least the following elements:

a. a validation plan that specifies the critical parameters to be assessed and the acceptable result thresholds for these parameters;
b. a documented methodology;
c. all results obtained, described clearly and with relevant interpretation;
d. a declaration of validation acceptance or rejection signed by the quality manager (QM) or a person authorised by the RP.

The principle of the "worst case" scenario should always be applied in the design of validation studies; this means that the most extreme conditions of the processing protocol should be evaluated with regard to their effects on the critical quality attributes of the tissue or cell product being prepared. For example, if an irradiation sterilisation process is used for a tissue graft, validation of the antimicrobial efficacy of the process should be done at the lower end of the acceptable dose range, while validation of the effects of the process on the structural properties of the tissue should be done at the upper end of the acceptable dose range. The equipment used for validation studies should be fully qualified, and measuring devices should be calibrated to traceable standards. For examples of good practice in the design of process validation studies, see Appendix 6. Example of process validation – tissue transportation and Appendix 7. Example of method validation – oocyte vitrification.

Where validation is based on data from published studies, the relevant publications should be filed as part of the validation record. In this case, the TE should demonstrate that they can effectively reproduce the published process with the same results in their facility (operational validation). Copies of the relevant SOP and the results of the operational validation should be recorded, to demonstrate that the process is equivalent to that applied in the scientific literature.

Where specific steps have been significantly modified or adapted, separate validation should confirm that these changes have not invalidated the method. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

If validation is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment (i.e. for well-established processing procedures), data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred. It should be demonstrated that, where a vigilance system was already in place at the time, clinical users were informed of the procedure for reporting adverse reactions. There should be a signed declaration of validation acceptance or rejection by the QM or RP.

The procedures used to prevent or reduce contamination during processing may vary, depending on the type of tissue and how it is processed. Decontamination methods, such as antibiotic soaking, should be validated to demonstrate effectiveness against a range of contaminants similar to those commonly found on the tissues or cells in question. Such studies should be designed to ensure that residual decontaminants (e.g. antibiotics) do not affect the validity of the microbial tests carried out on the product. If the process includes a sterilisation or viral-inactivation step, process-specific validation studies should be completed to demonstrate the log reduction achieved by the process.

Subsequent to process validation and during routine processing, TEs should monitor tissue and cell quality according to a documented schedule and frequency. This verification will provide assurance of the continued capability of the process to produce finished tissues and cells that meet their required specifications. Relevant process trends (e.g. quality of incoming materials or components, in-process and finished product results, cases of non-conformance and defect reporting) should be collected and assessed to verify the validity of the original process validation and to confirm that quality control methods

are still fit for purpose. Documentation and tracking of patient outcomes constitutes a critical element of ongoing process verification. For new or significantly changed processes where significant potential risk is identified, a system to enable close clinical outcome monitoring should be agreed with clinical users.

9.11. References

1. Yates P, Thomson J, Galea G. Processing of whole femoral head allografts: validation methodology for the reliable removal of nucleated cells, lipid and soluble proteins using a multi-step washing procedure. *Cell Tissue Bank* 2005;6(4):277-85.
2. Lomas R, Drummond O, Kearney J. Processing of whole femoral head allografts: a method for improving clinical efficacy and safety. *Cell Tissue Bank* 2000;1(3): 193-200. https://doi.org/10.1023/A:1026512312385.
3. Klop A, Vester M. The effect of repeated freeze-thaw cycles on human muscle tissue visualised by post-mortem computed tomography. *Clin Anat* 2017 Sep; 30(6):799-804. https://doi.org/10.1002/ca.22917.
4. Pruss A, Seibold M, Benedix F. Validation of the 'Marburg bone bank system' for thermodisinfection of allogenic femoral head transplants using selected bacteria, fungi, and spores. *Biologicals* 2003 Dec;31(4): 287-94.
5. Leeming JP, Lovering AM, Hunt CJ. Residual antibiotics in allograft heart valve tissue samples following antibiotic disinfection. *J Hosp Infect* 2005 Jul; 60(3):231-4.
6. German Institute for Standardization (DIN). Chemical disinfectants and antiseptics, basic bactericidal activity – Test method and requirements (phase 1); EN 1040 (2006-03).
7. European Agency for the Evaluation of Medicinal Products. Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. CPMP/BWP/268/95. London, UK: Committee for Proprietary Medicinal Products, 1996.
8. Pruss A, Kao M, Gohs U. Effect of gamma irradiation on human cortical bone transplants contaminated with enveloped and non-enveloped viruses. *Biologicals* 2002 Jun;30(2):125-33.
9. Winters M and Lomas R. The principles of process validation and equipment qualification. In: *Tissue and cell processing: an essential guide*, ed. Fehily D, Brubaker SA, Kearney JN *et al.*, Wiley-Blackwell, August 2012, pp. 124-47.
10. Radiation sterilization of tissue allografts: requirements for validation and routine control. A code of practice. Vienna: International Atomic Energy Agency, 2008, available at www-pub.iaea.org/MTCD/Publications/PDF/Pub1307_web.pdf, accessed 7 April 2022.
11. Hoburg AT, Keshlaf S, Schmidt T. Effect of electron beam irradiation on biomechanical properties of patellar tendon allografts in anterior cruciate ligament reconstruction. *Am J Sports Med* 2010 Jun;38(6):1134-40.
12. Pruss A, Göbel UB, Pauli G. Peracetic acid-ethanol treatment of allogeneic avital bone tissue transplants – a reliable sterilization method. *Ann Transplant* 2003; 8(2):34-42.
13. Kainer MA, Linden JV, Whaley DN. Clostridium infections associated with musculoskeletal-tissue allografts. *N Engl J Med* 2004 Jun 17;350(25):2564-71.
14. Kuehnert MJ, Clark E, Lockhart SR. Candida albicans endocarditis associated with a contaminated aortic valve allograft: implications for regulation of allograft processing. *Clin Infect Dis* 1998 Oct;27(4):688-91.
15. Lepper P, Held T, Schneider E *et al.* Clinical implications of antibiotic-induced endotoxin release in septic shock. *Intensive Care Med* 2002;28:824-33. https://doi.org/10.1007/s00134-002-1330-6, accessed 7 April 2022.

Related material

Appendix 4 – Example of cleanroom qualification
Appendix 5 – Example of incubator qualification
Appendix 6 – Example of process validation – tissue transportation
Appendix 7 – Example of method validation – oocyte vitrification

Chapter 10. **Storage**

10.1. Introduction

Storage is the maintenance of tissues and cells for clinical application under appropriate controlled conditions and it occurs at various stages from procurement to clinical use. For the remit of this chapter, storage covers the stages from procurement to distribution. Storage must be controlled and documented to ensure that the critical properties of the tissues or cells are maintained during storage and that cross-contamination or loss of functionality, efficacy, clinical effectiveness and traceability is avoided.

The opportunity to store tissues and cells is also common during processing and brings great advantages:

a. preservation of the required properties of the biological material, making extended storage for future use possible;
b. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells;
c. reducing the risk of disease transmission by testing of infectious diseases and microbial contamination prior to release and clinical use, though this is only possible if the tissues and cells can be stored under defined conditions for an adequate time in order to await the test results;
d. performing and awaiting other quality-control results before release, e.g. cell counts, clonogenic assays for haematopoietic progenitor cells (HPC), residual moisture in lyophilised or dehydrated grafts.

This chapter provides generic guidance on the storage of tissues and cells carried out by tissue establishments (TEs). Further tissue- and cells-specific guidance is provided in Part B of this Guide.

10.2. Storage

10.2.1. General

Storage facilities for materials, tissues and cells are present in most TEs, procurement organisations, donation facilities and organisations responsible for human application (ORHA). These storage facilities must have policies and standard operating procedures (SOPs) for all processes that affect quality and safety.

Storage facilities must ensure that the equipment being used, the working environment, the process design and the qualification, validation and control conditions are in compliance with established quality and safety requirements (see Chapter 2). Storage (before, during and after any processing activities, as well as after release) must be carried out as defined in the specifications for the particular tissues or cells to guarantee their quality and safety. Organisations must have a dedicated area or equipment for quarantine (temporary storage) and a plan for back-up storage in the event that the primary storage area is unavailable. An inventorial audit of tissues and cells stored should be performed regularly.

If an organisation entrusts one of the stages of storage to a third party, a written agreement is needed between the TE and the third party. The TE

must evaluate and select third parties based on their ability to meet the established standards of quality.

The recommended time limits between procurement, processing and storage are described in the tissue- and cell-specific sections of this Guide (see Part B). These maximum times from procurement (or cardiac arrest) until processing and storage must be defined to maintain quality, safety and clinical effectiveness of the tissues and cells. Procurement, processing and storage times must be documented in the records for tissues and cells.

Reference samples of tissues and cells for quality control should be stored under the same conditions as the tissues and cells themselves (e.g. HPC and cord blood reference samples).

In the case of termination of all activities, the tissue establishment must have third-party agreements for transferring stored usable tissues and cells or for disposing of the unusable tissues and cells to another active tissue establishment, including quality, safety and traceability and data.

10.2.2. Methods of storage

Following processing, tissues and cells must be stored according to currently accepted good practice, based on the best available scientific evidence as appropriate, for tissues and cells. All procedures associated with storage of tissues and cells must be documented in SOPs.

10.2.3. Storage temperature

Refrigeration devices/incubators containing tissues and cells must be suitable for the intended use, and the procedures for monitoring such devices must be appropriate so that tissues and cells are maintained at the required storage temperature. Continuous monitoring and recording of temperature, together with suitable alarm systems, must be employed on all incubators, storage refrigerators, freezers and liquid nitrogen tanks (see Chapter 2). The functionality of the alarm systems must be checked regularly. Temperature ranges for storage of tissues and cells are shown in Table 10.1

10.2.4. Requirements for storage facilities

Facilities for storage must be dedicated to this activity, and must be designed, qualified and monitored to ensure appropriate conditions (see §8.2). Out-of-order equipment must be labelled accordingly.

A written sanitation programme should be available, describing the frequency and methods of cleaning. Additionally, a pest control system should be in place [1].

If tissues and cells are not stored at the manufacturing tissue establishment, storage may also be carried out at a storage facility.

Although storage facilities do not need to strictly follow the same environmental criteria as the procurement and processing facilities, once the tissues and cells have been processed and stored/banked, storage facilities should maintain a secure, clean and stable environment for long-term storage. The storage facility should be large enough for its intended purpose.

The room should be in a dry, cool, well-ventilated place, free from unnecessary heat sources. Where mechanical refrigerators and freezers are utilised, suitable air conditioning systems may be required to dissipate excess heat.

The necessary air-conditioning capacity for the storage area should be calculated on the basis of the actual heat load of the equipment and the environmental factors.

For security reasons, access to the storage facilities must be restricted to authorised personnel. In facilities where compressed gases are used, appropriate alarms for temperature control and low levels of oxygen must be in place, in case of liquid nitrogen leaks. In case of loss of electrical power, as a part of the

Table 10.1. **Temperature range for storage of tissues and cells**

Storage method	*°C minimum	*°C maximum
Cryopreservation	−196	−140
Deep frozen†	−80	−60
Frozen‡		<−15
Refrigerated‡	+2	+8
Cold or cooled‡	+8	+15
Room temperature‡	+15	+25
Organ culture	+28	+37

* Tissue-specific temperatures are indicated in the relevant tissue or cell chapters as well as the associated monographs.

† Based on general practice.

‡ Based on the *European Pharmacopoeia* [2].

general emergency plan, the storage facility should have an uninterrupted power supply (UPS) system linked to a backup generator for those devices where temperature cannot be maintained accordingly.

10.2.5. Selecting appropriate air quality for storage

In order to minimise the risk of environmental microbial contamination, certain areas such as corridors or open access from outdoors should be separated from the storage area by doors. Storage rooms can be equipped with an HVAC (heating, ventilation and air conditioning) system for maintaining the temperature and humidity by adequate air exchange.

10.2.6. Environmental monitoring

Similarly, as with the processing rooms, if storage is carried out based on the principles of GMP and defined in the in-house environmental hygiene programme, monitoring systems for storage rooms may need to be put in place. The frequency of monitoring, as well as the implementation of alarm limits, will depend on the degree of stringency needed and the principles of quality risk management. Microbiological contact-plate monitoring may be performed to demonstrate the degree of cleaning of the room (see §8.5.2 and §8.5.3).

10.2.7. Special safety measures for liquid nitrogen facilities

The organisation of the storage room should allow circulation and manipulation around the cryogenic tanks. The room must be clearly identified with pictograms indicating the dangers and also the location of personal protection equipment. The staff need to be specially trained and qualified for these working conditions.

Before entering the room, the staff must be able to ensure that ventilation is functioning correctly and be able to check the oxygen level. An adapted mechanical ventilation (extraction and fresh-air intake) system, working continuously, is mandatory, ensuring that under normal operating conditions the minimum oxygen concentration is kept above a minimal level. Continuous monitoring of the oxygen level must be ensured by detectors, checked and maintained to the supplier's recommendations. They must be placed in lower areas of the room in order to monitor any accumulation of nitrogen at floor level. The oxygen rate or alarm status must also be visible and audible outside the storage room.

Following nitrogen evaporation, leading to an oxygen level equal to or lower than 19 %, an alarm (visual and audible) must be activated and staff should then leave the room and await normalisation of the oxygen level. The alarm should be connected to a continuous monitoring station allowing alerts for the staff working in the room and the rescue or assistance of staff, if necessary. Systems must be in place to ensure that, when the oxygen level in the facility is lower than 19 %, the facility can only be entered by suitably trained and qualified personnel equipped with an independent air supply [3].

Tank-filling systems should be designed to minimise evaporation of nitrogen. They should be equipped with safety valves and rupture discs, and external degassing valves for long lines.

It is important to control condensation on the tanks and the formation of ice inside them. The floor should be covered with a material resistant to low temperatures and high loads, allowing easy movement of the tanks without shock. The tanks must be maintained and checked according to the manufacturer's guidance.

The entrance door of the room should be equipped with an oculus allowing surveillance from outside. Where the entirety of the room cannot be seen from the oculus, alternative surveillance systems, such as closed circuit television, should be used. The opening of the door should be outwards. Gloves with long sleeves able to protect against cold, cryoprotective aprons and shoes, as well as safety glasses (EN166) or visors protecting the face, must be made available to the staff. (For further details, see §8.2.)

10.2.8. Avoiding contamination and cross-contamination

Storage conditions must be designed to minimise the risk of mix-ups, contamination and cross-contamination of tissues, cells, supplies and reagents (see also §8.6) [4, 5, 6]. Areas designated for separate storage of cells and tissues in process, under quarantine, and released for distribution must be established and controlled. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during storage, with tissues or cells from another donor, unless they are pooled intentionally. Every effort should be made to avoid cross-contamination of material. It is advised that, where possible, tissues and cells should be stored in the vapour phase of liquid nitrogen, rather than by immersion.

If no alternative is available, tissues and cells immersed in liquid nitrogen should be double-

wrapped during storage or stored in a high-security primary container especially designed for liquid nitrogen (depending on the storage system, type of sample and after risk assessment).

This is highly important for storage with liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels, as well as to avoid cross-contamination among samples. The seals and the material employed must be qualified for their use at the designated storage temperature and for the conditions of use, to demonstrate that the packaging and labelling can retain their integrity under such conditions.

In principle, to avoid contamination of tissues and cells, the entry of personnel to the storage facilities should be reduced to a minimum.

Additionally, for periodical cleaning, a cleaning plan for the sanitation of the storage equipment should be implemented, depending on the type of equipment. During the cleaning process, a back-up unit should be used to provide the same safe conditions as the storage equipment. The emptying for cleaning and maintenance should be scheduled in advance and needs to be performed following an SOP.

10.2.9. Types of storage

10.2.9.1. *Quarantine storage*

All human tissues and cells that are stored before having determined their suitability must be kept under quarantine. The TE must operate a system that identifies tissues and cells throughout any phase of processing and clearly distinguishes released products from quarantined and discarded products. Quarantined tissues and cells must be physically separated and visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from released tissues. An SOP must describe how to categorise quarantined and released tissues and cells.

To maintain physical separation, specific areas or storage devices (or secured segregation within the device) must be defined for tissues and cells in quarantine, for tissues and cells that must comply with special criteria and for tissues and cells that have been released; a separate area must be dedicated to the storage of medium and other materials used during the process of preparation. Access to storage facilities must be restricted to authorised persons. If the storage devices are located in a shared facility with other users, they must be securely locked.

10.2.9.2. *Short-term storage*

Because of processing methods and the life span of cells, some viable tissues and cells can only be stored for a short period of time (e.g. corneas stored in organ culture for about 4-5 weeks).

10.2.9.3. *Long-term storage*

If tissues or cells need to be stored for a longer period, other strategies such as cryopreservation, deep-freezing or lyophilisation should be considered. The long-term storage method selected must be suitable for maintaining the critical properties of the tissue or cell in question.

Obtaining and analysing the critical clinical outcome data can provide evidence to be used to verify the safety and efficacy of the storage procedures.

10.2.9.4. *Storage at an organisation responsible for human application*

Organisations responsible for human application (ORHAs) have short- and long-term storage for tissues awaiting inspection, surplus or unused tissues and tissues recalled by TEs. Therefore, a system for identification of tissues and cells throughout any phase of the clinical application at the ORHA must clearly distinguish tissues and cells released from quarantine and/or discarded ones (see Chapter 13).

10.2.10. Expiry date

To ensure the maximum safety and quality of tissues and cells, it is mandatory to specify a maximum storage time with an expiry date for each type of tissue or cell and its storage conditions. The chosen maximum storage period for each individual type of tissue or cell should be validated, based on data from published studies, stability testing by the establishment or evidence-based facts (e.g. retrospective evaluation of the clinical results for tissues and cells supplied by the establishment), or it can be specified by national legislation. When determining the maximum storage period, several factors should be considered. These include (but are not limited to):

a. possible deterioration of the required properties of tissues and cells;
b. risks related to microbiology;
c. package integrity over time;
d. expiry of storage solutions;
e. stability at the storage temperature;
f. overall risk assessment of quality assurance: donor evaluation, donor testing (kits), quality criteria (viability, functionality after thawing), regulations.

When relevant for the type of tissue or cell, the time of procurement should also be indicated. In certain specific cases, it may be possible to prolong the expiry date, especially in the case of cryopreserved HPC (i.e. cord blood), reproductive tissues and cells for partner donation or in cases of fertility preservation (see Chapter 29 and Chapter 30), e.g. MAR/ART do not have expiry dates, and HPC are retested before release, even after decades of storage.

10.3. Disposal of human tissues and cells

There must be a documented policy for disposal of tissues and cells that are unsuitable for clinical use (e.g. not meeting quality criteria, withdrawal of permission from donor, death of autologous donor). Records must include details of date and involved personnel, with method of and reasons for disposal. The material should be handled appropriately and disposed of in a manner compliant with local guidelines on control of infection. Human tissues and cells and other hazardous waste items should be disposed of in such a manner as to minimise the hazards to the TE's personnel or the environment, and should be in conformity with applicable European, national and local regulations.

Disposal of human tissues and cells should be carried out in a manner that shows respect for fundamental rights and the human body. For HPC and autologous tissues and cells it is necessary to document that the conditions for disposal defined in the consent prior to collection have been met, including (where applicable) the option to transfer the tissues and cells to another facility if the designated recipient is still alive after the agreed storage period. Disposal of cellular therapy products must include a pre-collection written agreement between the storage facility and the designated recipient or the donor, defining the length of storage and the circumstances for disposal of cellular therapy products [7].

In case of MAR/ART, the donors and patients must declare in writing the destiny of their reproductive material when the maximum storage period has ended (see Chapter 29). A specific signed consent is needed for the acceptance of the final disposal of a given sample.

10.4. References

1. World Health Organization (WHO). Annex 9: Guide to good storage practices for pharmaceuticals; Technical Report Series, No. 908, 2003.
2. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*, General notices, general chapter 1. In: *Ph. Eur.* 10th edition. Strasbourg, France: Council of Europe; 2022.
3. EIGA-European Industrial Gases Association. Doc 44/18: Hazards of Oxygen-deficient atmospheres, available at www.eiga.eu/publications/?_sf_s=doc044, accessed 8 April 2022.
4. Tedder RS, Zuckerman MA, Goldstone AH *et al.* Hepatitis B transmission from contaminated cryopreservation tank. *Lancet* 1995 Jul 15;346(8968):137-40. https://doi.org/10.1016/s0140-6736(95) 91207-x.
5. Hawkins AE, Zuckerman MA, Briggs M *et al.* Hepatitis B nucleotide sequence analysis: linking an outbreak of acute hepatitis B to contamination of a cryopreservation tank. *J Virol Methods* 1996 Jun;60(1):81-8. https://doi.org/10.1016/0166-0934(96) 02048-4.
6. Cobo A, Bellver J, de los Santos MJ *et al.* Viral screening of spent culture media and liquid nitrogen samples of oocytes and embryos from hepatitis B, hepatitis C, and human immunodeficiency virus chronically infected women undergoing *in vitro* fertilization cycles. *Fertil steril* 2012;97(1):74-8. https://doi.org/10.1016/j.fertnstert.2011.10.006.
7. FACT–JACIE. *International standards for hematopoietic cellular therapy product collection, processing, and administration,* 7th edition. Barcelona: European Society for Blood and Marrow Transplantation, 2018.

Chapter 11. Principles of microbiological testing

11.1. Introduction

This chapter addresses a comprehensive strategy for the assessment of the microbiological quality and safety of human tissues and cells for human application. This outline of the test methods of the *European Pharmacopoeia (Ph. Eur.)* [1] takes into account the specific characteristics and limitations of human tissues and cells. The selection of these analytical methods should be based on a risk assessment that includes the initial microbial load (bioburden) of tissues and cells after procurement, the nature of the test sample, the availability of the test sample due to scarcity of tissues and cells, the ability to decontaminate or sterilise the final tissue or cell graft, the mode and route of application of tissue and cell to the recipient and other relevant factors. Alternatives to the standard *Ph. Eur.* methods may be used in accordance with the recommendations of *Ph. Eur.* Chapter 5.1.6: 'Alternative methods for control of microbiological quality'. Guidance on microbiological testing for particular tissue and cell processes is provided in tissue- and cell-specific chapters in Part B of this Guide.

All facilities that procure, process or store tissues and cells should have access to the services of a microbiology laboratory with a fully implemented quality management system and should have access to the advice of a suitably qualified expert microbiologist.

11.2. Testing

The approaches outlined in this section cover the microbiological testing of procurement, processing, storage and release of tissues and cells.

11.2.1. Sampling

Sampling for microbiological testing must be performed before processing starts, at any point from the end of the procurement process and the start of any processing protocols, and at the end of the processing protocols, immediately before final packaging of the tissue or cell graft. For tissues that do not undergo processing, there should be at least one sampling point prior to placing the graft in its final package. For some tissues and cells, pre-processing microbiology testing may not help to decrease the risk of microbiological transmission or to increase the safety of implantation [2, 3]. If an appropriately validated terminal sterilisation process is applied, parametric release allows tissue establishments to replace post-processing microbiological testing as a release criterion with acceptance criteria for the control of identified process parameters.

Because microbiological contaminants may be present inside the tissues and cells, as well as on the surface of tissues and cells, representative tissue or cell samples should be processed for microbiological testing [4]. If testing of a representative tissue or cell sample is not feasible owing to its nature, scarcity or size, sampling of the surface of tissues (swabs) or contact solutions (media used for storage, transport

or rinsing) may be appropriate for surrogate testing. If feasible, a representative sample of the adjacent tissues or cells could also be processed and used for testing. Even though tissues and cell samples available for testing might be limited, samples should be representative of the whole tissue or cell preparation and suitable to ensure that the chosen analytical method can be performed with acceptable sensitivity and specificity to prevent false negative or false positive test results (see *Ph. Eur.* 2.6.39).

11.2.2. Microbiological testing for bacteria and fungi

Testing for aerobic bacteria, anaerobic bacteria and fungi in tissue or cell samples under appropriate incubation conditions is the most accurate qualitative measure of microbiological contamination. Considering the nature of the procured tissues and cells and any subsequent processing step, the microbiological testing approach should follow the procedures outlined in the *Ph. Eur.*, specifically sections 2.6.1, 2.6.13 or 2.6.27. Deviations from such standards should be justified, and alternative test methods should be validated in accordance with *Ph. Eur.* 5.1.6. Factors such as samples containing antibiotics, very small sample amounts and/or specific micro-organisms known to represent a potential contamination risk may affect the sensitivity of tests leading, in the worst-case scenario, to false-negative results. In addition to the prescribed test strains, method suitability should also be demonstrated for tissue- and cell-associated contaminants, when not adequately covered by the test strains prescribed in the method [5].

Contamination with specific pathogenic micro-organisms should result in the discard of the tissues or cells unless treated with a sterilisation process validated to eliminate the infectivity of such organisms [5-9]. If applicable, a non-exhaustive list of pathogens whose presence necessarily excludes the procured tissue or cells from further processing should be established by each tissue establishment based on Table 11.1 and their own experience.

If release of the tissues or cells is necessary before the end of the required testing period, negative-to-date reading of the results may be carried out. In this case, intermediate results of the final testing in combination with intermediate or final results of in-process testing are used for tissue or cell release. The clinician using the relevant graft must be notified to decide if its application is clinically justified. If micro-organisms are detected after tissue or cell release, predefined measures such as identification and antibiotic sensitivity of the species should be carried out and information must be provided immediately to clinicians caring for the patient. Alternative rapid microbiological testing methods should be considered, especially for preparations of tissues and cells with a short shelf life. Independent of the applied method, the fitness for purpose of rapid microbiological testing methods should be shown according to *Ph. Eur.* 2.6.27 or 5.1.6. Deviations from these standards should be justified.

Table 11.1. **Micro-organisms that, if identified in pre-processing tissue or cell samples, should result in discard of tissues or cells unless treated with a sterilisation process validated to eliminate the infectivity of such organisms***

Staphylococcus aureus, Staphylococcus lugdunensis
Streptococcus spp., *Enterococcus* spp.
Clostridium spp.
Enterobacteriaceae (e.g. *Escherichia coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp., *Klebsiella* spp.)
Yeast and filamentous fungi (moulds)

* Except in specific cases (e.g. HPC transplantation) where the decision is life-saving and where antibiotics can be applied to the recipient. In those cases, a case-specific risk assessment should be performed.

11.2.2.1. *Processing using closed systems*

For cells and tissues in which a closed system is used, pre- and post-process testing should be carried out. In this context, in-process testing does not yield more information on the microbiological status of the cell or tissue graft.

11.2.2.2. *Processing with terminal sterilisation*

For cells and tissues that undergo a validated terminal sterilisation process, the requirements of *Ph. Eur.* 5.1.1 should be considered where methods of sterilisation are described. In particular, it should be shown that adequate precautions have been implemented to minimise the microbial contamination before sterilisation and that tissues and cells with an acceptably low degree of microbial contamination have been used as determined by regular bioburden testing (see §11.2.5). Procedures and precautions employed for sterilisation are to be such as to give a sterility assurance level (SAL) of $\geq 10^{-6}$ [10].

If the release of tissues and cells terminally sterilised in their final container is intended to rely on process data only (parametric release), and not on microbiological testing of the final tissue and cell graft, then validated procedures for all critical production steps and a fully validated sterilisation method should be applied. This approach includes validation of procurement of tissues or cells, transportation, washing, antibiotic treatment and other

processing steps, packaging and storage. In some countries authorisation by the Health Authority is needed for such an approach.

11.2.2.3. *Open processing without terminal sterilisation or decontamination*

Most tissues and cells are exposed to the direct environment at certain processing stages between procurement and packaging. Without terminal sterilisation, the environmental contamination risk during open processing should be avoided to the greatest possible extent. The requirements for microbiological sampling and testing are expected to be most stringent in these situations, and aseptic conditions should be maintained throughout processing.

In accordance with a tissue- or cell-specific risk assessment, microbiological testing must be performed at least pre- and post-processing, unless justified by tissue- or cell-specific risk assessment or scientific evidence. Absence of microbial contamination should be confirmed by microbiological testing using either of the *Ph. Eur.* methods 2.6.1 or 2.6.27, depending on the nature of the preparation, or alternative methods validated according to *Ph. Eur.* 5.1.6.

In addition, enumeration of contaminants or detection of specified micro-organisms in in-process samples can be achieved using methods *Ph. Eur.* 2.6.12 and 2.6.13 (as described in §11.2.5).

11.2.2.4. *Processing that includes decontamination steps*

Procedures applied for decontamination of tissues and cells are usually limited to approaches in which the vitality and functionality of the preparation can be maintained. For instance, treatment with antibiotics and anti-fungal agents is employed widely to achieve reduction of the microbial load in tissues and cells. *Staphylococci*, *Streptococci*, *Pseudomonas*, *Bacillus*, *Candida*, *Acinetobacter*, *Escherichia*, *Cutibacterium*, *Enterococci* and *Corynebacterium* are generally the most frequently isolated organisms [11] and several of these strains are often susceptible to antibiotic decontamination procedures [12]. Efficacy studies focusing on the usually expected microbial flora of tissues or cells, as well as the type and concentration of effective antimicrobial agents, should be carried out. Based on those studies, treatment schemes and an exclusion list of specific contaminants for the incoming material should be determined, based upon not only the category of tissue but also upon the method by which the tissue was processed.

Because of virulence mechanisms such as facultative intracellular parasitism, and persistence in a resting metabolic state without growth (which is reversible upon withdrawal of antimicrobial agents or stress conditions), micro-organisms can escape these treatments. Therefore, the efficacy of the decontamination procedure should be validated for every tissue or cell type. Any changes in the decontamination procedure require risk assessment and validation of similar (non-inferior) efficacy of the new procedure.

Further processing after the decontamination step should be conducted without antimicrobial agents, if possible. Microbiological test methods should be carefully evaluated with respect to possible inhibition of microbial growth due to such agents and, if needed, these agents should be removed from the sample matrix before microbiological testing or inactivated by suitable techniques (e.g. Resins, Penase) [13]. Microbiological testing must be performed as a minimum pre- and post-processing unless justified by tissue- or cell-specific risk assessment or scientific evidence.

11.2.3. Testing for mycoplasma and mycobacteria

Testing for specific infectious agents such as mycoplasma and mycobacteria should be carried out if they are known to constitute a relevant risk. To identify and assess the contamination risk of specific tissues or cells, a sufficient number of samples from different tissue or cell batches should be examined. Possible sources for mycoplasma and mycobacteria contamination include the cellular starting material itself or its procurement, animal- or human-derived raw materials such as untreated sera, personnel in the clinic or manufacturing site, and the entire processing method. Testing should be conducted at processing steps at which mycoplasma (*Ph. Eur.* 2.6.7) or mycobacteria (*Ph. Eur.* 2.6.2) contaminations would most likely be detected, such as after collection, but before washing steps.

Mycoplasma are cell-associated micro-organisms that may even locate within the cell, so testing should always include the tissues or cells. Mycoplasma can penetrate sterilising-grade filter membranes with a nominal pore size of ≤ 0.2 µm. They also lack a rigid cell wall, which makes them unsusceptible to antimicrobial agents that target the cell wall. Furthermore, many broad-spectrum antibiotics inhibit the proliferation of mycoplasma but do not kill them. Hence, elimination with antibiotics is difficult.

Contamination with mycoplasma represents a potential risk for the patient because of transfer of infectious microbial agents into a potentially immuno-

compromised patient, but the effect of mycoplasma contamination on the tissue or cell preparation might also be critical. Mycoplasma are known to alter cell function, leading to an alteration of gene expression, cellular signalling and metabolic activity.

11.2.4. Testing for pyrogens

Pyrogens are microbial components that lead to fever if applied parenterally. In some specific situations, pyrogen contamination may be responsible for unexplained graft failure, e.g. pancreatic islet transplantation [14]. The major pyrogen of concern is lipopolysaccharide (LPS) derived from the outer membrane of Gram-negative bacteria, the so-called bacterial endotoxins, but also other non-endotoxin pyrogen contaminations are relevant, e.g. bacterial flagellin. Pyrogen contaminations can originate from the donor material itself or from raw materials, solutions and instruments coming in contact with the tissues and cells during processing. Risk assessment of tissue and cell processing by tissue establishments should address potential risks of product contaminations with bacterial endotoxin and non-endotoxin pyrogens in respect to the intended use of the transplant (e.g. route of administration, transplant size). The raw materials should be of adequate microbiological quality with specified bacterial endotoxin limits.

In cases with a high bioburden status of starting material, testing for bacterial endotoxins might be warranted after elimination of viable microbes by decontamination or sterilisation steps. If risk assessment reveals the necessity of bacterial endotoxin control, methods *Ph. Eur.* 2.6.14 (Bacterial endotoxins) or 2.6.32 (Test for bacterial endotoxins using recombinant factor C) should be used. Depending on the risk assessment, testing of non-endotoxin pyrogens in addition to endotoxin or mixtures of both can be performed with the monocyte activation test (*Ph. Eur.* 2.6.30). Guidance for selection of a suitable test strategy is provided by *Ph. Eur.* 5.1.10. In this chapter the specified endotoxin limit is 5 IU per kg of patient weight per bolus or hour for intravenous drugs (*Ph. Eur.* Table 5.1.10–1), which is usually applied for all parenteral preparations.

11.2.5. Bioburden testing

Bioburden testing is the quantitative measure of the microbiological status (bioburden) of tissue and cells. The bioburden status of starting material (pre-processing) should be measured as part of the validation and monitoring of the efficacy of decontamination procedures and/or terminal sterilisation [15]. Regular bioburden testing should be carried out, based on a risk assessment if post-processing microbiological testing is replaced by parametric release or if post-processing microbiological test results are not available at the time of release. Although bacterial and fungal infection post-transplantation is rarely reported, some virulent microbes when present in high numbers on a tissue surface may form cytotoxic exoproducts such as proteases and toxins, which can have a deleterious effect on the cellular integrity or structural properties of the graft.

Bioburden is usually expressed as the total number and/or identity of micro-organisms associated with a specific sample determined by testing. Bioburden test results are also used by tissue establishments to define acceptance/rejection criteria for tissues and cells for further processing.

For the assessment of the bioburden status of the starting material, *Ph. Eur.* test methods 2.6.12 and 2.6.13 can be carried out. Tissues or cells can be tested by a representative tissue or cell sample suspended in a broth medium and, less commonly, by a culture swab or suspension of an initial wash of the specimen. Although swab-based methods have a low efficiency of recovery, generally less than 20 % [16, 17], they allow a crude estimation of viable microbial contaminants as heavy, moderate or light, and the identification of individual species helps to inform decisions as to the potential hazard of the contaminant. Rigorous sonication/mechanical shaking methods, exposing representative tissue samples to an extraction fluid with a surfactant, can increase the recovery efficiency [16]. (See also §11.3.3.)

11.3. Conditions and methods of microbiological testing

For each procedure, aerobic and anaerobic testing should be conducted under incubation conditions that are appropriate for the detection of tissue- or cell-specific bacteria and fungi (yeasts and moulds) as well as bacteria and fungi of environmental or clinical origin.

11.3.1. Sterility testing (*Ph. Eur.* 2.6.1)

Conditions for sterility testing are detailed in Table 11.2. Precautions should be taken against microbial contamination during a test (*Ph. Eur.* 2.6.1 and 5.1.9). At the least, sub-cultivation should be carried out in a GMP Grade A laminar airflow cabinet, properly disinfected before the test, and no other activity should be conducted at the same time. The preferred

Table 11.2. **Incubation conditions for sterility testing**

	Culture medium	Incubation temperature °C	Testing period
Aerobic	Soya-bean casein digest medium	20-25	14 days
Anaerobic	Fluid thioglycolate medium	30-35	14 days
Fungi	Soya-bean casein digest medium	20-25	14 days

Note: Fluid thioglycolate medium will also detect aerobic bacteria.

microbiological control procedure is dependent on the sample material.

11.3.1.1. *Membrane-filtration method*

This method uses membrane filters having a nominal pore size ≤ 0.45 µm whose effectiveness in retaining micro-organisms has been established.

11.3.1.2. *Direct inoculation method*

The direct inoculation method is suitable for solutions and tissue samples (i.e. solid substances). Sample quantities are listed in *Ph. Eur.* 2.6.1.

For both methods, microbial growth media are assessed macroscopically for evidence of microbial growth. During incubation, at least one intermediate reading and one final reading should be made. If the test carried out on the sample material results in turbidity of the culture medium, sub-cultivation should be carried out (see *Ph. Eur.* 2.6.1).

Fungi are detected along with aerobic bacteria in soya-bean casein digest medium (see *Ph. Eur.* 2.6.1). However, other media and/or incubation temperatures may be used, provided that the method is validated according to *Ph. Eur.* 5.1.6.

11.3.2. Microbiological testing using automated culture systems

This method is based on the method described in *Ph. Eur.* 2.6.27 supplemented with additional instructions for samples containing cellular material.

11.3.2.1. *Incubation conditions*

Samples containing cellular material may result in turbidity of the culture media immediately after inoculation. Use of validated automated culture systems, which do not rely on turbidity for pathogen detection, may be advantageous in this case.

Incubation in automated culture systems should be carried out over at least 7 days. The testing time can be adapted to specific requirements arising from the characteristics of the preparation. For example, if risk assessment identifies potentially slow-growing micro-organisms such as *Cutibacterium acnes* the testing time could be extended up to 14 days.

The temperature of incubation usually established for clinical microbiology (at 37 °C) might be too limited to sufficiently account for a broad range of contaminating micro-organisms found in the environment or in tissues and cells. Hence, the incubation conditions detailed in Table 11.3 are recommended as alternatives based on risk assessment, taking into account the expected microbial flora and environmental conditions. Testing times must be validated if incubation temperature deviates from the temperature recommended by manufacturer.

Preparations of tissues and cells with a short shelf life may be released based on an intermediate readout of the test before the test period is completed (negative-to-date result). In the case of a positive readout during the test period after release of the tissue or cell graft, identification of the microbial species and an antibiotic susceptibility profile must

Table 11.3. **Conditions for microbiological control testing according to *Ph. Eur.* 2.6.27***

	Aerobic incubation	Anaerobic incubation
Option 1	20-25 °C normally (automated system) 30-35 °C if necessary (automated system)	30-35 °C (automated system)
Option 2	35-37 °C (automated system) and, where relevant, additional incubation at a lower temperature (manual method)†	35-37 °C (automated system)
Option 3	30-32 °C (automated system)	30-32 °C (automated system)
Option 4	30-32 °C (automated system)	35-37 °C (automated system)

* According to *Ph. Eur.* 2.6.27, testing period is 7 days with an automated growth-based method and may be extended up to 14 days. Testing period is 14 days with a manual method.

† Where relevant, incubate in addition at 20-30 °C. Incubation can be done using commercially available microbiological media, either aerobic bottles intended for automated systems or tryptic soy broth (TSB).

be carried out and the information immediately forwarded to the caring physician.

11.3.2.2. *Sample volume*

For automated culture systems, recommended sample volumes from 100 µL up to 10 mL can be inoculated per culture bottle. Usage for testing of a small percentage part of total volume bears the risk of an increased sampling error, leading to false-negative results, especially if bioburden is low. Therefore, a larger amount of sample should be envisaged for inoculation (if applicable and appropriately validated).

11.3.2.3. *Samples without anti-microbiological additives*

Aerobic and anaerobic culture media bottles without any neutralising additives of microbial growth inhibitor should be used.

11.3.2.4. *Samples with anti-microbiological additives*

Aerobic and anaerobic culture media bottles with resin or activated charcoal neutralising additives of microbial growth inhibitor should be used (if membrane filtration cannot be carried out due to the nature of the sample).

The type, amount and mixture of anti-microbial growth agents used during procurement and processing or used for patient treatment (and therefore present in samples for microbiological testing) is highly variable. Culture media bottles containing neutralising or adsorbing substances are established for the testing of patients' blood with therapeutic doses of a limited number of antibiotics or antimycotics. Therefore, such samples should be validated very thoroughly for residual anti-microbial activity to prove the suitability of the chosen method.

11.3.2.5. *Period between inoculation of culture bottles and incubation in an automatic culturing system*

Inoculated culture media bottles should be placed into the automated culture system as soon as possible; if a delay occurs, they should be maintained at 15-20 °C [18]. If a delay period of 12 hours is exceeded, the results of the automated culture system should be verified by subculture unless otherwise validated. For some automated systems and delayed kinetic culture media bottles, manufacturer's specifications mention as acceptable a delay up to 48 hours at room temperature. However, given the variability in terms of delay time, pre-incubation temperature and type of micro-organism, a delay exceeding 12 hours should be validated.

11.3.3. Microbial enumerations tests (*Ph. Eur.* 2.6.12 and 2.6.13)

The tissue-associated bioburden of aerobic mesophilic bacteria and fungi can be quantitatively enumerated either by membrane filtration or plate-count methods according to *Ph. Eur.* 2.6.12. The preferred microbiological recovery procedure is dependent on the type of sample. Different mechanical techniques can be used, alone or associated, to extract micro-organisms of the product before filtration or plate-count method (e.g. sonication, crushing, blending, shaking). The addition of a surfactant, such as polysorbate 80, can improve the extraction. The extraction method should be validated. A correction factor can be calculated from the validation data. The tissue-containing jar can further be sonicated for 5 minutes (47 kHz) followed by mechanical shaking for 30 minutes (200 strokes/minute) on a linear reciprocal shaker [16].

11.3.3.1. *Membrane-filtration method*

An appropriate volume of the extraction fluid is filtered through at least two appropriate filters (e.g. 0.45 µm pore size nitrocellulose filter). One filter should be transferred to the surface of a casein soya-bean digest agar plate for determination of the total aerobic microbial count (TAMC) and one filter to the surface of a Sabouraud-dextrose agar plate for determination of the total combined yeasts/moulds count (TYMC). The casein soya-bean digest agar should be incubated at 30-35 °C for 3-5 days and the plate of Sabouraud-dextrose agar at 20-25 °C for 5-7 days. In addition, anaerobic bacteria can be enumerated by transfer of a filter to an appropriate medium plate (e.g. thioglycolate agar) and anaerobic incubation at 30-35 °C for 3-5 days. The microbial count can be calculated as colony-forming units (CFU) per unit weight or volume (gram or millilitre) or surface area of tissues and cells.

11.3.3.2. *Plate-count methods*

At least two Petri dishes for each level of sample dilution for each medium are prepared, either by the pour-plate method or by the surface-spread method. Plates of casein soya-bean digest agar, Sabouraud-dextrose agar and anaerobic medium agar are incubated as stated above. Those plates should be counted that show the highest number of colonies less than 250 for TAMC and 50 for TYMC, corresponding to a given dilution. The arithmetic mean per culture medium of the counts is used to calculate the number of CFUs per unit weight or volume (gram or millilitre) or surface area of tissues and cells.

For determination of the absence or limited

occurrence of specified micro-organisms, test conditions are described in *Ph. Eur.* 2.6.13, in which the growth-promoting, inhibitory and indicative properties of specific media are described.

11.3.4. Alternative methods for control of microbiological quality (*Ph. Eur.* 5.1.6)

Alternative microbiological testing methods based on novel technologies that provide automated, rapid and more sensitive microbiological results, as compared with classic or conventional testing methods, may be used to control microbiological quality of pre-processing samples, in-process samples or final graft samples, in particular for preparations with a short shelf life. Validation of such alternative microbiological testing methods (examples in Table 11.4) should comply with requirements of *Ph. Eur.* 5.1.6, including validation for the intended use and demonstration of equivalence or non-inferiority compared with the compendia method.

Table 11.4. **Alternative methods for control of microbiological quality**

Growth-based methods	Electrochemical
	Gas consumption/production
	ATP bioluminescence
	Turbidimetry
Direct methods	Cytometry • solid phase • flow cytometry
Cell components methods	Phenotypic
	Genotypic • RT-PCR • Genetic fingerprinting

11.4. Validation or verification of microbiological testing methods

Microbiological test methods listed in general chapters of *Ph. Eur.* do not require full validation. Only alternative methods need to be fully validated in accordance with *Ph. Eur.* 5.1.6 prior to use. However, every test laboratory needs to verify that it is able to perform the test according to recommendations of Chapter 2 of this Guide. Furthermore, method suitability should be demonstrated for every tissue and cell preparation to be tested. Even small changes in the tissue and cell processing might result in interference with the microbiological test procedure. Therefore, method suitability should be re-demonstrated after any changes in the processing of the tissue and cell preparation. Similarly, changes in the test procedure itself require re-demonstration of its suitability. Any deviation from these rules needs to be justified.

11.4.1. Growth-promotion testing

Each batch of the microbial culture medium used for microbiological testing should be tested for its growth-promoting capacities as well as being used to test for the microbial strains listed in the relevant *Ph. Eur.* chapters. In general, it is recommended to include in the assays any possibly relevant microbial contaminants from the respective tissue or cell preparation or the environment – for instance, *Cutibacterium acnes* and *Micrococcus* spp. – because of their specific growth properties.

Frequently, growth-promotion testing has been performed by the vendor of microbial media using set suitable micro-organisms and documented by the certificate for each particular medium batch. In this case growth promotion need not be repeated by the end user, provided transport and storage conditions are properly documented and are in accordance with the requirements of the vendor.

11.4.2. Method suitability

The method should be verified in the presence of the intended sample material (e.g. transport medium, tissues or cells). The basis for method verification is the 'method-suitability test' laid down in the relevant chapters of *Ph. Eur.*

The same conditions should be chosen as for routine testing (e.g. culture conditions, sample type, sample amount). The method-suitability test should be undertaken using the bacterial and fungal species indicated in the relevant chapters of *Ph. Eur.* For microbiological methods not described in *Ph. Eur.*, validation is required, as described in *Ph. Eur.* 5.1.6.

It is recommended to complement the microbial spectrum by tissue-specific and/or contaminating micro-organisms, if found to be applicable to the process, such as Cutibacterium acnes and *Micrococcus* spp., which are typical skin contaminants.

For instance, *Cutibacterium acnes* is not readily accessible to skin disinfection due to its prevalence in the sebaceous glands, and detection of this species in tissue preparations is not unusual. *Cutibacterium acnes* grows under anaerobic or microaerophilic conditions as a 'slow-grower' and is associated with a particularly long detection time, so it may be included in method validation or verification.

The sensitivity of the chosen method should be shown, as described in the relevant chapters of *Ph.*

Eur., by experimental studies. Applicability of this method should be assessed in connection with its impact to ensure microbial safety of the tissues and cells.

Each micro-organism species should be tested to evaluate the capacity of the method to allow a good detection of the relevant micro-organisms. The efficacy of the method to extract the micro-organism should be repeated with different samples (minimum 3) with at least one strain. A strain of a bacterial aerobic germ is commonly chosen for this step, showing the robustness of the method.

For comparison, a positive control (without tissues or cells) should be included in the test for each test strain.

Inoculated media should be incubated under the conditions applied in routine testing (temperature, duration) and checked for growth at regular intervals.

Test assays and controls should be evaluated at predetermined intervals during and at the end of the testing period. Samples for subculture should be taken from positive detected tests as quickly as possible. In the case of microbial growth, the micro-organisms should be identified.

If inhibition of microbiological growth by the sample material is identified at validation, the method should be adapted in an appropriate way, for instance, using a higher volume of the culturing media or addition of binding or enzymatic substances that inhibit anti-microbiological agents.

11.4.3. Documentation and interpretation of results

All materials used and working steps undertaken should be documented. Interpretation of results should include at least the following factors:

a. assessment of the growth of micro-organisms in the presence of the tissues or cells to be tested and in controls;
b. specification of the microbial count for evaluation of the method;
c. period of time until a positive result has been detected for test assays and positive controls;
d. proof of identity of inoculated micro-organisms.

For negative controls or test assays without detection of micro-organisms, the total testing period and results of the subculture (including the methods used) should be specified.

11.5. Microbiological examination of donors

Although they do not forecast what will be the microbiological status of the tissues, microbiological blood cultures can be a useful tool to give a picture of the microbiological status of the donor, especially for the diagnosis of infections in deceased donors [19] of tissues and cells, in addition to the required serological examinations. In the case of living donors, see Chapter 24 – Haematopoietic progenitor cells from bone marrow and peripheral blood for more information.

If available, the results might be also helpful information while assessing a potential contamination from the procurement team and environment, as well as cross-contamination between tissue or cell preparations or to evaluate the efficacy of antimicrobial treatment, if applicable.

Blood samples should be of sufficient quantity, collected properly within a suitable timeframe, prepared/stored appropriately to ensure proper testing and accompanied by relevant clinical information. A clear protocol should be followed that addresses skin disinfection, the amount of blood obtained and the number of blood cultures. Skin disinfection is an important prerequisite for reliable results to avoid secondary contamination of the sample. At least a two-step alcoholic disinfection (preferably accompanied by sporicidal disinfection) should be done. Larger quantities of blood as well as several independent blood cultures improve the probability of detecting an infection in the donor. It is recommended to take at least 2-4 blood cultures (each aerobic and anaerobic), ideally in different vessels. The blood cultures should be incubated for ≥ 5 days in order to give time for the potential micro-organisms to grow.

Blood samples for culture can be obtained:

- Before circulatory arrest. The results of blood cultures using samples collected before circulatory arrest provide useful information about the clinical status of the donor (e.g. infections with objectionable organisms) and can be a useful supplementary tool for evaluation of donor suitability, and the quality and safety of specific tissues and cells, especially if the tissues and cells are not terminally sterilised [15, 20-25].
- After cardio-circulatory arrest (in which case the organs, tissues and cells may be at a higher risk of endogenous microbiological contamination), the information provided by blood cultures may be questionable due to agonal spread and *post mortem* bacterial translocation.

Manipulation of the deceased donor can lead to dissemination of micro-organisms from the lung and visceral organs to the heart. However, published data suggest that neither agonal nor *post mortem* spread can be expected to produce false-positive cultures if the body is appropriately cooled, the tissue procurement is performed within 24 hours after death and the samples for microbiological culture are collected early during tissue procurement, with minimum possible manipulation of the deceased donor [15, 20].

Nevertheless, a positive culture, in the particular context of organ, tissue or cell procurement, may signify contamination or cross-contamination during procedures, indicating problems with *post mortem* microbial spread, recovery techniques and/or environment. These possibilities should be evaluated.

11.6. Interpretation of results and actions to be taken

In general, source material that demonstrates contamination should be rejected unless the preparation undergoes decontamination and/or terminal sterilisation, and the detected bioburden can be reliably inactivated or removed by the intended procedure, or if it is justified by exceptional clinical circumstances (refer to Part B for specific examples).

Contaminated source material should be rejected if processing includes decontamination (but not terminal sterilisation) and if risk assessment considering the intended route of administration cannot exclude risk to the recipient even if adequate anti-microbial treatment is initiated. Such source materials should be evaluated on the basis of an exclusion list for objectionable micro-organisms and bioburden status. The decontamination procedure should be shown to be suitable to remove or destroy the type and number of contaminants allowed in the source material. In particular, multi-drug resistant micro-organisms and possible toxin-producing micro-organisms, as well as yeasts and filamentous fungi, need to be evaluated carefully and, if appropriate, the tissues and cells should be rejected (see Table 11.1).

In the case of locally acquired contamination or a local infection, the microbial result applies only to the tissue where the contamination was detected and to tissues that could have been cross-contaminated. If bacteraemia, septicaemia (anamnestic or blood culture) or any other distribution of the objectionable micro-organisms (during procurement, storage, transport or manufacturing) cannot be excluded, other tissues should be rejected.

For contaminated autologous preparations, or preparations received from a specific allogeneic donor, where a repeated procurement cannot be conducted or involves a high degree of risk and it is considered clinically necessary to proceed with the implantation, a risk assessment based on the urgency of the application, judgment of infection risk and treatment options should be conducted. In any application of such preparations, measures should include full identification of the contaminating micro-organisms and their antibiotic susceptibility profile, as well as adequate prophylaxis of the donor/recipient if the tissues or cells are used.

11.7. References

1. European Directorate for the Quality of Medicines and HealhCare (EDQM). *European Pharmacopoeia* (*Ph. Eur.*), 10th edition. Council of Europe, 2019-22.
2. European Eye Bank Association. Statement on pre-processing microbiology testing in eye banks, available at www.eeba.eu/news/news-details/eeba-statement-on-pre-processing-microbiology-testing-in-eye-banks, accessed 8 April 2022.
3. Fuest M, Plum W, Salla S *et al.* Conjunctival and intraocular swabs for the microbiological assessment of donor corneas. *Acta Ophthalmol* 2016 Feb;94(1):70-5. https://doi.org/10.1111/aos.12796.
4. Stones DH, Krachler AM. Against the tide: the role of bacterial adhesion in host colonization. *Biochem Soc T* 2016 Dec 15;44(6):1571-80. Review.
5. Domanović D, Cassini A, Bekeredjian-Ding I *et al.* Prioritizing of bacterial infections transmitted through substances of human origin in Europe. *Transfusion*. 2017 May;57(5):1311-17. https://doi.org/10.1111/trf.14036.
6. AATB. *Standards for tissue banking*, 13th edition. McLean VA, USA: American Association of Tissue Banks, April 2016.
7. Martinez OV. Microbiological screening of cadaver donors and tissues for transplantation. Chapter 5. In: Phillips G, ed. *Advances in tissue banking*, Vol. 6. Singapore: World Scientific Publishing 2003;7:143-55.
8. Pellet S, Kearny J, Dziedzic-Goslawska A *et al. Standards for skin banking*. Vienna: European Association of Tissue Banks, 1996.
9. Superior Health Council Belgium. Practical recommendations on microbiological control of human body material for human application with maximal protection of the microbiological safety. HGR8698, 2014.
10. ISO 11737-1. Sterilization of medical devices – Microbiological methods – Part 1: Determination of a population of microorganisms on products.
11. Brubaker S, Lotherington K, Zhao J *et al.* Tissue recovery practices and bioburden: a systematic review.

Cell Tissue Bank 2016 Dec;17(4):561-71. https://doi.org/10.1007/s10561-016-9590-5.

12. Pitt TL, Tidey K, Roy A *et al.* Activity of four antimicrobial cocktails for tissue allograft decontamination against bacteria and *Candida* spp. of known susceptibility at different temperatures. *Cell Tissue Bank* 2014 Mar;15(1):119-25. https://doi.org/10.1007/s10561-013-9382-0.
13. Skenderi Z, Giurgola L, Gatto C *et al.* Increased sensitivity of microbiological testing of cornea organ culture medium by additional resin treatment. *BMJ Open Ophthalmol*. 2018 Nov 10;3(1):e000173. https://doi.org/10.1136/bmjophth-2018-000173.
14. Vargas F, Vives-Pi M, Somoza N *et al.* Endotoxin contamination may be responsible for the unexplained failure of human pancreatic islet transplantation. *Transplantation* 1998 Mar 15:65(5):722-7. https://doi.org/10.1097/00007890-199803150-00020.
15. Lobmaier IVK, Vege A, Gaustad P, Rognum TO. Bacteriological investigation – significance of time lapse after death. *Eur J Clin Microbiol Infect Dis* 2009;28(10):1191-8.
16. Kowalsky J, Mosley G, Merrit K, Osborne J. Assessment of bioburden on human and animal tissues: Part 1 – Results of method development and validation studies. *Cell Tissue Bank* 2012;13:129-38.
17. Varettas K, Taylor P. Bioburden assessment of banked bone used for allografts. *Cell Tissue Bank* 2011 Feb;12(1):37-43. https://doi.org/10.1007/s10561-009-9154-z.
18. Willems E, Smismans A, Cartuyvels R *et al.*; Bilulu Study Group. The preanalytical optimization of blood cultures: a review and the clinical importance of benchmarking in 5 Belgian hospitals. *Diagn Microbiol Infect Dis* 2012 May;73(1):1-8.
19. Palmeire C, Egger C, Prod'Hom G, Greub G. Bacterial translocation and sample contamination in postmortem microbiological analyses. *J Forensic Sci* 2016 Mar;61(2):367-74. https://doi.org/10.1111/1556-4029.12991.
20. Morris JA, Harrison LM, Partridge SM. Post-mortem bacteriology: a re-evaluation. *J Clin Pathol* 2006 Jan;59(1):1-9. https://doi.org/10.1136/jcp.2005.028183.
21. Martinez OV, Malinin TI, Valla PH, Flores A. Postmortem bacteriology of cadaver tissue donors: an evaluation of blood cultures as an index of tissue sterility. *Diagn Microbiol Infect Dis* 1985 May;3(3):193-200. https://doi.org/10.1016/0732-8893(85) 90031-8.
22. Riedel S. The value of post-mortem microbiology cultures. *J Clin Microbiol* 2014;52(4):1028-33.
23. Saegeman V, Verhaegen J, Lismont D *et al.* Influence of post-mortem time on the outcome of blood cultures among cadaveric tissue donors. *Eur J Clin Microbiol Infect Dis* 2009;28(2):161-8.
24. Segur JM, Almela M, Farinas O *et al.* Bone contamination and blood culture in tissue donors. *Ann Transplant* 2005;10(2):11-13.
25. Vehmeyer S, Bloem R, Deijkers R *et al.* A comparative study of blood and bone marrow cultures in cadaveric bone donation. *J Hosp Infect* 1999;43(4):305-8.

Chapter 12. **Release, distribution and import/export**

12.1. Introduction

This chapter sets out the procedures for the release and distribution, import and export of tissues and cells that are deemed suitable for clinical application; it also describes the requirements for distribution of tissues and cells and defines recommended controls for their import and export.

Each tissue establishment (TE) must have a documented policy on tissue and cells release that describes which specifications should be met and when, how and by whom these are verified. These specifications must include all criteria considered by the TE to be essential for the safety and quality of the tissues and cells. In general, only when all specifications have been evaluated and met can the tissues and cells be distributed for clinical application.

The term 'distribution' should be understood to mean transport and delivery of tissues or cells to the organisation responsible for human application of tissues and cells (ORHA). 'Transport' is meant as the act of transferring a tissue or cellular product between facilities under the control of suitably trained, designated and authorised staff at the distributing and receiving facilities. 'Shipment' is a type of transport where the transfer of the tissues or cells from the distributing to the receiving facility is carried out by means of a contract with a third party, usually a specialised logistics company. The entire distribution chain must be validated appropriately, including the equipment used, to ensure the maintenance of critical transport or shipment conditions.

The terms 'import' and 'export' should be understood to include all processes and procedures that facilitate the entry or exit of tissues and cells, regardless their stage of processing, to/from a single country. Import/export controls must ensure that the quality and safety of the tissues or cells are in compliance with this Guide.

Tissues and cells can be transferred by a TE:

- to an ORHA within the same country, where they will be received and stored until clinical application (i.e. distribution);
- to another TE within the same country for local distribution.
- to another TE within the country for processing into tissues and cells for clinical application;

Cross-border movement of tissues and cells includes transfers:

- to a TE outside the country (i.e. export);
- from another country to a clinical facility or TE in the country (i.e. import).

For transfers of tissues or cells between countries that are within the European Union (EU), usually referred as 'distribution', the legislation does not require import/export controls to be in place provided that these tissue and cell preparations come from a duly authorised TE and their processes have also been authorised by the national Competent Authority. However, several EU member states opt to apply more stringent requirements than those in the directives and consider this movement in the same way as import/export involving countries outside the EU (referred to as 'third countries'). Written agree-

ments might be needed between countries in some member states (see §12.5.5).

12.2. Release

12.2.1. Release procedure

Release is the act of certifying compliance of a specific tissue or cells, or a batch of tissues or cells, with a pre-defined set of requirements and specifications. Before any tissues and cells are released, all relevant records (including current declaration forms, donor records, medical records, processing and storage records, and post-processing quality-control test results) must have been reviewed, approved and documented as acceptable by an authorised and trained person according to the relevant local SOP and national regulations. There must be an SOP that details the specifications, circumstances, responsibilities and procedures for the release of tissues and cells.

At the time of release, donor records and tissue- or cell-processing records must be reviewed to ensure that the material is suitable for clinical use and implantation. The review should include:

a. approval of donor eligibility by the responsible person (RP) or designated person;
b. consideration and approval of the processing and storage record (including environmental monitoring records);
c. final evaluation of the label and container to ensure traceability, accuracy and integrity;
d. results of screening tests on incoming material and in-process controls;
e. specifications for final release of tissues and cells based on testing results used to determine final release (e.g. quality controls such as viability or cells count, microbiology test results, biological activity);
f. all the same checks if the tissue or cells are designated for autologous use;
g. in case of deviating test results, the decision of the RP and the treating physician whether the tissue or cells can be used, but in any case the final decision has to be justified and documented.

There must be a documented system in place, supervised by the RP, for ratifying that tissues and/or cells meet the appropriate specifications for quality and safety. The RP, or personnel designated by the RP, must register (in writing or digital format) the review and fulfilment of all legal requirements, tissue and cells specifications and quality-release criteria as defined by the TE, thereby releasing the tissues and cells for storage in an inventory of tissues and cells that are available for human application. If release cannot be approved, the tissues or cells must be discarded or can be made available for research/educational use, if a specific consent for research/educational use was given.

Released tissues must be clearly distinguishable (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) and preferably be physically separated from quarantined and discarded tissues. The TE must provide clinical users with instructions for handling and using the tissue or cells.

12.2.2. Exceptional release

In exceptional circumstances, a TE may agree with the treating physician from the ORHA that tissues or cells that do not meet the standard TE criteria for release can be released and used in a specific patient. Such a release should be based on a risk–benefit analysis taking into consideration the alternative options for the patient and the consequences of not providing the tissues or cells, and this analysis requires documented informed consent by the treating physician. For more information, see Chapter 13.

12.2.3. Risk assessment

A documented risk assessment approved by the RP must determine the fate of all stored tissues and cells following the introduction of any new donor-selection or testing criterion or any significantly modified processing step that enhances safety or quality. Guidance on risk assessment is provided in Chapter 3.

12.2.4. Disposal of human tissues and cells

There must be a documented policy for disposal of tissues and cells that are unsuitable for clinical use. Records should include details of date and involved personnel, with method of and reasons for disposal. The material should be handled appropriately and disposed of in a manner compliant with local control-of-infection guidelines. Human tissues, cells and other hazardous waste items must be disposed of in such a manner as to minimise the hazards to the TE's personnel or the environment, and should be in conformity with applicable European, national and local regulations.

Disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and the human body. For HPC and autologous tissues and cells it is necessary to document that the

conditions for disposal defined in the consent prior to collection have been met, including (where applicable) the option to transfer the tissues and cells to another facility if the designated recipient is still alive after the agreed storage period (see Chapter 24).

In case of MAR/ART, the donors and patients must declare in writing the destiny of their reproductive material when the maximum storage period has ended (see Chapter 29). A specific signed consent is needed for the acceptance of final disposal of a given sample.

Disposal of cellular therapy products must include a pre-collection written agreement between the storage facility and the designated recipient or the donor, defining the length of storage and the circumstances for disposal of cellular therapy products [1].

12.3. Transport

The choice of mode of transport should take into account any general regulations governing transport of biological substances and any specific handling, storage or transport recommendations provided by the TE.

Critical transport conditions, such as temperature and time limit, must be defined to ensure maintenance of the required properties of tissues or cells [2]. When transport is carried out under storage conditions, the impact on transport time is minimal. Unfrozen products are usually transported refrigerated (2 to 8 °C), or cooled (8 to 15 °C) or at room temperature (15 to 25 °C); frozen products are transported deep-frozen (– 80 to – 60 °C in dry ice), frozen (< – 15 °C with ice packs) or cryopreserved (< – 140 °C in liquid nitrogen vapour phase). When the transport device does not allow the temperature conditions to be maintained over time, a time limit must be validated to guarantee that the storage conditions during transport do not affect the quality of the product.

For unfrozen products, such as bone marrow or mobilised peripheral blood, there are conflicting recommendations for storage and transportation – e.g. 4 °C *versus* room temperature [3, 4, 5] – so the transplant centre is usually requested to define the transport conditions they wish to be applied. But in any case, the TE must validate the best recommended shipping temperature for their products. For cells and tissues potentially contaminated during the procurement, refrigerated transportation is generally recommended in order to prevent the risk of bacterial proliferation.

If the tissues or cells require specific environmental conditions, the capacity of the transport container to maintain the required environmental conditions, and the length of time that these conditions can be maintained by the transport container, must be determined by validation and documented. For instance, if liquid nitrogen is used to maintain very low temperatures, the dry-shipper must contain sufficient absorbed liquid nitrogen to maintain the storage chamber temperature < – 140 °C for a defined period of time, at least 48 h beyond the expected time of arrival at the receiving facility. In cases where the tissue or cells during transport could be exposed to a wide range of outdoor temperature (e.g. winter *versus* summer) the cycling temperature validation profiles should be used for validation of the transport container [6]. Where temperature control is critical, data loggers should be used to monitor temperature during transport or shipment, with data downloaded from the device providing a graph to show that temperature was within the acceptable range at all times. Temperature indicators can be also used to indicate exposure to extremes of temperature.

Containers/packages must be secured and labelled appropriately (see Chapter 15).

Written agreements should be in place for the shipment of tissues and cells. In EU member states a written agreement must be signed between the shipping company and the TE to ensure that the required conditions will be maintained. This document must describe what should happen if the tissues or cells are damaged or lost during shipment (see also Chapter 3) and must cover a requirement that any related serious adverse events should be identified and reported to the Health Authorities (see Chapter 17).

12.4. Allocation

The allocation of tissues and cells should be guided by clinical criteria and ethical norms. The allocation rules should be equitable, externally validated and transparent.

The procedures for distribution of tissues and cells by authorised TEs must comply with the criteria laid out in the sections below.

It is mandatory for EU member states to have procedures in place for the management of requests for tissues and cells. The rules for allocation of tissues and cells to certain patients or healthcare institutions must be documented, and made available in appropriate circumstances, in the interests of transparency.

12.4.1. Visual examination

Packaged tissues or cells must be examined visually for appropriate labels, expiry date, container

integrity and security, and any evidence of contamination prior to being dispatched (see Chapter 15).

12.4.2. Medical competence

Distribution for clinical application should be restricted to hospitals, physicians, dentists or other qualified medical professionals and must comply with all applicable national regulations.

12.4.3. Documentation

The place, date and time of pick-up and delivery (including time zone where relevant) and identity of the persons handing over and receiving the tissues and cells must all be recorded, and this record should be maintained in the TE from which the tissues or cells are distributed (see Chapter 2).

Any transportation must be accompanied by specific documentation attached to the package (see Chapter 7, Chapter 13 and Chapter 15).

12.4.4. Recall and return procedures

An effective recall procedure must be in place in every TE, including a description of the responsibilities and actions to be taken in the case of a recall. This must include procedures for the notification of the relevant Health Authority/ies and all the facilities/institutions potentially affected by the recall.

A documented system must be in place for the handling of returned products, including criteria for their acceptance into the inventory, if applicable.

For further information, see Chapter 16.

12.5. Import and export

12.5.1. Underlying principles

Import and export between countries should be done only through legally authorised tissue establishments that can guarantee that they have sufficient competence to evaluate the safety and quality of tissues and cells from donation until distribution, and also can guarantee that they have adequate systems to meet traceability requirements. They should be specifically authorised for one or more of the following:

a. import and/or export of human tissues and/or cells intended for human application;
b. import and/or export of tissues or cells intended for the manufacture of medicinal products derived from human tissues and/or cells (with the exception of tissues/cells that have been substantially manipulated, such as cell-lines or cell banks);
c. import and/or export of procured human material intended for processing, storage or banking in a TE or cell establishment in their country.

As a general rule, if organisations responsible for human application, manufacturers of advanced therapy medicinal products, clinical practitioners or individuals identify a need to import tissues or cells, they should organise this through a written agreement with a licensed TE in their own country. Third-party agreements must specify the terms of the relationship and the relevant responsibilities, as well as the protocols to be followed, to meet the required performance specifications.

12.5.2. Import

TEs that wish to import tissues or cells should be able to demonstrate that the need cannot be adequately met by comparable material available from sources within their country or that there is another justifiable reason for the import. They should also be able to justify the import in terms of accessibility, quality, speed of supply, risk of infection, quality of service, cost-effectiveness or scientific or research needs. They should ensure that any material intended for import is consistently sourced under the legal and ethical requirements of their country and the exporting country. If the importing TE cannot satisfy itself that ethical standards are in place in the country of origin, the tissues or cells should not be imported.

The safety and quality characteristics of the tissues or cells to be imported must be equivalent to those in place within the importing country. Imports should be accepted only from countries that have established procedures to authenticate the legitimacy of exporters and the provenance of the donated material they supply. Exporter TEs should be asked to provide evidence of compliance with the regulations that they are required to observe before any orders are placed with them.

Companies that act as distributors, often also carrying out import and export activities, have responsibilities equivalent to those of TEs for ensuring the equivalent requirements for safety and quality, for maintaining traceability and for having adequate vigilance systems in place. Fulfilment of these requirements implies having suitably trained, designated and authorised staff (including those with medical expertise) to evaluate donor-selection criteria and reports of adverse incidents and reactions.

See also §12.5.5 below for EU requirements for import.

12.5.2.1. *Routine importation*

The importing TE should assess whether the supplying TE complies with the quality and safety recommendations in this Guide and should document that assessment, which includes respect for the fundamental ethical principles of consent, non-remunerated donation, anonymity and respect for public health. The evaluation should include at least the following:

a. the general quality and safety systems at the exporting establishment, including organisational chart, staff training, facilities, processing methods, validation studies, traceability and biovigilance systems, licences and accreditation (including lab certification/authorisation) and donor blood testing;
b. a review of the safety and quality of individual dispatches of tissues or cells (i.e. confirmation of donor consent, verification of donor sample testing and the results, donor eligibility records, description of the tissue or cells, transportation arrangements, etc.).

Potential language barriers should be considered and a common language agreed upon for all donor and tissue- and cell-related documentation.

A service-level agreement or contract between the exporting and importing TEs that clearly defines roles and responsibilities is a basic requirement. Agreed procedures for the transport of the tissues and cells from the country of origin to the TE in the importing country must form part of the contract and must specify the methods to be followed to ensure maintenance of the required environmental conditions, of the package integrity and of compliance with agreed timeframes. Such transportation should be direct, without intermediate stops when possible, using an approved courier. The courier or transportation service must provide records of pick-up and delivery to the TE so that complete traceability is ensured.

The agreement must specify how tissues and cells will be identified. Unique identifying codes must allow traceability and a formal and unambiguous identification of all tissues and cells (see Chapter 15).

Agreements between importing TEs and suppliers in other countries should include provisions for the performance of audits at the exporting facility and should require that any changes to authorisation status be immediately communicated to the importing TE.

12.5.2.2. *'One-off' importation*

There may be cases where exceptional or one-off importation is necessary for a single patient. In these cases, the importing TE should ensure that there exists a documented evaluation of the safety and quality of the tissues or cells being imported. The importing TE should keep the documentation obtained from the supplying TE for the time period specified in national regulations (e.g. 30 years in EU member states).

In limited cases (e.g. in emergency situations or for immediate transplantation) the import of certain tissues and cells may be directly authorised by a Health Authority, which should take all the necessary measures to ensure that imported tissues and cells respect the national quality and safety standards.

12.5.3. Customs and security clearance

For clearance of Customs, all tissues and cells supplied from abroad require a clear description of the content of the consignment and its destination and must be labelled as described in Chapter 15. It is important that frozen tissues or cells, which are usually packed in dry ice or stored in a dry-shipper, as well as fresh cells and tissues for urgent medical need, must not be delayed at border crossings. Viable tissues and cells for clinical use must not be exposed to irradiation devices; instead they should be subject to a visual inspection, without impairing viability.

However, it should be noted that a study published in 2002 concluded that even 10 passages through the hand-luggage control system resulted in no harm to haematopoietic progenitor cells (HPC) and lymphocytes in terms of viability and potency. Interestingly, the radiation dosage of passage through the hand-luggage control system is of 1.5 + 0.6 μSv compared to a radiation dose of 60 μSv received by the HPC during a 10 h flight [7]. The lack of data on long-term effects suggests that, in line with the precautionary principle, the non-irradiation rule should be followed for the time being. Therefore, it may be expedient for the importer to inform Customs in advance of a prospective consignment and any enquiries by Customs should be answered promptly. The agreement with the exporter should define responsibilities for meeting the cost of transport, refrigeration and/or storage at a Customs facility for any items that may be detained pending Customs enquiries.

12.5.4. Acceptance at the tissue establishment

Each importing establishment must have a documented procedure and specifications against

which each consignment of tissues and cells, together with its associated documentation, is verified for compliance with the written agreement in place with the exporter. Any non-compliance should be reported to the exporter. Consignments should be examined for any evidence of tampering or damage during transport.

Tissues and cells must be stored in quarantine in an appropriate secure location under defined conditions until they, along with their associated documentation, have been verified as conforming to requirements. The acceptance or rejection of received tissues and cells must be undertaken and documented in accordance with the guidance shown in Chapter 13.

12.5.5. EU requirements for importing tissues and cells

In April 2015, a new implementing directive on procedures for verifying equivalent standards of quality and safety of imported tissues and cells was adopted by the EU. Commission Directive 2015/566/EU stipulates that tissues and cells must be imported into the EU by an importing TE authorised for such imports by competent authorities. An importing TE is defined in the directive as:

> a tissue bank or a unit of a hospital or another body established within the Union which is a party to a contractual agreement with a third country supplier for the import into the Union of tissues and cells coming from a third country intended for human application.

Directive 2015/566/EU also lays down the obligations of the importing TEs and the competent authorities of EU member states who must verify that imported tissues and cells meet quality and safety standards equivalent to those in place in the EU legislation for tissues and cells. These new requirements aim to facilitate the exchange of tissues and cells with non-EU countries while ensuring that high standards of quality and safety are applied whatever the origin of the imports.

The procedures laid down in the new directive mirror closely the verification systems already in place within the EU. That is, procedures on the authorisation and inspection of importing TEs are laid down, specifying the information and documentation that must be provided or made available to Health Authorities in EU member states when TEs apply for import authorisations. Such information and documentation relates to the importing TE itself and the non-EU country suppliers it plans to use as a source of tissues and cells.

Another key element of the 2015 directive concerns the need for written agreements between importing TEs and their non-EU-country suppliers. Several minimum requirements for such agreements are listed in the text with a view to ensuring that the roles and responsibilities of each party are clear and fully undertaken to ensure that equivalent quality and safety standards are met. Annexes to the directive describe the minimum requirements in the information and documentation to be provided by importing TE applicants when applying to be accredited, designated, authorised or licensed for the purpose of import activities, the content of the authorisation certificate for the importing TE and the information to be provided regarding the third-country supplier.

According to Directive (EU) 2015/565 the TE has the obligation to assign a Single European Code (SEC) on tissue and cells distributed or imported for human application in the EU or exported from the EU (see §15.2.3).

The directive allows a limited number of exceptions to certain procedures for situations where certain tissues and cells are imported on a one-off basis. A 'one-off import' is defined in the directive as the import of any specific type of tissue or cell which is for the personal use of an intended recipient or recipients known to the importing tissue establishment and third country supplier before the importation occurs. Such an import of any specific type of tissue or cell must normally not occur more than once for any given recipient. Imports from the same third country supplier taking place on a regular or repeated basis must not be considered to be 'one-off imports'.

Those tissues and cells imported under direct authorisation of the competent authority of an EU member state (i.e. in emergency situations or for immediate transplantation) are not affected by the new procedures. An 'emergency' is defined in the directive as any unforeseen situation in which there is no practical alternative other than to urgently import tissues or cells from a third country into the Union for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import.

In the EU, distribution and shipment of all cells classified as advanced therapy medicinal products (ATMPs) are within the responsibility of a marketing authorisation holder and supervised by national/EU authorities for medicinal products.

Where an EU country imports from a non-EU

country and the ultimate destination is a different EU member state, then the tissues or cells should fulfil the quality and safety requirements of both EU countries (i.e. with one EU country acting as the point of entry into the EU and the other as the final receiver of the tissues or cells).

12.5.6. Export

Tissues or cells should not be exported if there is an unmet clinical need for the material in the country of origin. Exported material should be procured, used, handled, stored, transported and disposed of in accordance with the consent that has been given by the donor. Tissues and cells should be exported only to countries that have proper controls on the use of donated material. They should be exported only for the purposes for which they can lawfully be used in the country of destination, and exporters should satisfy themselves beforehand that the human tissues and/or cells will be used for a *bona fide* clinical application or research.

TEs must ensure that the quality and characteristics of the tissues and cells to be exported are equivalent to those of the tissues and cells implanted in their own country and are required in the country of destination.

12.6. International co-operation

For some transplant patients, including sensitised patients, it may be difficult to find a match within their own country. In these cases, co-operation between countries is necessary and in some cases it may be necessary to search worldwide to identify suitable donors, e.g. the World Marrow Donor Association harmonises search and distribution worldwide. International co-operation and exchange of tissues and cells is necessary to increase the chances of providing tissues and cells for patients in life-threatening situations. For these reasons, it is important to ensure that there is good co-operation between organisations that allocate internationally, and that procurement and processing centres worldwide use the same standards [1, 8]. Registries should be in place for all imported and exported tissues and cells to ensure transparency in the process.

12.7. References

1. FACT–JACIE. International standards for hematopoietic cellular therapy product collection, processing, and administration, 7th edition. Barcelona: European Society for Blood and Marrow Transplantation; 2018.
2. Pamphilon DH, Selogie E, Szczepiorkowski ZM. Transportation of cellular therapy products: report of a survey by the cellular therapies team of the Biomedical Excellence for Safer Transfusion (BEST) collaborative. *Vox Sang* 2010 Aug 1;99(2):168-73. https://doi.org/10.1111/j.1423-0410.2010.01329.x. Epub 2010 Mar 10.
3. Antonenas V, Garvin F, Webb M *et al.* Fresh PBSC harvests, but not BM, show temperature-related loss of CD34 viability during storage and transport. *Cytotherapy* 2006;8(2):158-65. https://doi.org/10.1080/14653240600620994.
4. Hahn S, Sireis W, Hourfar K *et al.* Effects of storage temperature on hematopoietic stability and microbial safety of BM aspirates. *Bone Marrow Transplant* 2014;49(3):338-48. https://doi.org/10.1038/bmt.2013.176. Epub 2013 Nov 4.
5. World Marrow Donor Association (WMDA). International Standards for Unrelated Hematopoietic stem cell donor registries, available at https://wmda.info/wp-content/uploads/2021/01/WMDA-2020-Standards_AM1_Jan2021-1.pdf, accessed 7 July 2022.
6. Rasche A, Prasath A, Hutchins C *et al.* WMDA guidelines for validation of transport containers used for the distribution of haematopoietic stem cell (HSC) products, 2018, available at https://share.wmda.info/download/attachments/322174994/20181018-WGQR-Guidelines%20validation%20transportation%20containers.pdf, accessed 9 April 2022.
7. Petzer AL, Speth HG, Hoflehner E *et al.* Breaking the rules? X-ray examination of hematopoietic stem cell grafts at international airports. *Blood* 2002 Jun 15; 99(12):4632-3.
8. Netcord–Fact International Standards for Cord Blood collection, banking, and release for administration, 7th edition, 15 October 2019.

Chapter 13. Interaction between tissue establishments and organisations responsible for human application

13.1. Introduction

An organisation responsible for human application (ORHA) is a healthcare facility that carries out human application of tissues or cells. The type and frequency of interactions between a tissue establishment (TE) and an ORHA may vary depending on the types of tissues and cells, geographical location and clinical environment. It is necessary to have a collaborative approach with well-defined roles and responsibilities to be mutually beneficial to all stakeholders.

Tissues and cells for clinical application must be ordered by a clinician or other authorised person at the ORHA. On arrival at an ORHA, the responsibility for maintaining the quality-assurance chain is transferred to that organisation. The ORHA must store and handle tissues and cells correctly according to the instructions of the supplying tissue establishment (TE). The ORHA must also maintain traceability and biovigilance, which includes responsibility for immediately reporting serious adverse reactions and events to the TE and the Health Authority, participation in the investigation and, where required, implementation of corrective and preventive actions [1, 2, 3].

13.2. Decisions on using and ordering tissues and cells

Clinicians intending to use tissues and cells with human application must be aware of the risks associated with the application of tissues and cells and give careful consideration to the risks and benefits of the procedure to the intended recipient. They should consider the feasibility and availability of alternative options, including associated risks. This must be explained to the patient in order for them to take a decision on whether to undergo the procedure and give informed consent.

Tissues and cells for allogenic application are donated for the benefit of patients in need and are often in short supply; therefore, only the required amount should be ordered, to minimise the likelihood of wastage. Healthcare professionals responsible for the storage and preparation of human tissues and cells for clinical application should receive timely and appropriate training to ensure their compliance with all applicable technical and legal requirements that assure the quality and safety of the supplied tissues and cells.

13.3. Choosing a supplier of tissues or cells

ORHAs must comply with national regulations and obtain tissues and cells for human application from a TE authorised by the Health Authority to distribute tissues and cells for clinical use. It is strongly recommended that ORHAs should obtain tissues or cells directly through a TE. If that is not possible, and the ORHA uses an organisation that mediates distribution between TE and ORHA, the ORHA should verify that the distributor is

authorised appropriately by a Health Authority and that the distributor has an agreement signed with the TE that processed those tissues and cells. It is recommended that the ORHA select a TE supplying tissues and cells on a non-profit basis from voluntary unpaid donations as the provider. Health Authorities must also authorise, where appropriate, the direct distribution of tissues and cells to ORHAs for immediate clinical application from abroad. Co-operating with authorised TEs ensures that the donors of tissues or cells have been selected and tested correctly, and that all quality system requirements are in place for the procurement, processing, storage and distribution of tissues or cells.

To ensure that the quality and safety standards and the respective responsibilities of TEs and ORHAs are clearly set out and fully understood by both parties, there should be a formal service-level agreement (SLA) or contract in place between the supplying TE and the ORHA. These written agreements should be signed, dated and reviewed regularly (as defined by the parties concerned), but sooner if changes are required. They must comply with relevant laws and regulations. Where an ORHA and the supplying TE are within the same healthcare institution, responsibilities should be specified in the overall quality-system documentation.

Service-level agreements should include:

a. contact details for relevant persons in both parties, including the TE's Responsible Person (RP);
b. procedures for ordering, including the method of assuring relevant quality and quantity parameters of ordered tissues and cells;
c. procedures for the delivery of tissues or cells, including liability for transport;
d. a statement that storage and preparation of tissues and cells for human application at the ORHA must comply with all relevant and specific instructions provided by the TE, including adherence to expiry dates;
e. procedures at the ORHA for the lawful disposal of unused tissues or cells or remnants of tissues and cells after human application;
f. procedures, if permitted, for the return of tissues or cells to the TE, or to use for research;
g. responsibility for maintaining traceability and biovigilance, including procedures for the timely reporting and investigation of adverse reactions and adverse events, including 'near misses', and procedures for the management of tissue and cell recalls and look-backs;
h. procedures, where permitted, for reporting of relevant clinical outcome data relating to the quality, safety and efficacy of the applied tissues or cells by the ORHA to the TE (see §13.14);
i. the option for TE and ORHA to conduct an audit of the other party.

Where novel tissues and cells are to be supplied by a TE, or where novel clinical applications of processed tissues and cells are intended, authorisation by the Health Authority is needed. The extent of clinical follow-up needed to evaluate the efficacy of the applied tissues and cells should also be agreed between the Health Authority, TE and ORHA (see Chapter 18).

Tissue establishments should distribute gametes, embryos and germinal tissues only to other authorised TEs or ORHAs for human application under the supervision of a clinician or other appropriate healthcare personnel.

In most cases, procured tissues and cells require processing and storage at the TE before their distribution to ORHAs for human application. In specifically authorised cases of direct distribution, procurement organisations send procured tissues and cells directly to the ORHA for immediate transplantation without any intermediate steps such as processing or storage.

Before requesting tissues or cells, the ORHA should confirm that the supplying TE, or the procurement organisation in the case of direct distribution, is compliant with all relevant legal and technical standards and requirements for the lawful provision of tissues and cells that are safe and of appropriate quality. In the case of procurement of haematopoietic progenitor cells (HPC) or lymphocytes for unrelated allogeneic use, there is no direct interaction between the procurement organisation and the ORHA before cell procurement. This means that the ORHA does not directly select the procurement organisation that will procure the HPC or lymphocytes. Suitable donors are identified through donor registries. The registry is responsible for ascertaining whether the procurement organisation complies with appropriate quality and safety standards, including traceability of the procured cells.

13.4. Importing tissues or cells from other countries

If an ORHA needs to import tissues or cells from another country, it is good practice to make arrangements through a local TE to locate and communicate with the exporting TE. In the EU, tissues and cells from a third country outside the EU must be imported through a TE authorised for importation by an EU Health Authority. Commission Directive

566/2015/EU sets out the procedures for verifying the equivalent standards of quality and safety of tissues and cells imported from third countries. The only exceptions to this rule are tissues and cells imported for direct distribution or for cases where there is an urgent clinical need. In the former, imported tissues and cells may be distributed directly for immediate clinical application provided the supplier is authorised for this activity. Urgent clinical cases include any unforeseen situation where there is no alternative other than to urgently import tissues and cells from a third country for immediate application to a known recipient or known recipients whose health would be seriously endangered without such an import. In both of these cases, the Health Authority must authorise the import directly.

The importing and exporting TEs must liaise with each other to ensure that the equivalent standards of quality and safety required by the importing TE are applied. In the EU, any TE that is authorised by a Health Authority in its own member state may provide tissues or cells directly to ORHAs in other member states. However, some member states have implemented more stringent regulations that require formal import procedures to be followed, even if the tissues and cells come from another EU member state. It is important to be aware of the national legislation in place for the importation of tissues or cells from another country.

13.5. Exceptional release

In exceptional circumstances, an ORHA may agree with a TE that tissues or cells that do not meet the normal release criteria can be applied in a specific individual on the basis of a risk–benefit analysis, taking into consideration the alternative options for the individual and the consequences of not providing the tissues or cells. The risk assessment should be documented and shared with the treating clinician about the reason for the exceptional release of tissues or cells. The TE's RP or nominated deputy/individual should liaise with the recipient's clinician to discuss the exceptional release so that the treating clinician can make a decision by performing a risk–benefit analysis for the intended recipient. These discussions and conclusions must be documented. The treating clinician must inform the recipient, where possible, as part of the normal consenting procedure of the intention to use tissues or cells under exceptional release (see §13.6).

13.6. Recipient consent

Although donors are carefully selected and tested, there remains an albeit small risk of an adverse reaction in a recipient of tissues or cells. Recipients must therefore be made aware both of the risks and benefits of the intended treatment to be able to give informed consent. The Notify Library is an invaluable source of information for clinicians when evaluating the risks associated with the human application of tissues and cells [3]. An additional important source of information on possible adverse outcomes and their frequency may also be based on specific types of tissues and cells and their specific applications, e.g. registries, if they exist; see Chapter 17 for more information.

Where the collection of clinical follow-up data is proposed, recipients may need to provide consent for the sharing and secondary use of their data in accordance with national legislation and guidance. In the EU, the General Data Protection Regulations (GDPR, Regulation EU 2016/679) provide certain exemptions from the need for consent for the collection and use of such data under Articles 6.1 (e) and 9.2 (h). However, recipients must be informed about the collection and use of their clinical data and, although they do not need to give consent, they do have the right to refuse to allow their data to be collected and used. However, their therapeutic care will remain unchanged. Moreover, they can request to have their data withdrawn at any time even though they may not initially have refused their permission.

The information given to a prospective recipient should include at least the following:

a. a description of any adverse outcomes that have been reported for the given type of tissue or cell application;
b. an estimate of the frequency of the adverse outcomes described;
c. whether the treatment is consolidated or if it involves novel methods of processing/clinical application;
d. information on alternative treatments, if available.

Once the appropriate information has been given, the recipient, if willing to proceed, should then consent to the treatment, according to national requirements. The recipient should confirm :

a. that the risks associated with the human application of the tissues or cells have been explained and the information has been understood;
b. that they accept the risks in light of the potential benefits.

A specific consent form must be signed by the recipient in the case of any novelty. This novelty could be due to changes introduced either at the TE, such as introduction of new processing methodology, or at the ORHA, such as introduction of new clinical application procedures for the tissues or cells (see Chapter 4 and Chapter 18).

13.7. Centralised *versus* devolved management of tissues and cells

Tissues and cells are either delivered directly to the relevant department or operating theatre in an ORHA (i.e. devolved management of tissues and cells) or they are delivered to a single, dedicated location under the direct supervision of an authorised healthcare professional (i.e. centralised management of tissues and cells). The advantage of devolved management is that the relevant department or unit in the ORHA with the appropriate specialist knowledge takes responsibility for the tissues and cells, whereas under centralised management there may be a more uniform approach to biovigilance and traceability and to ensuring compliance with quality and safety requirements for storage, handling and inventory control of the tissues and cells.

Regardless of the model applied for the management of human tissues and cells, all activities associated with receipt, storage, handling and follow-up should be incorporated into the existing quality-management system of the ORHA. The roles and tasks of officially designated persons should be clearly specified in standard operating procedures (SOPs).

13.8. Checks by ORHA on accepting delivery

When tissues and cells are received by an ORHA, appropriate personnel should verify and record that:

a. the tissues or cells received correspond to what was ordered and to the information in the accompanying documentation, which must be complete and legible;

b. the shipping containers and primary containers are labelled with the required information – including, where appropriate, the Single European Code (SEC) – and that labels are affixed and legible (see Chapter 15). Separate accompanying documents must provide any other relevant information that is not on the primary container label;

c. the shipping container and primary container are intact;

d. the specified expiry dates of tissues or cells have not been exceeded;

e. the transport temperature range was monitored or maintained adequately and is acceptable. For tissues or cells that are transported at low temperatures, maintenance of the required transport temperature can be confirmed by data readout from a temperature logger placed in the shipping container or by a residual coolant in the container (e.g. water ice for refrigerated tissues or cells and dry ice or vapour/liquid nitrogen for frozen tissues or cells). The supplying TE should be able to provide, on request, a validation report to show that the required temperature can be maintained in the shipping container for a period of time that exceeds the maximum duration of transport.

Where the delivered tissues or cells do not comply with the above requirements, the ORHA must liaise with the TE to decide the correct course of action, which could include disposal of the tissues and cells or their return to the TE.

The TE must provide documentation demonstrating that the tissues or cells comply with all the TE's quality and safety standards and legal requirements for donation, testing and banking, along with specific information about the characteristics of the tissues and cells required by the end-user clinician.

13.9. Package insert/instructions and temporary storage before use

Once tissues or cells have been distributed by a TE for clinical use, appropriate storage and handling become the responsibility of the ORHA. Instructions must be available in the package insert that accompanies the tissues or cells, describing the appropriate storage conditions and the proper handling procedures to be followed before clinical application. These instructions must be followed precisely by the ORHA, as specified in the contract with the TE (see points *a* to *i* listed in §13.3).

Tissues and cells are stored under various temperature conditions, depending on their type, method of preservation and packaging. Where a specific storage temperature is necessary from receipt to clinical application, the storage device (e.g. refrigerator, freezer, liquid nitrogen storage tank, incubator) should be regularly maintained and calibrated and

should be secure, i.e. with restricted access. It should be dedicated to the storage of healthcare products and cleaned according to a defined protocol and frequency. It should have functional alarms, and emergency back-up storage capacity should be present. Storage procedures should address the steps to be taken if the temperature is outside defined limits or in the event of equipment/power failure. Failure to monitor and maintain controlled temperatures can result in waste of a precious resource and, if tissues or cells are used, serious adverse outcomes due to deterioration in their quality. All records pertaining to storage temperatures must be retained for at least 10 years after clinical use, disposal or expiry date.

During the storage of tissues and cells at the ORHA before clinical application, they must be kept together with their associated documentation or else the documentation must be reliably linked to the tissues or cells and easily accessible. The accompanying documentation must specify the presence of particular additives or reagents that may adversely affect the recipient (e.g. antibiotics, allergens). If there is no package insert accompanying the tissues or cells, they should not be used.

Some EU member states regard short-term storage of tissues or cells at an ORHA as a licensable activity that requires specific authorisation from a Health Authority. Therefore, it is important to be aware of the national legislation in place for the storage of tissues or cells at an ORHA.

13.10. Inspection of the container, documentation and tissues or cells prior to application

Before human application, the container/packaging must be inspected and the accompanying documentation must be reviewed and confirmed to be complete and legible. The label should be checked and compared with the description on the package insert to confirm that the material is indeed what was ordered for the patient and is what is shown on the label. The packaging and the contents should be inspected for any signs of damage during transport. Where temperature during transport and storage at the ORHA is critical, there should be confirmation that the required temperature has been maintained.

In the case of tissues, the graft should be examined once the container has been opened to confirm that the anatomical characteristics are as shown on the label (e.g. left *versus* right femur, aortic *versus* pulmonary heart valve).

Tissues to be used in surgery should be specified and their use documented in the surgical checklist.

13.11. Preparation of tissues or cells before use

Instructions for opening the container or package, and any required manipulation or reconstitution (e.g. thawing, washing, rehydration), as well as information on expiry dates after opening/manipulation and the presence of any potentially harmful residues or reagents that may adversely affect the recipient (e.g. antibiotics, ethylene oxide), must be provided on the label or in the documentation accompanying the tissues or cells.

The handling instructions provided by the TE for the preparation of tissues and cells for human application should be followed precisely. Any departure from the instructions provided by the TE is at the discretion of the clinical user, who must take full responsibility for any adverse outcome resulting from not adhering to the instructions provided by the TE.

13.12. Surplus or unused tissues or cells

Tissues or cells remaining from a clinical procedure must not be used in another patient; any residue should be discarded as clinical or anatomical waste, in accordance with national rules, or returned to the supplying TE for appropriate and lawful disposal. Similarly, a single unit of tissues or cells (e.g. two halves of femoral head delivered in one container) must not be used in more than one separate patient. Activities that are routinely performed to finally prepare tissues and cells just before their clinical application, e.g. shaping of tendons or bone grinding for impaction grafting, are not considered as processing and do not require notification to the RP at the supplying TE.

Tissues or cells provided to one ORHA should not, in general, be sent to another ORHA for clinical application. Within the EU, this would be defined as distribution and it would require specific authorisation. However, such transfer of tissues and cells may be acceptable where the TE manages the process and the quality and safety requirements of the tissues and cells are not in any way compromised.

Certain types of tissues (e.g. bone graft) that are received and not subsequently used in one recipient or department of an ORHA may be occasionally reallocated to a different recipient or department in the same ORHA, If this is the case, safety, quality and traceability measures should be put in place with

clear documentation. The details of such activity must be specified in the overall quality-system documentation and in an SLA (see §13.3). There may be nationally established rules for such circumstances.

The documentation that accompanies the tissues and cells should specify whether they can be returned to the TE if not opened or used, e.g. if the patient is not well enough for surgery or if surgery is cancelled for another reason. Tissue establishments that do accept the return of unused and unopened tissues or cells must be able to confirm that the required storage conditions have been maintained, that the packaging has not been tampered with, and that the quality and safety of the tissues and cells has not been compromised.

13.13. Traceability

Coding and traceability are addressed fully in Chapter 15 and Chapter 16. In the EU, ORHAs are required to maintain traceability records from the point of receipt of the tissue until 30 years after clinical use. These records (mandatory in the EU) must include:

a. identification of the supplying TE or procurement centre (for tissues and cells directly distributed);
b. identification of the clinician/end user/facility;
c. type of tissues or cells;
d. unique product identification;
e. identification of the recipient;
f. date of application or disposal.

Details of the tissues or cells applied should be in the recipient's record and in the record of the treatment room or operating theatre where they have been applied. However, these records alone are not adequate to permit rapid tracing of patients who might be at risk from a particular donation or processing batch. The ORHA should also have an electronic or paper log where all received, transplanted and discarded tissues or cells are recorded. This should provide a robust two-way audit trail to facilitate rapid identification of tissues and cells in the case of a recall by the TE or the Health Authority, or identification of recipients where the TE has been notified of a serious adverse reaction or serious adverse event that may have implications for one or more recipients treated at the ORHA. Careful consideration should be given to where and how this log will be archived for the required period, and the person(s) responsible for its maintenance and safe storage should be clearly identified and documented.

Some TEs require the ORHA to return a traceability form or card providing details that will be sufficient to unambiguously identify the recipient and the applied tissues and cell. A copy of the documentation of these details should be retained in the recipient's medical record. Returning the card does not release the ORHA from its responsibility to maintain the above-mentioned traceability records for 30 years after clinical use or final disposal. Where cards or forms are returned to the TE, the manner of documentation should adhere to national data-protection regulations and should ensure that confidential information is stored in secure systems and that the recipient's privacy is not compromised in any way.

It is highly recommended that when individuals who have been treated with human tissues or cells are discharged from an ORHA, their discharge documentation should specifically mention this fact. Hence, general practitioners looking after the patient in the longer term will be able to associate unexpected symptoms with possible transmission or other reactions from the tissues or cells applied. Moreover, general practitioners should be advised to report any suspicious or unusual findings to the ORHA.

13.14. Recipient follow-up and clinical outcome registries

Depending on the healthcare system, a routine clinical follow-up of the tissue or cell recipient is performed either by the ORHA or by another healthcare organisation. The extent and duration of this routine clinical follow-up should, where possible, be standardised for each tissue and cell product and application.

Clinical follow-up data are to be kept at the ORHA in the recipient's records and may be submitted to national/international clinical outcome registries. If clinical follow-up data are collected, see section 13.6 and Chapter 17 for discussion of the implications for recipient consent. In some countries there may be legal obligations to collect clinical follow-up data. In addition to registries for which clinical outcome data entry is mandatory, there are registries based on voluntary reporting of clinical data. Some of them are national, others international; some are maintained by scientific or professional associations whereas others are held by Health Authorities.

Clinical outcome registries provide real-world data that may give a more realistic overview of outcomes compared with single-centre studies. While randomised clinical trials are considered to provide the highest level of evidence, it is not always possible to apply the results more generally outside the strict inclusion/exclusion criteria of such studies. Regis-

tries fulfil an important role in allowing studies on large data sets that can be used to determine recipient outcomes for a wide range of conditions, evaluating factors that influence clinical outcome or that may increase the risk of adverse events, and for validation of TE protocols and practices [5]. Registries have been established for several tissue and cell products including HPC, medically assisted reproduction and corneal transplantation.

TEs should have a policy for introducing new or novel tissues or cells into clinical use. The level of clinical follow-up should be determined by risk assessment using standard methodology such as the Euro GTP II tool. See Chapter 18 for more information.

13.15. Adverse events and adverse reactions

Vigilance and surveillance (V&S) is addressed in Chapter 17. However, it should be noted that effective V&S relies heavily on all healthcare professionals involved, from procurement through to application.

Serious adverse reactions (SARs) may be detected during or after procurement in living donors or after application in recipients of procured tissues or cells. As SARs in recipients might result from many diverse factors associated with the clinical procedure or with the recipient's underlying condition, clinicians might not consider the applied tissues or cells to be the cause of or a contributory factor to the SAR. However, there is an obligation, legal in the EU, for ORHAs to report known or suspected SARs to the supplying TE, which then must report to the Health Authority. ORHAs are key players in the identification of relevant factors in SARs and in implementation of the Vigilance investigation. Health professionals at ORHAs have to be trained in vigilance and should collaborate with others to achieve the desired outcomes.

Serious adverse events (SAEs), if detected by the ORHA, must also be reported to the TE and the Health Authority.

TEs that supply tissues and cells should provide ORHAs with clear instructions on how to report SARs and SAEs, preferably using standardised documentation. In general, any suspected adverse reaction or event should be reported immediately by the ORHA to the TE that supplied the tissues or cells, before it is confirmed or investigated, to enable the TE to take appropriate precautionary actions to prevent harm to other recipients and to involve the TE in the investigation process. The ORHA has a key role in supporting and contributing to the TE's investigation of suspected adverse reactions and events.

13.16. Management of recalls and reviews

There are various reasons why a TE may recall tissues or cells that were distributed to an ORHA. A recall may be related to the receipt of new information on the donor's medical or behavioural history that implies a risk of disease transmission, or it may be related to the discovery of an error in processing or a fault or contaminant in a reagent or solution used in processing. It may be instigated by the TE or required by the Health Authority.

When a TE issues a recall, it will be necessary to trace very quickly all the recipients of the particular batch (or donation) of tissues or cells implicated. The existence of a centralised logbook or electronic database that maintains a two-way audit trail of tissues and cells received, with dates of use or disposal and identification of recipients, will greatly facilitate conducting a recall, so that appropriate actions may be taken. In many of the most significant cases of disease transmission arising from tissue and cell transplantation, it has not been possible to trace the fate of some of the tissues supplied for clinical use. This could leave some patients at risk and without appropriate follow-up and treatment. In these situations, centralised management of tissues and cells in the ORHA should facilitate effective action.

A review may also be required as part of an investigation of the safety of particular tissues or cells that have been applied to patients in the past. It may require recalling patients for additional testing or other investigations. Again, maintenance of a two-way audit trail is essential for effective identification of potentially affected patients.

13.17. References

1. SoHO V&S. Guidelines for healthcare professionals on vigilance and surveillance of human tissues and cells, Part 1: Tissues, available at www.notifylibrary.org/sites/default/files/SOHOV%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%201%20Tissues_0.pdf, accessed 9 April 2022.
2. SoHO V&S. Guidelines for healthcare professionals on vigilance and surveillance of human tissues and cells, Part 2: Cells, available at www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%202%20HPCs_0.pdf, accessed 9 April 2022.

3. Eisenbrey AB, Eastlund T. *Hospital tissue management: a practitioner's handbook*. Bethesda MD, USA: American Association of Blood Banks, 2008.
4. Notify Library. The global vigilance and surveillance database for medical products of human origin, available at www.notifylibrary.org, accessed 9 April 2022.
5. Armitage WJ, Claesson M. National corneal transplant registries. In Hjortdal J, ed., *Corneal transplantation*, pp. 129-38, New York: Springer, 2016.

Chapter 14. **Computerised systems**

14.1. Introduction

Computerised systems are playing an ever-increasing part in the management of business operations, including those related to healthcare. Tissue establishments (TEs) and donor registries may use a wide range of computerised systems. These can range from simple stand-alone computer systems that use a software package to track and trend data to fully integrated systems that control a range of processing steps and present data that will allow release of tissues and cells for clinical applications. In some cases, these systems are relied upon to record consent and donor identity. Computerised systems may also have a role in managing the quality system documentation, and controlling the facility (premises) or ensuring that the required environmental conditions, such as air-pressure differentials or particle counts, are maintained (e.g. a building-management system).

Errors and malfunctions of computer systems can go unnoticed and might have serious consequences [1]. Changes in software must be managed carefully to ensure that data have not been corrupted or reorganised in a manner that changes their meaning or impact. A review conducted in 2010 reported the discovery of a systematic error in the documentation of wishes of organ donation that had probably occurred in 1999 and which potentially affected the records of > 900 000 individuals.

Computerised systems help to bring efficiency to processes. However, if they record critical information with an impact on donation, processing and release of tissues and cells, they must be selected and validated just like any other piece of critical equipment [2].

14.2. Planning the implementation of a computerised system

Figure 14.1 describes the different steps in implementing a computer system. It illustrates the design flow and documents related to specific phases (life-cycle documentation), together with the division of responsibilities between supplier/vendor and user with regard to testing, user instructions, maintenance, system improvements and access to source code. Diagram A reports the models for system software categories 4-5 and diagram B reports the simplified model for system software category 3 (as in Table 14.1). Before implementation of a computerised system at a TE, it is advisable that the user has close contact with their information technology (IT) department, or an IT consultant independent of any supplier of computerised systems.

The TE needs to:

a. define the system by generating a written description of the functions that it is designed to carry out, and all human interactions, i.e. functional and non-functional requirements, to define the user requirements specifications (URS). The URS will be the basis for subsequent testing and verification of the developed/supplied system. A list of minimal requirements for the computerised system includes (but is not limited to):

Table 14.1. **An approach to verification and control of computerised systems by system category**

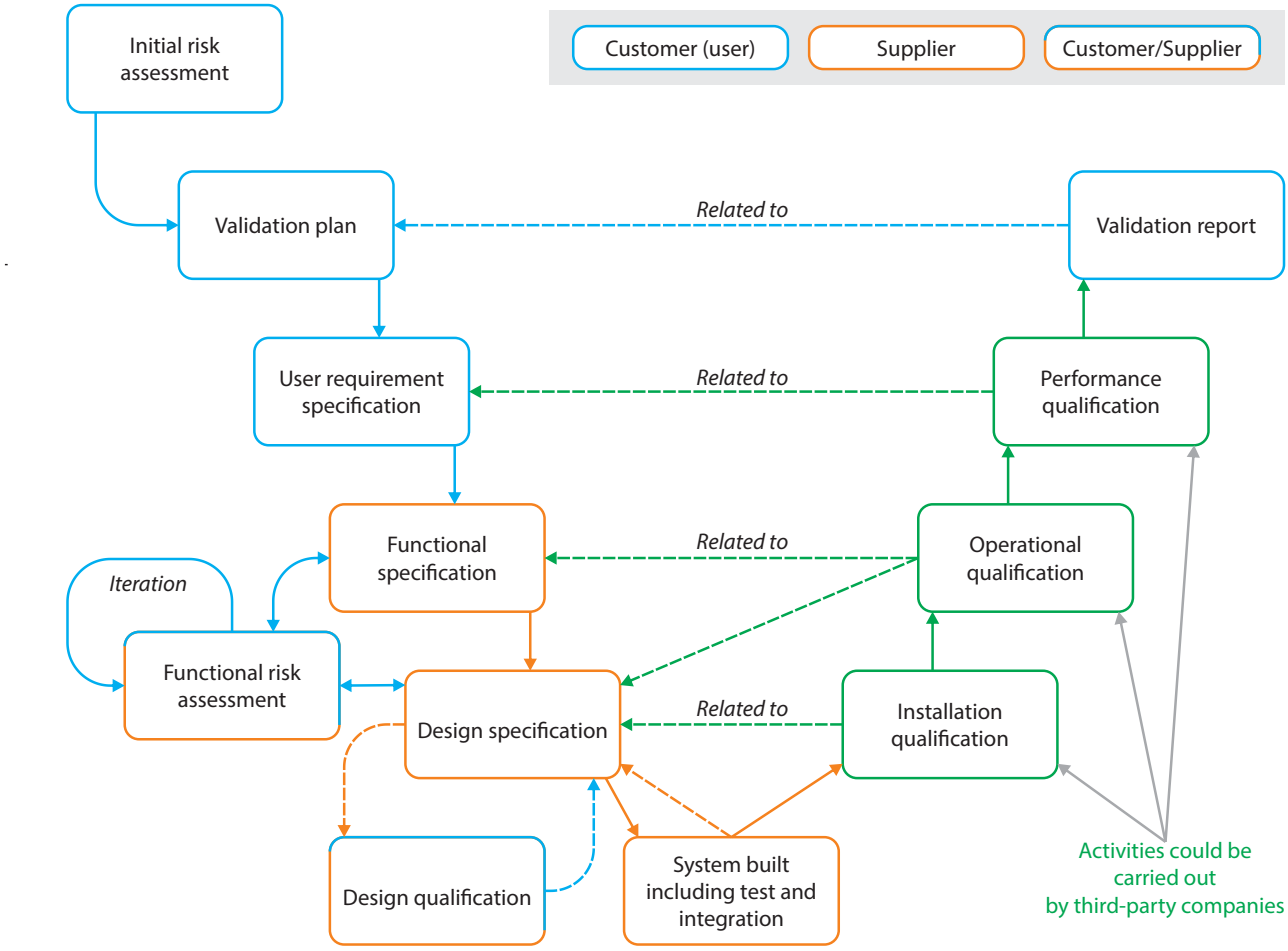
Category	Description	Typical examples	Typical approaches
Infrastructure software (Cat.1)	• Software on which applications are built • Software used to manage the operating environment	• Operating systems • Database engines • Statistical packages • Spreadsheets • Network monitoring tools • Scheduling tools • Document version control tools	• Record version number and verify correct installation by following approved installation procedures
Non-configured (Cat.3)*	• Software cannot be configured to suit the specific process, but working parameters can be set to suit the intended use.	• Firmware-based application • Commercial off-the-shelf software packages • Instrument software (e.g. software associated with machines used for testing bacteriology or serology, cell counters)	• Specify user requirements before selection • Risk-based approach to supplier assessment • Record version number and verify correct installation • Risk-based tests against requirements as dictated by use (for simple systems, regular calibration may substitute for testing) • Procedures in place for maintaining compliance and fitness for intended use
Configured (Cat.4)	• Software, often very complex, that can be configured by the user to meet the specific needs of the user's business process; software code is not altered	• Management system for donation, processing, storage and distribution of tissues and cells • Building-management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) • Clinical trial • Monitoring • Note: specific examples of the above system types may contain substantially customised elements	• Risk-based approach to supplier assessment • Demonstrate supplier has adequate quality management system • Some life-cycle documentation retained only by supplier (e.g. design specifications) • Configuration documentation supplied to the customer • Record version number and verify correct installation • Risk-based testing to demonstrate application works as designed in a test environment • Risk-based testing to demonstrate that the application works as designed within the routine environment • Procedures in place for maintaining compliance and fitness for intended use • Procedures in place for managing data
Custom (Cat.5)	• Software custom-designed to suit business process	Varies, but may include: • Internally or externally developed management systems for donation, processing, storage and distribution of tissues and cells • Internally or externally developed process control applications • Spreadsheet macro (i.e. database spreadsheet for clinical trial monitoring)	Same as for 'Configured' above, but also: • More rigorous supplier assessment, with possible supplier audits • Possession of full life-cycle documentation (as indicated in Figure 14.1)

*In GAMP4, firmware applications represented category 2, which is now integrated into category 3.

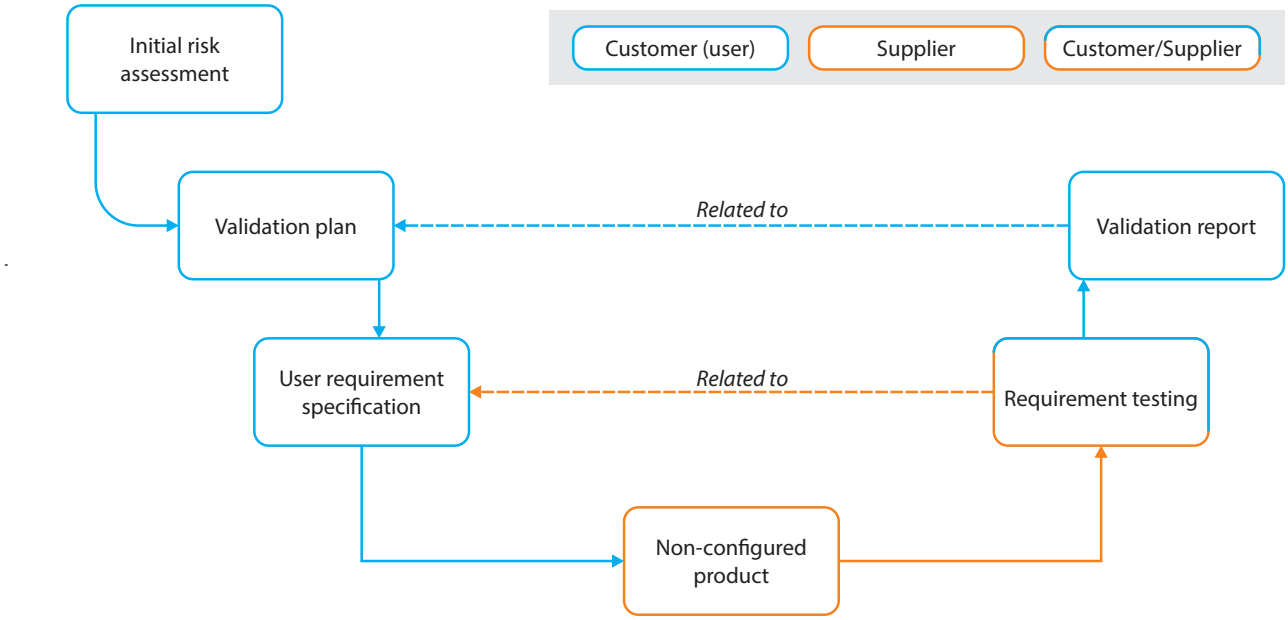
Source: International Society for Pharmaceutical Engineering. Good Automated Manufacturing Practice (GAMP) 5 [10].

Figure 14.1. **'V' model diagrams for computer system implementation**

A. Models for system software categories 4-5



B. Simplified model for system software category 3



Dashed lines indicate relationships between testing and specification documents. Section A (top) describes system software categories 4-5; section B describes category 3 (categories as in Table 14.1).

i. the need to manage calculations and printouts (e.g. reports and labels),
ii. the need for data protection, e.g. personal access to the system or parts of the system (see §14.12),
iii. the duration of and options for record storage (in general in the EU, 10 years is required for quality-system-related data and 30 years for traceability-related data),
iv. backup conditions ensuring data restoration,
v. the need to interface with other computerised systems/registries (social security registries, administrative systems, financial systems), as well as system availability over time,
vi. the need for encryption in case information is transferred over an open network,
vii. fulfilment of the applicable legal requirements if the computerised system is intended to be used for a purpose specified in the definition of a "medical device" (in the EU, this is defined in the medical devices regulation EU 2017/745),
viii. the need for audit trails (registration of GMP-relevant changes or data deletion),
ix. read-only access of Quality Assurance to the computerised system and its audit trail(s),
x. handling of data originating/accumulating from measurements,
xi. e-archives for long-term storage of data,
xii. the need to handle archive migration to other TEs in compliance with data protection and national regulatory authority directives;

b. evaluate the different systems available and choose one that meets the established requirements (though the degree of user-friendliness and maintenance should also be taken into consideration);
c. audit the developer/manufacturer to ensure that they can provide a product that meets regulatory requirements on the basis of a risk assessment (see Chapter 2).

These steps should ensure that the user has all the necessary information about the system to be purchased and that the IT department or IT consultant has received the relevant technical information. It is recommended that the developer/supplier of the computerised system receives proper information about integrated systems that have to be linked to the system to be purchased. This course of action also minimises the need for 'work around' by the user (which can be a source of error).

The computer system that manages the activities of a TE usually includes hardware, software, peripheral devices and documentation such as manuals and standard operating procedures (SOPs). For further information, refer to the International Organization for Standardization ISO/IEEE 12207:2017, ISO/IEC/IEEE 29148:2018, ISO/IEC 27001:2013 and ISO/IEC 27007:2011 [3, 4, 5, 6].

14.3. Qualification and testing

The guidance in Chapter 2 on the qualification of new equipment should be taken into account. The qualification of computerised systems in a TE should be incorporated in the general validation plan of the centre, which should include:

a. the identity of the computerised systems and interfaces that are subject to qualification;
b. a brief description of the verification strategies for different categories of computerised systems, as well as other qualification activities;
c. an outline of the protocols and related test procedures for all qualification activities of the computer system (the reporting requirements for documenting the qualification exercises and related results should also be defined);
d. the identity of the responsible persons of the computerised system, and their responsibilities as part of the qualification programme.

The level of qualification required for computerised systems is dependent on the criticality of the systems to the quality and safety of the tissues and cells. Therefore, a criticality rating based on a risk assessment should be applied to all computerised systems in place. The method of verification of these critical systems depends on the type/category of software used. Table 14.1 gives some examples with suggested approaches to verification.

Verification should be commensurate with level of risk, intended use and potential implications of malfunction to quality and safety.

Before qualification of a newly installed computerised system can be carried out, a full set of documentation that is as detailed as necessary to ensure appropriate operation of the system must be in place. The documentation should include:

a. a detailed specification (inventory) of the hardware, software and peripheral devices, including their environmental requirements and limitations;
b. diagrams or flowcharts of the system's operations that describe all component interfaces, a network diagram and all database structures (e.g. file sizes, input and output formats) – if applicable: i.e. for system software categories 4 and 5;

c. SOPs that describe how the system is used. The user should develop the SOPs, based on the instructions for use provided by the software developer and the internal procedures of the establishment. In particular, SOPs should address all manual and automated interactions with the system, including:
   i. routine backup, maintenance and diagnostic procedures, including assignment of responsibilities,
   ii. safety leading indicators [5, 6, 7],
   iii. 'work-arounds' for system limitations,
   iv. procedures for handling errors, including assignment of responsibilities,
   v. procedures for handling disasters and contingency planning, including assignment of responsibilities,
   vi. procedures for database amendments by formal request by authorised persons,
   vii. procedures for verification of a change,
   viii. a training system that includes manuals, documentation and procedures for training.

Verification documents and the results of tests undertaken and approved by the supplier/vendor or developer of the system must be part of the documentation supplied to the user. The user can then carry out tests according to a predefined and documented test plan [8]. Types of risk to consider include inadequate design of a system, errors that may occur in use (errors of use or system defects) and loss or distortion of data [9]. Testing should involve the entire system, and in the manner in which it is expected to perform routinely in the establishment. Testing may be done by a third party but, in that case, must also include personnel from the TE. The organisation for ownership, system management, maintenance and support, and the plan for regular internal revisions, should be included in the quality-management system (see Chapter 2). If the operating system and/or firmware platform are subject to changes, including but not limited to patching or updating for security reasons, an analysis should be performed to evaluate whether those changes could affect the system, and a revision of the quality system should be done to assure the procedures (see §14.4).

The following types of basic testing are examples of what should be conducted initially and when new versions of the software are installed:

a. *Functional testing of components*
   Initial qualifications are usually carried out by supplier/vendor, who will provide the user with documentation related to the tests performed. At minimum, documents should include:
   i. details of the methods employed to conduct verifications and testing of requisites stated in the URS document,
   ii. qualification documents with results of tests (test scripts) for each functionality, including test procedure, expected result, test result, acceptance criteria, and
   iii. conformity statement with relevant signatures.
   iv. For traceability and to facilitate quality assurance review and follow-up, it is recommended that any supporting documentation (e.g. print screens) be included to verify the specific test case.

b. *Data migration*
   The process for data migration should be defined, documented and tested appropriately. This should ensure full maintenance of traceability, including archiving of data (if necessary).

c. *Environmental testing (installation and verification instructions, or IVI)*
   In the actual operating environment, functional tests are carried out to demonstrate that:
   i. the software systems work appropriately with the hardware,
   ii. all applications of the software perform appropriately with the software operating system,
   iii. appropriate information passes correctly through system interfaces, including appropriate data transfer to or from other laboratory and automated (e.g. serology testing, cell counting) systems (if applicable),
   iv. accessories such as barcode scanners perform as expected with the barcodes in use (if applicable),
   v. printed reports are formatted appropriately and correctly,
   vi. personnel are trained and use the system correctly,
   vii. the system performs appropriately at peak production times and with the maximum number of concurrent users,
   viii. backups restore data in a correct and regular way.

Moreover, monitoring of personnel usage training should be performed periodically as part of the quality system.

14.4. Change control

In case of changes in the software, the verification status needs to be re-established. If re-verification analysis is needed, it should be based on risk assessment and conducted not only for verification of the individual change, but also to determine the extent and impact of that change on the entire computerised system.

Changes should be handled following the V diagram in Figure 14.2. Depending on the system, it may be desirable to have a test version of the computerised system containing the same data (mirrored).

14.5. Maintenance and scheduled operations

Data should be checked periodically and systematically by qualified IT personnel to identify and remove unwanted data (e.g. duplicate records) and to ensure that data entries are stored accurately and appropriately. Manual entry of critical data requires independent verification by a second authorised person, and this step can be part of the software requirements.

Please refer to ISO/IEC 14764 for further guidance on maintenance [9].

Security should cover at least:

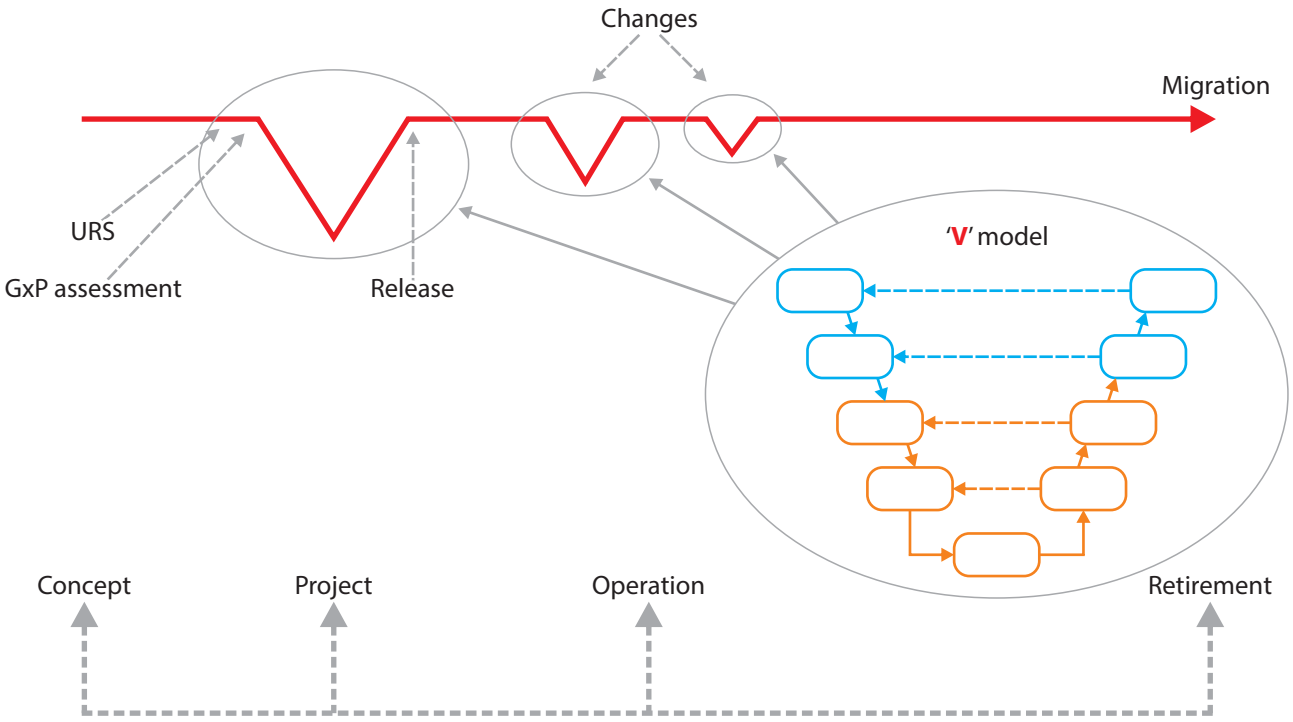
a. an adequate change history of the system, for both software and hardware (when necessary);
b. periodically altering electronic passwords and removing unnecessary or outdated access;
c. creating records of all data changes (i.e. an audit trail), including a retained record of previous data and the reason for the change;
d. appropriate use of malware (e.g. computer virus) protection programs;
e. control of administrative security access to ensure that only authorised personnel can make changes to the software, system configuration and data;
f. regular testing to verify the appropriate integrity and accuracy of backed-up data;
g. consider if a permanent storage (e-archive) of specific data is necessary.

14.6. Quality assurance

The data-processing system should be considered as critical equipment within the quality-assurance programme, which as a minimum should:

a. ensure the ongoing accuracy and completeness of all documentation on equipment, software maintenance and operator training;
b. undertake audits periodically to verify appropriate accomplishment of all performance

Figure 14.2. **Handling of software life-cycle activities**



Supplier may provide knowledge, experience, documentation and services throughout life-cycle.

URS: user requirements specifications. GxP: good [specialism] practice. Orange: user. Blue: supplier.
Source: modified from ISPE Good Automated Manufacturing Practice (GAMP) 5 [10].

tests, routine maintenance, change procedures, data-integrity checks (including audit-trail checks), error investigations and operator-competence evaluations.

Appendix 26 contains an example of a checklist that can be used for internal or external audits.

14.7. Industry guidance for verification of computerised systems

The most common industry guide used for verification of computerised systems is that from the International Society for Pharmaceutical Engineering (ISPE) [10]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [11].

14.8. Regulations and official guidance on verification of computerised systems

Regulation of computerised systems is well established in the pharmaceutical industry, with EU Good Manufacturing Practices (GMP) [12] acting as the regulatory reference in the EU. The advice of the OECD Working Group on Good Laboratory Practice on applying these principles to computerised systems is also recommended [13]. Inspectors in the EU also use the Pharmaceutical Inspection Co-operation Scheme Guidance (PIC/S) [14]. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [15]. These regulatory documents can be useful sources of reference for TEs.

If a computerised system replaces a manual operation, there should be no decrease in product quality, process control or quality assurance, as well as no loss of data. There should be no increase in the overall risk of the process [12].

The PIC/S document also lists the critical items that an inspector should consider during inspection and is a valuable tool for TEs since it details the minimum requirements that should be in place [14]. Appendix 26 contains a checklist adapted from ISO/IEC 27007:2011 and the guidance document of the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

14.9. Infrastructure

Infrastructure is necessary in order to guarantee the correct data handling between work stations hosting the computer system and the relevant server(s). Infrastructure includes but is not limited to communication by physical lines (e.g. ethernet), switches and routers. Correct design of a computer system must consider the use of suitable tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability and behavioural-modelling tools). With increasing availability of flexible working solutions, some infrastructure limitations may ensue from the use of personal work stations.

14.10. Failure of the system

For computerised systems that support critical processes, provision (e.g. disaster recovery or business contingency plan/procedure) should be made to ensure continuity of support for those processes in the event of a system breakdown (e.g. a manual or alternative system). The time required to enact alternative arrangements should be based on risk assessment and should be appropriate for the particular system and the business process it supports. These arrangements should be documented and tested adequately [12]. Testing of these alternative systems and their ability to retrieve data should be assessed periodically (based on risk assessment).

14.11. Electronic signature

Records may be signed electronically. According to Annex 11 of EU GMP [12], all electronic signatures are expected to:

a. have the same impact as handwritten signatures within the boundaries of the organisation;
b. be permanently linked to their respective record;
c. include the reason, the time and date that they were applied.

14.12. Data protection

Critical and sensitive data must be protected from unauthorised information modification and from unauthorised information access/release. Procedures for personal data protection must comply with national legal requirements or, for EU countries, with the requirements defined in Regulation EU 2016/679 and Directive 2010/45/EU on the Protection

of natural persons with regard to the processing of personal data and on the free movement of such data.

Appropriate technical and organisational measures must be taken to guarantee a level of security appropriate to the risk, measured against the context and purposes of the processing. The factors which may be analysed to determine the appropriateness of the measures include degree of data sensitivity, the risks to data subjects in the event of a breach, and the costs involved in implementing specific types of security measures. The latter may include:

a. encryption or pseudonymisation;
b. measures to ensure the confidentiality, integrity and resilience of processing systems;
c. methods which enable the timely access to, restoration of or availability of personal data in the event of an incident;
d. regular tests and evaluation to ensure that the measures implemented meet their desired objective of maintaining security of data processing.

All personal data stored in computerised systems must be stored in a secure manner, with access available only to authorised personnel. The system should ensure data inalterability, and an audit trail with registration of data access and modifications, including date and identification of personnel executing modifications. For those applications in which all users should not have identical authority, some scheme is needed to ensure that the computer system implements the desired authority structure.

14.13. Archiving

Critical data must be archived in a long-term stable medium and placed 'off site' physically or as an e-archive backup at a location remote from the hardware, to ensure secure storage. Backup is essential for disaster recovery, and a common approach, one that keeps data safe in almost any failure scenario and is easy to remember, is the 3-2-1 backup rule: there should be at least three different versions of your data over different periods of time stored on two different pieces of media, one of which is placed off-site [16].

Archived critical data should be checked for accessibility, readability and integrity. If changes are made to the system (e.g. new computer equipment or software is installed), then the ability to retrieve archived data must be ensured and tested [12]. Archiving should be conducted using secure software methods such as databases compliant with ACID (atomicity, consistency, isolation and durability) requirements, that guarantee data integrity. Files should be stored in databases, if possible in a time-durable format. Among formats more commonly used are software-encrypted files and CRC (cyclic redundancy check)-secured files, that require dedicated software to be managed. As this characteristic may condition future retrieval, an ISO-standardised version of the Portable Document Format (PDF), called PDF/A [17], has been implemented to ensure document reproduction using any device in years to come, as the format is independent of hardware and software platforms.

14.14. References

1. Borycki E, Dexheimer JW, Hullin Lucay Cossio C *et al.* Methods for addressing technology-induced errors: the current state. *Yearb Med Inform* 2016 Nov 10;(1): 30-40. https://doi.org/10.15265/IY-2016-029, accessed 7 July 2022.
2. Leveson N. *Engineering a safer world: systems thinking applied to safety*. Cambridge MA: MIT Press; 2012.
3. ISO/IEC/IEEE (International Organization for Standardization/International Electrotechnical Commission/Institute of Electrical and Electronics Engineers) 12207:2017. Systems and software engineering – Software life cycle processes.
4. ISO/IEC/IEEE 29148:2018. Systems and software engineering – Life cycle processes – Requirements engineering.
5. ISO/IEC 27001:2013 Information technology – Security techniques – Information security management systems – Requirements.
6. ISO/IEC 27007:2011 Information technology – Security techniques – Guidelines for information security management.
7. Leveson N. A systems approach to risk management through leading safety indicators, available at http://sunnyday.mit.edu/papers/leading-indicators-final.pdf, accessed 10 April 2022.
8. IEC (International Electrotechnical Commission) 62366. Medical devices: application of usability engineering to medical devices.
9. ISO/IEC 14764. Software engineering – Software life cycle processes – Maintenance.
10. International Society for Pharmaceutical Engineering. Good Automated Manufacturing Practice (GAMP) 5: a risk-based approach to compliant GxP computerised systems, available at www.ispe.org/gamp-5, accessed 10 April 2022.
11. British Committee for Standards in Haematology. *Guidelines for the specification, implementation and management of information technology (IT) systems in hospital transfusion laboratories*, June 2017, available at https://b-s-h.org.uk/media/15774/transfusion-jones-specification-implementation-and-

management-of-information-technology-systems-in-hospital-transfusion-laboratories.pdf, accessed 10 April 2022.

12. European Commission. Good Manufacturing Practices, Vol. 4: Human and veterinary, Annex 11: computerised systems, available at https://ec.europa.eu/health/document/download/8d305550-dd22-4dad-8463-2ddb4a1345f1_en, accessed 10 April 2022.
13. OECD Working Group on Good Laboratory Practice. *Application of GLP principles to computerised systems*, Advisory Document No. 17 (ENV/JM/MONO (2016) 13), available at www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono (2016) 13&doclanguage=en, accessed 10 April 2022.
14. PIC/S (Pharmaceutical Inspection Co-operation Scheme). Good practices for computerised systems in regulated GxP environments (PI 011-3), available at www.picscheme.org, accessed 10 April 2022.
15. FDA (United States Food and Drug Administration). Title 21 Code of Federal Regulation (CFR) Part 11, Electronic records; electronic signatures—scope and application, available at www.fda.gov/regulatoryinformation/guidances/ucm125067.htm, accessed 10 April 2022.
16. Ruggiero P, Heckathorn MA. Data backup options. United States Computer Emergency Readiness Team (US-CERT), 2012, available at https://us-cert.cisa.gov/sites/default/files/publications/data_backup_options.pdf, accessed 10 April 2022.
17. ISO 19005-1. Document management – Electronic document file format for long-term preservation – Part 1: Use of PDF 1.4 (PDF/A), available at www.pdfa.org/publications/?wpv_view_count=3934-TCPID3755&wpv_sort_orderby=post_date&wpv_sort_order=desc&wpv_paged=7, accessed 10 April 2022.

Related material

Appendix 26. Checklist for revision of computerised systems

Chapter 15. **Coding, packaging and labelling**

15.1. Introduction

The quality and safety of tissues and cells is dependent not only on the way they are procured or processed, but in the way they are coded, packaged and labelled before being sent to an organisation responsible for human application [1, 2]. The World Health Organization (WHO) has published an *aide-mémoire* on the key safety requirements (including storage, packaging and labelling) for essential minimally processed human cells and tissues for transplantation, and some countries have adopted legal requirements to ensure that human tissues and cells are appropriately packaged, labelled and coded [3]. These steps are also addressed in the EU tissues and cells legislation. This chapter offers guidance on the coding of tissues and cells, and their packaging and labelling requirements are discussed.

15.2. Coding

With increasing movement of tissues and cells across borders, the capacity to uniquely identify them is essential. This can be achieved by coding that facilitates tracing the tissues and cells from donor to recipient and vice versa while respecting data protection and confidentiality rules.

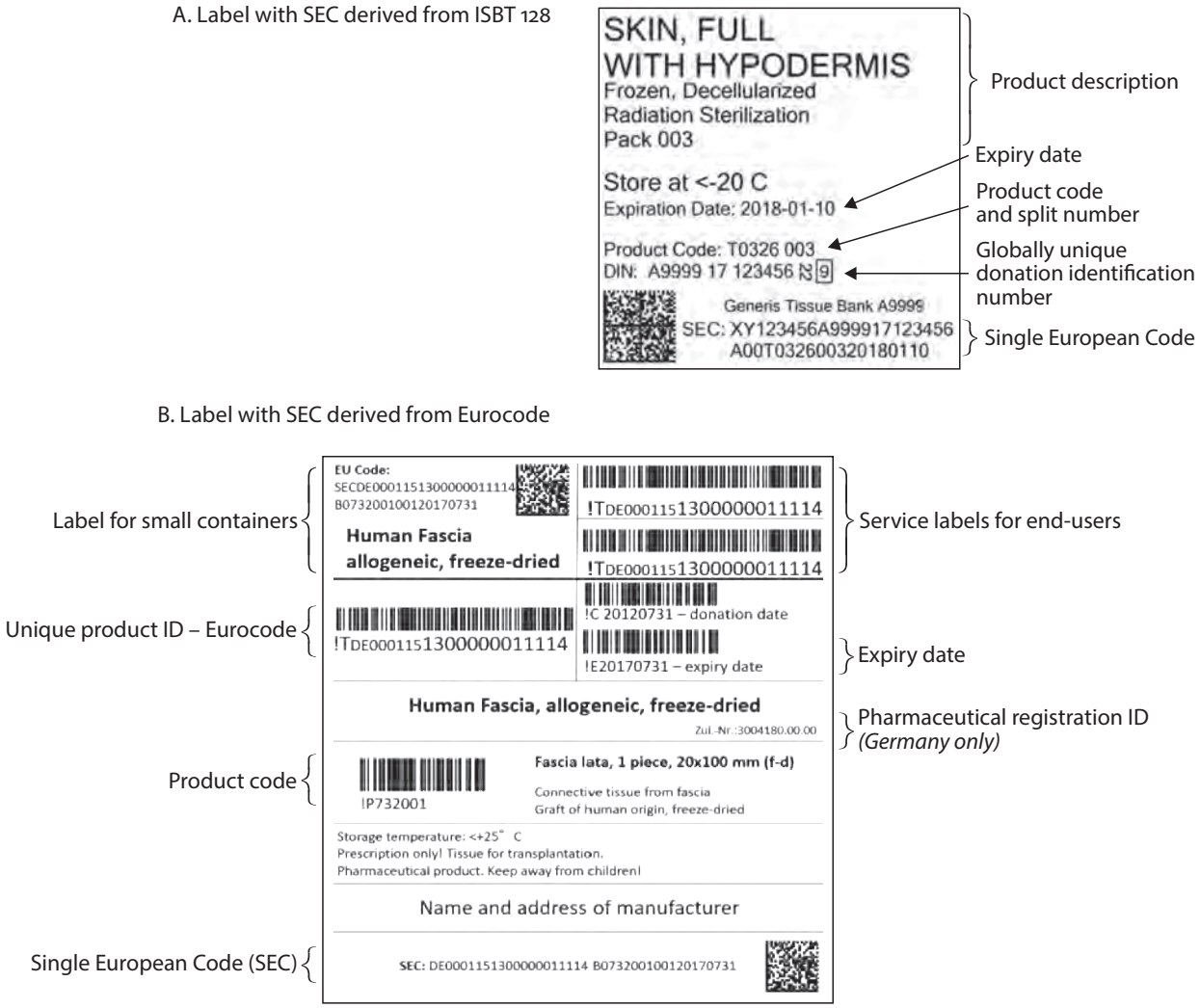
Coding started with the development of local coding systems applied in individual tissue establishments, but in the last two decades there has been significant movement towards the use of national and international coding standards, building on the longer and more consolidated experience in blood transfusion.

15.2.1. ISBT 128

ICCBBA (the International Council for Commonality in Blood Banking Automation) manages ISBT 128 [4, 5, 6], which is the most widely used information standard for medical products of human origin, including tissues and cells. ICCBBA is a not-for-profit, non-governmental organisation in official relations with the WHO, and ISBT 128 is endorsed by 21 scientific and professional organisations. The standard is developed and maintained with input from more than 250 volunteer experts in the fields of transfusion and transplantation from around the world and provides a structured product terminology with more than 2 500 defined cell and tissue codes. In January 2020, the ICCBBA standardised the terminology of almost all types of cells and tissues and based it on the concepts of Class, Modifiers and Attributes [4, 5].

Due to the growing number of cell donors worldwide, the World Marrow Donor Association has developed a unique global donor identifier to guarantee safe and unequivocal donor identification: the Global Registration Identifier for Donors (GRID) [7]. In September 2017, ICCBBA issued a new ISBT 128 standard [7, 8] describing its rules.

Figure 15.1. **Examples of finished tissue product labels with the Single European Code**



15.2.2. Eurocode

Eurocode International Blood Labelling Systems e.V. (Eurocode IBLS) [9] is a not-for-profit association under German law. Eurocode IBLS manages the coding standard Eurocode, which is an ISO15418-listed information standard providing worldwide unique identifiers for labelling blood products, cells and tissues to enhance security in blood transfusion and cell and tissue transplantation. Today it is used in various countries, including Germany and Austria.

15.2.3. Single European Code for tissues and cells

In 2015, the European Commission adopted Directive 2015/565/EC, amending Directive 2006/86/EC [10] as regards certain technical requirements for the coding of human tissues and cells, and establishing the EU Coding Platform and the Single European Code. The EU Coding Platform (https://webgate.ec.europa.eu/eucoding) provides access to the EU Tissue Establishment Compendium, the EU Tissues and Cells Products Compendium (EUTC) and a code-translator application. The directive introduces the obligation on tissue establishments to affix a Single European Code (SEC) on tissues and cells distributed or imported for human application in the EU or exported from the EU [11]. The directive also sets out the requirements for its application (including exceptions) and the general obligations of tissue establishments, competent authorities and the European Commission.

The SEC provides for standardisation across the EU. The permitted product coding systems are ISBT 128, Eurocode and the EUTC (Figure 15.1). The ISBT 128 and Eurocode product descriptions are mapped to the high-level product description codes provided by the EUTC (see §15.2.3.3 below).

15.2.3.1. *Application of the Single European Code*

Except for the exemptions described later, application of the SEC [11, 12, 13] by EU tissue establishments is mandatory for all tissues and cells distributed for human application. When tissues and cells are

released for circulation (e.g. to other tissue establishments, third parties, manufacturers of advanced therapy medicinal products), the primary package must include a unique identification number or code and the donation identification sequence (DIS) (see Table 15.1). If the container is too small to include the DIS on the label, the DIS must be included in the accompanying documentation.

There are some general exemptions to the requirement for application of the SEC code. These include partner donation of reproductive cells, tissues and cells distributed directly for immediate transplantation to the recipient (e.g. HPC), and tissues and cells imported from non-EU countries into the EU in cases of emergency that are authorised directly by the Health Authorities. EU member states may also allow exemptions for tissue and cells other than partner gamete donation, when these tissues and cells remain in the same centre or when tissues and cells that are imported from non-EU countries into the EU remain within the same healthcare facility from importation to application (provided that the healthcare facility is a tissue establishment authorised to import tissues and cells).

Application of the SEC does not preclude additional application of other codes in accordance with the national requirements of EU member states.

Countries already using existing coding systems compatible with the SEC requirements (i.e. ISBT 128, Eurocode) with a standard for barcoding and other forms of machine readability can continue using those systems while incorporating the new legal requirements. There will also be the potential of making the SEC machine-readable in the future. The use of machine-readable barcode labels will ensure the accuracy of records, as manual transcription errors will not occur, and the machine output can easily be entered into electronic databases.

Tissues or cells imported from third countries for distribution in the EU must also be labelled with the SEC (unless the EU member state applies the exemption above). The importing tissue establishment is responsible for the application of the SEC on the product and in the accompanying documentation (double coding/labelling with both the original code and the SEC).

The DIS (see Table 15.1) must use the tissue establishment number allocated to the importing tissue establishment in the EU Tissue Establishment Compendium. Imported tissues or cells that are already labelled with a globally unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS) must use this as the unique donation number. If the imported tissues or cells do not carry an identifier from one of these systems, the importing tissue establishment must assign its own unique number. The importing tissue establishment must retain traceability mapping between the identifier they have assigned and the original identification of the imported tissues or cells. Consideration must be given to the possibility that the original identifier may not be unique if products are received from more than one source where the suppliers have used local donation numbering systems; it is quite possible that the same identifier may be used by different suppliers to identify completely different donations. The traceability mapping must therefore include both the original identifier and the supplier identification.

If the imported tissues or cells are already labelled using ISBT 128 or Eurocode, the product code from the original label may be used in the product identification sequence (see Table 15.1) provided that this code is listed in the EU Product Compendium. In all other cases, the importing tissue establishment must assign a product code from one of the three product coding systems (EUTC, ISBT 128 or Eurocode) that is listed in the EU Product Compendium and most accurately describes the imported tissues or cells.

Split numbers carried by imported tissues or cells that do not exceed three alphanumeric characters can be used directly in the SEC. If the imported tissues or cells carry a longer split number, or where no split number is provided, the importing tissue establishment must assign a new split number with a maximum of three alphanumeric characters to ensure uniqueness of the SEC. Particular care needs to be taken where the product code being assigned to

Table 15.1. **Single European Code for tissues and cells**

Donation Identification Sequence			**Product Identification Sequence**			
Tissue establishment code		Unique donation number	Product code		Split number	Expiry date (yyyymmdd)
ISO country code	Tissue establishment number		Product Coding System identifier	Product number		
2 alphabetic characters	6 alpha-numeric characters	13 alpha-numeric characters	1 alphabetic character	7 alpha-numeric characters	3 alpha-numeric characters	8 numeric characters

Source: Annex VII of EU Directive 2015/565.

the imported tissues or cells is more generic than the original product; for example, bone rings and bone dowels imported with the same donation number where each product is identified by a product code assigned by the supplier and with a split number of 001. The importing tissue establishment applies the SEC using the EUTC code of MUSCULOSKELETAL, BONE, SHAPED GRAFT, which means that the two different original product codes have now been mapped to one EUTC code. It is, therefore, no longer possible to use the allocated split numbers as this would result in duplication and the importing tissue establishment must assign a new split number and retain records to map back to the original identifiers.

15.2.3.2. *Structure of the Single European Code*

The SEC is a unique identifier that consists of two elements: a donation identification sequence that indicates the origin of the tissue or cells, and a product identification sequence that describes the type of tissue or cells. Further details are specified in Annex VII to the directive (see Table 15.1).

15.2.3.2.1. Donation identification sequence

The coding system must identify each donation event because donors can potentially donate tissues and cells on several occasions (e.g. an individual may donate gametes and HPC when alive and corneal tissue after death). Each tissue establishment authorised in an EU member state must use the tissue establishment number allocated in the EU Tissue Establishment Compendium which, in combination with the International Organization for Standardization (ISO) country code, will create the tissue establishment code.

Each tissue establishment must assign a unique number for the donation based on the donation identification system in place in their country. Donation numbers with fewer than 13 characters will be padded with leading zeros in the SEC. The unique donation number may be created locally by the tissue establishment, centrally (by a Health Authority) or globally as a unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS).

Taken together, these codes will ensure that each donation event will have a unique donation identification number that can be used to label each tissue. In the case of pooling of tissues and cells, a new donation identification number must be allocated to the final product.

15.2.3.2.2. Product identification sequence

The product identification sequence consists of the assigned product code, a split number (if applicable) and the expiry date of the product (if applicable) in ISO standard format (yyyymmdd). For tissues and cells without a defined expiry date, the expiry date must be 00000000. The product code includes an identifier of the coding system used ('E' for EUTC, 'A' for ISBT 128 and 'B' for Eurocode) followed by the appropriate product number corresponding to the tissue/cell type.

As explained above, and also taking into account the coding practices used by the EU member states, the SEC provides a flexible solution by allowing tissue establishments to use one of three product coding systems (EUTC, ISBT 128, Eurocode) for which all tissue and cell codes have been included in the EU Tissue and Cell Product Compendium. Tissues and cells in the three product coding systems are mapped to each other to ensure that a tissue or cell code in the SEC can be 'translated' irrespective of the system used. EUTC provides only the basic nomenclature, but ISBT 128 and Eurocode include more detailed product information (e.g., EUTC may represent a product type such as a tendon, whereas the other two systems may specify whether the tendon is whole, shaped or irradiated).

EU member states may decide to permit tissue establishments to use only one product coding system (EUTC, ISBT 128 or Eurocode), or more than one in parallel.

The SEC on the label attached to each product will be in eye-readable format and preceded by the abbreviation 'SEC'. The DIS and product identification sequence must be separated by a single space or as two successive lines. Using ISBT 128, a data structure is available to allow the SEC to be machine-readable.

The coding system for traceability of reproductive tissues and cells started to be obligatory from April 2017. Now, tissue establishments distributing reproductive tissue and cells must provide the SEC within the transport documentation. For reproductive samples stored before April 2017 or within the transitional period, centres may need to create the SEC manually by combining the donation identification number with the product identification number.

During the manual creation of the sequence, and taking into consideration the importance of the number of digits of the SEC, double witnessing protocols have to be applied if in-house automation is not possible during the SEC generation.

An example of SEC for reproduction cells can be found in the article by Alteri *et al.* [14].

15.2.3.3. *EU Coding Platform*

The EU Coding Platform introduced by Directive 2006/86/EC (as amended by Directive 2015/565/

EC) is the major tool for implementing the SEC requirements. It is an IT platform hosted by the Commission and it contains the EU Tissue Establishment Compendium and the EU Tissue and Cell Product Compendium [15].

a. The EU Tissue Establishment Compendium is the register of all tissue establishments that are authorised, licensed, designated or accredited by each EU member state's competent authority or authorities; it contains the information about these tissue establishments along with their corresponding tissue establishment codes. The EU Tissue Establishment Compendium is hosted by the European Commission and maintained by the member states' competent authorities. Each competent authority is responsible for the accuracy of the entries for the tissue establishments that they have licensed or authorised and for keeping these entries up to date.

b. The EU Tissue and Cell Product Compendium is the register of all types of tissues and cells circulating in the Union and the respective product codes under the three permitted coding systems (EUTC, ISBT 128 and Eurocode IBLS).

Acknowledging the existence of product coding systems already in use in the EU, Directive 2015/565/EC allows the use of ISBT 128 [16] and Eurocode [17] coding systems, and has put in place bilateral agreements with their managing organisations (i.e. ICCBBA and Eurocode IBLS) to ensure that updated product codes are regularly made available and included in the EU Tissue and Cell Product Compendium.

The EUTC tissue and cell product coding system was developed by the European Commission for tissue establishments not using the other two coding systems. The EUTC covers all types of tissues and cells along with high-level terminology and their corresponding product codes. A mapping of the more detailed ISBT 128 and Eurocode product codes to the generic EUTC codes is also provided on the EU Coding Platform. Each tissue and cell product must be assigned a specific code, which identifies and describes that product. The information in the SEC can be decoded by the code-translator application in the EU Coding Platform to obtain text that describes the tissues or cells and their origin.

These tools are publicly available and free of charge. Therefore, the EU tissues and cells product coding system used by EU member states may also be used by other countries. Further information on the SEC and its application can be found on the European Commission's website [11, 12, 13].

15.3. Packaging and labelling

Packaging of tissues and cells has an important role during all procedures, starting from procurement, through the processing and storage steps, to distribution and human application. Adequate packaging minimises the risk of contamination of tissues and cells, protects the persons involved in transportation and aids retention of required characteristics and biological functions.

Ensuring the traceability of all tissues and cells from the donor to the recipient is a responsibility shared by procurement centres, tissue establishments (TEs) and organisations responsible for human application (ORHAs). All of these participate and contribute actively to safeguarding, in a continuous manner, the tracking of the tissues and cells through from procurement to human application. Accurate tracking of tissues and cells allows reliable data to be scientifically assessed for potential risks to the donor, to the procurement and processing operations, and to the storage, transport and clinical use of donated material. Traceability is addressed in depth in Chapter 16. An essential aspect of ensuring accurate traceability is clear and complete labelling of tissues and cells at all stages. The system of identification for donors and recipients must be aligned with the packaging and labelling system of tissues and cells in such a way that a connection between tissues and cells, the source and the recipients exists at all times.

Labels must be attached to packaging that has been validated to demonstrate that it maintains the required properties of the tissues and cells and ensures integrity. This part of the chapter addresses good practice in packaging and labelling at all stages from donation to implantation.

15.3.1. General concepts

Packaging and labelling operations must be considered an integral part of the activities of procurement organisations and TEs. They must be included in the training of personnel and specified in all relevant procedures. Although this chapter establishes specific recommendations for packaging and labelling for the procurement and processing phases, they should equally apply to intermediate phases, such as in-process steps, in which all materials, containers, equipment and tissues and cells must be adequately identified at all times. In addition, tissues and cells procured or processed for research purposes

should be clearly identified as such on their packages and labels (e.g. 'FOR RESEARCH USE ONLY' or 'NOT FOR CLINICAL USE').

There should be written procedures describing the receipt, identification, quarantine, sampling, examination, testing and release of packaging and labelling materials, as well as the handling of such materials.

Premises and procedures for the packaging and labelling of tissues and cells must be designed to prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or, where unavoidable, adequate additional safeguards should be put in place.

Primary packaging and labelling of tissues or cells must be done in an environment specified in standard operating procedures (SOPs).

For EU member states, the requirements for packaging and labelling of tissues and cells are detailed in Annex IV of Directive 2006/17/EC, Annex II of Directive 2006/86/EC and Directive 2015/565/EC.

15.3.2. Packaging of tissues and cells

Packaging includes all operations, including primary and secondary packaging, which procured or processed tissues and cells have undergone from the start, during processing or as final packaging. Packaging aims to protect tissues and cells, and to present them to the operator (in initial or in-process packaging) or to the clinical end user (in final packaging) in a suitable manner. The type of substance of human origin and its intended use will determine the requirements needed to carry out a packaging operation in a safe manner.

Special consideration must be given to the primary packaging that will be in direct contact with tissues and cells. Containers intended to be used as primary packaging should be submitted to visual inspection before use and, if single-use containers are unavailable, the need for applying an adequate cleaning process should be assessed along with suitable sterilisation methods such as irradiation or autoclaving of materials and containers. If the cells/tissues are stored in liquid nitrogen, they must be double-bagged to prevent cross-contamination during storage [18] or stored in validated high-security packaging especially designed for liquid nitrogen (see Chapter 10, §10.1.4). The packaging materials should be stored in a clean area. In this case, the materials and the conditions under which packaging takes place must be carefully specified, assessed and approved before use. Processing facilities must establish and document validated packaging protocols.

Storage containers must be appropriate for the type of tissue or cells, the temperature and method of storage, and the intended application. They must withstand sterilisation (where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport. Each tissue or cell container must be examined visually for damage or evidence of contamination before distribution for clinical use and by the end user.

15.3.3. Labelling of tissues and cells

Written procedures must be established and followed to ensure correct labelling. Each labelling phase for all tissues or cells must be documented. Tissues and cells must be labelled during all phases of procurement, processing, storage and distribution. Labelling must be clear, legible, indelible and unique.

Before labelling a unit of donated or processed tissues and cells, the container must be inspected for evidence of impurities, defects, broken seals or contamination that could compromise the quality, integrity or safety of the product.

Labels attached to the containers should identify and describe the contents. The description should characterise the tissues and cells, and reflect key aspects of their maintenance and use. Standard nomenclature and standard international units of measurement must be used to describe the tissues and cells, and the processing they have undergone (see §15.2 on coding).

Identification should provide information on traceability that links the tissues and cells to the tissue establishment of origin and, ultimately, the donor. When tissues or cells are to be distributed internationally, language barriers should be considered, and information translated or coded to ensure understanding.

For autologous or directed donations, the name or identifier of the intended recipient must be included in the label. Further guidance on traceability is provided in Chapter 16.

The production of labels must be controlled. When applicable, reconciliation of labels that have been edited, used or returned/rejected must be undertaken according to written procedures. All excess labels containing quality or traceability information must be destroyed or maintained in a secure manner, when necessary, to prevent mix-ups. Obsolete, unused labels must be destroyed according to written procedures.

It is highly recommended to undertake labelling and packaging simultaneously, in a continuous

process, to reduce the risk of mix-ups or cross-contamination. Before application to the container, printed labels must be carefully examined to ensure that the information they contain conforms to the corresponding tissues or cells. The results of this examination should be documented at identified critical stages. Labels must be designed to adhere firmly to the container under all anticipated storage and transport conditions. The label applied must not be removed, altered or obscured. A sufficient area of the container must remain uncovered to permit inspection of the contents, whenever possible.

Where additional labels are applied to packaging, an automated verification step to ensure the correct match between container label and package label is recommended.

For processing of batches that include large numbers of individual final units, a representative printed label should be included in the processing batch record.

In the European Union, the requirements for final labelling of tissues and cells for distribution are detailed in Annex II.E of Directive 2006/86/EC. Following the adoption of Directive 2015/565/EC, the label also needs to include the SEC and, for imported tissues and cells, the country of procurement and the exporting country (if different from the procurement country).

15.4. Sample and documentation labelling

All key cell and tissue samples for testing or archiving and all related documents must be labelled in a legible, indelible and unique manner that ensures traceability to the donor and the associated donations. A record of the time and place the sample was taken must be included on the label or in accompanying documentation.

15.5. Management of packaging and labelling materials

Selected packaging material must be able to withstand the storage temperature (ambient temperature, refrigeration, freezing, cryopreservation) and the sterilisation procedure (if this is to be applied) that are needed to preserve the required characteristics of the tissues or cells and, if applicable, biological function. Additionally, the shipping container must be able to maintain this environment for an appropriate amount of time during transport. Primary packaging and transport containers used for tissues and cells should be validated for this purpose, and they must be suitable for use with human materials (see Chapter 2). Selection of packaging, or a combination of packaging systems, should result in a sealed environment that prevents leaks.

As a general rule, labels should be machine-printed for clarity. They should be printed with ink that does not run or otherwise become unreadable when exposed to water or other liquids. Labels must maintain integrity and remain attached to primary packages and transport containers at the storage temperatures.

All printed labels for primary packaging, secondary packaging and for documentation intended to accompany the tissue or cell product should be stored in access-controlled areas.

Management of packaging and labelling materials must include the following elements:

a. there must be written specifications for all packages and labels used for tissues and cells;
b. there must be documented procedures describing the receipt, identification, quarantine, sampling, examination, testing, release and handling of both packaging and labelling materials;
c. a version control system should be in place to guarantee use of the current approved version. If a change of version occurs with regard to labels, inserts or packages, the actions needed to ensure that only the latest version is attached to the tissue or cells should be described in a written manner;
d. the suitability of packaging material, containers and labels for their intended purpose must be documented.

15.6. Packaging and labelling for procurement operations

15.6.1. Primary packaging and labelling for procurement operations

'Primary packaging' refers to the materials that will come into direct contact with the tissues and cells and are, therefore, considered to be 'critical'. The selected materials should not leach harmful chemicals, they should be capable of being sterilised by a safe method (if required) and they should be sealable, leakproof and traceable.

After procurement, all tissues and cells must be packaged in a manner that minimises the risk of contamination and must be stored at temperatures that preserve the required characteristics and biological functions of the tissues and cells.

Packaging must also prevent contamination

Table 15.2. **Labelling of the primary container**

Minimum requirements
As a minimum, the primary container must include a unique donation identification number or code. The information listed in this table in **bold print** must be included on the label if space permits or, if there is insufficient space on the primary container label, the information must be included in a separate sheet accompanying the primary container. Information listed in normal print must be included either on the label or in accompanying documentation, which must include the unique donation identification number or code specified on the primary container.
Labelling of procured tissues and cells
• **unique donation number or code** • **type of tissues or cells** • **date (and, where possible, time) of procurement** • identification of the procurement organisation
Any accompanying tissue or blood samples for testing must be accurately labelled to ensure identification with the donor and must include a record of the time and place the specimens were taken.
Labelling of tissues and cells from a tissue establishment released for circulation to another operator for further processing
• **unique donation number or code and, for tissue establishments in the EU, the donation identification sequence (DIS) from the Single European Code (SEC). For tissues or cells imported from outside the EU, the DIS must be applied by the tissue establishment responsible for import.** • **identification of the originating tissue establishment** • **type of tissues or cells** • **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone). If an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'** • date of circulation (in accompanying documentation to avoid having to re-label the primary container) • biological tests/assessments carried out on the donor and the results • presence of potential harmful residues (e.g. antibiotics, ethylene oxide)
Final labelling of tissues and cells released for distribution to an organisation responsible for human application
• **unique donation number or code and, for tissue establishments in the EU, the SEC; for tissues or cells imported from outside the EU, the SEC must be applied by the tissue establishment responsible for import** • **types of tissues or cells and lot or batch number where applicable** • **expiry date and, where relevant, time (in UTC if the tissues or cells are to be shipped to another time zone); if an expiry date has not been defined, the expiry date must be recorded in the SEC as '00000000'** • description (definition) and, if relevant, dimensions/volume of the tissue or cell product • date of distribution (in accompanying documentation to avoid having to re-label the primary container) • biological tests/assessments carried out on the donor and the results • presence of potential harmful residues (e.g. antibiotics, ethylene oxide) • morphological and functional data, where relevant • biological investigations carried out on the donor and the results • a statement limiting use of the tissues or cells to specific health professionals • a statement, as applicable, that the tissues or cells may not be sterilised or re-sterilised • a statement that it is the responsibility of the organisation responsible for human application to maintain the tissues or cells according to specified storage conditions and to follow instructions for opening the container, package and, where relevant, any required manipulation/reconstruction • instructions for reporting serious adverse reactions and/or events
Information to be included on all labels
• **for autologous donations, the label must state 'FOR AUTOLOGOUS USE ONLY'** • **for directed donations, the label must identify the intended recipient** • **when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning 'BIOLOGICAL HAZARD' must be included** • for imported tissues or cells, the country of procurement and, if different from the country of procurement, the exporting country • nature of additives (if used) • storage conditions required to maintain the quality and safety of the tissues or cells • instructions for opening the container, package and, where relevant, any required manipulation/reconstitution • expiry date after opening/manipulation

through exposure to those persons responsible for handling and transportation of the tissues and cells.

Procured tissue must be inspected and recognised appropriately before packaging and labelling to avoid mix-ups. Each tissue must be packed separately in sterile packaging as soon as possible after recovery. Double or triple wrapping may be necessary, depending on the tissue-specific requirements. Musculoskeletal tissues and skin may be packed in sterile, transparent polymer foil (though additional cotton wrapping can be used) or in containers with or without transport medium. Corneas must be placed in sterile transparent containers with medium, whereas heart-for-heart valves, amniotic membrane,

skin, or cartilage for cell cultures must be packed in sterile containers with transport medium. Whole eyes must be stored separately in moist chambers. Composition of the transport medium for a particular type of tissue must maintain the biological properties of such tissues and may include antibiotics and antimycotics validated by type and concentration.

Procured cell products are mostly packaged in disposable bags. These bags are also double wrapped before the product is transported. Reproductive tissues and cells are mainly packed and transported in straws or tubes, either in culture medium or cryopreserved.

A unique identification number or code must be allocated to the donation and to donated tissues and cells during procurement, or at the end of the recovery process, to ensure appropriate identification of the donor and traceability of all donated material.

The minimum information that should be present in a primary label is described in Table 15.2. If any of the information listed in Table 15.2 cannot be included on the primary package label, it must be provided in accompanying documentation inside the transport container. Small containers, such as straws, must be labelled at least with a unique identification number or code (e.g. treatment code, donation number or similar) and this identifier must be provided on the accompanying documentation. Table 15.2 lists the required information that should be provided either on the label or in accompanying documentation.

15.6.2. Secondary packaging and labelling for procurement operations

If secondary packaging is used after procurement, it should comply with the same requirements as those established for primary packaging. If labels with all the required information are not attached to the primary packaging they should be attached to the secondary packaging, which should be closed and sealed, ensuring that any unique identification number on the primary label is present on the label for the secondary pack and on accompanying documentation.

15.6.3. Outer container packaging and labelling for procurement operations

Packaged tissues and cells must be shipped in a container that is suitable for the transport of biological materials and maintains the safety and quality of the tissues or cells. Temperature conditions between recovery and processing must be appropriate for the type of tissue or cell to preserve the required characteristics and biological functions (i.e. temperature and duration of transport to the tissue establishment where the tissue processing will take place). The container must be closed fully with a tamper-evident seal and not opened until the procured tissues or cells are received by the tissue establishment.

When tissues or cells are shipped from the procurement site to the tissue establishment, the transport container must be labelled with the information described in Table 15.3.

Table 15.3. **External labelling of the shipping container**

For transfer of procured tissues or cells from the procurement organisation to a tissue establishment
• identification of the originating procurement organisation, including name, address and telephone number of a contact person • identification of the tissue establishment destination, including name, address and telephone number of a contact person
For transfer of tissues or cells from a tissue establishment to another operator for further processing
• identification of the originating tissue establishment, including name, address and telephone number of a contact person • identification of the other operator destination, including name, address and telephone number of a contact person
For transfer of tissues or cells from a tissue establishment to an organisation responsible for human application
• identification of the originating tissue establishment, including name, address and telephone number of a contact person • identification of the organisation responsible for human application destination including name, address and telephone number of a contact person
Information to be included on all shipping labels
• a statement that the package contains HUMAN TISSUES/CELLS and the warning HANDLE WITH CARE • where living cells are essential for successful human application, the warning DO NOT IRRADIATE must be added • when tissues or cells are from a donor known to be positive for a relevant infectious disease marker, the warning BIOLOGICAL HAZARD must be added • date and time at the start of shipping • shipping conditions relevant to the quality and safety of the tissues or cells (e.g. DO NOT DELAY, KEEP COOL, KEEP IN UPRIGHT POSITION, DO NOT FREEZE) • when shipping by air, it is mandatory under International Air Transport Association (IATA) regulations that an IATA Time- and Temperature-sensitive Label is attached to the outside of the shipping container. The lower half of the label must indicate the permitted external temperature range in degrees Celsius (see §15.9)

15.6.4. Procurement package insert

It is recommended that the documentation accompanying the procured tissues or cells indicates, where applicable, that they are in a state of 'quarantine' to ensure that it is clear that a final review regarding their release for distribution and use has not been completed. See Chapter 7 for full guidance on the requirements for procurement documentation.

15.7. Packaging and labelling of tissues and cells

Labelling of tissues or cells during intermediate phases of processing must be applied to all packaging materials and containers to assure identification at all times.

15.7.1. Primary packaging and labelling

Primary packaging and labelling refers to the materials that will come into direct contact with tissues and cells, and the requirements in this regard are described in section 15.6, with a special focus on the radiation-resistance of packaging material for tissue that will be sterilised by irradiation. The expiry date will be determined not only by the properties of the tissues and cells but also by the integrity and stability of the packaging and labelling materials, among other factors.

Packaging and labelling procedures must be done to prevent cross-contamination or mix-ups. Simultaneous operations should be avoided or adequate measures should be taken to ensure that no cross-contamination or mix-ups occur [19].

Facilities where packaging or labelling operations have taken place should be checked before starting any other operation to guarantee that all previous materials have been removed.

Printed labels must be examined carefully to ensure that the information contained conforms to the corresponding tissues or cells. Results of this examination should be documented. A printed label, representative of those used, should be included in the processing records.

Unused and already printed labels must be destroyed according to written procedures.

The information that needs to be on the primary package label is detailed in Table 15.2.

If the primary container is too small to host a label with all the required information (as may be the case with gametes and embryos), the minimum information on the primary container needs to be a unique identification number or code. This unique identification number or code and the other required information must be included in an accompanying document.

The additional information that must be provided either on the label or in accompanying documentation is described in Table 15.2.

15.7.2. Secondary packaging and labelling

'Secondary packaging' and 'labelling' refer to materials that are not intended to come into direct contact with the tissues and cells. Special consideration must be given when primary and secondary packaging and labelling are designed to be kept together until the moment of use. If secondary packaging is not sterile, it should be clarified in the package instructions that the outside of the primary package is also not sterile and should not be placed within the sterile field during clinical application.

15.7.3. Outer container packaging and labelling

When tissues or cells are shipped for distribution, every transport container must be guaranteed to maintain the conditions needed for the specific tissue or cell type. Containers must provide adequate protection against deterioration or contamination of tissues and cells that may occur during storage and transportation. Containers should be cleaned before use to ensure that they are suitable for their intended use. These containers should not alter the quality, safety or efficacy of the tissues or cells. Records should be maintained for each shipment of labels and packaging materials showing receipt, examination or testing, and whether accepted or rejected. For transport, the shipping container must be labelled with all the same information as specified in Table 15.3.

15.7.4. Package insert

A 'package insert' refers to the supplementary information (associated with tissues and cells) that cannot be placed on labels. Critical information for the clinical user must be provided.

15.8. Customs clearance

For clearance of customs, all tissues and cells crossing borders require a clear description of the content of the consignment, its destination and intended use. The paperwork sent with the consignment should include the World Customs Organization Tariff Number for Human Tissue for Transplantation. It is important that the transport of frozen or cryopreserved products packed in dry ice or

stored in a dry-shipper must not be delayed at border crossings. If the goods are being transported by air, packages must be labelled with the appropriate International Air Transport Association (IATA) codes: UN1845 for dry ice or UN1977 for liquid nitrogen in a dry-shipper, and UN3373 for shipment of biological substances by air [20, 21]. Therefore, it may be expedient for the importer to inform customs of a prospective consignment, and any enquiries by customs should always be answered promptly (see Chapter 11). For tissue or cell transport, the agreement with the shipping tissue establishment should define responsibilities for meeting the cost of transport and storage under appropriate conditions at a receiving facility for any items that may be detained pending customs enquiries.

15.9. References

1. Ashford P. Traceability. *Cell Tissue Bank* 2010 Nov; 11(4):329-33. https://doi.org/10.1007/s10561-010-9195-3.
2. Euro-GTP. Good tissue practices, available at www.eurogtps.com. For guidance, see http://eurogtps.com/Portals/0/pdf/Euro%20GTP%20Final%20Delivery.pdf, accessed 10 April 2022.
3. WHO. Human cell and tissue transplantation. WHO information, available at www.who.int/health-topics/transplantation#tab=tab_2, accessed 13 April 2022.
4. ICCBBA. ISBT 128 Standard terminology for Medical Products of Human Origin for use with product description code database (Version 7.63, November 2022), available at https://www.isbt128.org/standard-terminology, accessed 29 November 2022.
5. Ashford P, Rydman K, Sparks A *et al.* Standard terminology for reproductive tissue and cell products for use in ART. *Hum Reprod Open* 2019 Mar 29;2019(2): hoz005. https://doi.org/10.1093/hropen/hoz005. eCollection 2019.PMID: 30949588.
6. ICCBBA. ISBT 128 Standard Technical Specification (Version 6.1.0, December 2021), available at www.iccbba.org/uploads/fa/52/fa5290615f2297b8726bccb3fe8f54d6/ST-001-ISBT-128-Standard-Technical-Specification-v6.1.0.pdf, accessed 11 April 2022.
7. WDMA. Why a global donor identifier? (GRID) https://wmda.info/professionals/optimising-search-match-connect/why-global-identifier/ accessed 10 April 2022.
8. ICCBBA. ISBT 128 Standard Global Registration Identifier for Donors: ION Database and GRID Rules (Version 3.2.0, September 2017), available at www.iccbba.org/uploads/bc/01/bc010620c138b6d40c7ec26e5b55ad22/ST-015-ISBT-128-Standard-Global-Registration-Identifier-for-Donors-ION-Database-and-GRID-Rules-v3.2.0.pdf, accessed 10 April 2022.
9. Eurocode IBLS – Technical Specification V2.1 (2016), available at www.eurocode.org/guides/structures/EurocodeTechnicalSpecification-2-1-0.pdf, accessed 10 April 2022.
10. European Commission. Directive 2015/565 of 8 April 2015 amending Directive 2006/86 as regards certain technical requirements for the coding of human tissues and cells, available at http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1475052881122&uri=CELEX:32015L0565, accessed 10 April 2022.
11. European Commission. Single European Code for tissues and cells, available at https://ec.europa.eu/health/blood-tissues-cells-and-organs/implementation/single-european-code-sec-tissues-and-cells_en, accessed 10 April 2022.
12. European Commission. Information for competent authorities and tissue establishments on the implementation of the Single European Code for tissues and cells, available at https://ec.europa.eu/health/sites/health/files/blood_tissues_organs/docs/sec_cas_tes_en.pdf, accessed 10 April 2022.
13. European Commission. Single European Code (SEC) questions and answers, available at https://ec.europa.eu/health/system/files/2019-12/sec_qa_en_0.pdf, accessed 10 April 2022.
14. Alteri A, Vermeulen N, Rugescu IA *et al.* Coding in medically assisted reproduction: the status of the implementation of the Single European Code for reproductive cells and tissues. *Hum Reprod Open* 2020; 2020(3):hoaa027. https://doi.org/10.1093/hropen/hoaa027.
15. Caramia V, Ghirardini A, Di Ciaccio P *et al.* From the EU legislation to the application of the Single European Code: support for the implementation. *Transfus Med Hemother* 2017 Nov;44(6):391-4.
16. Ashford P, Delgado M. ISBT 128 standard for coding medical products of human origin. *Transfus Med Hemother* 2017 Nov;44(6):386-90.
17. Knels R, Stüpmann K, Pruß A *et al.* Coding of tissue and cell preparations using Eurocode. *Transfus Med Hemother* 2017 Nov;44(6):401-5.
18. Tedder RS, Zuckerman MA, Goldstone MH *et al.* Hepatitis B transmission from contaminated cryopreservation tank. *Lancet* 1995;346:1370140.
19. Schroeter J, Schulz T, Schroeter B *et al.* Implementation of the Single European Code in a multi-tissue bank. *Transfus Med Hemother* 2017 Nov;44(6):396-400.
20. International Air Transport Association. DGR handling labels, available at www.iata.org/publications/store/Pages/dgr-handling-labels.aspx, accessed 10 April 2022.
21. International Air Transport Association. IATA perishable cargo regulations, chapter 17 (amended by Resolution 607 in 2012), available at www.iata.org/publications/store/Pages/

perishable-cargo-regulations.aspx, accessed 10 April 2022.

Chapter 16. **Traceability**

16.1. Introduction

Clinical application of tissues and cells brings great benefits for patients. There are, however, rare (but important) risks associated with such clinical use, including graft/application failure, donor-transmitted infections, malignancies and genetic conditions. The concept of traceability is the means of linking a donor with recipients, or with offspring born through medically assisted reproduction (MAR), and with all information about the transferred tissues and cells from donation to clinical outcome and follow-up.

Traceability means the ability to locate and identify the tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal, which also implies the ability to identify the donor and the tissue establishment (TE) receiving, processing or storing the tissue/cells, and the ability to identify the clinicians at the medical facility applying the tissue/cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues/cells [1].

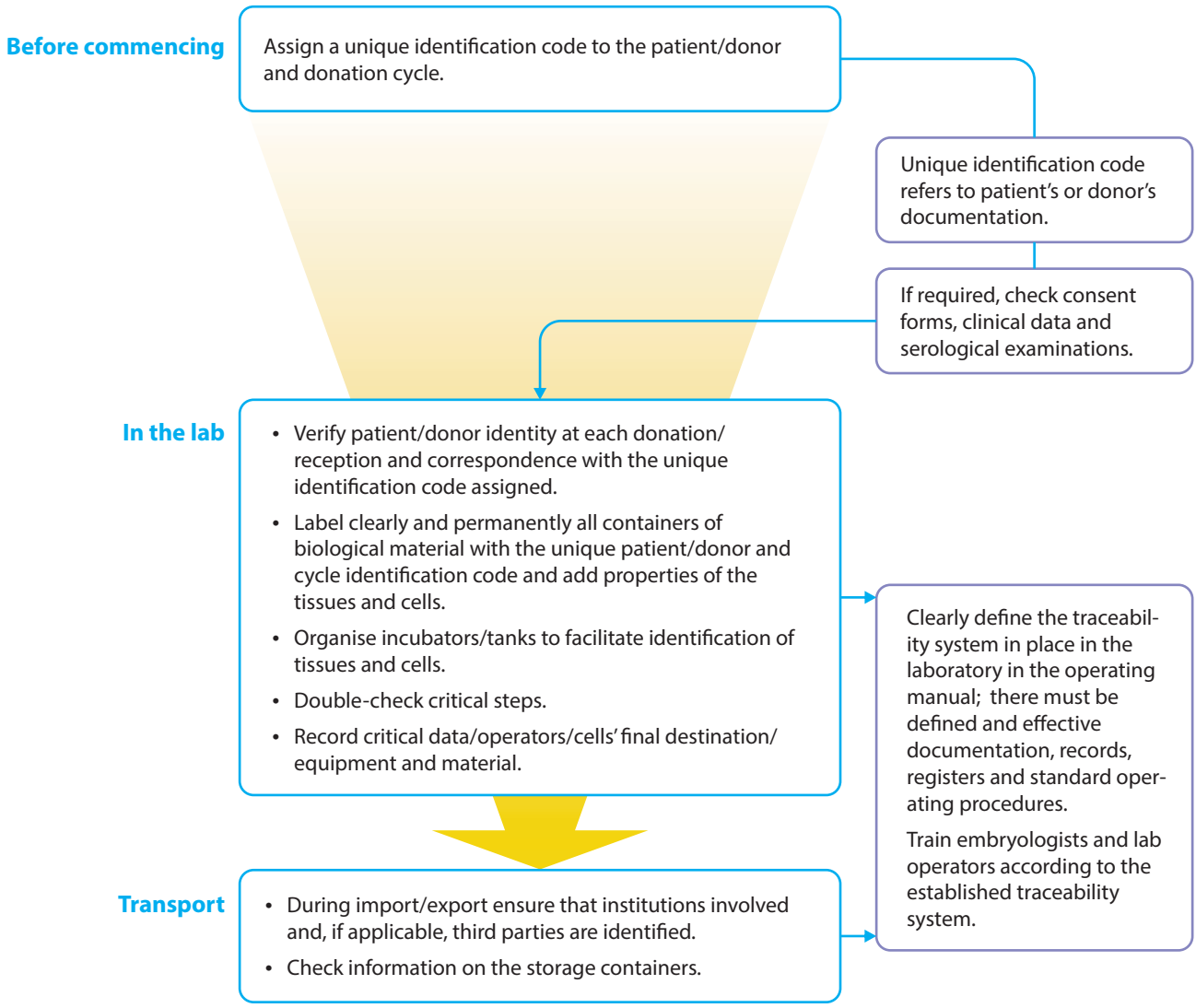
The increased transportation of grafts across national boundaries has made traceability difficult and sometimes impossible [1]. It is therefore essential to facilitate rapid action to prevent harm when links in the safety and quality chain are found to have been compromised. Apart from quality and safety, traceability is also crucial for ethical reasons, as it allows legitimate donation with proper consent to be verified for every tissue or cell product.

The system of traceability is inseparable from, and in practice dependent on, the coding system (see Chapter 15). Effective traceability and biovigilance in the global context depend on the use of globally unique identification for all donated biologic products [2].

The need to comply with traceability requirements should not compromise the need to guarantee anonymity between donor and recipient (or newborn), depending on the type of donation and the national legislation enforced. Records must be kept by the entities involved in the donation, procurement, processing, storage, distribution and application of tissues and cells to ensure compliance with safety requirements, but records should never allow the disclosure of confidential information to unauthorised persons.

Human error, equipment failure or other adverse events that cannot be predicted may affect quality, safety or effective use of tissues and cells at any stage, potentially increasing the risk to recipients and offspring. In the case of deceased donors, procurement teams are provided with a medical history at short notice, and additional information about the donor at a later stage may have implications for the safety and quality of tissues procured from those donors. Use of defective equipment, poor-quality consumables, contaminated solutions or defective testing kits may only come to light after the tissues and cells have been processed and transplanted. This means that traceability, from donation through to end use, is essential to determine which tissues or cells could potentially be affected by additional infor-

Figure 16.1. **Example of traceability system in medically assisted reproduction**



Source: ESHRE revised guidelines for good practices in IVF laboratories (2015) [4].

mation or adverse incidents. For MAR, traceability does not stop when the tissues and cells reach the recipient. The health of the children born as a result of MAR treatment must be followed up, so that data on follow-up of pregnancies and children's health are included in the chain of traceability.

TEs play a special role in assuring traceability, collecting the data that guarantee the ability to locate and recall tissues and cells or inform the applying clinicians and recipients, once the establishment becomes aware of information that may have implications for their quality and safety. TEs are responsible for communication with other entities, such as organ transplant units, and with other TEs (including MAR centres) involved in the procurement/collection or processing of additional tissues and cells, or cells from shared donors.

The time interval between detecting a risk to the quality and safety of tissues and cells and preventing them from being used in patients has been referred to as the 'traceability window period' [3]. Recalls can be due to inappropriate evaluation of donors, positive serology tests in the donor, contamination of tissues or cells, infection in recipients of other tissues donated by an individual donor and other risks introduced during the processing or storage of tissues or cells. The increasing global circulation of tissues and cells for clinical use, the fact that several tissue products can originate from one donor who may also donate organs, or that many children may be born from one sperm donor, and the existence of international markets for equipment, consumables and additives all add to the need for robust systems of traceability.

Records and procedures required to maintain traceability must be kept long after the clinical use of products (see §16.2.f), allowing personnel to track and trace all steps associated with the tissues and cells long after their clinical application, making adequate biovigilance and follow-up procedures possible. Traceability underpins biovigilance (see Chapter 17).

Within each TE, investigation of adverse events and adverse reactions, and of deviations from standard procedures, can be carried out only if a system of traceability is in place. Many establishments share practices and standards, and effective investigations can help to improve them. Hence, in addition to biovigilance, ongoing quality improvement – of procedures relating to procurement, processing, donor testing, storage and distribution of tissues and cells – also benefits from good systems of traceability.

Traceability requirements are often defined in legal obligations and may include the ability to report the precise number of units and recipients, for use as denominator data in the evaluation of adverse occurrence frequency at national and international level. Traceability must encompass all the data associated with the final destination of tissues and cells distributed by third parties, including records of the final distribution and application of imported and/or exported units (see Figure 16.1 for an example of traceability through unique identification and coding).

16.2. How traceability works

Traceability is the 'thread' that joins all the pieces of critical information together, from the moment that a potential donor is identified until the moment when the tissues or cells are applied to the recipient or discarded. This means traceability is a concept allowing: (i) tracing of procedures when donors and/or recipients show any adverse reaction that could be linked with procurement of the tissues and cells and/or the quality of the tissues or cells distributed, respectively (see Chapter 6); (ii) tracking the fate of recipients; (iii) tracking follow-up of the health of MAR children; (iv) tracking those units associated with incidents detected after distribution or clinical application; and (v) tracing the tissues and cells available to be re-tested if new risks become evident after release, e.g. from procurement, processing, testing, handling, storage or transport.

TEs must ensure that data protection and confidentiality measures are in place, in accordance with the national and European data protection laws. Many organ donors are also tissue and cell donors, so it is important that effective links are in place between organ-procurement organisations and TEs.

The following are the key requirements of an effective traceability system:

a. *Unique identification*
At each stage in the pathway, from donor to recipient or child conceived as a result of MAR treatment, each TE must have records of the donor, the donation and the donation samples, and must ensure that they are identified and labelled uniquely within their own organisation. While uniqueness can be ensured without difficulty within one organisation, the risks of duplication are increased when tissues, cells, samples or records move from one organisation to another. For example, duplicate identifiers may result when samples are sent to a testing laboratory or when tissues or cells are sent to a hospital because each receiving establishment may assign its own identifier. This risk can be eliminated if a global standard is used to identify samples or tissue products. Within the European Union (EU), the Single European Code (SEC) will help to address this need (see Chapter 15). The SEC allows, within its structure, incorporation of the international coding systems ISBT 128 and Eurocode.

b. *Double witnessing protocols*
The implementation of specific policies, such as double-checking protocols, is receiving more and more interest. Because manual witnessing is associated with human errors due to the occurrence of conscious automaticity, involuntary automaticity, ambiguous accountability and stress, different models for witnessing the identification of samples and patients have been proposed, such us barcoding or radiofrequency [5]. Risk assessment of all steps in all processes should be performed to identify where double witnessing is needed.

c. *Safe transfer of critical information*
The traceability trail depends on the accurate transcription of critical identification information. Manual transcription errors can cause disruption of the traceability trail. Use of a qualified electronic transfer system for critical information (bar codes or other machine-readable codes) is recommended. If manual transcription is used, double checking of data should be implemented. Electronic storage of data – in well-protected databases for easy and quick access by authorised personnel – is preferred to paper-based information collections.

d. *Timeliness*
If a risk is identified, it should be possible to rapidly trace all implicated products or all potentially affected recipients and children conceived through MAR. A delay could result in harm to patients or children conceived through

MAR. Systems need to be quickly accessible, with efficient links between organisations to reduce the 'traceability window period'.

e. *Clarity of responsibilities at interfaces between organisations*
To guarantee traceability, TEs should distribute tissues and cells to other TEs, to organisations responsible for human application (ORHAs) or to healthcare professionals who have responsibility for clinical application (and not directly to recipients). It is essential that each organisation in the chain clearly understands its responsibilities for traceability. It is notable that, in the published high-profile cases of viral transmission during transplantation, hospitals were often not able to trace all recipients [6]. Maintaining traceability is one of the key legal and technical responsibilities of an ORHA (see Chapter 13). TEs must define responsibilities and procedures prior to the distribution of tissues, cells, gametes or embryos to those organisations.

f. *Long-term storage of secure records*
For effective reviews, traceability data need to be maintained for long periods of time; therefore secure premises equipped with adequate protection systems are necessary. For example, in the EU, all information related to traceability must be maintained for 30 years after application or the expiry date of the tissues and cells. Data that are critical to the safety and quality of tissues and cells, including records of equipment used and materials such as consumables coming into contact with those tissues or cells, should be kept so as to ensure access to the data for at least 10 years after clinical use of the product, its expiry date or disposal. Organisations need to consider the impact of the obsolescence of technology and ensure that records remain quickly accessible. There is a need for regular management review of data storage, with a proactive approach to prevent obsolescence.

g. *Traceability provisions*
The location of traceability records may change when organisations are closed or merged, or if they cease activities relating to donor selection, donor testing, procurement, processing, distribution or transplantation. In such cases, there should be an effective link between the new location of the data and the previous location, and provision must be made to prevent loss of traceability information, for example by signing contracts with other TEs for taking care of these data in critical situations.

h. *Traceability audits*
Organisations should include audits of traceability from donor to recipient and vice versa as part of ongoing quality management. The traceability trail may encompass data stored in several organisations.

The EU definition of traceability is provided in the Glossary (Appendix 3) of this Guide.

16.3. Which records must be traceable?

All records must be legible and indelible, protected from unauthorised amendments, stored securely and readily retrievable. Establishments should conduct regular audits of records to ensure that they are accurate and comprehensive. Good practice requires that there is trail of amendments to written and digital records and that these are authenticated and dated. Computer records should be maintained in a validated system (see Chapter 14) and there must be procedures to back up electronic records to prevent loss, corruption and unauthorised access or amendment. Records must be shown to be reliable and a true representation of the events. Records may be handwritten or transferred to another qualified system, such as a computer or microfilm. Records should be maintained of critical equipment and consumables, including the lot numbers and expiry dates of additives, cryoprotectants and packaging materials used during procurement and processing. The TE must also retain temperature records, analyser printouts and relevant environmental monitoring records for viable and non-viable particles. If tissues and cells have been imported, it is important that TEs ensure that the traceability chain is retained and that the records for traceability (see §16.3) are accessible.

There must be a system of record keeping for all activities associated with tissues and cells. These robust systems must ensure secure identification of:

a. the donor and all records associated with the donor and their medical and behavioural history,
b. the donation (tissues or cells procured/collected from the donor),
c. all records associated with processing, storage and distribution of the final products, and related events,

d. all samples taken from the donor or from the tissues/cells for the purposes of testing for quality and safety,
e. the clinical application and recipient(s) of the tissues or cells,
f. the health of the resulting child(ren) and any adverse data on pregnancies (for MAR treatment).

16.3.1. Records of identification, donor tests and clinical evaluation of the donor

Besides the information defined in Chapter 5 – Donor evaluation, TEs must keep in their records at least the following data:

a. donor identity,
b. age, sex, medical and behavioural history of the donor,
c. outcome of clinical and physical examinations for the donors,
d. completed haemodilution algorithm (where applicable),
e. consent/authorisation form,
f. relevant clinical data, laboratory test results and the results of any other tests carried out,
g. for deceased donors, results of the autopsy (if carried out) or preliminary verbal report,
h. for haematopoietic progenitor cell (HPC) donors, the donor's suitability for the chosen recipient (see Table 16.1 for an example),
i. for unrelated HPC donations, where the organisation responsible for procurement has limited access to recipient data, the ORHA or the physician should be provided with the relevant donor data to confirm suitability.

In addition, the donor testing records must be accessible at the laboratory (in-house or at a contracted laboratory) and contain at least:

a. date and time donor blood samples were taken,
b. date of receipt of the blood sample at the testing facility,
c. record of each test kit used to test donor blood sample (i.e. manufacturer, lot number, expiry date),
d. results of donor testing, including repeat testing (if applicable).

Accessibility authorisations and the responsibilities associated with record keeping and reporting, of both TEs and testing laboratories, should be properly defined through a technical and legal written agreement (for technical agreements with testing laboratories, see §6.4).

16.3.2. Records of procurement of tissues and cells

Besides the information defined in Chapter 7 – Procurement, the organisation undertaking procurement must produce procurement reports and provide them to the TE. The procurement report must contain at least:

a. the identification data of the TE receiving the tissues or cells,
b. donor identification data (including how and

Table 16.1. **Traceability of unrelated haematopoietic progenitor cells (HPC) donor and recipient data**

	Donor centre	National registry	WMDA	Collection centre	Tissue establishment	Transplant centre patient
Activities	Consent Testing Donor follow-up	Listing Donor and patient follow-up	Listing	HPC collection	Product labelling, processing and release	Infusion Patient follow-up
Donor data*	ID code Identity	ID code Identity of National Registry donors only	ID code only	ID code Identity	ID code Product code (e.g. SEC)	ID code only
Patient data*	ID code Identity	ID code Identity	ID code	ID code Identity	ID code Identity	ID code Identity

WMDA: World Marrow Donor Association.
The identity and privacy of all patients and donors are protected throughout the process of HPC donation and transplantation (identity = name).
*Donor/patient data: anonymous contact between patient and donor is allowed post-transplantation only through registry.

by whom the donor was identified) and records of testing of the donor,
c. description and identification of procured tissues and cells (including samples for testing),
d. identification of the person who was responsible for the procurement session, including his/her written or digital authentication,
e. date, time (start and end, if relevant) and location of the procurement and standard operating procedure used,
f. description of the physical area where procurement took place, including environmental conditions at the procurement site (where relevant),
g. for deceased donors, storage conditions of the deceased donor, i.e. refrigerated (or not) and time of start and end of refrigeration,
h. manufacturers and lot numbers of reagents and transport solutions used,
i. any incidents that occurred during procurement,
j. equipment used during procurement.

16.3.3. Records of processing of tissues and cells

Besides the information defined in Chapter 9 – Processing, Chapter 10 – Storage and Chapter 12 – Release, distribution and import/export, the organisation undertaking processing must keep at least the following records:

a. tissues and cells received and evaluation of their suitability,
b. standard operating procedures used to process the tissues and cells,
c. equipment used during processing with full maintenance records,
d. records of consumables used during processing (manufacturer, lot number, storage conditions of consumables – if appropriate – and expiry date),
e. records of sterilisation or decontamination, if applicable,
f. records of cryopreservation and freezing protocols, if applicable,
g. records of environmental monitoring (temperature monitoring, microbial monitoring and particle counts as appropriate),
h. records of product testing, including microbial testing,
i. any incidents that occurred during processing.

16.3.4. Records of storage and distribution of tissues and cells

Besides the information defined in Chapter 12 – Release, distribution and import/export, organisations undertaking storage of tissues or cells must keep at least the following records:

a. storage location and a transfer record if storage locations change,
b. date placed in storage,
c. date removed from storage,
d. records of storage temperature including details of nitrogen storage (e.g. vapour phase above liquid nitrogen),
e. any incidents that occurred during storage.

In addition, when the tissues or cells are transported or distributed to hospitals or clinics for application, TEs must keep the following records:

a. name of party responsible for distribution,
b. identification of the establishment, courier or individual who transported the tissues and cells at any stage between procurement and end use (clinical application),
c. packaging records (e.g. records of the dry-shipper used),
d. time and date of distribution of tissues and cells,
e. time and date of delivery of tissues and cells,
f. identification of the receiving establishment, clinician or ORHA,
g. any incidents that occurred during distribution.

16.3.5. Records of clinical application of tissues and cells

Besides the information defined in Chapter 13, the ORHA must keep at least the following records:

a. identification of the supplier TE,
b. identification of the clinician or ORHA,
c. type(s) of tissues and cells,
d. product identification,
e. identification of the recipient,
f. date of clinical application,
g. any incidents that occurred during clinical application,
h. any adverse reactions or adverse events in the recipient,
i. health outcomes of children born following MAR.

Systems must be in place to assure the follow-up of tissue and/or cell recipients and children conceived after MAR treatment. Such follow-up can be

achieved only if a close working relationship exists between all stakeholders: that is, the TE, ORHA, MAR centre and parent(s) involved.

Some national standards require the ORHA to provide the supplying TE with details of the patient to whom the tissues or cells were clinically applied. Whether this information is sent to the TE or not, it is essential that the end user maintains these records and keeps the chain of traceability intact from the beginning to the very end.

16.4. References

1. Strong M, Shinozaki N. Coding and traceability for cells, tissues and organs for transplantation. *Cell Tissue Bank* 2010;11(4):305-23. https://doi.org/10.1007/s10561-010-9179-3.
2. Traceability, an absolute pre-requisite for MPHO safety. Notify Booklet 18. Notify library. Rome: Italian National Transplant Centre, 2016, available at www.notifylibrary.org/content/18-traceability-absolute-pre-requisite-mpho-safety, accessed 11 Apr 2022.
3. Ashford P. Traceability. *Cell Tissue Bank* 2010 Nov;11(4):329-33. https://doi.org/10.1007/s10561-010-9195-3.
4. De los Santos MJ, Apter S, Coticchio G *et al.* Revised guidelines for good practice in IVF laboratories (2015). *Hum Reprod* 2016 Apr;31(4):685-6. https://doi.org/10.1093/humrep/dew016.
5. De los Santos MJ, Ruiz A. Protocols for tracking and witnessing samples and patients in assisted reproductive technology. *Fertil Steril* 2013 Dec;100(6):1499-1502. https://doi.org/10.1016/j.fertnstert.2013.09.029.
6. Tugwell BD, Patel PR, Williams IT *et al.* Transmission of hepatitis C virus to several organ and tissue recipients from an antibody-negative donor. *Ann Intern Med* 2005 Nov;143(9):648-54. https://doi.org/10.7326/0003-4819-143-9-200511010-00008.

Chapter 17. **Biovigilance**

17.1. Introduction

This chapter provides general guidance on the implementation of good vigilance and surveillance (V&S) practice by all those involved in the process from donation through banking to clinical use, which includes medically assisted reproduction (MAR), assisted reproductive technology (ART) and haematopoietic progenitor cells (HPC), including regulators and Health Authorities. The tissue- and cell-specific chapters in Part B provide additional specific guidance on vigilance in those fields; in particular, Chapter 29 and Chapter 30 detail, respectively, several specificities within MAR and fertility preservation vigilance.

In a tissue establishment (TE), the quality management system focuses on preventing errors and maintaining a consistent standard of agreed specification for tissues and cells released for clinical application. However, residual or unexpected risks or procedural errors occasionally result in failures, disease transmissions or situations in which donors or patients are exposed to risk, even if not harmed. A programme of V&S is essential for ensuring the quality and safety of tissues and cells for human application. Reporting of these incidents presents important learning opportunities that can help all procurement organisations, TEs, cell therapy, MAR facilities and clinical users (not only those involved in the incident/problem in question) to improve their processes and to achieve higher standards of safety and quality at all levels: from TEs to donors and recipients [1, 2].

17.1.1. Biovigilance phases

Biovigilance is the systematic monitoring of serious adverse reactions and events (SAREs) from the selection of the donor to the follow-up of the recipient, with the objective of making the application of different substances of human origin (SoHO) safer and more effective. There are several stages (phases) in a biovigilance system:

a. Detection. The first stage is to detect a biovigilance case that could be described as an adverse event or reaction. Depending on the case and the system in place at national level, the following steps can be done in parallel. Each case should be collected.

b. Reporting/notification. After detecting the case, it must be reported or notified to the Health Authority even if the investigation is not concluded. If there is a suspicion that other centres, TEs, suppliers, donors, recipients or children in MAR could be affected or involved, they have to be alerted rapidly by the TE or by the Health Authority to prevent further complications.

c. Investigation/evaluation. Every single case (at least every serious case) must be investigated and evaluated by the TE and Health Authority, with the collaboration of all parties involved and also a group of relevant multidisciplinary professionals with experience in infectious and malignant or genetic diseases, quality control and quality management, as well as professionals with experience in the use of the tissue involved in the case.

d. Management. During the investigation or once the investigation is finished, it is important to decide how its findings should be managed, depending upon what kinds of actions have been decided on. Occasionally, an SAE (Serious Adverse Event, e.g. discovery of an infectious diseases marker as positive or a tumour found after tissues or cells were used) does not inflict immediate harm on the recipient, but requires follow-up during a specified period of time to detect if the recipient develops any problem related to the SAE (for example, seroconversion). The period of time should be established between TE and clinicians, taking into account the SAE (tumoral, infectious etc.) and according to the data recorded when the patient developed signs or symptoms. In that case, what was at the beginning defined as an SAE is converted later to an SAR (Serious Adverse Reaction).

e. Closing the notification/report. Finally, the case will be closed and the final report must include both corrective and preventive measures and therapeutic measures if applicable. This final report should detail how to act on similar occasions in the future in order to prevent the recurrence of the SARE. It is worth noting that learning is an important benefit derived from biovigilance, and feedback to all involved parties should be given.

17.2. Definitions

17.2.1. Adverse events

An adverse occurrence can be classified into 'adverse events' (AEs), which are process failures that might expose a recipient or living donor to risk or to a loss of any irreplaceable autologous tissues or cells or to a loss of any highly matched allogeneic tissues or cells, and 'adverse reactions' (ARs), which are adverse outcomes that have indeed occurred with harm to a donor, a recipient or a child born through MAR procedures. An adverse event may or may not cause an adverse reaction. Similarly, an adverse reaction may or may not be related to an adverse event.

According to European Union (EU) definitions, a 'serious adverse event' (SAE) in the present context is any untoward occurrence associated with the procurement (including donor selection), testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to a life-threatening, disabling or incapacitating condition for the patient or that might result in prolonged hospitalisation, morbidity or death. In MAR, the unintended mix-up of gametes or embryos is considered an SAE.

Although adverse events may occur at all stages from donor selection to distribution of tissues and cells, many of them are not severe and may be managed through the quality management system (QMS) of the TE. Conversely, SAREs are rare. Therefore, there are significant benefits associated with consolidating V&S data on regional, national or international scales and on an integrated system for the various substances of human origin (SoHO), because all such substances are exposed to risks from donation to transplantation (from breaches of ethical, legal and safety standards).

17.2.2. Adverse reactions

A 'serious adverse reaction' (SAR) is an unintended response, including a communicable disease, in the donor or in the recipient, associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating, or which results in, or prolongs hospitalisation or results in morbidity.

The follow-up of living donors after donation should ensure that, if a condition not known at the time of donation occurs to the donor that may have an impact on the recipient, it is clearly identified. In such cases there should be a documented procedure to notify the recipient's physician of this condition. This is not necessarily an adverse event. Conversely, when the recipient's physician detects an impact on the recipient, this must be reported to the TE. The same also applies to the potential long-term influence of any treatment provided for the procurement (e.g. mobilisation with cytokines or hormonal stimulation), in which case pharmacovigilance should also be involved.

In summary, an adverse reaction is an incident whereby a living donor, a recipient or a fetus or child created through IVF or intra-uterine insemination using donor gametes has been harmed, whereas an adverse event is an incident that results in a risk of harm, although no harm may actually occur. Those that are classified as 'serious' should be notified to Health Authorities, in accordance with national or regional requirements.

These definitions are also reflected in the World Health Organization (WHO) Notify Library for V&S of medical products of human origin (MPHO) [3]. Adverse occurrence type is categorised in the library as follows:

a. harm to recipient,

b. harm to donor,
c. harm to fetus or offspring,
d. risk of harm.

If products containing tissues or cells are classified as advanced therapy medicinal products (ATMPs) in the EU, biovigilance is applied in the first steps of the process (selection of the donor, collection and controls of the collected products) and pharmacovigilance in the rest of the process (see 17.6 – Vigilance co-ordination).

17.3. Management and quality of vigilance

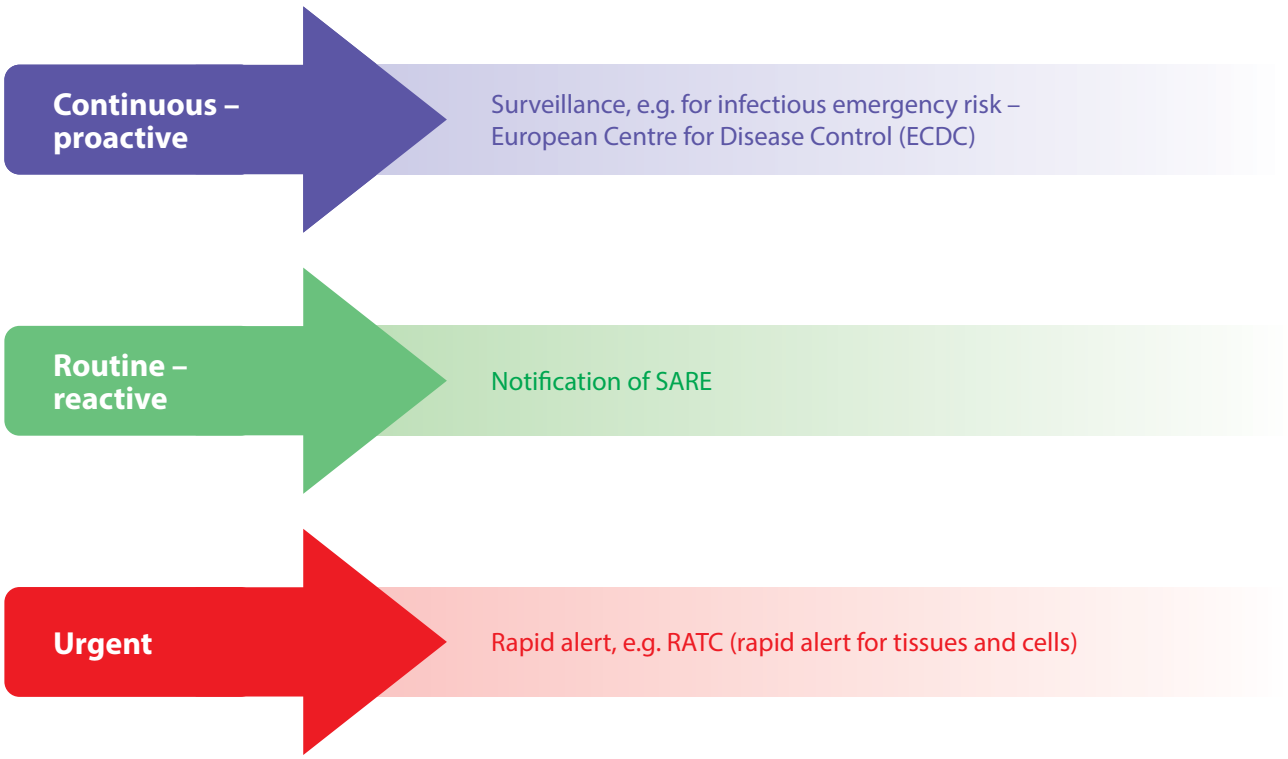
17.3.1. Vigilance

The organisation of the vigilance system, as well as the role of the various parties involved, should be defined and broadly communicated within the TE. Health Authorities are encouraged to draw up guidelines for vigilance systems, notification forms, surveillance methods, acceptable risk criteria and examples of SAREs for tissues and cells that should be reported to them. Appropriate communication and co-ordination between procurement organisations, TEs and organisations responsible for human application (ORHAs) are essential for an efficient vigilance system. Organisations or bodies involved in activities based on tissues and cells (including clinical users) should have standard operating procedures (SOPs) in place that describe how to detect, collect, report, investigate and communicate notifications for adverse reactions and events (AREs).

The co-ordinator (officer at local, hospital, regional level etc.) in charge of biovigilance should be clearly identified in order to receive a notification, co-ordinate investigations, gather experts from different fields and establish with the experts and the TE the measures to be implemented. It is recommended that the QMS and V&S systems, both of which contribute to risk-management policy, should be co-ordinated at TE level according to guidelines established by the Health Authority and under the direct responsibility of the Responsible Person (RP). Implementation of computerised and integrated systems for collection and management of ARE data is encouraged to facilitate the assessment, synthesis of the data, and preparation and dissemination of reports.

This chapter focuses on the procedures for detection, collection, reporting, alert, investigation, management, evaluation and closure of AREs that may occur from the time of selection of the donor until clinical application and follow-up. All AREs and non-compliances involving any party (clinical users, donors, patients or third parties) and including those with minor consequences, should be documented and reviewed regularly within the QMS of the TE. Each report or communication should be considered for classification as an SARE and should be managed as

Figure 17.1. **Categories of biovigilance scenarios**



such if it meets the criteria described in this chapter. This allows trends to be monitored and actions to be taken to continually improve quality and safety.

Three categories of biovigilance scenario can be described, depending on the type of measures and the speediness of the actions that can be taken into consideration: urgent communication, routine notification and proactive monitoring of possible SARs/SAEs (see Figure 17.1). These categories are explained further in this chapter.

17.3.2. Surveillance

The term 'surveillance' denotes the follow-up of organs, cells or tissue recipients or living donors, with or without SARE, to provide indicators and information on stratification of risks. Furthermore, an active surveillance system should monitor all follow-up, not only for some specific expected serious reactions or events. When a surveillance system is implemented, periodic analyses can show if there is an upward trend of SAREs, AEs or ARs, more or less systematically occurring and expected. These should be reported to the Health Authority, a root cause analysis should be initiated, and corrective measures should be implemented.

Routine monitoring of clinical outcomes is part of the surveillance system. Clinical teams should set up a system with follow-up on grafts and recipients post-transplant (e.g. registries), as well as living donor outcomes, in order to monitor the results and to identify currently unknown risk factors. This monitoring should be complemented by an active surveillance system for well-known adverse reactions. Unfortunately, in the case of tissues, there are very few transplant registries at European level. One of those registries is the ECCTR (European Cornea and Cell Transplantation Registry), which aims to build a common assessment methodology and establish an EU web-based registry to assess and verify the quality and efficacy of corneal transplantation in ophthalmic surgery [4]. Importantly, registries can help to identify both known and previously unidentified long-term effects of cell or tissue transplantation.

In the field of MAR, the European Society of Human Reproduction and Embryology (ESHRE) has set up a European IVF Monitoring Programme (EIM) that, among others, collects data on perinatal outcomes, risks and complications of MAR treatments. Although they are outside the scope of the vigilance system, because this focuses only on the 'undesired and unexpected SAR', any 'known ARs' should be evaluated further in order to exclude their occurrence being caused by a systematic error (e.g. incorrect handling of heart-for-heart valves during procurement).

The expected frequency of these 'known SARs' can be defined by the relevant experts or professional bodies, based on local experience and evidence based on literature data (e.g. expected rate of arterial thrombosis after the use of cryopreserved arteries). When the frequency of these complications increases in one centre beyond a threshold of pre-defined rates, a notification of 'suspected SAR' must be made to the Health Authority. The root cause analysis has to be performed by the local investigation team in order to determine the reasons for this deviation from the acceptable rate. This investigation falls within the framework of the vigilance system, and should trigger corrective measures and improve the quality and safety of care to patients. But such surveillance should also identify whether good results are achieved by risk-avoiding behaviour of an institution or by true best clinical practice being applied.

When a TE asks clinicians specifically about the presence of SAR for each tissue or cell implanted, the number of cases reported is higher than the percentage collected by the Health Authority [5, 6]. For this reason, it is important to reinforce the surveillance and registries, to avoid under-reporting.

The widespread use of active surveillance systems will be a step-by-step process that still requires healthcare professionals to obtain consensus views on some important points, including issues like the definition of serious adverse reactions and events, as well as the description of their appropriate monitoring.

17.3.3. Surveillance for new risks

Surveillance programmes should also include an activity of scanning for new risks that have not been recognised previously or have not yet occurred. New risks may be related to new donor selection criteria, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing, or new inheritable pathogenic DNA variants that MAR donors have not been tested for. Newly emerging infectious diseases, for which targeted testing can be carried out or which might imply the need to exclude certain donors, represent an example of one type of new risk. The European Centre for Disease Prevention and Control (ECDC) monitors the epidemiology of diseases in Europe and publishes a weekly *Eurosurveillance* report that provides useful data to support the development of donor-selection policy. Moreover, the ECDC has recently been mandated to initiate risk

assessments on particular epidemic agents, infectious diseases or new *in vitro* diagnostic techniques in the field of tissues and cells. This is what we call the proactive biovigilance category (surveillance).

17.4. Adverse reactions

Adverse reactions must be detected, reported, investigated and evaluated in terms of:

a. severity,
b. imputability,
c. probability of recurrence or frequency, and
d. consequences.

Several symptoms or situations can suggest that an adverse reaction might have occurred in a recipient of tissue or cells and should, therefore, be seen as 'triggers' for an adverse reaction report. Note that, in certain circumstances, clinicians may knowingly transplant an infective donation (e.g. *Cytomegalovirus*-positive bone marrow); in such cases, patients should be informed about the benefits and the additional risks using an informed consent, and there should be specific follow-up. Clinical and biological monitoring, as well as prophylactic or pre-emptive treatment, should comply with existing recommendations or regulatory requirements, where they exist. Below are examples of reportable adverse reactions (for more information, see the chapters in Part B on each specific tissue) [with abbreviated descriptions in square brackets]:

a. suspected harm in living donor related to procurement [Donor harm];
b. unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial, parasitic, fungal, prion) [Infection from donor];
c. suspected transmitted infection (viral, bacterial, parasitic, fungal, prion) possibly due to contamination or cross-contamination by an infectious agent in the procured tissues, cells or associated materials, between procurement and their clinical application [Infection from infected/contaminated tissues and cells] – this could be during processing (e.g. if the previous donor processed was infected and the cleanroom was not disinfected) or during storage (e.g. if the tissues and cells were stored with only one bag in liquid nitrogen);
d. immunological reactions, including allergic reactions, graft-*versus*-host disease, rejection, haemolytic reactions or other immunological reactions [Immunology];
e. malignant disease possibly transferred by the tissues or cells (donor-derived, process-associated or other) [Malignancy];
f. unexpectedly delayed or absent engraftment, or graft failure (including mechanical failure) [Failure];
g. toxic effects to tissues and cells or associated materials [Toxicity];
h. unexpected immunological reactions due to tissue or cell mismatch [Mismatch];
i. aborted procedure involving unnecessary exposure to risk, e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun [Undue risk];
j. suspected transmission of genetic disease by transplantation or gamete/embryo donation [Genetic abnormality];
k. suspected transmission of other (non-infectious) illness [Other transmission];
l. transfusion-associated circulatory overload in haematopoietic progenitor cell (HPC) transplantation [Volume overload];
m. neurological reaction [Insult];
n. severe febrile reaction [Fever];
o. other [Other].

Efficient systems for rapid quarantine or recall of unsafe tissues or cells must be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk. Important learning outcomes from each adverse reaction should be communicated appropriately to all professionals involved.

17.4.1. Detection of adverse reactions

Effective V&S relies heavily on all health professionals involved, from donor selection to clinical application and follow-up, namely:

a. medical staff (including surgeons) involved in tissue- and cell-procurement activities who might become aware or informed of additional safety information on donors during their follow-up;
b. staff and personnel carrying out procurement of tissues and cells;
c. clinical users who should pay attention to adverse outcomes and be aware when such outcomes might be associated with the clinical use of tissues or cells;
d. physicians caring for children born after non-partner MAR/ART treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of gametes/embryos from that donor;

e. any other TE staff involved in any procurement, processing, tissue distribution or follow-up information step;
f. other vigilance systems (e.g. haemovigilance, vigilance of organs, material/device vigilance, pharmacovigilance) when issues of concern are detected that might affect the safety of tissues or cells for transplantation.

Adverse outcomes might result from many diverse factors associated with the surgical procedure or the patient's underlying condition. Hence, clinicians might not consider the tissues or cells (including MAR) that were applied to be a possible source of the adverse outcome. TEs that supply tissues and cells should encourage procurement organisations and clinical users of tissues and cells to always consider whether adverse outcomes might have been associated with the donation process or caused by the tissues or cells applied (see §17.4.3 – Investigation and assessment of adverse reactions) so that similar occurrences are prevented in the future.

For most types of well-established clinical application of tissues and cells, detailed reporting of clinical outcome by the clinical user to the TE is required only in those exceptional circumstances in which there is suspicion of an untoward adverse reaction. However, reporting of the clinical progress of tissue and cell recipients to the TE might also be required for all highly matched, life-saving transplants such as HPC infusions, or when novel tissue or cell processes have been applied or new types of tissues or cells are being transplanted. This routine clinical follow-up is considered as part of surveillance.

An important part of vigilance is detecting donation complications (also considered to be adverse reactions) in living donors that might be associated with the donation process in some way. For example, adverse reactions may be detected after stimulation treatment in living donors and recipients (see Chapter 24 – Haematopoietic progenitor cells from bone marrow and peripheral blood and Chapter 29 – Medically assisted reproduction).

17.4.2. Serious adverse reactions reporting

17.4.2.1. *Clinicians to tissue establishments*

TEs that supply tissues and cells should provide organisations representing clinical users with clear instructions on how to report adverse reactions, preferably using standardised documentation. In general, suspected adverse reactions should be reported immediately by the clinical users to the TE that supplied the tissues or cells before investigation or confirmation of an SAR. This approach allows the TE to take appropriate precautionary actions to prevent harm to other patients, if needed, and start the investigation process. Clinical users should be encouraged in the surveillance of all types of suspected adverse reactions (serious and non-serious) which might be related to the tissues and cells from the supplying TE, to allow filtering of those considered to be serious and reportable to an authority at a later stage. Administrative barriers for reporting of adverse events by clinicians should be minimised if possible.

Specifically, in MAR, patients (and, if appropriate, partner) undergoing non-partner donations are important stakeholders when reporting of SARs is concerned. Clinicians offering treatments that involve the use of donor gametes or donor embryos should inform and encourage patients (and, if appropriate, partner) that, if any disease is detected in their child, they must report back to the MAR/ART centre. Patients (and, if appropriate, partner) should be clearly informed of their registration obligations concerning diseases in children. It is in the interest of all (future) patients and couples using donor gametes that SARs in gametes donors are quickly notified in order to be able to quarantine material from these specific donors and prevent further spread of a particular disease or condition. It is imperative to note that not all diseases in these children are directly related to the donor. Therefore, a careful risk assessment is needed that takes into account the type of disease (chromosomal, multifactorial, single-gene or mitochondrial disorders, communicable and non-communicable diseases) and the possibility of (genetic) testing of the child, biological parent and/or gametes donor, as well as global prevalence and genetic predisposition. A similar approach applies to HPC transplantation (see §24.10.2.1.12).

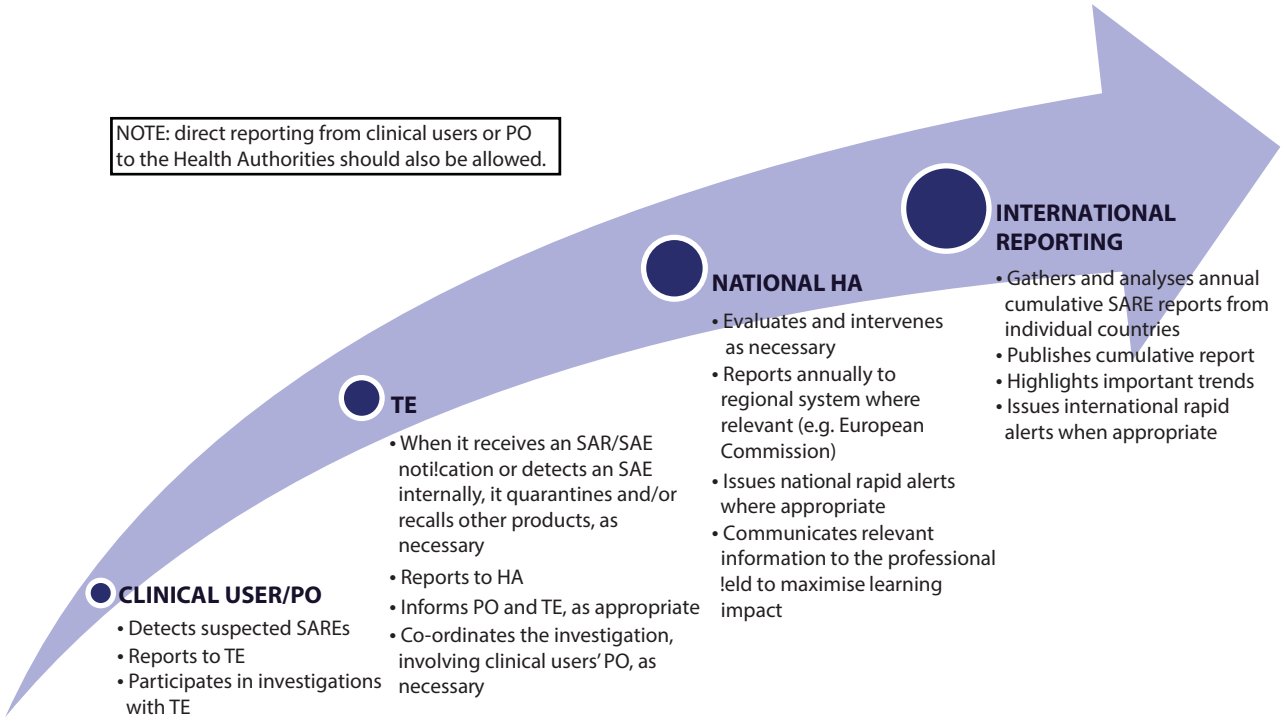
17.4.2.2. *Procurement organisations to tissue establishments*

Health professionals and procurement organisations should report adverse reactions in living donors and organ and tissue recipients to the TE, even if the adverse reaction is only suspected to be donation-derived, so that the broader implications for other centres and donors can be considered without delay. For this reason, it is important to link all the different vigilance systems, especially organ and tissue systems (see §17.6 – Vigilance co-ordination).

17.4.2.3. *Reporting to regional/national programmes*

TEs must report information on SARs to Health Authorities (see Figure 17.2 – Reporting flow for serious adverse events/reactions). In the EU, all SARs

Figure 17.2. **Reporting flow for serious adverse events/reactions**



HA: Health Authority; EU: European Union; PO: Procurement Organisation; SAE: Serious Adverse Event; SAR: Serious Adverse Reaction; SAREs: Serious Adverse Reactions and Events; TE: Tissue Establishment.

related to quality or safety that meet the descriptions of 'serious', 'life-threatening' or 'death' must be reported to the Health Authorities. The TE is responsible for providing clinical user organisations and critical third parties with clear instructions, forms and guidance on how to notify adverse reactions in accordance with national or local requirements. Reporting and management of adverse reactions should be incorporated in the quality system of the TE, with one or more SOPs that describe the process for acknowledgement of notifications, investigation, follow-up on corrective and preventive actions and reporting to the Health Authorities if criteria are met. Moreover, a specific procedure should enable rapid action, if needed, to be taken by all affected organisations to protect the safety of recipients (see Figure 17.1 – Categories of biovigilance scenarios).

This may involve tissue and cell quarantine, recall and look-back in patients who have already had implicated tissues or cells applied. These actions may need to be taken by organisations other than the one that received the original notification. For example, the organ procurement organisation will play a central part when the deceased donor was an organ and tissue donor.

Figure 17.3 shows a series of actions that might need to be taken in a report of suspected transmission from a deceased donor of organs and tissues. It makes clear that communication with other organisations that might need to quarantine implicated tissues or cells, or conduct recalls or look-backs, should be quick and effective.

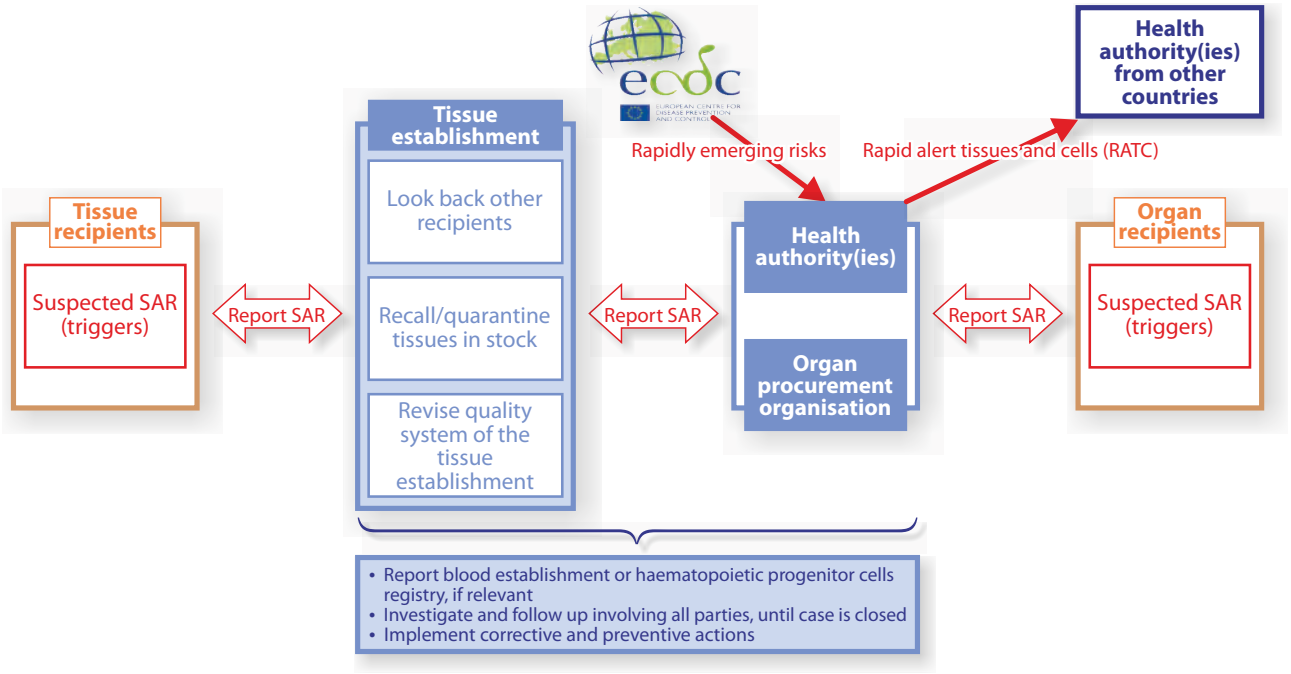
Although reporting of SARs should, in general, be co-ordinated and centrally reported by TEs at a national level, it is recommended that national V&S programmes allow direct reporting from clinical users or even patients to Health Authorities. There are some examples in pharmacovigilance of patients directly reporting outcomes of adverse events. The idea with this initiative is to have a full range of self-reporting rather than patients' reports only being collected by clinicians. The project is called Prosper (Patient Reported Outcome Safety Event Reporting) [7]. In the case of tissues and cells, patients should be advised to contact healthcare professionals in the event of any potential symptom which is not expected.

17.4.2.4. *International reporting*

If SARs are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all the stakeholders involved (clinicians, TEs and Health Authorities) in each of the countries concerned are informed and participate, as necessary, in the investigation and follow-up actions.

In the case of HPC, WMDA and national HPC registries have implemented a global reporting

Figure 17.3. **Example of an adverse reaction involving multiple parties**



system because HPC products have specific characteristics (for more information see Chapter 24).

In the EU, according to Directive 2006/86/EC, member states must submit to the European Commission an annual report on the notification of Serious Adverse Reactions and Events (SAREs) compiled at national level by the Health Authorities. In return, the European Commission submits a summary of the reports received which is publicly available on their website [8]. Such international reporting allows for trend analyses on the basis of consolidated data and for sharing of the lessons learned. This directive also states that Health Authorities must make this report available to TEs.

The latest data available, from the 2020 SARE exercise (data 2019), were published by the commission on 08 November 2021. In the summary of the European Commission of the 2020 annual reporting of serious adverse reactions and events for tissues and cell using the data collected from 2019, a total of more than a million tissues and cells were distributed (393 625 non-reproductive and 966 690 reproductive). In total, 306 SARs were reported (156 non-reproductive and 150 reproductive) and 949 SAEs were reported (689 non-reproductive and 260 reproductive). Unfortunately, still not all countries report their complete data although the number of notifications is increasing regularly since the first European published report.*

In order to put into context the numbers of SAEs and SARs related to the use of tissues and cells of human origin, it is essential to collect harmonised and accurate activity data. These data would also be critical in assessing how many tissues and cells are available in a given country or region and how many are regularly used, in an effort to achieve self-sufficiency both at the level of each country and at European scale, so that transplantation requirements can be consistently met. In an attempt to streamline and harmonise data collection exercises in Europe, in the framework of a co-operation Grant Agreement between the European Commission and the Council of Europe/EDQM and with the support of the main professional societies and existing registries in the field, the EDQM co-ordinated the identification of a minimum dataset that, if regularly collected by member states, would serve the above-mentioned objectives [9]. The exercise entailed reaching an agreement on the parameters, units and expected quality of the data to be collected, as well as making recommendations on who should be accountable for the collection and validation of the data and who should ensure dissemination among all relevant stakeholders. It was agreed that all stakeholders identified in this exercise should collect and record the identified data. This would lead to important improvements in the data available at national and European level for transparency and biovigilance purposes, as well as supporting policy decisions in relation to supply and self-sufficiency.

* https://ec.europa.eu/health/publications/summary-2020-annual-reporting-serious-adverse-reactions-and-events-tissues-and-cells_en.

17.4.2.5. *Follow-up of the donors of tissue and cells*

The follow-up of donors of tissue and cells is important for the early detection and reporting of SAREs. Short-term follow-up is essential to ensure recovery from the donation procedure. Long-term follow-up is desirable to enable any long-term effects of the donation to be identified. The nature and duration of this follow-up will depend on the type of donation, the nature of the intervention and its potential impact on the individual's health [10]. Adapted and robust registries should be developed by professionals in order to trace and assess these data. We can consider follow-up as an example of the continuous-proactive category of biovigilance (see Figure 17.1).

Completed donor follow-up has been defined as physical, phone or laboratory contact at a given time point [11]. There should be written SOPs for follow-up of donors. For related donors, the responsible person for the follow-up should be the physician undertaking the assessment of the donor for the donation. For unrelated donors, the follow-up should be the responsibility of the relevant donor registry, if present.

After donation, the living donor (related or unrelated) should also be followed up, by documented procedures. This follow-up will depend on the type of donation, and the length of follow-up should reflect the guidance of the professional body. This follow-up ensures that, if a new condition occurs that may have an impact on the recipient, proper action can be taken; it also ensures that, if SARs occurred to the donor, the procurement procedure will be improved. This should be clearly documented. No matter how extensive the testing that is performed prior to donation, the donor can develop diseases not known at the time of donation. These can be newly discovered infectious diseases or malignancies. These conditions may have been transmitted to the recipient as well. There is no clear evidence regarding malignancies, but haematological malignancies within one year of donation may have been present in the graft and transmitted to the recipient. In these cases, the recipient should be specifically monitored for the presence of the disease and if possible, preventive steps should be taken.

17.4.3. Investigation and assessment of adverse reactions

Depending on the level at which the adverse reactions occurred, certain measures have to be taken before starting the investigation. The first precautionary measure is to quarantine any other remaining tissues or cells from the same donor. If tissues or cells have already been distributed, it must be determined where or to whom they are allocated and consider the possibility of recalling if still an option. These measures are intended to minimise the number of recipients exposed to the same reaction.

a. The first step in the investigation is to determine the severity. A 'severity scale' can be used to decide whether a particular adverse reaction is an SAR that needs to be reported to the Health Authorities. The scale shown in Table 17.1 is used in the EU. It was proposed by the project European Union Standards and Training for the Inspection of Tissue Establishments (EUSTITE) [12] for vigilance for tissues and cells and is based on the scale used for haemovigilance.

Adverse reactions in recipients of tissues or cells should be investigated by a multidisciplinary team that can carry out an independent investigation; the team should also include the

Table 17.1. **Severity scale for adverse reactions**

Not reportable	Insignificant	No harm to the recipient or living donor, and considered to be reportable as an event rather than a reaction according to EU directives
	Non-serious	Mild clinical consequences that do not necessitate hospitalisation and/or do not result in long-term disability or consequences for the recipient or living donor
To be reported	Serious	Adverse reaction resulted in: • hospitalisation or prolongation of hospitalisation and/or • persistent or significant disability or incapacity and/or • medical or surgical intervention to preclude permanent damage or impairment of a body function and/or • evidence of a serious transmissible infection and/or • birth of a child with a serious genetic disease after ART with non-partner gametes or donated embryos
	Life-threatening	• The living donor or recipient required major intervention after procurement or application of tissues or cells (vasopressors, intubation, transfer to the intensive care unit) to prevent death and/or • There is evidence of a life-threatening transmissible infection
	Fatal	Death in a living donor or a recipient of tissues or cells

Table 17.2. **Scale describing possible outcomes of an imputability investigation**

	Criteria adapted from Eustite-SoHO V&S [14, 15]	**Criteria for infectious and malignant transmissions, adapted from the Disease Transmission Advisory Committee [16]**
Not assessable	Insufficient data for imputability assessment	Insufficient data for imputability assessment
0. Excluded	Conclusive evidence beyond reasonable doubt for attributing an adverse reaction to alternative causes	Suspected transmission and fulfilment of at least one of the following conditions: • clear evidence of an alternative cause • the appropriate diagnostic tests carried out have failed to document infection by the same pathogen in any recipient from the same donor • laboratory evidence that the recipient was infected with the same pathogen or had a tumour before the application of organs, tissues or cells
1. Possible	The evidence is indeterminate for attributing an adverse reaction to the quality/safety of tissues and cells, to the donation process or to alternative causes	Either • suspected transmission and • laboratory evidence of the pathogen or tumour in a single recipient or • data suggest a transmission but are not sufficient to confirm it
2. Probable	The evidence is clearly in favour of attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	Not only are the following two conditions met: • suspected transmission and • laboratory evidence of the pathogen or tumour in a recipient but also at least one of the following conditions is met: • laboratory evidence of the same pathogen or tumour in other recipients • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, such evidence must indicate if the same recipient was negative for the pathogen involved before transplantation
3. Definite; certain	The evidence is conclusive beyond reasonable doubt for attributing the adverse reaction to the quality/safety of tissues and cells (for recipients) or to the donation process (for donors)	All the following conditions are met: • suspected transmission • laboratory evidence of the pathogen or the tumour in a recipient • laboratory evidence of the same pathogen or tumour in other recipients (if multiple recipients) • laboratory evidence of the same pathogen or tumour in the donor If there is pre-transplant laboratory evidence, it should be noted that the same recipient was negative for the pathogen before transplantation

This table can be adapted to other tissues or cells and for MAR in order to take into account the specificities of each type of product.

clinician who transplanted the tissues or cells, the TE that provided them and, in more serious cases, the Health Authority in that country. Efficient co-ordination of the investigation is critical to the rapid implementation of effective corrective actions. If relevant, experts in particular fields (e.g. viral transmission) should also be invited to participate in the investigation of the adverse reaction.

b. The second step is to assess imputability. The investigation should focus on establishing the level of imputability (i.e. the extent to which the tissues or cells used clinically can be considered to have caused the adverse reaction). The scale provided in Table 17.2, developed by Eustite, can be applied to describe the outcome of an imputability investigation. It proposes that all adverse reactions be graded in terms of imputability. Table 17.2 also recommends specific approaches to the establishment of imputability for suspected infectious or malignant transmissions, as proposed by Garzoni and Ison in the context of transplantation [13]. Imputability grades might change during an investigation and should, in general, be assigned at the point of initial notification and again at the completion of the adverse reaction investigation. The evaluation of imputability should be based on scientific or clinical data. The ECDC, the WHO or other sources of epidemiological and risk information may be useful to support the process. Consideration should also be given to the practice of archiving pre-transplant samples for transplant recipients to support imputability investigations (including the possibility of genetic material from donors in MAR).

TEs have to review:

1. all the reagents, substances etc. that were in contact with the tissues and cells during processing, checking the expiry date and the sterility;
2. the tissues processed in the same room on the same day, the day before and the day after;
3. donor medical and social history;

4. all the microbiological results of the donor and of each specific graft;
5. the microbiological checks of the processing rooms.

The ORHA that uses the tissues or cells needs to determine whether other patients were operated on in the same operating room and those patients' clinical and microbiological conditions. This approach is not advised for recipients of gametes or embryos (see Chapter 29).

c. The final step is the impact assessment, which establishes the probability or likelihood of recurrence of the SAR, taking into account the consequences to the individual, or the system or the supply, selecting the worst-case scenario in each category. Using the impact matrix allows determination of the level of corrective and preventive action to be taken according to the Health Authority (see the impact assessment tool given in Appendix 27).

17.5. Adverse events

Adverse events can occur at any moment from donor selection to clinical application. However, it should be emphasised that an adverse event may not always produce a subsequent reaction in the recipient.

Non-compliances with the quality system should be documented and investigated as part of the internal QMS. On occasions, however, a particular non-compliance may be of such importance that it should be considered as an SAE and reported through the vigilance system. Some examples of SAEs are (for more information, check the specific tissue chapter in Part B):

a. Final result of a negative-to-date release with a cultured cornea was reported as positive; no adverse reaction detected in the cornea recipient.
b. *Aspergillus* was detected in an incubator used for storing tissues and cells that had been distributed. This could be attributed to a processing step.
c. Loss of all embryos during handling; the patient requires a new cycle of IVF.
d. Skin donor not tested for malaria although donor resided in a malaria-endemic area.
e. Lack of liquid nitrogen, in a liquid nitrogen refrigerator containing several tissues, resulting in thawing of the tissues. This example could have been related to storage.
f. A frozen bone arrives at the hospital and the bag is broken, this being discovered before patient is anaesthetised and before the surgical procedure has begun.

17.5.1. Detection of serious adverse events

For effective detection of adverse events, all relevant stakeholders must be aware of their responsibilities for identifying errors or unexpected results. This includes all staff in TEs and procurement organisations, those working in organisations such as testing laboratories that provide 'third party' services to TEs, and clinical users who may also detect errors at the point of clinical use. Following the SoHO V&S project in the EU, some Competent Authorities now promote the reporting of what are called near misses, meaning those errors that have real potential to provoke a serious adverse reaction, which either does not take place (by chance or preventive remedial action) or does not have consequences for the patient, the system or the staff.

17.5.2. Serious adverse event reporting

According to instructions from the European Commission to EU member states for annual vigilance reporting, deviations from SOPs in TEs (or other adverse events) that have implications for the quality and safety of tissues and cells should result in an SAE report to the Health Authority if one or more of the following criteria [17] apply (see also Figure 17.1).

a. Inappropriate tissues/cells have been distributed for clinical use, even if not used;
b. The event could have implications for other patients or donors because of shared practices, services, supplies or donors;
c. The event resulted in loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;
d. The event resulted in the loss of a significant quantity of unmatched allogeneic tissues or cells.

The criteria adopted for reproductive tissues and cells are:

a. inappropriate gametes, embryos or germinal tissues have been released for clinical use, even if not used;
b. the event could have implications for other patients or donors because of shared practices, services, supplies, critical equipment or donors;
c. any type of gamete or embryo misidentification or mix-up of gametes or embryos;

d. the event resulted in a loss of traceability of gametes or embryos;
e. contamination or cross-contamination;
f. accidental loss of gametes, embryos, germinal tissues (e.g. breakdown of incubators, accidental discard, manipulation errors) resulting in a total loss of chance of pregnancy for one cycle.

17.5.3. Investigation and assessment of serious adverse events

Despite the fact that SAEs, by definition, have not (or not yet) involved harm to recipients or donors, the impact of an SAE can be significant if considered in a broader way. The impact assessment tool given in Appendix 27 can also be applied to SAEs to help reach a decision on the response required. Appendices 28, 29, 30 and 31 provide examples that may serve as guidance.

17.6. Vigilance co-ordination

Co-ordination between various systems of vigilance (e.g. organ and haemovigilance, material/device vigilance, pharmacovigilance) should be in place at the local level (TE) and at the Health Authority level.

The European Medicine Agency defines pharmacovigilance as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem". The relevant legal texts and guidelines are described on the pharmacovigilance web page of the European Commission [18]. Donation, procurement and testing of the tissues and cells used to prepare an ATMP are regulated in the EU by directives on tissues and cells. Consequently, good communication between biovigilance and pharmacovigilance systems is essential to facilitate effective investigation and corrective/preventive actions if ATMPs are associated with adverse outcomes.

The lack or omission of the exchange of information can put more patients or the same recipients at risk. Sometimes it is someone within the system who discovers a problem and needs to inform the Health Authority. For example, if there is problem with a bag used for cryopreserved tissues or cells and that problem altered the characteristics of the product, or if such a bag was stored at −196 °C and after some time this bag was broken, then the medical device vigilance system should be informed. The same caution should apply when faced with an SAR or SAE with organs if it involves tissues which were retrieved, or vice versa; in either case, all the corresponding vigilance systems should be informed. Sharing information between vigilances and health professionals might be really important, notably because some materials or devices used in the preparation, shipment or other steps of the process from procurement to graft are manufactured by only one supplier and a quality defect or a problem can have huge consequences on SoHO activities.

Each country decides how to connect all the vigilance systems at national and international level. There are some initiatives to join organs, tissues and cells in one system, although not all professionals agree on this due to the differences in terms of risk assessment [19]. In any case, the systems must be linked, connected and able to exchange information in both directions.

17.6.1. Rapid alerts

In some circumstances, a particular event or reaction requires rapid communication nationally or internationally to facilitate urgent action, such as a recall of products or critical materials or the quarantine of tissues or cells (see Figure 16.1). In that case a communication system must be available at all times. Rapid alerts should only be issued in exceptional circumstances. The following criteria have been identified in the SoHO V&S project [20] as triggers for rapid alerts within or between EU member states:

a. an ARE of a serious or potentially serious nature;
b. potential risk to other individuals or other TEs;
c. wider public health implications;
d. rapid intervention needed (preventive or corrective measures, urgent communication).

Within the EU, a system for rapid alerts – called Rapid Alerts for Tissues and Cells (RATC) – is hosted by the European Commission and enables the Competent Authorities of EU member states to rapidly share urgent information on risks to patients where that information has consequences in more than one EU member state [21]. In February 2013, this system was moved to a new secure internet platform where all rapid alerts are generated and shared, with access restricted to Competent Authorities. The reports summarising the launched alerts every year are published on the EC webpage [22].

17.7. Vigilance communication: education and training

17.7.1. 'No blame' culture

Effective communication at all levels of the results of vigilance systems is fundamental to ensure the benefits of these programmes. Regular feedback to health professionals is critical in supporting the continued notification of AREs. All stakeholders, Health Authorities, TEs and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients and donors. It should be accepted that mistakes happen and that no programme of transplantation or MAR is risk-free. Programmes of training and awareness should be organised to encourage reporting. The message should be promoted that reporting and disseminating V&S information can result in positive improvements for donors and patients, as well as feedback to health professionals.

17.7.2. Vigilance experience and feedback

Health Authorities and professional societies should publish the results of their programmes without identifying individual centres, hospitals or people. Those TEs or hospitals directly involved in specific incidents should also consider publishing their experience to alert others to the means by which they detected and confirmed the event or reaction.

The Notify Library is an initiative launched by the WHO and supported by the Italian National Transplant Centre (CNT) that has gathered information on documented adverse occurrences in transplantation and assisted reproduction. It has reviewed cases to identify general principles supporting detection and investigation. The database has been constructed from the information gathered and is accessible on a dedicated website [3, 23]. The database is maintained and updated on this platform and is intended as a communication hub for institutions and organisations worldwide collaborating in the facilitation of access to V&S information to improve safety and efficacy.

17.7.3. Educational training and workshops

When SoHO are used, there is always a risk that needs to be considered if something happens afterwards that can be related to the quality of the tissue or cells used and that needs to be communicated. Health Authorities should encourage biovigilance awareness in all professionals involved in tissues and cells at any step (from donation to implant). To achieve this awareness, it is necessary to educate and train the professionals about the benefits of implementing a biovigilance system. This can be done with a high-quality educational programme and with well-organised workshops, disseminating the message in meetings, publishing reports with anonymised data, etc.

The purpose is to stimulate reporting in an appropriate manner while avoiding over-reporting, which can collapse the system. Professionals need guidance about what to communicate, when and to whom. Healthcare providers need to have confidence in this system, which is why it is important that the reporting system is non-punitive and confidential. Reporting and further analysis are very useful tools for learning how to avoid mistakes and other errors; in the end, the resulting analysis is beneficial for the safety of donor and patients.

Workshops – using real cases for discussion, describing how to investigate them and defining the possible causes of SAREs summarised in the final reports – can help in professionals' daily work.

17.8. References

1. Kohn LT, Corrigan JM, Donaldson MS (eds). *To err is human: building a safer health system*. Washington DC: National Academy Press, 2000.
2. Leape LL. Reporting of adverse events. *N Engl J Med* 2002;347(20):1633-8.
3. Notify Library, available at www.notifylibrary.org, accessed 11 April 2022.
4. European Cornea and Cell Transplantation Registry, available at www.ecctr.org, accessed 11 April 2022.
5. Gauthier AS, Castelbou M, Garnier MB *et al.* Corneal transplantation: study of the data of a regional eye bank for the year 2013 and analysis of the evolution of the adverse events reported in France since 2010. *Cell Tissue Bank* 2017;18(1):83-9.
6. Ikeda K, Ohto H, Okuyama Y *et al.* Adverse events associated with infusion of hematopoietic stem cell products: a prospective and multicenter surveillance study. *Transfus Med Rev* 2018;32(3):186-94.
7. Banerjee AK, Okun S, Edwards IR *et al.* Patient-reported outcome measures in safety event reporting: PROSPER consortium guidance. *Drug Saf* 2013;36(12):1129-49.
8. European Commission. Tissues and cells – reports on implementation, available at https://ec.europa.eu/health/blood-tissues-cells-and-organs_en, accessed 11 April 2022.
9. Harmonising activity data collection exercises in the field of tissues and cells in Europe, available at www.edqm.eu/en/

transplantation-areas-work#Harmonisation-of-data-collection, accessed 11 April 2022.

10. den Exter A (ed.). *International health law and ethics*. Antwerp/Apeldoorn: Maklu, 2009:311-12.
11. Brown RS Jr, Smith AR, Dew MA. Predictors of donor follow-up after living donor liver transplantation. *Liver Transplant* 2014;20:967-76.
12. Fehily D, Sullivan S, Noel L, Harkin D. Improving vigilance and surveillance for tissues and cells in the European Union: Eustite, SoHO V&S and Project Notify. *Organs Tiss Cells* 2012;15(2):85-95.
13. Garzoni C, Ison MG. Uniform definitions for donor-derived infectious disease transmissions in solid organ transplantation. *Transplantation* 2011;92(12): 1297-300.
14. Eustite Project. Vigilance and surveillance of tissues and cells in the European Union. Final Recommendations, June 2012, available at www.notifylibrary.org/background-documents#European-Union-Standards-and-Training-for-the-Inspection-of-Tissue-Establishments-Project-(Eustite), accessed 11 April 2022.
15. SoHO V&S. Guidance for competent authorities: communication and investigation of serious adverse events and reactions associated with human tissues and cells, available at www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Communication%20and%20Investigation%20Guidance.pdf, accessed 11 April 2022.
16. US Department of Health. Disease Transmission Advisory Committee, available at https://optn.transplant.hrsa.gov/members/committees/disease-transmission-advisory-committee/, accessed 11 April 2022.
17. SoHO V&S. Guidance on vigilance and surveillance in assisted reproductive technologies in the European Union. Work Package 5, Deliverable 5, available at www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Guidance%20on%20V%26S%20in%20ART%20in%20the%20European%20Union.pdf, accessed 11 April 2022.
18. European Commission. EU pharmacovigilance system, available at https://ec.europa.eu/health/medicinal-products/pharmacovigilance_enec.europa.eu/health/human-use/pharmacovigilance/index_en.htm, accessed 11 April 2022.
19. Pruett TL, Blumberg EA, Cohen DJ *et al.* A consolidated biovigilance system for blood, tissue and organs: one size does not fit all. *Am J Transplant* 2012; 12(5):1099-101.
20. Fehily D, Uhrynowska-Tyszkiewicz I, Creusvaux H *et al.* Vigilance: lessons learned from the tissue and cell experience in the European Union. Part 1: reporting and communication. *Organs Tiss Cells* 2013 Nov;16(3): 165-73.
21. European Commission. Tissues and cells, available at https://health.ec.europa.eu/blood-tissues-cells-and-organs/tissues-and-cells_en, accessed 29 November 2022.
22. European Commission. Rapid Alert System: Tissues and Cells, latest updates, available at https://ec.europa.eu/health/latest-updates/summary-2020-activities-rapid-alert-system-human-tissues-and-cells-ratc-and-human-blood-and-blood-2021-04-16_en, accessed 11 April 2022.
23. Fehily D, Strong DM, Minutoli D *et al.* Sharing vigilance experience and knowledge globally: a preliminary overview of the Notify Library. *Organs Tiss Cells* 2013;16(2):117-25.

Related material

Appendix 27. Serious adverse reaction or event: impact assessment form
Appendix 28. Example of serious adverse reaction (SAR) for non-reproductive tissues and cells
Appendix 29. Example of serious adverse reaction (SAR) for reproductive tissues and cells
Appendix 30. Example of serious adverse event (SAE) for non-reproductive tissues and cells
Appendix 31. Example of serious adverse event (SAE) for reproductive tissues and cells

Chapter 18. Introduction of novel processes and clinical applications

18.1. Introduction

Advances in basic science, technology and medicine create opportunities for the development of novel tissue or cell preparation processes (including changes to donor selection, procurement, processing, storage and distribution methodologies) or clinical applications. There are several key elements that should be considered to ensure the quality, safety and efficacy of novel processes and applications and, thus, the safety of donors and recipients:

a. Clinical need should be the predominant driver for the development of novel processes and applications for tissues and cells.

b. The involvement of, and close co-operation between, three stakeholders – tissue establishments (TEs), clinicians representing organisations responsible for human application (ORHAs) and Health Authorities – is essential to ensure that the above-mentioned principles (i.e. quality, safety and efficacy) are addressed. A clear structure identifying the responsibilities of each party, and how the different parties interact with each other, must be established and documented.

c. Comprehensive risk analysis should underpin the development and evaluation of novel processes and applications. This risk analysis should consider both the risks and potential clinical benefits of the novelty. Evaluation may comprise *in vitro*, *in vivo* and, where indicated, clinical evaluation and patient follow-up according to the level of risk identified.

Some processes and applications, while new to a specific TE, are not novel *per se* as they have been well consolidated by others. For those processes, Part D of this Guide includes monographs appropriate to different processes and applications for substances of human origin. Monographs are useful tools for TEs and Health Authorities, providing the minimum criteria to ensure the quality of different types of tissues and cells; they are tools that can be used by TEs to design appropriate validation studies for new processes.

18.2. Regulatory considerations

When a TE is developing a novel preparation process, or if it plans to provide tissues or cells for a new clinical application, it should consider whether the process or therapy might lead to a regulatory classification of the tissues or cells as medicinal products, or as advanced therapy medicinal products (ATMPs) or as medical devices. If this is the case, the regulatory framework for the authorisation of the relevant product type will be applicable in the EU (for further information, see Chapter 34). In these cases the regulatory requirements of the tissue and cell legislation for donation, procurement and testing still apply. TEs should engage with their Health Authority at an early stage of the product development cycle, in

order to establish in advance which is the applicable legal (regulatory) framework in their country.

This chapter is based on the principles and guidance developed by three EU-funded projects.

a. *VISTART*
This EU Joint Action (Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation) proposed regulatory principles for Health Authorities for the appraisal and approval of clinical evaluation protocols for blood, tissues and cells prepared with new processing methodologies [1]. These VISTART principles aim to guide stakeholders in the development and implementation of novel preparation processes or clinical applications, ensuring compliance with regulatory and technical requirements, and they propose an approach whereby the degree of risk associated with the novelty is linked to an appropriate clinical follow-up plan.

b. *EuroGTP II*
This project (Good Practices for demonstrating safety and quality through recipient follow-up) developed good-practice guidance for TEs for evaluation of the safety, quality and efficacy of tissue and cellular therapies and products. The key outputs from Euro GTP II included a systematic mechanism and interactive assessment tool to identify and evaluate risk, and defined methodologies that can be used to mitigate risk [2] (see also Chapter 3).

c. *GAPP*
GAPP Joint Action (facilitating the Authorisation of Preparation Process for blood and tissues and cells) focused on facilitating the development of a common and optimal approach for Competent Authorities to assess and authorise preparation processes in blood and tissues establishments (BEs and TEs) [3]. This project developed Good Practice Guidelines for Health Authorities for preparation process authorisation (PPA) for blood, tissues and cells, and three annexes: 1. authorisation changes in donation, procurement and collection, processing, preservation, storage and distribution; 2. assessing the quality and safety of donor testing, microbial inactivation/reduction and sterilisation steps as part of PPA; and 3. assessing clinical data as part of PPA. The EuroGTP II tool and methodologies are recognised by the GAPP project as a suitable mechanism for performing this type of evaluation based on the ViSTART principles.

18.3. Interaction between key stakeholders

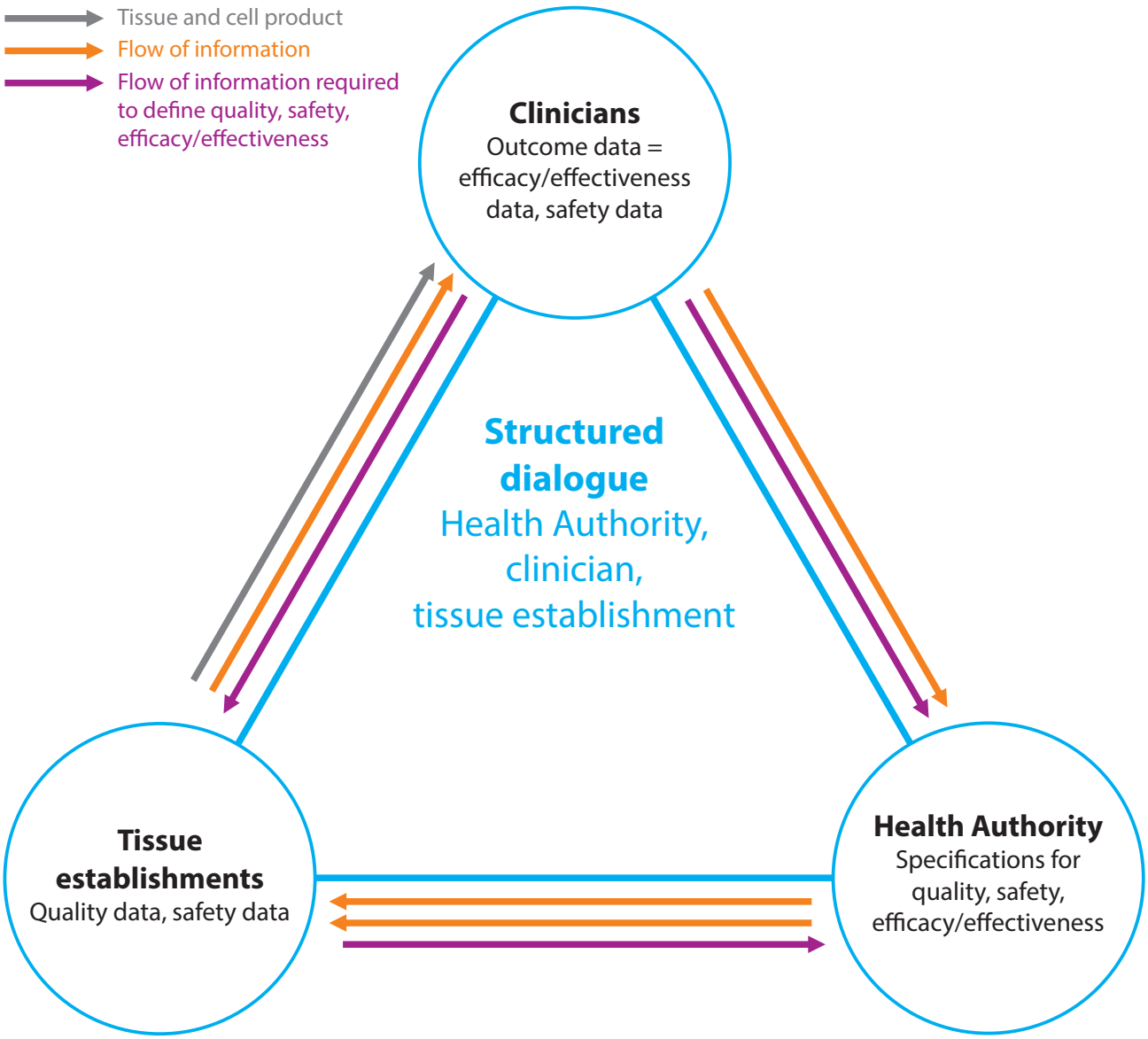
When introducing novelties in the field of tissue and cells, three main stakeholders should interact closely. These are the TEs, the clinicians in the ORHA and Health Authorities. They each contribute their specific expertise to establish a structured, safe and efficient approach to the introduction of new tissue and cell preparations and clinical applications. As consideration of the risks, both to the product effectiveness and to the safety of the patient and donor, is critical, consultation with all of these stakeholders is essential. There should be a formal agreement between the TE and the clinicians/ORHA clearly specifying their respective roles and responsibilities when setting up clinical evaluations. Moreover, the TE and clinicians/OHRA may contribute information for completing the preparation-process dossier to request a formal authorisation/agreement to their Health Authorities. Figure 18.1 describes schematically a structured flow of information between TE, clinicians/ORHA and Health Authorities.

18.3.1. Responsibilities of the tissue establishment

The TE is responsible for ensuring that the quality and safety of tissue and cell products meet the regulatory requirements and technical specifications necessary for release for clinical application. In this context, quality and safety result from the donor selection, the procurement of tissues and cells, the testing and the preparation processes. Preparation processes must be performed in compliance with the tissue and cell Good Practice Guidelines (Part C) and the EU Tissue and Cells directives requirements.

Two different perspectives currently apply to the meaning of the term 'quality' within the field of tissue and cell processing and both should be addressed by the set of quality-control parameters used to characterise the tissues and cells resulting from the preparation process: quality may be seen as the fulfilment of a specific set of standards, characteristics and requirements as predefined by the preparation process, i.e. compliance of the tissue or cell product with its specifications (tissue and cell monographs). Quality may also be seen as an indicator of the safety and efficacy of the tissue or cell product. The critical parameters for novel tissue or cell preparation processes should cover both quality perspectives.

Figure 18.1. **Flow of information between tissue establishments, clinicians and Health Authorities**



The safety profile of tissues and cells covers biological (infectious, immunological), physical (e.g. morphological appearance, integrity, elasticity) and/or chemical (e.g. toxicological, residual traces of reagents) properties. The safety of novel preparation processes or clinical applications results from a careful and comprehensive risk analysis. The methodology to perform a risk-based analysis of tissue and cells preparation processes and clinical applications proposed by the EuroGTP II project [2] takes into account risks related to donor characteristics, procurement process and environment, preparation process and environment, reagents, reliability of microbiology testing, storage conditions, transport conditions, the presence of unwanted cellular material and the complexity of the preparation/application method (see Chapter 3).

Risk analysis should be based on current evidence derived from known preparation processes, processing steps and products that are comparable to the tissues and cells obtained with novel preparation methods and/or new clinical applications. In circumstances where evidence is lacking, due to the grade of novelty and uncertainty, an analysis should be done to estimate the risks. The EuroGTP II tool and methodologies are recognised as a suitable method for performing this type of evaluation. The TE should use *in vitro* and (where feasible) *in vivo* testing to investigate and mitigate any potential risks prior to clinical application. The TE should include the risk analysis and supporting information in the preparation-process dossier to submit to Health Authority for approval (see §18.3.3). The results of these tests are a pre-requisite to initiate a clinical investigation or follow-up of a well-defined patient cohort, as a first step of the clinical efficacy and safety validation of these new products, before a routine application. The

safety and efficacy of the tissue and cells product can only be confirmed by clinical outcome data.

18.3.2. Responsibilities of the clinicians/ organisations responsible for human application

The key role of the clinician is ensuring product safety and efficacy in the context of clinical application of tissue and cell products. The clinician is responsible for obtaining appropriate patient consent for application, and for collecting clinical outcome data from patients. Clinical outcome data should be gathered from a well-defined patient cohort to demonstrate clinical efficacy and safety of the novelty.

The extent to which clinical outcome data are required to verify the safety and efficacy of novel tissues or cells depends on the level of risk of the novelty. A systematic analysis of literature evidence may be used to define the extent of clinical follow-up required. In this case, the methodology used must be documented. The clinical evaluation plan, where feasible, should have a statistical evaluation to ensure the right number of patients treated, the right parameters assessed and the end points to generate data to support the safety and efficacy of the new product. The principles of Good Clinical Practice and the Declaration of Helsinki must be integral to the design and performance of clinical evaluation. The clinical outcome data must be shared between the clinician and the TE and forwarded to the Health Authorities by means of a clearly structured process. This is in addition to the routine biovigilance reporting procedures, which are mandatory.

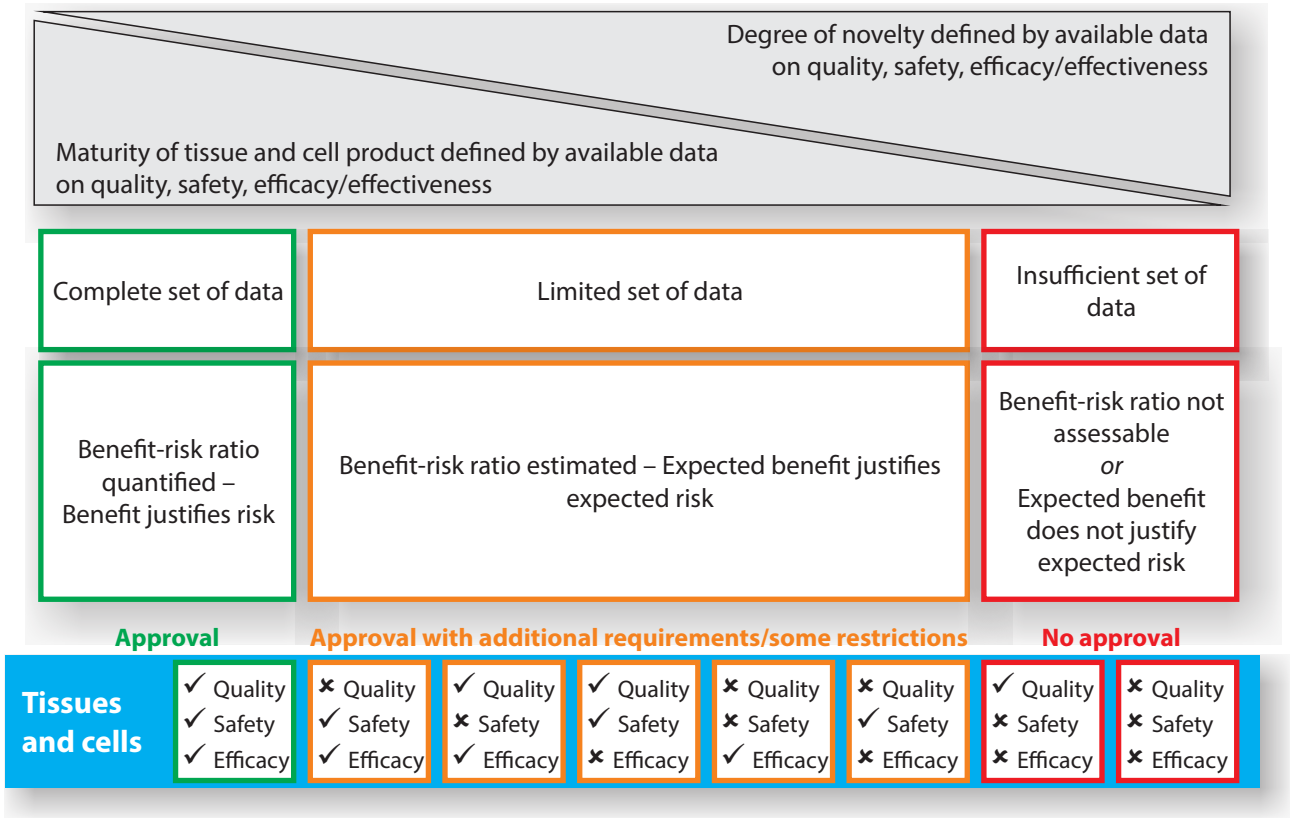
If long-term clinical outcome data are required for the demonstration of clinical efficacy/effectiveness, then national, European or international registries might be a useful tool to obtain sufficient clinical information. The quality of the data should be assessed before being included on registries to improve reliability.

18.3.3. Responsibilities of the Health Authorities

Regulation of tissue and cells by independent bodies, e.g. Health Authorities, is important to ensure quality, safety, efficacy/effectiveness of tissue and cell products [1]. Regulation focuses on two key elements:

- data-driven, risk–benefit assessment of tissues and cells, based on well-established specifications;

Figure 18.2. **Regulatory models of risk assessment for novel preparation processes and clinical applications**



✓ Level of safety, quality, efficacy/effectiveness is accepted.

✗ Level of safety, quality, efficacy/effectiveness does not meet the required criteria.

- risk-based decision-making on the approval of preparation processes or clinical applications by the Health Authorities.

As novel tissue or cell products, inherently by definition, have limited clinical data relating to quality, safety and efficacy, it can be challenging to assess their benefits and risks. Health Authorities can only approve tissue and cell products for routine clinical use based on sufficient data relating to safety and quality. In cases when the Health Authority determines that it has insufficient data to provide full authorisation, they might approve the tissue and cell for restricted clinical use, providing a conditional authorisation, if the expected benefit justifies the expected risk; see Figure 18.2.

In the case of innovative preparation methodologies or new applications, the normal authorisation procedure might need to be enhanced with associated clinical follow-up requirements, depending on the assessed risk. This approach allows regulatory requirements to be balanced with timely access for patients to novel tissues and cell therapies – for example, authorisation might be granted on the condition that the TE and clinicians provide novel tissue and cell therapies for clinical application only to limited numbers of selected recipients, possibly in the context of an ethically approved clinical evaluation, or only to a limited number of named clinicians – so that the data needed to guarantee a comprehensive assessment of safety and quality can be systematically generated and reported by the TE to the Health Authority.

At that point, if deemed appropriate, the Health Authority can issue a full approval. A final proof of the quality, safety and efficacy of tissues and cells can only be provided by favourable clinical outcome data. Consequently, data resulting from clinical evaluation constitute the basis for a fully data-based, risk-dependent decision whether full approval of the novel preparation process or clinical application may be granted. Figure 18.2 summarises models of authorisation for novel preparation processes and clinical applications for tissues and cells.

18.4. Life-cycle management of novelties and registries of consolidated practices

Because novel tissues and cells are typically prepared, regulated and applied in the context of a continuously evolving 'state of the art', effective and efficient life-cycle management is of high importance. Life-cycle management comprises management of knowledge, change and uncertainty at the interface of the TE, clinician and the Health Authority. Thus, clinical follow-up of patients and biovigilance surveillance, i.e. the analysis of clinical outcome data and adverse reactions, is essential. Life-cycle management of tissues and cells also comprises a close regulatory oversight of the entire donation–distribution–application chain, requiring a close interaction between Health Authority, ORHA and TE.

Life-cycle management of novel preparation processes or new clinical applications requires a broad and comprehensive data-based framework. Continuous review of preparation processes, clinical monitoring of recipients and review of up-to-date clinical outcome data all contribute to maintaining product quality and safety, and establishing an efficacy/efficiency profile. Such data-based frameworks require consideration of geographic, temporal and technical aspects: instead of local or regional overview of products, a much broader approach should be considered, utilising the tissue and cell monographs included in Part D of this Guide and by utilising existing European databases, e.g. the European Cornea and Cell Transplantation Registry (ECCTR), the European Society for Blood and Marrow Transplant (EBMT), Patient Registry and European IVF Monitoring (EIM), whenever possible. The co-operation between stakeholders to improve data collection, data processing, data analysis and data sharing will provide reliable information to inform patients about their treatments.

18.5. References

1. VISTART Joint Action (Vigilance and Inspection for the Safety of Transfusion Assisted Reproduction and Transplantation). Deliverable 5.4, "Principles for Competent Authorities for the evaluation and approval of clinical follow-up protocols for blood, tissues and cells prepared with newly developed and validated processing methodologies", available at https://webgate.ec.europa.eu/chafea_pdb/health/projects/676969/outputs, accessed 12 April 2022.
2. EuroGTP-II Project. Good practices for demonstrating safety and quality through recipient follow-up, available at www.goodtissuepractices.eu/, accessed 12 April 2022.
3. GAPP Joint action. Facilitating the authorisation of preparation process for blood, tissues and cells, available at www.gapp-ja.eu, accessed 12 April 2022.

Part B. Specific requirements for substances of human origin

Chapter 19. **Ocular tissue**

19.1. Introduction

Ocular tissues procured from deceased donors are used for treating loss of vision caused by irreversible corneal disease or trauma, as well as for reconstructive and glaucoma surgery.

The cornea is the principal refractive component of the eye. Good vision depends on its transparency and a smooth, spherical corneal surface. The cornea is also part of the ocular surface and must therefore be strong enough to withstand the intraocular pressure and help protect the delicate inner structures of the eye.

19.1.1. Corneal transplantation

A corneal transplant (keratoplasty) is an operation to remove all or part of a diseased or damaged cornea and replace it with healthy donor tissue. In Europe, the main indications for corneal transplantation include:

- Fuchs endothelial corneal dystrophy (FECD) – failure of the corneal endothelium, the monolayer of non-dividing cells lining the inner surface of the cornea, that controls stromal hydration and thus maintains corneal transparency.
- Keratoconus – a connective tissue disorder resulting in thinning and degeneration of the corneal stroma, which normally accounts for 90 % of corneal thickness, and distortion of corneal shape.
- Pseudophakic bullous keratopathy (PBK) – failure of the corneal endothelium as a consequence of previous cataract or other intraocular surgery.
- Infection, such as *Herpes simplex* keratitis (HSK) – mainly treatment of post-infectious scars, but acute infection may require a therapeutic keratoplasty.
- Regraft for a failed corneal transplant.

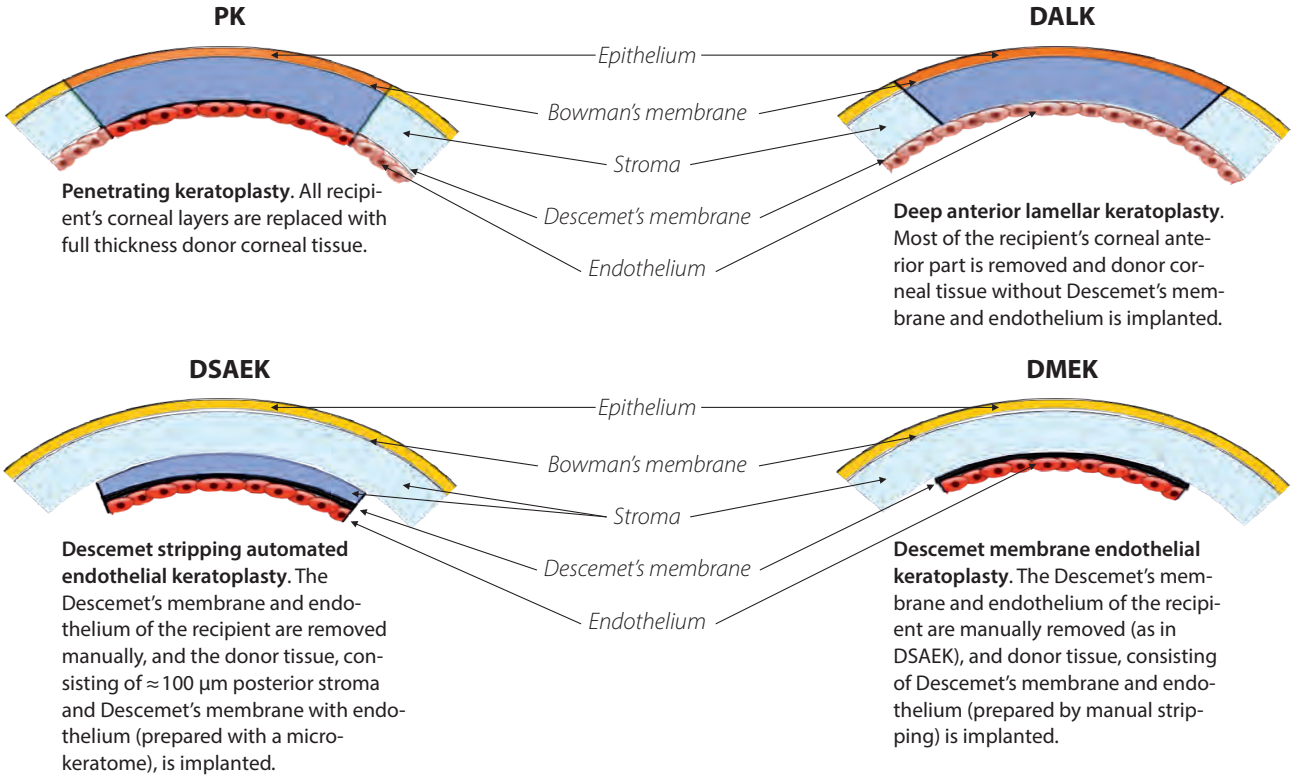
These conditions may all be treated with a full-thickness corneal transplant (penetrating keratoplasty, PK); however, current practice is to replace, where possible, only the dysfunctional part of the cornea [1, 2, 3] as illustrated in Figure 19.1.

Endothelial keratoplasty (EK) is the method of choice for endothelial dysfunction such as FECD or PBK. The lamellar graft, which consists of endothelium on its basement membrane (Descemet membrane), with or without a thin supporting layer of stroma, is inserted through a small incision into the anterior chamber of the eye and attaches to the posterior surface of the patient's cornea. The initial attachment is ensured by air or sulphur hexafluoride gas tamponade in the anterior chamber. The affected patients are mostly elderly and the advantages of EK over PK include much faster visual rehabilitation, lower rejection rates and, since there are no sutures required to hold the graft in place, negligible surgically induced astigmatism.

There are currently four techniques for preparing tissue for EK:

a. Descemet stripping endothelial keratoplasty (DSEK) – endothelium on Descemet mem-

Figure 19.1. **Different types of keratoplasties**



brane with a thin layer of stroma, prepared by manual dissection.

b. Descemet stripping automated endothelial keratoplasty (DSAEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by using a microkeratome.

c. Descemet membrane endothelial keratoplasty (DMEK) – endothelium on Descemet membrane without any stroma, prepared by manually separating Descemet membrane from the stroma.

d. Pre-Descemet membrane endothelial keratoplasty (PDEK) – endothelium on Descemet membrane with a thin layer of pre-Descemet stroma, prepared by injecting air to create a 'big bubble', which separates the graft from the bulk of the stroma.

For keratoconus, which typically affects younger patients, deep anterior lamellar keratoplasty (DALK) is an alternative to PK that replaces the full thickness of the stroma, leaving the recipient's endothelium intact. In PK, the immunological rejection against the endothelium is one of the main reasons for failure, and the advantage of DALK is that the patient's endothelium is maintained. Superficial stromal defects and scars can also be treated by anterior lamellar keratoplasty (ALK), without the need to replace the full thickness of the stroma.

Whereas a tissue for PK and EK requires a donor corneoscleral disc with a viable endothelium, a tissue for DALK and ALK does not; however, corneoscleral discs with an endothelium suitable for PK may be requested for DALK owing to the occasional need to switch procedure in the operating room from DALK to PK. Nowadays tissue for EK is mostly prepared in a tissue establishment (TE), which saves time for the surgeon and avoids the risk of damage to the tissue when prepared in the operating room. In this way risks of damage to the tissue are transferred to the TE, but the advantage is that this does not happen in the OR and a backup tissue can be arranged. Furthermore, TEs are more and more trained to prepare high-quality tissues for EK due to the sheer volume of their preparations.

Corneal transplant outcome – in terms of both graft survival and visual acuity – depends on the recipient disease, the presence of pre-operative risk factors, such as vascularised cornea, glaucoma and inflammation, and post-operative complications such as allograft rejection. In the absence of other risk factors, keratoconus and FECD are considered to be low-risk grafts with 4-year survivals of, respectively, 95 % and 88 % [4]. Regraft and PBK are more likely to undergo allograft rejection and/or failure, and are therefore considered high-risk grafts with 4-year survival of, respectively, 63 % and 63 % [4]. A major cause of graft failure is allograft rejection. Most rejec-

tion episodes can be successfully treated with topical (sometimes intraocular, subconjunctival, peribulbar or systemic) corticosteroids. In some cases, use of systemic immunosuppression (e.g. cyclosporine, tacrolimus, mycophenolate) is considered necessary to reduce the risk of immune reactions. The results from studies of human leukocyte antigen (HLA) matching to reduce the risk of rejection are not as conclusive as for organ transplantation, but matching may be beneficial for high-risk corneal grafts [5].

19.1.2. Further use of ocular tissue

If, for example, a patient has suffered multiple failed corneal grafts, a keratoprosthesis may be an appropriate alternative to a corneal graft [6]. Keratoprostheses are attached to a ring of corneal tissue from a donor, which is then sutured to the recipient's cornea after removal of the failed graft. Sclera and cornea can also be used in glaucoma surgery and for reconstructive surgery of the ocular surface. Limbal tissue, which contains a population of corneal epithelial progenitor cells, may be transplanted as a keratolimbal allograft (KLAL) to treat ocular surface disease caused by failure of the corneal limbus (limbal stem cell deficiency) [7].

There are other tissues and cells that are used in ocular surgery but not covered in this chapter:

- Limbal progenitor cells may be isolated from a corneoscleral disc and expanded *ex vivo* for treating ocular surface disease (Chapter 34) [8];
- Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease (Chapter 34) [9];
- Amnion is used for treating ocular surface conditions and as a support for limbal progenitor cells (see Chapter 20) [10];
- Autologous and allogeneic serum eye drops may be used for treating dry eye (Chapter 33) and/or persistent epithelial defects [11].

The following generic chapters of this Guide (see Part A) all apply to ocular tissue banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the specific requirements for ocular tissues that vary from the generic chapters in Part A of this Guide. Where differences are not specified, the requirements of the relevant generic chapter should be followed.

19.2. Donor evaluation

19.2.1. Tissue-specific exclusion criteria for ocular tissue donation

Acceptance and exclusion criteria for cornea donation that differ from the criteria for other tissues are based on the avascularity of the cornea and ocular-specific conditions that may affect the cornea.

19.2.1.1. *Selection criteria for ocular tissue other than cornea*

For any tissue or cells that are not derived from the avascular cornea, such as sclera, limbal tissue and limbal cells, the exclusion criteria for non-ocular tissues must be followed (see Chapter 5).

19.2.1.2. *Donor age*

Provided that corneas are examined to exclude those unsuitable for transplantation based on endothelial cell density and/or stromal abnormalities (see §19.7), the upper age limit for eye donors may be determined by the TE. The likelihood that corneas will be suitable for PK or EK does decline with increasing donor age but, where the endothelial cell density is considered sufficient, graft survival is little affected by donor age [12, 13]. The minimum donor age is more uncertain and should be determined by the TE because corneas from young children lack rigidity and have a high radius of curvature. Therefore, there is little demand for corneas from young

donors for transplantation; however, corneas from these donors may be important as a source of limbal grafts or limbal progenitor cells. Older donors (over 65 years) are often preferred for DMEK surgery, as grafts from older donors are easier to prepare and to unfold during surgery [14], although a clinical study does not suggest any clinical disadvantage of using donors younger than 55 years [15].

19.2.1.3. *Malignancies*

Haematological neoplasms, retinoblastoma and malignant tumours of the anterior segment are absolute contraindications to cornea donation. Donors with certain malignant diseases may be evaluated and considered for avascular cornea donation but not for vascularised ocular tissues (i.e. limbal tissue, limbal stem cells or sclera). A report of metastatic cholangiocarcinoma cells found in the sclera and sclerocornea interface, but not avascular cornea, supports this [16]; there has been a report of malignant cutaneous melanoma (MCM) metastases in peripheral, but not central, avascular cornea [17]. The Medical Standards of the Eye Bank Association of America recommend the exclusion of donors with a history of melanoma with known metastatic disease [18], while the European Eye Bank Association recommends that, in the case of donors with malignant diseases and a potential risk of metastasis formation in the anterior ocular segment, a thorough slit-lamp examination of the globe or the corneo-scleral disc focused on possible metastasis must be undertaken in the eye bank [19].

The incidence of metastases from non-ocular tumours to the anterior segment of the eye is extremely low; however, corneas must be excluded where there is evidence of anterior segment metastases from the slit-lamp examination of the eye or the corneoscleral disc [19, 20, 21]. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

19.2.1.4. *Infections*

Individuals with localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) are excluded from donation of ocular tissues. This exclusion includes those with a known history of past ocular *Herpes* infection. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

Individuals with uncontrolled systemic infections should in general be excluded from donation. Individuals suffering from bacterial septicaemia may be considered for cornea donation, provided that the corneas are stored by organ culture and the medium tested for microbial contamination before transplantation. Donors colonised with multidrug-resistant bacteria need a thorough risk assessment before they may be accepted as donors.

19.2.1.5. *Diabetes mellitus*

Diabetes mellitus increases the risk of unsuccessful DMEK graft preparation [22]. However, this risk may be related to the severity of the diabetes, and an algorithm has been proposed to allow grafts to be prepared from corneas from donors with mild disease [23].

19.2.1.6. *Eye diseases*

The following exclude cornea donation:

a. ocular inflammation and infection (see §19.2.1.4);

b. autoimmune disease, e.g. sarcoidosis, rheumatoid arthritis, if there is ocular involvement.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

a. corneal disorders including keratoconus, keratoglobus and epithelial and stromal dystrophies. As these diseases typically are bilateral, both eyes should be excluded;

b. corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea. As these diseases typically are unilateral, only the affected eye must be excluded. The utmost care must be taken to correctly identify the affected eye/cornea to avoid the risk of procuring/processing the affected eye/cornea.

The following exclude cornea donation for EK and PK, but not necessarily for DALK:

a. endothelial disease: either primary, such as Fuchs endothelial corneal dystrophy; or secondary, such as bullous keratopathy.

19.2.1.7. *Previous intraocular or anterior segment surgery*

The following exclude cornea donation:

- previous ocular surgery that would prejudice graft outcome;
- receipt of a corneal, scleral or limbal allograft.

The following exclude cornea donation for PK or DALK, but not necessarily for EK:

- refractive corneal surgical procedures, including radial keratotomy, synthetic lamellar inserts and laser refractive surgery. As these procedures typically are performed bilaterally, both eyes should be excluded.

19.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure ocular tissue safety. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as a tailored EDQM Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk-reducing measures, as needed.

19.3.1. *Post mortem* time

Ocular tissues should be procured from donors as soon as possible after cardiac arrest, preferably within 24 h; however, Health Authorities or local practice may allow procurement up to 72 h after cardiocirculatory arrest for organ-cultured corneas. A recent study has documented the safety of this practice in corneal tissue procurement [24]. For EU member states, a blood sample for the mandatory tests for transmissible disease must be obtained from the donor within the first 24 h after death (see Chapter 6).

19.3.2. Procurement team

Ocular procurement personnel must be appropriately clothed and must apply aseptic technique to minimise the risk of contamination of the tissue to be removed and also to protect personnel. Usually, this requires hand disinfection, the wearing of sterile gowns and gloves and the use of face masks or protective masks.

19.3.3. Procurement procedures

The ocular surface is exposed to the environment and, after death, there is no blinking and no tear film. Therefore, it is likely to be contaminated by environmental micro-organisms. For these reasons, a classified area with a specified air quality is not required for ocular tissue procurement, but other guidance given in Chapter 7 does apply. In case of concomitant skin donation, procurement of skin before ocular tissue is recommended (as described in Chapter 21 §21.3.1).

The donor's eyelids and surrounding skin area should be cleaned with an antiseptic solution and a sterile drape (eye sheet) placed over the face leaving the eyes exposed. The donor's eyes should be flushed with an appropriate sterile solution to remove debris, mucus and foreign matter from the cornea and conjunctival sac. An antiseptic solution suitable for cleaning the ocular surface prior to intraocular surgery, or a broad-spectrum antibiotic solution, should be applied. After insertion of a lid speculum, peritomy is performed, preferably leaving a frill of conjunctiva at the limbus to avoid damage to the limbal progenitor cell niche.

The subsequent procedure depends on whether the eye is to be enucleated or just the corneoscleral disc procured by *in situ* excision. Advantages of *in situ* excision include: reduced death-to-preservation time since the corneoscleral disc is placed in storage medium immediately following procurement; potentially improved cosmetic reconstruction of the donor; and it may be more acceptable to some relatives or next of kin than enucleation. However, procurement by enucleation is simpler, with less risk of harm to the corneal endothelium; and enucleated eyes provide sclera, for glaucoma or reconstructive surgery, and retina, optic nerve, lens and iris for research. Furthermore, a whole eye can be rinsed with physiological salt solution and disinfected easily with, for example, povidone-iodine. Also, trephining the corneoscleral disc is better controlled in the lab environment inside the TE. There is no reported evidence of a difference in corneal quality or clinical outcome between these two procurement methods.

19.3.3.1. *Procurement of the whole eye*

The lateral extraocular muscle is located and lifted with a muscle hook and clamped with artery forceps close to its point of attachment to the sclera. The muscle is divided distally, leaving the artery forceps in place to stabilise and steady the eye. The remaining rectus muscles are then lifted in turn with the muscle hook and divided close to the sclera. It is not necessary to divide the oblique muscles. The eye is then gently lifted and the optic nerve severed using curved enucleation scissors. After enucleation, the whole eye should be placed, cornea uppermost, in a fixed position in a moist chamber and transported to the TE refrigerated in ice. Broad-spectrum anti-

biotics may be used to further minimise the risk of bacterial contamination.

19.3.3.2. *Procurement of corneoscleral discs*

After peritomy, sclerotomy is performed, maintaining a wide scleral rim (about 4 mm) around the cornea. The corneoscleral disc is then gently lifted away from the eye without folding, to avoid damage to the endothelium. After excision, the corneoscleral disc should be immersed, endothelium uppermost to avoid the risk of damage, in an appropriate corneal storage solution that may contain antibiotics and antimycotics. Unless the cornea is to be transferred directly to organ culture at the TE, it is recommended that, when possible, the container be a corneal viewing chamber or have a flat bottom and adequate optical properties to facilitate subsequent assessment by slit lamp and specular microscopy.

For light microscopy evaluation, other relevant containers and evaluation methods may be used.

19.3.3.3. *Procurement of scleral tissue*

Scleral tissue is prepared in the TE from the whole eye after excision of the corneoscleral disc.

19.3.4. Reconstruction of the donor

The aim is to mimic as closely as possible the original profile of the donor's closed eyes. After enucleation, the orbit should be filled with an appropriate prosthesis or other suitable material. The eye lids are then closed to restore the appearance of the donor.

19.4. Temporary storage and transportation to the tissue establishment

Whole eyes should be stored and transported in a moist chamber at 2 to 8 °C. The time from procurement to processing at the TE should not exceed 48 h.

Corneoscleral discs procured by *in situ* excision may be placed in a hypothermic storage solution or in a room-temperature storage medium or solution (designated by the manufacturer). In both cases, the manufacturer's recommendations for storage temperature should be followed or the temperature conditions during transport should be validated.

19.5. Processing, preservation and storage

19.5.1. Processing facilities

In selecting an appropriate air-quality specification for ocular tissue, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered.

19.5.2. Cornea processing methods

When corneoscleral discs have been procured by *in situ* excision, they will already be in a storage medium on arrival at a TE and may not require further processing unless they are to be transferred to organ culture. When whole eyes are received by a TE, they should be subjected to a cleaning protocol to reduce the bioburden on the ocular surface before excision of the corneoscleral disc; for example, rinsing in sterile saline and immersion in a disinfectant such as povidone-iodine or chlorhexidine [25]. Further processing of corneoscleral discs to prepare grafts for EK may be undertaken in the TE [26, 27].

19.5.3. Cornea storage methods

a. *Hypothermic storage at 2 to 8 °C*
   i. For whole eyes in moist chambers, storage times of < 48 h are recommended for procedures where a viable corneal endothelium is required. This may be extended to 72 h for other purposes.
   ii. For corneoscleral discs in storage medium, the manufacturer's recommendations should be followed for storage temperature and for maximum storage time, which can vary up to 21 days [28, 29, 30]. Corneas prepared in a TE for DSAEK or for DMEK can be shipped to hospitals in hypothermic storage media.

b. *Organ culture at 28 to 37 °C*
   i. A storage time of up to 4-5 weeks is typical for organ culture, although successful transplants after 7 weeks have been reported [31]. It is at the discretion of the Responsible Person (RP) or medical director to approve prolonged storage times, provided that the procedure has been validated. An inspection of the endothelium is mandatory at the end of the storage period and then the transplant can be assigned to the proper kind of surgery based on the cell density. Renewal of the storage medium using aseptic procedures during the storage period is at

the discretion of the RP/medical director and may depend on the manufacturer's recommendations.

ii. To reverse the stromal oedema that occurs during organ culture, corneas are transferred to a medium, the transport or 'deswelling' medium, containing a macromolecule to increase oncotic pressure and induce an efflux of water from the stroma. The cornea may be kept at 15 to 37 °C for up to 4-7 days, at the discretion of the RP and depending on the medium used [32, 33]. A cornea that is released to the surgeon for a DMEK procedure, to be prepared by the surgeon in the OR, may also be sent without pre-thinning.

iii. Organ-cultured corneas can be prepared in the eye bank for DSAEK after pre-thinning in deswelling medium, or for DMEK with or without pre-thinning [34]. The DSAEK grafts may be laid back on the anterior stroma to provide additional support during transport in deswelling medium [35]. DSAEK grafts are prepared after mounting the corneoscleral disc in a pressurised artificial anterior chamber followed by cutting away the anterior stroma using a microkeratome. Clinical results after graft preparation using a femtosecond laser have been found inferior when compared with microkeratome-cut DSAEK grafts [36, 37]. Clinical quality-control studies comparing DSAEK grafts prepared in an eye bank with DSAEK grafts prepared by the surgeon immediately before surgery have not identified differences in early complications (graft detachment), primary graft failure or endothelial cell density two years after surgery [38]. DSAEK grafts possibly should be cut as thin as possible because visual acuity is better after grafting with a thin graft compared with a thick graft [39].

iv. Of relevance for eye banks, DMEK grafts can be prepared by manual dissection, pneumatic dissection, or hydrodissection. A no-touch technique, without direct physical tissue manipulation during tissue preparation, may be an ideal approach to minimise graft damage [40]. Clinical results after eye-bank- and surgeon-prepared DMEK grafts seem similar [41]. For DMEK, the graft may be supplied rolled in the final diameter direct into medium or attached, either in the centre or at the periphery, and laid back on the stroma and stored in normal medium with or without deswelling properties [26, 42] although medium without deswelling properties seems superior [43]. Pre-prepared grafts for DSAEK and DMEK may be shipped to hospitals in medium at room temperature [26]. Pre-loaded grafts for both DSAEK and DMEK can also be provided in order to minimise the time and effort needed for tissue preparation in the theatre [44, 45].

v. Although not yet implemented in TE practice, corneal storage under controlled positive pressure (bioreactor) and continuous medium renewal, thereby avoiding corneal swelling, can improve endothelial survival during culture. This technique may become a significant technical advancement of the organ culture technique [46].

c. *Storage of non-viable corneal tissue*

i. Corneoscleral discs or pieces of cornea for glaucoma or reconstructive surgery may be stored in ethanol (≥ 70 % v/v) or glycerol for extended periods. Corneal tissue may also be irradiated and stored in albumin, cryopreserved or frozen [47].

19.5.4. Sclera processing and storage

After excision of the corneoscleral disc from the eye, sclera is prepared using aseptic techniques by removing the intraocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). Sclera may be stored – whole, or divided into smaller, individually packaged pieces – in ethanol (≥ 70 % v/v) or glycerol, or fixed in formalin, freeze-dried, frozen or kept in physiological solution or medium with antibiotics. Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days).

19.6. Microbiological testing

Testing may be carried out before processing by swabbing the eye before excision of the corneoscleral disc; however, there appears to be little predictive value in this procedure [48, 49]. At the discretion of the transplanting surgeon, any corneoscleral tissue and storage medium remaining after preparation of the graft may be sent for microbiological testing; although there appears to be little predictive value from this [50], it can be helpful for the investigation of post-operative endophthalmitis. For further information, refer to Chapter 11 on the principles of microbiological testing.

a. *Hypothermic storage of corneas*
Taking a sample of medium for microbiological testing during hypothermic storage of corneas is not standard practice but may be required by national guidelines. Due to low temperature and limited time of storage, sampling of storage medium as a surrogate for direct microbiological testing of tissue may be made immediately upon arrival at the TE.

b. *Organ-culture storage of corneas*
Since corneoscleral discs intended for transplants requiring viable cells cannot be sterilised, microbiological testing of samples of organ-culture medium taken during corneal storage must be undertaken to test for microbial contamination. If an external validated microbiological laboratory is used, the laboratory should be aware of the type and concentration of anti-microbiological components added to the culture media. Microbiological media for bacteria and fungi should be inoculated and incubated at appropriate temperatures. A minimum corneal storage period of at least 3 days is required before taking samples for microbiological testing. In addition to microbiological testing, the culture medium should be inspected regularly for turbidity and change in pH (e.g. change in colour of phenol red in the medium), which may indicate microbial contamination. It is recommended to keep the organ-culture medium for at least a week after transfer of the corneoscleral disc to transport medium to allow additional monitoring for signs of contamination.
Further microbiological testing should, if possible, be carried out whenever a cornea is re-exposed to the environment, for example after endothelial assessment and transfer of the cornea into the transport medium or after preparation of corneas for DSAEK or DMEK in a TE. However, given the restricted time a cornea may remain in this medium, it is possible that growth of micro-organisms may not be detected before the cornea is transplanted. A negative-to-date release is possible, as described in Chapter 11. If growth is detected, the surgeon must be informed immediately to prevent transplantation of the tissue. If the transplantation has taken place, the identification and sensitivities of the contaminating micro-organisms must be established as soon as possible in order to help the surgeon's post-operative management of the recipient. The fellow cornea should be re-evaluated and the donor risk should be re-assessed, whereafter the fellow cornea may be discarded, e.g. in case of contamination with *Candida* spp. If the fellow cornea has already been transplanted, the transplanting surgeon should be informed, and the patient monitored.

c. *Sclera*
Depending on the method of storage, for example refrigerated in saline, microbiological testing should be carried out after processing. Storage in ethanol (≥ 70 % v/v), glycerol (≥ 85 % v/v) or gamma irradiation of the tissue may render microbiological testing unnecessary (see Chapter 11).

19.7. Quality control and cornea evaluation

Quality-control tests on corneal grafts should consider at least the following minimum quality criteria:

a. no evidence of microbiological growth (aerobic or anaerobic bacteria, yeast or fungi);
b. endothelial characteristics;
c. morphology and integrity of the cornea layers;
d. diameter of clear central area of cornea.

Depending on the specific use of the cornea, it is necessary to document the appearance of:

a. epithelium (it may partially detach or reduce in thickness during storage);
b. stroma, which should have no central opacities or scars; the stroma of organ-cultured corneas may be hazy but should be transparent after reversal of stromal oedema in transport medium;
c. endothelium.

The quality-control tests to be carried out include the following:

a. *Gross examination*
i. Abnormalities of the external globe;
ii. Signs of previous surgery of the anterior segment;
iii. Epithelial abrasions, retention of excessive orbital tissue or laceration of the globe;
iv. Epithelial defects;
v. Stromal opacities – a mild arcus senilis with a defined clear central zone may be acceptable; the minimal diameter of the clear zone is at the discretion of the RP/medical director;

vi. Abnormal corneal shape (keratoconus, micro- or megalocornea);
vii. Condition of the anterior chamber (shape, evidence of blood);
viii. Abnormalities, such as the pterygium extending over the optical zone of the cornea.

b. *Slit-lamp evaluation*
i. Slit-lamp examination of whole eyes and corneoscleral discs is recommended by the European Eye Bank Association [19].
ii. It facilitates exclusion of pathological changes to the epithelium or stroma, such as scars, oedema, significant arcus, striae, epithelial defects, endothelial guttae or disease, infiltrates or foreign bodies, and anterior segment tumours or metastases.

c. *Microscopic evaluation of corneal endothelium*
i. The aim is to provide an estimate of endothelial cell density and a qualitative assessment of the appearance of the endothelium.
ii. This evaluation must be applied to all corneas intended for PK or EK in order to minimise the risk that factors such as low endothelial cell density may have a negative influence on graft survival [12].
iii. For corneas stored by hypothermia, this assessment is typically at the start of storage.
iv. If the corneoscleral disc is not in a corneal viewing chamber, it needs to be turned over so that the endothelium is facing downwards to allow observation by specular microscopy through the base of the container.
v. It should then be returned to the endothelium-uppermost position to avoid the risk of subsequent damage.
vi. For organ-cultured corneas, this endothelial assessment can be both at the start and at the end of the storage period; assessment at the end of storage, shortly before the cornea is transferred to the transport medium, is considered to be essential, whereas assessment at both the start and end of storage allows endothelial cell loss during storage to be determined.

d. *There are two main methods used for endothelial evaluation by microscopy:*
i. Specular microscopy. This method allows direct examination of the endothelium without staining; however, the appearance of the endothelial cells varies with temperature, type and time of preservation and the storage medium used. It is recommended that cold-stored corneas are warmed to room temperature to enhance the quality of the endothelial image.
ii. Transmitted light microscopy (bright field or phase contrast). If necessary, to enable cell counting, brief exposure to hypotonic sucrose solution (1.8 % w/v) or short exposure (few seconds, max. 4 minutes) to either balanced salt solution (BSS) or 0.9 % (w/v) NaCl is possible to make endothelial cell borders visible. The exposure time to these solutions must be limited. Prior use of a stain such as trypan blue (0.06-0.4 %) will help to identify dead cells and areas of denuded Descemet membrane.

e. *Contraindications to use of corneas for PK or EK include:*
i. low endothelial cell density – the minimum endothelial cell density is set at the discretion of the RP/medical director but is typically 2 000 to 2 500 cells/mm^2;
ii. moderate to severe signs of polymegathism and pleomorphism;
iii. significant (> 25%) endothelial cell loss during organ culture;
iv. abnormalities such as guttae;
v. central stromal scars or opacities (may be acceptable for EK, depending on cause and depth);
vi. presence of dead endothelial cells – corneas with scattered, isolated dead cells are acceptable, whereas corneas with larger areas of dead or missing cells are not.

f. *For eye-bank-prepared tissues for DSAEK or DMEK, quality control also includes:*
i. gross inspection for larger variations in the thickness of DSAEK grafts and edge ruptures in DMEK grafts;
ii. measurement of the overall diameter of the prepared graft;
iii. for DSAEK grafts, measurement of the thickness of defined areas of the graft by indirect ultrasound or optical coherence tomography.

19.8. Corneal transplant registries

Corneal transplant registries, such as those in Australia, the Netherlands, Sweden and the UK, provide an invaluable resource to validate the quality and safety of transplanted corneas. Registries also allow investigation of donor and recipient factors influencing graft survival, post-operative complications (including immunological rejection and serious adverse reactions) and visual outcome [4, 12, 51].

Although randomised clinical trials (RCT) are considered to provide the highest level of evidence, they are costly and complicated to set up, they can be undermined by changes in clinical and surgical practice during the course of the study, and it is not always straightforward to generalise beyond the specific inclusion/exclusion criteria of an RCT, especially when corneal transplantation outcomes and the risk of post-operative complications are influenced by many factors.

Registries, while not without pitfalls, rely on large datasets to reduce selection bias. They provide a broad overview across multiple transplant units and an evidence base that does not always reflect the optimism generated by the excellent results from single-centre studies [52, 53]. In addition to evaluating the outcome of established techniques and monitoring the uptake and success of new processing and surgical techniques, such as endothelial keratoplasty, registry data can also be used for validating eye-bank processes and storage methods in terms of clinical outcome measures rather than simply relying on *in vitro* laboratory measures of quality and safety [12, 54, 55]. A recent project, part funded by the EU and led by the European Society for Cataract and Refractive Surgery, has established a European Cornea and Cell Transplant Registry (ECCTR), building on the existing registries in the Netherlands, Sweden and the UK. The registry is based on donor and recipient factors registered at the time of surgery and a follow-up two years after surgery. The registry is open for all surgeons or transplant centres (see www.ecctr.org).

19.9. Biovigilance

Serious adverse reactions (SARs) for corneal transplants include:

a. primary graft failure (corneal transplant never cleared);
b. local infection (endophthalmitis or other serious ophthalmic infection);
c. graft failure due to a defect in the donor tissue, which was out of date, scarred or marked by incisions from previous surgery;
d. transmission of malignancy (possibly attributable to the transplanted tissue);
e. transmission of systemic infection (possibly attributable to the transplanted tissue).

Serious adverse events (SAEs) include:

a. wrong tissue supplied for the intended surgical procedure;
b. tissue supplied was damaged or showed signs of unacceptable previous surgery;
c. tissue supplied beyond its expiry date;
d. infection detected in organ-culture medium after the cornea was supplied to the surgeon.

Partial or complete graft detachment after EK is not uncommon; although the reported incidences vary, they are often in the range of 5-10 % for DSAEK procedures and 10-30 % for DMEK procedures [56]. Fortunately, graft detachment is most often successfully treated by re-bubbling (repeated air or SF6 tamponade of the anterior chamber of the recipient). It has not been possible to ascribe this serious adverse event to donor factors such as donor endothelial cell density, donor age or method of preservation [57, 58]. Consequently, graft detachment is mainly considered a surgical complication.

The Notify Library includes some well-documented cases of adverse reactions and adverse events in transplantation of ocular tissue; for example:

- A case of donor-to-recipient transmission of the *Herpes simplex* virus (HSV) by cornea transplantation was confirmed by polymerase chain reaction-based DNA fingerprinting of donor and recipient HSV strains (Record Number 429);
- A case of a transplant-acquired diagnosis of rabies is supported by temporal association of the recipient's illness, lack of other exposure to rabies and the retro-orbital pain of the recipient of the corneal transplant (Record Number 20);
- A case of transmission of T-cell lymphoma is described, whereby molecular analyses were used to detect the same alleles in HLA-DQα testing of the recipient and donor of the graft (Record Number 338);
- A case of donor ocular tissue being examined and then shipped to the eye bank with a contact lens on the cornea, thereby highlighting the importance of *in situ* inspection before recovery (Record Number 720);
- A case of metastases from a cholangiocarcinoma in the vascularised limbal region of a corneoscleral disc. There was no evidence of transmission to the recipient of the avascular corneal graft. The authors recommended that tissue from donors with a history of malignancy should not be used for limbal allografting (Record Number 1663) [16].

Further cases of adverse outcomes associated with ocular tissue can be found in the Notify Library at www.notifylibrary.org. The database is publicly ac-

cessible and can be searched by the substance type, adverse occurrence type and record number. A relatively recent report, Notify Library record number 1681, concerns the identification of metastases in the peripheral, but not central, avascular cornea from a donor with malignant cutaneous melanoma (see §19.2.1.3) [17]. The implications for donor-selection criteria have been considered by the Eye Bank Association of America Medical Advisory Board, resulting in the exclusion of donors with a history of melanoma with known metastatic disease [18].

Examples of two different SAR notification reports for ocular tissues used in France and by the NHS (UK) are included as Appendix 32 and Appendix 33.

19.10. Patient follow-up

Patients undergoing corneal transplantation need systematic and regular clinical follow-up in order to comply with international standards for patient care. Follow-up may take place at the corneal transplant centre exclusively or as shared care between the transplant centre and primary or secondary eye clinics or general ophthalmology practices. Regular routine follow-up is needed to recognise complications, such as suture-loosening, rejection episodes or an increase in intraocular pressure. If unnoted and untreated these events ultimately will result in graft failure and/or loss of vision.

The frequency of follow-up visits is individualised according to the type of corneal transplant procedure, the indication for surgery and presence of possible risk-factors in the recipient. Low-risk procedures are, for example, EK for Fuchs endothelial corneal dystrophy, while high-risk procedures include re-grafting of patients with ongoing ocular inflammation. High-risk procedures need closer follow-up.

Systematic longer-term follow-up at 1 and 2 years after corneal transplantation is typically performed in many transplant centres, either as part of an internal quality programme and/or as part of participation in a national or international corneal transplant registry. After 2 years, lifelong annual or biannual clinical follow-up visits at an ophthalmologist are often recommended for all corneal transplant recipients.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most registries record the main clinical indication to surgery, which typically is performed to 1. improve visual acuity, 2. relieve the patient from pain or 3. preserve the integrity of the eye (tectonic keratoplasty). At the follow-up it is, first of all, registered whether the graft is functional (clear). Reasons for non-clarity include primary graft failure, failure due to immunological graft rejection, late endothelial failure or recurrence of disease (for example, if the graft was performed for tectonic reasons in an eye with uncontrolled keratitis). Registries such as most national registries and the ECCTR provide definitions (SOPs) of clinical and quality criteria to improve agreement of the registration in different corneal transplant centres.

Primary graft failure is typically defined as lacking clearance of the graft and cornea after surgery. Although this may be caused by a real failure of the graft itself, for example caused by errors in the TE related to endothelial cell evaluation and counting or wrong handling during transport, it is most often caused by surgical complications or recurrence of disease in the recipient. For example, primary graft failure is extremely rare after penetrating keratoplasty for uncomplicated cases such as keratoconus and Fuchs endothelial dystrophy, while it is more common in EK performed by surgeons in their learning phase.

Visual acuity is typically registered at follow-up, but this measure may be confounded by other co-existing eye diseases such as age-related macular degeneration and pre-existing glaucoma damage but also of the refractive optical quality of the graft, which in penetrating and deep lamellar keratoplasty is determined by the specific performance of the operating surgeon.

Systematic patient follow-up and participation in registries is important for corneal transplant centres, not only for providing the best treatment of individual patients at the level of international standards, but also for comparing own results to international standards.

Although it is obligatory to report SARs to the national CA, it is often international personal contacts in corneal societies or groups which actually realise and address rare serious adverse reactions. Recently, several cases of fungal infection were recognised after EK and also reported as SARs to the respective CA but it was not until these cases were presented at a small group meeting of European corneal surgeons that actions were taken. Investigations were initiated and appropriate initiatives to prevent further reactions considered [59]. Therefore, international collaboration and exchange of single case reports on top of the obligatory SAR reports should be supported at the level of clinicians taking care of corneal transplant procedures.

19.11. Developing applications for patient treatment

The Bowman Layer lies between the epithelial basement membrane and the stroma. It can be dissected from donor corneas and inserted into the mid stroma of corneas with advanced keratoconus to help strengthen and flatten the patient's cornea [60].

Decellularised stroma can be used as a scaffold or for transplantation for corneal scars/ulcers. Decellularised porcine stroma is being used clinically, and development of human decellularised stroma is in progress [61]. Retinal pigment epithelial cells derived from human embryonic stem cells, induced pluripotent stem cells, umbilical cord, fetal brain or bone marrow are being investigated for the treatment of age-related macular degeneration [62].

Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease [9].

Stromal corneal lenticules, obtained as a surgical by-product of Small-Incision Lenticule Extraction (SMILE) refractive technique, or potentially prepared in the TE, may be used in the future for tectonic or refractive purposes. Intrastromal corneal lenticule implantation (additive keratoplasty), allowed by the minimally invasive femtosecond laser technology, may offer new therapeutic options and expand the surgeon's armamentarium for treating corneal diseases [63, 64].

19.12. References

1. Tan DT, Anshu A, Parthasarathy A, Htoon HM. Visual acuity outcomes after deep anterior lamellar keratoplasty: a case-control study. *Brit J Ophthalmol* 2010;94:1295-9.
2. Dapena I, Ham L, Melles GR. Endothelial keratoplasty: DSEK/DSAEK or DMEK – the thinner the better? *Curr Opin Ophthalmol* 2009;20:299-307.
3. Kruse FE, Schrehardt US, Tourtas T. Optimizing outcomes with Descemet's membrane endothelial keratoplasty. *Curr Opin Ophthalmol* 2014;25:325-34.
4. Williams KA, Keane MC, Coffey NE *et al.* (eds). *Australian Corneal Graft Registry 2018 Report*. Adelaide: Snap Printing; 2018, available at https://dspace.flinders.edu.au/xmlui/handle/2328/35402, accessed 12 April 2022.
5. van Essen TH, Roelen DL, Williams KA, Jager MJ. Matching for human leukocyte antigens (HLA) in corneal transplantation – to do or not to do. *Prog Retin Eye Res* 2015;46:84-110.
6. Chang H-YP, Luo ZK, Chodosh J *et al.* Primary implantation of Type I Boston Keratoprosthesis in nonautoimmune corneal diseases. *Cornea* 2015;34:246-70.
7. Ilari L, Daya SM. Long-term outcomes of keratolimbal allograft for the treatment of severe ocular surface disorders. *Ophthalmology* 2002;109:1278-84.
8. Rama P, Matuska S, Paganoni G *et al.* Limbal stem-cell therapy and long-term corneal regeneration. *N Engl J Med* 2010;363:147-55.
9. Kinoshita S, Koizumi N, Ueno M *et al.* Injection of cultured cells with a ROCK inhibitor for bullous keratopathy. *N Engl J Med* 2018;378(11):995-1003.
10. Dua HS, Gomes JA, King AJ, Maharajan VS. The amniotic membrane in ophthalmology. *Surv Ophthalmol* 2004;49:51-77.
11. van der Meer PF, Seghatchian J, de Korte D. Autologous and allogeneic serum eye drops. The Dutch perspective. *Transfus Apheresis Sci* 2015;53:99-100.
12. Armitage WJ, Jones MN, Zambrano I *et al.* The suitability of corneas stored by organ culture for penetrating keratoplasty and influence of donor and recipient factors on 5-year graft survival. *Invest Ophth Vis Sci* 2014;55:784-91.
13. Writing Committee for the Cornea Donor Study Research Group, Mannis MJ, Holland EJ *et al.* The effect of donor age on penetrating keratoplasty for endothelial disease: graft survival after 10 years in the Cornea Donor Study. *Ophthalmology* 2013;120:2419-27.
14. Heinzelmann S, Hüther S, Böhringer D *et al.* Influence of donor characteristics on descemet membrane endothelial keratoplasty. *Cornea* 2014;33:644-8.
15. Schaub F, Enders P, Zachewicz J *et al.* Impact of donor age on descemet membrane endothelial keratoplasty outcome: evaluation of donors aged 17-55 years. *Am J Ophthalmol* 2016;170:119-27.
16. Yao X, Lee M, Ying F *et al.* Transplanted corneal graft with metastatic cholangiocarcinoma to the donor eye. *Eye and Contact Lens* 2008;34:340-2.
17. Campanelli M, Misto R, Limongelli S *et al.* A donor cornea with metastatic cells from a cutaneous malignant melanoma. *Cornea* 2013;32(12):1613-16.
18. Eye Bank Association of America. Medical Standards. Washington DC, USA: Eye Bank Association of America.
19. European Eye Bank Association, Minimum medical standards, available at www.eeba.eu/files/pdf/EEBA%20Minimum%20Medical%20Standards%20Revision%205%20Final.pdf, accessed 12 April 2022.
20. Dubord PJ, Evans GD, Macsai MS *et al.* Eye banking and corneal transplantation communicable adverse incidents: current status and project NOTIFY. *Cornea* 2013;32:1155-66.
21. McGeorge AJ, Vote BJ, Elliot DA, Polkinghorne PJ. Papillary adenocarcinoma of the iris transmitted by

corneal transplantation. *Arch Ophthalmol* 2002;120: 1379-83.

22. Greiner MA, Rixen JJ, Wagoner MD *et al.* Diabetes mellitus increases risk of unsuccessful graft preparation in Descemet membrane endothelial keratoplasty: a multicenter study. *Cornea* 2014;33:1129-33.
23. Williams RS, Mayko ZM, Friend DJ *et al.* Descemet membrane endothelial keratoplasty (DMEK) tissue preparation: a donor diabetes mellitus categorical risk stratification scale for assessing tissue suitability and reducing tissue loss. *Cornea* 2016;35:927-31.
24. Hofmann N, Wittmershaus I, Salz A-K, Börgel M: Cornea procurement and processing up to 72 hours: no risk for cornea transplant quality. *Transfus Med Hemother* 2021 Feb;48(1):3-11. https://doi.org/10.1159/000510588.
25. van Luijk CM, Bruinsma M, van der Wees J *et al.* Combined chlorhexidine and PVP-I decontamination of human donor eyes prior to corneal preservation. *Cell Tissue Bank* 2012;13:333-9.
26. Price FW Jr, Price MO. Evolution of endothelial keratoplasty. *Cornea* 2013;32(Suppl 1):S28-S32.
27. Lie JT, Birbal R, Ham L *et al.* Donor tissue preparation for Descemet membrane endothelial keratoplasty. *J Cataract Refract Surg* 2008;34:1578-83.
28. McCarey BE, Meyer RF, Kaufman HE. Improved corneal storage for penetrating keratoplasties in humans. *Ann Ophthalmol* 1976;8:1488-92, 1495.
29. Lindstrom RL, Kaufman HE, Skelnik DL *et al.* Optisol corneal storage medium. *Am J Ophthalmol* 1992; 114:345-56.
30. Parekh M, Salvalaio G, Ferrari S *et al.* A quantitative method to evaluate the donor corneal tissue quality used in a comparative study between two hypothermic preservation media. *Cell Tissue Bank* 2014;15: 543-54.
31. Madzak A, Hjortdal JO. Outcome of human donor corneas stored for more than 4 weeks. *Cornea* 2018; 37:1232-6.
32. van der Want HJL, Pells E, Schuchard Y *et al.* Electron microscopy of cultured human corneas: osmotic hydration and the use of dextran fraction (Dextran T500) in organ culture. *Arch Ophthalmol* 1983;101: 1920-6.
33. Redbrake C, Salla S, Nilius R *et al.* A histochemical study of the distribution of dextran 500 in human corneas during organ culture. *Curr Eye Res* 1997;16:405-11.
34. Yoeruek E, Hofmann J, Bartz-Schmidt K-U. Comparison of swollen and dextran deswollen organ-cultured corneas for Descemet membrane dissection preparation: histological and ultrastructural findings. *Invest Ophthalmol Vis Sci* 2013;54:8036-40.
35. Jhanji V, Pollock GA, Mackey AL *et al.* Histopathological evaluation of anterior lamellar corneal tissue-on/-off storage conditions on DSAEK donor tissue after storage in organ culture. *Curr Eye Res* 2012;37:155-8.
36. Cheng YY, Schouten JS, Tahzib NG *et al.* Efficacy and safety of femtosecond laser-assisted corneal endothelial keratoplasty: a randomized multicenter clinical trial. *Transplantation* 2009;88:1294-302.
37. Hjortdal J, Nielsen E, Vestergaard A, Søndergaard A. Inverse cutting of posterior lamellar corneal grafts by a femtosecond laser. *Open Ophthalmol J* 2012;6:19-22.
38. Ragunathan S, Ivarsen A, Nielsen K, Hjortdal J. Comparison of organ cultured precut corneas versus surgeon-cut corneas for Descemet's stripping automated endothelial keratoplasty. *Cell Tissue Bank* 2014; 15:573-8.
39. Dickman MM, Kruit PJ, Remeijer L *et al.* A randomized multicenter clinical trial of ultrathin Descemet stripping automated endothelial keratoplasty (DSAEK) versus DSAEK. *Ophthalmology* 2016; 123:2276-84.
40. Birbal RS, Sikder S, Lie JT *et al.* Donor tissue preparation for Descemet membrane endothelial keratoplasty: an updated review. *Cornea* 2018;37:128-35.
41. Menzel-Severing J, Kruse FE, Tourtas T. Organ-cultured, pre-stripped donor tissue for DMEK surgery: clinical outcomes. *Br J Ophthalmol* 2017;101:1124-7.
42. Deng SX, Sanchez J, Chen L. Clinical outcomes of Descemet membrane endothelial keratoplasty using eye bank-prepared tissues. *Am J Ophthalmol* 2015;159: 590-6.
43. Abdin A, Daas L, Pattmöller M *et al.* Negative impact of dextran in organ culture media for pre-stripped tissue preservation on DMEK (Descemet membrane endothelial keratoplasty) outcome. *Graefes Arch Clin Exp Ophthalmol* 2018 Nov;256(11):2135-2142. https://doi.org/10.1007/s00417-018-4088-4.
44. Ruzza A, Parekh M, Ferrari S *et al.* Preloaded donor corneal lenticules in a new validated 3D printed smart storage glide for Descemet stripping automated endothelial keratoplasty. *Br J Ophthalmol* 2015;99:1388-95.
45. Parekh M, Ruzza A, Ferrari S *et al.* Preloaded tissues for Descemet membrane endothelial keratoplasty. *Am J Ophthalmol* 2016;166:120-5.
46. Garcin T, Gauthier AS, Crouzet E *et al.* Innovative corneal active storage machine for long-term eye banking. *Am J Transplant* 2019;19:1641-51.
47. Chae JJ, Choi JS, Lee JD *et al.* Physical and biological characterization of the gamma-irradiated human cornea. *Cornea* 2015;34:1287-94.
48. European Eye Bank Association. Statement on pre-processing microbiology testing in eye banks, available at www.eeba.eu/news/news-details/eeba-statement-on-pre-processing-microbiology-testing-in-eye-banks, accessed 12 April 2022.

49. Fuest M, Plum W, Salla S *et al.* Conjunctival and intraocular swabs for the microbiological assessment of donor corneas. *Acta Ophthalmol* 2016;94:70-7.
50. Everts RJ, Fowler WC, Chang DH, Reller LB. Corneoscleral rim cultures: lack of utility and implications for clinical decision-making and infection prevention in the care of patients undergoing corneal transplantation. *Cornea* 2001;20:586-9.
51. Claesson M, Armitage WJ, Fagerholm P, Stenevi S. Visual outcome in corneal grafts: a preliminary analysis of the Swedish Corneal Transplant Register. *Br J Ophthalmol* 2002;86:174-80.
52. Patel SV, Armitage WJ, Claesson M. Keratoplasty outcomes: are we making advances? [editorial]. *Ophthalmology* 2014;121:977-8.
53. Armitage WJ, Claesson M. National corneal transplant registries. In: Hjortdal J (ed.), *Corneal transplantation*, ch. 10. Cham, Switzerland: Springer; 2016:129-38.
54. Rijneveld WJ. Donor tissue for corneal transplantation: trust and control. PhD thesis (ISBN 978-9-08659428-3), Amsterdam: Vrije Universiteit 2010, available at https://research.vu.nl/en/publications/donor-tissue-for-corneal-transplantation-trust-and-control, accessed 12 April 2022.
55. Pels E, Rijneveld WJ. Organ culture preservation for corneal tissue: technical and quality aspects. *Dev Ophthalmol* 2009;43:31-46.
56. Meta-analysis of postoperative outcome parameters comparing Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty. *Cornea* 2017;36:1445-51. https://doi.org/10.1097/ICO.0000000000001384.
57. Hovlykke M, Ivarsen A, Hjortdal J. Venting incisions in DSAEK: implications for astigmatism, aberrations, visual acuity, and graft detachment. *Graef Arch Clin Exp Ophthalmol* 2015;253:1419-24.
58. Parekh M, Leon P, Ruzza A *et al.* Graft detachment and rebubbling rate in Descemet membrane endothelial keratoplasty. *Surv Ophthalmol* 2018;63(2):245-50.
59. Lau N, Hajjar Sesé A, Augustin VA *et al.* Fungal infection after endothelial keratoplasty: association with hypothermic corneal storage. *Br J Ophthalmol.* 2019 Oct;103(10):1487-90.
60. Van Dijk K, Liarakos VS, Parker J *et al.* Bowman layer transplantation to reduce and stabilize progressive, advanced keratoconus. *Ophthalmology* 2015;122:909-17.
61. Zhang M-C, Liu X, Jiang D-L *et al.* Lamellar keratoplasty treatment of fungal corneal ulcers with acellular porcine corneal stroma. *Am J Transplant* 2015;15:1068-75.
62. Ramsden CM, da Cruz L, Coffey PJ. Stemming the tide of age-related macular degeneration: new therapies for old retinas. *Invest Ophthalmol Vis Sci* 2016;57:ORSFb1-3.
63. Mastropasqua L, Nubile M, Salgari N *et al.* Femtosecond laser-assisted stromal lenticule addition keratoplasty for the treatment of advanced keratoconus: a preliminary study. *J Refract Surg* 2018;34:36-44.
64. Trias E, Gallon P, Ferrari S,*et al.* Banking of corneal stromal lenticules: a risk-analysis assessment with the EuroGTP II interactive tool. *Cell Tissue Bank* 2020;21:189-204.

Related material

Appendix 32. Serious adverse reaction: notification form – for ocular tissues (Agence de la Biomédecine, France)
Appendix 33. Serious adverse reaction or event: notification form – for ocular tissues (NHS, UK)

Chapter 20. **Amniotic membrane**

20.1. Introduction

Amniotic membrane (AM) allografts have been used in different medical specialties since the early 20th century. Histologically, AM is the innermost, semi-transparent layer of the fetal membranes (amnion and chorion), formed by a single layer of cuboidal epithelial cells (epidermis-like cells), that is attached to a thick basement membrane and an avascular stromal matrix consisting of scattered mesenchymal stromal cells in a collagen scaffold. The amnion contains no blood vessels, lymphatic vessels or nerves. It has some unique properties. A number of mechanisms have been suggested to explain the beneficial effects of AM, on the basis of its biological composition.

Clinical and experimental data have shown [1-6] that AM provides a compatible substrate for cell growth, facilitating migration and differentiation of epithelial cells, supporting maintenance of the original epithelial phenotype and having low or no antigenicity.

20.1.1. Properties of amniotic membrane

Overall, AM is mainly formed by three types of components: structural collagen and extracellular matrix, biologically active cells and a large number of important regenerative molecules. Collagen types I, III, IV, V and VII and specialised proteins (including laminin and fibronectin) have been identified in the amniotic basement membrane and stroma. Laminin and fibronectin are particularly effective in facilitating epithelial cell adhesion. The presence of a rich extracellular matrix and collagen endows the stroma with anti-inflammatory properties, which arise from the entrapment of inflammatory cells, the presence of various growth factors and the inhibition of protease activity and decreased lipid peroxidation. In addition, AM has other biological properties that include anti-adhesive, antibacterial and anti-fibrosis effects, along with the ability to decrease scarring and neovascularisation, enhance wound healing and reduce pain [7-9]. Moreover, AM contains various growth factors (e.g. epidermal growth factor, basic fibroblast growth factor, hepatocyte growth factor, keratinocyte growth factor and transforming growth factors) and cytokines (e.g. interleukin 6 and 8) [10-12].

These characteristics have led to the use of AM for a wide range of ophthalmic indications (e.g. corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, traumatic macular hole, chemical or thermal burns) and in the treatment of a broad variety of pathological conditions, including management of burns (as a temporary or permanent wound dressing), repair of skin lesions of different aetiologies (e.g. vascular ulcers, epidermolysis bullosa, radiation burns), in surgery (e.g. orthopaedic, nerves, genitourinary tract, head) and in intra-abdominal and reconstructive surgery as well as oral and maxillofacial surgery [13-21].

In addition, the special structure and biological properties of AM make it an important potential source for scaffold material [22].

Stem cells derived from AM have been demon-

strated to display multilineage potential and immunomodulatory properties [23, 24].

The fetal membranes, amnion and chorion, can be procured either separately or together with placenta. In this chapter, when discussing procurement, the term 'placenta/fetal membranes' is used to mean 'not processed', whereas 'AM' here means 'processed in a tissue establishment'.

The following generic chapters (Part A) of this Guide apply to AM banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the additional specific requirements for AM.

20.2. Donor recruitment and evaluation

Prior to full-term delivery, potential donors are approached to ascertain whether they would be willing to donate their placenta/fetal membranes (see Chapter 4). A trained nurse or healthcare professional will discuss the procurement process and complete the consent and medical and behavioural lifestyle assessment. General criteria for donor evaluation are described in Chapter 5. The potential donor should be evaluated before giving birth and after full consent, also having been informed that procurement will take place only if the delivery is without any complications.

Placenta/fetal membranes should be collected only from living donors, after a full-term pregnancy.

20.2.1. Specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 5, there are some specific conditions that exclude placenta/fetal membranes donation. The diseases of the female genital tract or other diseases of the donor or unborn child that present a risk to the recipient include but are not limited to:

a. significant local bacterial, viral, parasitic or mycotic infection of the genital tract, especially amniotic infection syndrome;
b. (known) malformation of the unborn/newborn;
c. premature rupturing of membranes;
d. endometritis;
e. meconium ileus;
f. vaginal delivery or emergency caesarean delivery, if a terminal sterilisation is not performed.

Individual tissue establishments (TEs) may have additional exclusionary criteria.

20.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure the safety of amniotic membrane grafts. Risks are often multifactorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed, as well as a tailored EDQM Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk reducing measures, as needed.

20.3.1. Procurement facility and procurement team

Donor placenta/fetal membranes are procured by clinical staff at obstetrics units after delivery. AM could be contaminated by normal vaginal flora during vaginal delivery; therefore, procurement under aseptic conditions after elective caesarean section is to be preferred. If placenta/fetal membranes are procured during vaginal delivery, different sterilisation procedures [25-27] should be applied to the processed

AM (e.g. sterilisation by gamma irradiation). Staff undertaking procurement must be dressed appropriately for the procedure to minimise the risk of contamination of the procured tissue and any hazard to themselves. Further details can be found in Chapter 7.

20.3.2. Temporary storage and transportation to the tissue establishment

Placenta/fetal membranes should be stored at appropriate temperatures to maintain the structural and biological properties of AM.

The storage and transport time of procured placenta/fetal membranes should be kept as short as possible (the recommended maximum time is 24 h) and a temperature of + 2-8 °C should not be exceeded. If AM is processed within less than 2 h after the delivery, the placenta/fetal membranes may be transported at room temperature [28].

Procured placenta/fetal membranes should be placed in a sterile, pre-labelled container. If the transport exceeds 2 h an appropriate transport medium (or decontamination solution) should be used [29]. The sterile packaging should then be placed inside an adequate outside container to be transported to the TE. Individual TEs should validate the composition of the transport medium and determine if antibiotics are required.

The temperature during transport to the TE must be maintained. Temperature stability should be guaranteed by the container, conditions of transport used and for the time interval before processing. A temperature-recording unit (data logger) should be enclosed in the container to record temperature at ≤ 30-minute intervals unless the transport system has been previously validated to maintain the temperature within the required limits for the required transport time.

20.4. Processing and storage

20.4.1. Receipt of placenta/fetal membranes at the tissue establishment

Upon receipt, the procured tissue should be stored in a temperature-controlled refrigerator at + 2-8 °C to ensure that the refrigeration process is not interrupted. Processing should be carried out within 24 h after procurement [29].

20.4.2. Processing facilities

In selecting an appropriate air-quality specification for processing amniotic membrane, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered.

It is appropriate that processing of AM should take place in a controlled environment with defined air quality (see Chapter 8), especially for cryoprotected AM where there is less opportunity for microbial inactivation.

Within the EU, tissues exposed to the environment without subsequent microbial inactivation must be processed in environments with an air quality equivalent to GMP Grade A, with a background environment of at least Grade D.

20.4.3. Processing and preservation methods

Processing must not change the physical and biological properties of AM so as to make it unacceptable for clinical use. TEs may use different processing and preservation methods, according to their own standard operating procedures (SOPs) and mandatory regulations. The methods used must be in line with current best practice and must be validated in accordance with the guidance given in Chapter 2 and Chapter 9.

Processing of AM generally includes the mechanical detachment of fetal membranes (after being previously separated from placenta, when applicable), according to a documented SOP. Fetal membranes easily split into an amnion and a chorion leaflet, separated by a jelly-like intermediate layer. The chorion is discarded or kept for further processing (see section 20.9.2) and then the amnion should be rinsed several times in sterile saline until blood residues are removed completely. During processing, AM may be decontaminated by soaking in antibiotic/antimycotic solution. The incubation temperature and the composition of decontamination cocktails must be defined, after validation, by each TE. Following the decontamination step and rinsing procedure, AM should be spread on a suitable carrier membrane (e.g. nitrocellulose), or fine mesh gauze for easier handling and if it needs to be cut into multiple pieces. Depending on the intended clinical use, both sides of amnion (epithelial and stromal) can be placed directly on the selected carrier.

Consistent with the defined preservation method, AM grafts may be decontaminated or sterilised by irradiation. Sterilisation methods should be validated for the initial estimated level of bioburden [25-27]. AM grafts should be packaged in sterile containers and labelled as advised in Chapter 15. Maximum storage time will depend on the preservation method and should be defined and validated [28, 30]. There are several methods of AM prepara-

tion and preservation, as below. In this chapter, the expression 'cryoprotected AM' refers to frozen AM in the presence of cryoprotectants.

20.4.3.1. *Cryoprotected amniotic membrane*

AM can be preserved in culture medium containing glycerol or dimethyl sulphoxide (DMSO), which is used to protect cells against freezing injury. AM intended to be cryoprotected may be decontaminated by soaking in antibiotic/antimycotic solution.

Following packaging, AM grafts are stored at – 80 °C or in liquid or vapour phase of nitrogen at temperatures below – 140 °C (cryopreserved) [31-33] but, in the case of cryopreservation, only after being previously submitted to a controlled-rate freezing procedure. Prolonged storage up to 5 years has been used after storage at – 140 °C. Most TEs consider the maximum storage time at – 80 °C to range between 1 and 2 years [34]; no evidence found for longer expiration date.

20.4.3.2. *Deep-frozen amniotic membrane*

If cell viability is not to be maintained, the processed AM can be deep frozen without addition of a cryoprotective agent or controlled-rate freezing. AM intended to be deep frozen may be decontaminated by antimicrobial solution or sterilised [28]. Following packaging, AM grafts should be stored below – 80 °C. Most TEs consider the maximum storage time at – 80 °C to range between 1 and 2 years; no evidence found for longer expiration date.

20.4.3.3. *Heat-dried amniotic membrane*

The processed AM is dried overnight in an oven at + 40 ± 2 °C, then packed and sterilised. Storage should be at room temperature [29].

20.4.3.4. *Air-dried amniotic membrane*

The processed AM is air-dried overnight in a laminar-flow hood. It can then be packed and sterilised. Although high temperatures are not applied using this method, some properties of the amnion are lost or altered due to dehydration. Air-dried irradiated AM grafts should be stored at room temperature [35, 36].

20.4.3.5. *Lyophilised (freeze-dried) amniotic membrane*

The processed AM is rapidly frozen at – 50 °C to – 80 °C. Then it is vacuum-dried using a freeze-drying device. Water from the tissue is extracted through sublimation until a final water content of 5-10 % is obtained. Following packaging, AM grafts may be sterilised by irradiation even if alterations to AM were demonstrated [37]. This preservation method induces minimal changes in the properties of the AM, and the product can be stored at room temperature [38] for a long period of time without deterioration. The expiration time should be validated due to different packaging system and residual water.

20.4.3.6. *Glycerolised amniotic membrane*

Glycerolisation is a preservation method combined with the antimicrobial properties of high concentrations of glycerol. Since glycerol permeates more slowly than water, there will be an initial efflux of water when the glycerol is added. However, as glycerol begins to permeate the tissue, water will re-enter. At the end of the glycerolisation process, the final water activity (a_w) is about 0.3, which is known to minimise lipid peroxidation and reduce other degradation reaction rates to very low levels. Rather than dehydrating the tissue, as is commonly assumed, it has been demonstrated with skin that glycerolisation results in the effective sequestration of water [39-40]. Typically, 85 % (*v/v*) glycerol is used to preserved AM, which can then be stored at + 2-8 °C for up to two years, although it does lose some of its biological properties [41].

AM intended to be glycerolised may be decontaminated by soaking in antimicrobial solution or sterilised by irradiation.

20.4.3.7. *Amniotic drops/suspension*

For some clinical applications where a liquid form of AM (injectable or drops) is required, the AM should be sliced into small pieces through pulverisation, micronisation or morselisation. The small pieces obtained should be immersed in a suitable certified liquid for the clinical application or freeze dried [42-52].

The amniotic suspension is widely used for different applications, especially for orthopaedic pathology [18, 53-56].

20.4.3.8. *Amniotic membrane extract*

There are various methods for the preparation of amniotic membrane extract, without consistent standardisation. The steps are: washing isolated human AM, slicing into small pieces, and submerging in liquid nitrogen. The mixture is homogenised and centrifuged, and then the supernatant is collected. One of the main applications of amniotic membrane extract is for ophthalmic use [57-58].

20.5. Quality control

During procurement and processing of AM, reliable macroscopic examination of the donor fetal membranes should be undertaken to exclude visible pathological changes and ensure structural integrity of the tissue (to provide barrier function). Samples for detecting aerobic and anaerobic bacteria and fungi should be obtained from the transport/storage medium or from the initial washings of the AM, and from pieces of the tissue obtained both before and after antibiotic decontamination step. Microbiological testing for the detection of bacteria and fungi should be carried out according to the procedures described in Chapter 11.

These approaches cover the minimum standards to control microbiological safety (see Table 11.3). Deviations from such standards should be justified, and the suitability of the intended test method must be demonstrated. Factors such as samples containing antibiotics or very small sample amounts may affect the sensitivity of tests leading, in the worst case, to false-negative results. Where samples taken before antibiotic/antimycotic decontamination yield microorganisms that are considered pathogenic and highly virulent (see Table 11.1), the tissue cannot be approved for clinical use. Tissue showing heavy or confluent bioburden growth should also be rejected. After decontamination, tissue is not deemed suitable for transplantation if the samples taken for microbiological testing show signs of any microbial growth.

20.6. Distribution

The TE must ensure that distribution of AM grafts is carried out under controlled conditions. General considerations can be found in Chapter 12.

For cryopreserved AM, distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. The expiration date of AM stored in liquid nitrogen and shipped in dry ice must be shorter. For frozen AM grafts, distribution should be in dry ice. Transport temperatures of cryoprotected or deep-frozen AM above − 60 °C must be avoided, to ensure the stability of the product and maximum safety for the recipient.

Heat-dried, air-dried and freeze-dried AMs can be distributed at room temperature, whereas glycerolised AM should be transported at + 2-8 °C.

20.7. Biovigilance

Serious adverse events (SAEs) and reactions (SARs) must be recorded, reported and investigated according to the relevant national regulations to Health Authorities for tissues and cells, as described in Chapter 17.

The Notify Library includes some documented cases of adverse occurrences. Examples of SAEs include:

- LN2 ran out from an LN2 tank containing AM (as a result, all tissues thawed),
- Loss of significant quantity of AM grafts due to storage at inadequate temperature (e.g. in a case of equipment failure),
- High level of microbial contamination of procured AM (reflecting the hygiene conditions of the delivery room).

20.8. Patient follow-up

Amniotic membrane application (AMA) is performed for many indications. Some indications are fairly standardised, such as in pterygium surgery where the membrane is used to cover the bared sclera [59], but most indications are for a variety of more complex ocular surface diseases, such as non-healing epithelial defects, deeper corneal ulcers and small perforations, ocular surface burns caused by chemical agents and fireworks [60], and as an adjunctive in simple limbal epithelial transplantation (SLET) procedures.

All patients undergoing AMA need close follow-up in order to monitor healing, adjust topical eye drop treatment, change of contact lens and removal of sutures.

While no national clinical registers on patient follow-up include AMA efficacy, the usefulness of the procedure has been reviewed in several publications. The use of amniotic membranes in routine pterygium surgery compared with a free autologous conjunctival auto-transplantation was evaluated in a Cochrane review. The authors concluded that the efficacy concerning recurrence was inferior using AMA [61]. In another review, it was concluded that in treatment of corneal ulcers, AMA was efficient in the majority of cases [62], whereas the efficacy of AMA is less clear in treatment of severe ocular burns. Heterogeneity of disease presentation, variations in treatment, undefined criteria for treatment success and failure, and non-uniform outcome measures are some of the factors complicating the search for clear evidence regarding treatment of severe ocular burns [63].

Serious adverse reactions are rare after AMA and may be very difficult to identify due to the severe indications for surgery. For example, AMA may be used to treat a tiny corneal perforation in order to

avoid a corneal transplant procedure, but the perforation may enlarge due to progression of the original disease eventually making corneal transplantation necessary.

AM is widely used in extra-ocular applications with encouraging results. Human amniotic membrane has been used for centuries as a biological wound dressing.

The first reported use of amniotic membrane in burn wounds was by Sabella in 1913 [64], shortly after Davis reported on the use of the material in skin transplantations in 1910 [65]. Many advantages of amniotic membrane as a wound dressing have been reported, most notably prevention of infection [66-69], alleviation of pain [68-69], acceleration of wound healing [66, 68, 70], and good handling properties [71].

In recent years, there has been an increasing body of literature addressing the use of amniotic membrane in chronic wounds and burns, with important outcomes and demonstration of AM safety and efficacy [72-73], also for paediatric patients [74]. AM has been used in burns for more than 100 years, and the further beneficial effects of HAM in burns have been demonstrated for pain relief, fluid loss and scar reduction and, especially in developing countries, low costs. The key role for the revival of AM in reconstructive surgery has been the development of long-term preservation techniques to overcome limitations of immunogenicity and infectiosity [75].

AM is widely used also for wound management, including chronic ulcers, venous stasis ulcers, post-operative or post-traumatic chronic wounds, and post-surgical wound dehiscence. AM assists in the healing process through a number of physical, biochemical and molecular biological pathways to promote regenerative healing while simultaneously reducing scar formation [76]. Serena *et al.* demonstrated that venous leg ulcers treated with allograft had a significant improvement in healing at 4 weeks compared with multilayer compression therapy alone [77]. Haugh *et al.* performed a meta-analysis examining randomised controlled trials comparing amniotic tissue products with standard of care in nonhealing diabetic foot ulcers; the conclusion indicates that the treatment of diabetic foot ulcers with amniotic membrane improves healing rates in diabetic foot ulcers. Further studies are needed to determine whether these products also decrease the incidence of subsequent complications, such as amputation or death, in diabetic patients [78].

For the new applications, such as the treatments of maxillofacial and oral pathology [79-82], orthopaedic diseases [18, 83, 84], plastic surgery [85], general surgery (e.g. urology, recto-vaginal, cardiovascular applications) [86] and other innovative uses, there are many cases in humans, but more high-level clinical trials are required. AM gives promising results and the scientific literature supports use of placental membranes to reduce inflammation, pain and scarring.

20.9. Developing applications for patient treatment

20.9.1. De-epithelialised amniotic membrane

AM can be used either with the amniotic epithelium (intact AM) or without it (denuded AM), and a huge number of experimental studies have been performed with AM as a scaffold [87].

Several methods have been described for denuding AM [88], such as:

- Dispase method,
- EDTA method,
- Trypsin-EDTA method,
- Urea method,
- Ethanol method,
- Thermolysin method,
- Hypotonic buffer, SDS and nuclease method.

20.9.2. Amnion-chorion membrane

So far, literature hardly differentiates between amnion and chorion grafts. However, based on the finding that amnion and chorion present different content of growth factors and cytokines [89], different effects from amniotic and chorionic grafts can be expected [90].

Amnion-chorion membrane, that includes the amnion and chorion layers inclusive of the spongy layer, has been used as a wound covering for different aetiology. The chorionic membrane is the outermost layer of placental tissue, consisting of the chorionic mesenchyme and trophoblast layer that encapsulates the amniotic tissue and embeds into the mother's endometrium during development. As previously described for AM, amnion-chorion grafts also contain a wide variety of growth factors and cytokines relevant to tissue repair [91-92].

20.10. References

1. Fetterolf DE, Snyder RJ. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. *Wounds* 2012;24(10):299-307.
2. Akle CA, Adinolfi M, Welsh KI *et al.* Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. *Lancet* 1981;2:1003-5.

3. Burgos H, Herd A, Bennett JP. Placental angiogenic and growth factors in the treatment of chronic varicose ulcers: preliminary communication. *J Royal Soc Med* 1989;82(10):598-9.
4. Galask RP, Snyder IS. Antimicrobial factors in amniotic fluid. *Am J Obstet Gynecol* 1970;106:59-65.
5. Gatto C, Giurgola L, D'Amato Tothova J. A suitable and efficient procedure for the removal of decontaminating antibiotics from tissue allografts. *Cell Tissue Bank* 2013;14:107-15. https://doi.org/10.1007/s10561-012-9305-5.
6. Gruss JS, Jirsch DW. Human amniotic membrane: a versatile wound dressing. *Can Med Assoc J* 1978; 118(10):1237-46.
7. Shimmura S, Shimazaki J, Ohashi Y, Tsubota K. Antiinflammatory effects of amniotic membrane transplantation in ocular surface disorders. *Cornea* 2001;20(4):408-13.
8. Liu T, Zhai H, Xu Y *et al.* Amniotic membrane traps and induces apoptosis of inflammatory cells in ocular surface chemical burn. *Mol Vis* 2012;18:2137-46.
9. Kim JS, Kim JC, Na BK *et al.* Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. *Exp Eye Res* 2000;70(3):329-37.
10. Hao Y, Ma DH, Hwang DG *et al.* Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea* 2000;19(3):348-52.
11. Talmi YP, Sigler L, Inge E *et al.* Antibacterial properties of human amniotic membranes. *Placenta* 1991; 12:285-8.
12. Wolbank S, Hildner F, Redl H *et al.* Impact of human amniotic membrane preparation on release of angiogenic factors. *J Tissue Eng Regen Med* 2009 Dec;3(8): 651-4.
13. Koller J, Orsag M. Skin grafting options at the Burn and Reconstructive Surgery Department of the Faculty Hospital in Bratislava. *Acta Chir Plast* 2006;48: 65-71.
14. Maral T, Borman H, Arslan H *et al.* Effectiveness of human amnion preserved long-term in glycerol as a temporary biological dressing. *Burns* 1999;25:625-35.
15. Martinez Pardo ME, Reyes Frias ML, Ramos Duron LE *et al.* Clinical application of amniotic membranes on a patient with epidermolysis bullosa. *Ann Transplant* 1999;4:68-73.
16. Mermet I, Pottier N, Sainthillier JM *et al.* Use of amniotic membrane transplantation in the treatment of venous leg ulcers. *Wound Repair Regen* 2007;15(4):459-64. https://doi.org/10.1111/j.1524-475X.2007.00252.x.
17. Kesting MR, Wolff K-D, Nobis CP, Rohleder NH. Amniotic membrane in oral and maxillofacial surgery. *Oral Maxillofac Surg* 2014;18(2):153-64.
18. McIntyre JA, Jones IA, Danilkovich A, Vangsness CT Jr. The placenta: applications in orthopaedic sports medicine. *Am J Sports Med* 2018;46(1):234-247.
19. Zhang ZY, Yang J, Fan ZH *et al.* Fresh human amniotic membrane effectively promotes the repair of injured common peroneal nerve. *Neural Regen Res* 2019 Dec;14(12):2199-2208.
20. Amer MI, Abd-El-Maeboud KH. Amnion graft following hysteroscopic lysis of intrauterine adhesions. *J Obstet Gynaecol Res* 2006 Dec;32(6):559-66.
21. Eichberg DG, Ali SC, Buttrick SS, Komotar RJ. The use of dehydrated amniotic membrane allograft for augmentation of dural closure in craniotomies and endoscopic endonasal transphenoidal surgeries. *Br J Neurosurg* 2018 Oct;32(5):516-20.
22. Niknejad H, Peirovi H, Jorjani M *et al.* Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cell Mater* 2008;15:88-99.
23. Ilancheran S, Michalska A, Peh G *et al.* Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod* 2007;77(3): 577-88. https://doi.org//10.1095/biolreprod.106.055244.
24. Insausti CL, Blanquer M, Garcia-Hernández AM *et al.* Amniotic membrane-derived stem cells: immunomodulatory properties and potential clinical application. *Stem Cells Cloning* 2014;7:53-63. https://doi.org//10.2147/SCCAA.S58696.
25. International Organization for Standardization (ISO). Sterilization of health care products – Radiation – Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices. Ref: ISO 11137-1: 2006.
26. International Organization for Standardization (ISO). Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose. Ref: ISO 11137-2: 2013.
27. Mrázová H, Koller J, Kubišova K *et al.* Comparison of structural changes in skin and amnion tissue grafts for transplantation induced by gamma and electron beam irradiation for sterilization. *Cell Tissue Bank* 2015; 17(2):255-60. https://doi.org//10.1007/s10561-015-9536-3.
28. Tyszkiewicz J, Uhrynowska-Tyszkiewicz I, Kaminski A, Dziedzic-Goclawska A. Amnion allografts prepared in the Central Tissue Bank in Warsaw. *Ann Transplant* 1999;4(3-4):85-90.
29. EuroGTP Project, available at www.goodtissuepractices.eu, accessed 13 April 2022.
30. Russo A, Bonci P, Bonci P. The effects of different preservation processes on the total protein and growth factor content in a new biological product developed from human amniotic membrane. *Cell Tissue Bank* 2012;13(2):353-61. https://doi.org/10.1007/s10561-011-9261-5.
31. Lee SH, Tseng SC. Amniotic membrane

transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997;123:303-12.

32. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. *Br J Ophthalmol* 1998;82:235-40.
33. Hennerbichler S, Reichl B, Pleiner D *et al.* The influence of various storage conditions on cell viability in amniotic membrane. *Cell Tissue Bank* 2007;8(1):1-8.
34. Jirsova K, Jones GLA. Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review. *Cell Tissue Bank* 2017 Jun;18(2):193-204.
35. Von Versen-Höynck F, Hesselbarth U, Möller DE. Application of sterilised human amnion for reconstruction of the ocular surface. *Cell Tissue Bank* 2004; 5(1):57-65.
36. Singh R, Gupta P, Kumar P *et al.* Properties of air dried radiation processed amniotic membranes under different storage conditions. *Cell Tissue Bank* 2003;4:95-100. https://doi.org/10.1023/b:catb.0000007030.72031.12.
37. Paolin A, Trojan D, Leonardi A *et al.* Cytokine expression and ultrastructural alterations in fresh-frozen, freeze-dried and γ-irradiated human amniotic membranes. *Cell Tissue Bank* 2016 Sept;17(3):399-406.
38. Rodriguez-Ares MT, Lopez-Valladares MJ, Tourino R *et al.* Effects of lyophilization on human amniotic membrane. *Acta Ophthalmol* 2009;87(4):396-403. https://doi.org/10.1111/j.1755-3768.2008.01261.x.
39. Ross A, Kearney JN. The measurement of water activity in allogeneic skin grafts preserved using high concentration glycerol or propylene glycol. *Cell Tissue Bank* 2004;5(1):37-44.
40. Huang Q, Pegg DE, Kearney JN. Banking of non-viable skin allografts using high concentration of glycerol or propylene glycol. *Cell Tissue Bank* 2004; 5(3):3-21.
41. Rejzek A, Weyer F, Eichberger R, Gebhart W. Physical changes of amniotic membranes through glycerolization for the use as an epidermal substitute. Light and electron microscopic studies. *Cell Tissue Bank* 2001; 2:95-102.
42. Bonci P, Bonci P, Lia A. Suspension made with amniotic membrane: clinical trial. *Eur J Ophthalmol* 2005; 15(4):441-5.
43. Cheng AMS, Chua L, Casas V, Tseng SCG. Morselized amniotic membrane tissue for refractory corneal epithelial defects in cicatricial ocular surface diseases. *Transl Vis Sci Technol* 2016;5(3):9.
44. Choi JA, Choi J-S, Joo C-K. Effects of amniotic membrane suspension in the rat alkali burn model. *Mol Vis* 2011;17:404-12.
45. Choi JA, Jin H-J, Jung S *et al.* Effects of amniotic membrane suspension in human corneal wound healing *in vitro*. *Mol Vis* 2009;15:2230-8.
46. Guo Q, Hao J, Yang Q *et al.* A comparison of the effectiveness between amniotic membrane homogenate and transplanted amniotic membrane in healing corneal damage in a rabbit model. *Acta Ophthalmologica* 2011;89(4):e315-19.
47. Hawkins B. The use of micronized dehydrated human amnion/chorion membrane allograft for the treatment of diabetic foot ulcers: a case series. *Wounds* 2016;28(5):152-7.
48. Joubert R, Daniel E, Bonnin N *et al.* Retinoic acid engineered amniotic membrane used as graft or homogenate: positive effects on corneal alkali burns. *Invest Ophthalmol Vis Sci* 2017;58(9):3513-18.
49. Shahriari HA, Tokhmehchi F, Reza M, Hashemi NF. Comparison of the effect of amniotic membrane suspension and autologous serum on alkaline corneal epithelial wound healing in the rabbit model. *Cornea* 2008;27(10):1148-50.
50. Stachon T, Bischoff M, Seitz B *et al.* [Growth factors and interleukins in amniotic membrane tissue homogenate]. *Klin Monbl Augenheilkd* 2015;232(7):858-62.
51. Stachon T, Wu M-F, Bischoff M *et al.* [Amniotic membrane suspension and autologous serum – are they important for wound healing?]. *Klin Monbl Augenheilkd* 2017;234(8):1015-18.
52. Wu M-F, Stachon T, Langenbucher A *et al.* Effect of amniotic membrane suspension (AMS) and amniotic membrane homogenate (AMH) on human corneal epithelial cell viability, migration and proliferation *in vitro*. *Curr Eye Res* 2017;42(3):351-7.
53. Castellanos R, Tighe S. Injectable amniotic membrane/umbilical cord particulate for knee osteoarthritis: a prospective, single-center pilot study. *Pain Med* 2019 Nov 1;20(11):2283-91.
54. Buday MC, Ozturk M. Evaluation of folded amniotic membrane and injectable amniotic membrane pieces as soft tissue filler materials. *Auris Nasus Larynx* 2019 Jun;46(3):451-6.
55. Hanselman AE, Tidwell JE, Santrock RD. Cryopreserved human amniotic membrane injection for plantar fasciitis: a randomized, controlled, double-blind pilot study. *Foot Ankle Int* 2015 Feb;36(2):151-8.
56. Farr J, Gomoll AH, Yanke AB *et al.* A randomized controlled single-blind study demonstrating superiority of amniotic suspension allograft injection over hyaluronic acid and saline control for modification of knee osteoarthritis symptoms. *J Knee Surg* 2019 Nov; 32(11):1143-54.
57. Mahbod M, Shahhoseini S, Khabazkhoob M *et al.* Amniotic membrane extract preparation: what is the best method? *J Ophthalmic Vis Res* 2014;9(3):314-19.
58. Murri MS, Moshirfar M, Birdsong OC *et al.* Amniotic

membrane extract and eye drops: a review of literature and clinical application. *Clin Ophthalmol* 2018 Jun 18;12:1105-12.

59. Clearfield E, Muthappan V, Wang X, Kuo IC. Conjunctival autograft for pterygium. *Cochrane Database Syst Rev* 2016 Feb 11;2:CD011349. https://doi.org/10.1002/14651858.CD011349.pub2.
60. Meller D, Pires RT, Mack RJ *et al*. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology* 2000 May;107(5):980-9; discussion 990.
61. Clearfield E, Hawkins BS, Kuo IC. Conjunctival autograft versus amniotic membrane transplantation for treatment of pterygium: findings from a Cochrane Systematic Review. *Am J Ophthalmol* 2017 Oct;182:8-17. https://doi.org/10.1016/j.ajo.2017.07.004. Epub 2017 Jul 19.
62. Liu J, Li L, Li X. Effectiveness of cryopreserved amniotic membrane transplantation in corneal ulceration: a meta-analysis. *Cornea*. 2019 Apr;38(4):454-62. https://doi.org/10.1097/ICO.0000000000001866.
63. Clare G, Suleman H, Bunce C, Dua H. Amniotic membrane transplantation for acute ocular burns. *Cochrane Database Syst Rev.* 2012 Sep 12;2012(9):CD009379. https://doi.org/10.1002/14651858.CD009379.pub2.
64. Sabella N. Use of fetal membranes in skin grafting. *Med Rec NY*. 1913;83:478.
65. Davis JW. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Johns Hopkins Med J*. 1910;15:307-96.
66. Maral T, Borman H, Arslan H *et al.* Effectiveness of human amnion preserved long-term in glycerol as a temporary biological dressing. *Burns*. 1999;25:625–35.
67. Robson MC, Krizek TJ. The effect of human amniotic membranes on the bacteria population of infected rat burns. *Ann Surg.* 1973;177:144-9.
68. Robson MC, Krizek TJ, Koss N *et al.* Amniotic membranes as a temporary wound dressing. *Surg Gynecol Obstet*. 1973;136:904-6.
69. Ninman C, Shoemaker P. Human amniotic membranes for burns. *Am J Nurs*.1975;75:1468-9.
70. Salisbury RE, Carnes R, McCarthy LR. Comparison of the bacterial clearing effects of different biologic dressings on granulating wounds following thermal injury. *Plast Reconstr Surg.* 1980;66:596-8.
71. Quinby WC Jr, Hoover HC, Scheflan M *et al.* Clinical trials of amniotic membranes in burn wound care. *Plast Reconstr Surg.* 1982;70:711-17.
72. Herndon DN, Branski LK. Contemporary methods allowing for safe and convenient use of amniotic membrane as a biologic wound dressing for burns. *Ann Plast Surg* 2017 Feb;78(2 Suppl 1):S9-S10. https://doi.org/10.1097/SAP.0000000000000979.
73. Sawhney CP. Amniotic membrane as a biological dressing in the management of burns. *Burns* 1989 Oct; 15(5):339-42.
74. Branski LK, Herndon DN, Celis MM *et al.* Amnion in the treatment of pediatric partial-thickness facial burns. *Burns* 2008 May;34(3):393-9. Epub 2007 Oct 24.
75. Kesting MR, Wolff KD, Hohlweg-Majert B, Steinstraesser L. The role of allogenic amniotic membrane in burn treatment. *J Burn Care Res* 2008 Nov-Dec;29(6): 907-16. https://doi.org/10.1097/BCR.0b013e31818b9e40.
76. Fetterolf DE1, Snyder RJ2. Scientific and clinical support for the use of dehydrated amniotic membrane in wound management. *Wounds*. 2012 Oct;24(10):299-307.
77. Serena TE, Carter MJ, Le LT *et al.* A multicenter, randomized, controlled clinical trial evaluating the use of dehydrated human amnion/chorion membrane allografts and multilayer compression therapy vs. multilayer compression therapy alone in the treatment of venous leg ulcers. *Wound Repair Regen*. 2014 Nov-Dec; 22(6):688-93. https://doi.org/10.1111/wrr.12227. Epub 2015 Jan 8.
78. Haugh AM, Witt JG, Hauch A *et al.* Amnion membrane in diabetic foot wounds: a meta-analysis. *Plast Reconstr Surg Glob Open* 2017 Apr 25;5(4):e1302. https://doi.org/10.1097/GOX.0000000000001302. eCollection 2017
79. Ragazzo M, Trojan D, Spagnol L *et al.* Use of amniotic membrane in the treatment of patients with BRONJ: two case reports. *J Surg Case Rep.* 2018 Apr 20;2018(4): rjy073. https://doi.org/10.1093/jscr/rjy073. eCollection 2018 Apr.
80. Guarda-Nardini L, Trojan D, Montagner G *et al.* Human amniotic membrane positioning in the surgical treatment of temporomandibular joint degenerative disorder. *Case Rep Surg.* 2019 Mar 3;2019: 6037191. https://doi.org/10.1155/2019/6037191. eCollection 2019.
81. Martelloni M, Montagner G, Trojan D, Abate R. Amniotic membrane in palatal epithelial-connective tissue reconstruction. *Int Med Case Rep J*. 2019 Nov 19;12:349-54. https://doi.org/10.2147/IMCRJ.S213275. eCollection 2019.
82. Kar IB, Singh AK, Mohapatra PC *et al.* Repair of oral mucosal defects with cryopreserved human amniotic membrane grafts: prospective clinical study. *Int J Oral Maxillofac Surg.* 2014 Nov;43(11):1339-44. https://doi.org/10.1016/j.ijom.2014.07.018. Epub 2014 Aug 15.
83. Ang J, Liou CD, Schneider HP. The role of placental membrane allografts in the surgical treatment of tendinopathies. *Clin Podiatr Med Surg.* 2018 Jul;35(3): 311-21. https://doi.org/10.1016/j.cpm.2018.02.004. Epub 2018 May 4.
84. Zelen CM, Serena TE, Snyder RJ. A prospective, randomised comparative study of weekly versus biweekly application of dehydrated human amnion/chorion

membrane allograft in the management of diabetic foot ulcers. *Int Wound J.* 2014 Apr;11(2):122-8. https://doi.org/10.1111/iwj.12242. Epub 2014 Feb 21.

85. Fairbairn NG, Randolph MA, Redmond RW. The clinical applications of human amnion in plastic surgery. *J Plast Reconstr Aesthet Surg.* 2014 May;67(5): 662-75. https://doi.org/10.1016/j.bjps.2014.01.031. Epub 2014 Jan 31.
86. Ganatra MA. Amniotic membrane in surgery. *J Pak Med Assoc.* 2003 Jan;53(1):29-32.
87. Riau AK, Beuerman RW, Lim LS, Mehta JS. Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. *Biomaterials.* 2010 Jan;31(2):216-25.
88. Mamede AC, Carvalho MJ, Abrantes AM *et al.* Amniotic membrane: from structure and functions to clinical applications. *Cell Tissue Res.* 2012 Aug;349(2): 447-58.
89. McQuilling JP, Vines JB, Kimmerling KA, Mowry KC. Proteomic comparison of amnion and chorion and evaluation of the effects of processing on placental membranes. *Wounds* 2017;29(6):E38-42.
90. Go YY, Kim SE, Cho GJ *et al.* Differential effects of amnion and chorion membrane extracts on osteoblast-like cells due to the different growth factor composition of the extracts. *PLoS ONE.* 2017;12(8): e0182716, available at https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0182716, accessed 13 April 2022.
91. McQuilling JP, Kammer M, Kimmerling KA, Mowry KC. Characterisation of dehydrated amnion chorion membranes and evaluation of fibroblast and keratinocyte responses *in vitro*. *Int Wound J.* 2019 Jun;16(3): 827-40.
92. Kogan S, Sood A, Granick MS. Amniotic membrane adjuncts and clinical applications in wound healing: a review of the literature. *Wounds.* 2018 Jun;30(6):168-73.

Chapter 21. **Skin**

21.1. Introduction

Autologous skin is considered the gold standard for wound treatment and final wound closure. For large wounds, autologous thin split-thickness skin can be widely expanded (meshed) or transplanted as island grafts (Meek wall technique). The autologous skin grafts will grow out to close the wound.

In cases of shortage of autologous skin sources (especially in burns), donor skin is used to treat patients with extensive skin loss, for wounds with either split-thickness or full-thickness depth. Deep injuries lead to dermal damage, impairing the ability of the skin to heal and regenerate. Skin allograft basically acts as a temporary coverage, preparing the wound bed, reducing scarring, controlling pain, preventing infection and maintaining patient homoeostasis by reducing loss of fluids, proteins and heat through the burn wound. For these reasons, donor skin is critical (and often life-saving) in the treatment of severely burned patients, after escharectomy.

In addition, allogeneic skin is considered to be an excellent biological dressing for the treatment of other types of skin defects such as ulcers (venous, decubitus), diabetic foot, surgical wounds, exfoliative skin disorders such as toxic epidermal necrolysis (Lyell's syndrome) and congenital epidermolytic skin disease. In these cases, skin allografts promote re-epithelialisation and formation of granulation tissue, shorten healing time, control pain and protect important structures (e.g. tendons, bones, cartilage, nerves) [1]; they are also successfully used as skin substitutes that incorporate the dermal component into the wound bed, guiding a more physiological healing process, having the ability to be integrated into the wound bed of full-thickness burns or leg ulcers.

Allogeneic skin can also be placed on top of the autologous skin (the 'sandwich technique') to protect it from mechanical damage, dehydration and infection. After 7-10 days the allogeneic skin can be removed from the wound [2, 3, 4].

These factors explain the consistent demand for skin allografts by burn centres and reconstructive surgery units, where their capacity to 'take' and integrate into the wound bed is exploited. In the past, allogeneic skin was used sometimes to replace the lost dermis ('Cuono technique') [5], but the donor cells and hairs still present may cause inflammatory reactions with a negative effect on the final scar formation. Nowadays acellular dermis is available. Several tissue banks have developed this type of skin graft that is more suitable as a dermal equivalent.

The shortage of allogeneic skin grafts has promoted the development of skin-replacement products, and many research teams have focused on biomaterials for skin substitution in wound healing. In the last 30 years, a huge number of biological, semisynthetic and synthetic skin/dermal substitutes have been developed with the aim of producing an artificial skin that is able to replace human skin completely, but an ideal skin substitute has not yet been realised. A further logical development of this research involves the use of stem cells to re-populate the dermal matrix and reproduce 'physiological' skin, but to date there is no ideal skin-replacement product available based on stem cells.

The following generic chapters of this Guide (see Part A) all apply to skin banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the additional specific requirements for skin.

21.2. Skin-specific donor evaluation

21.2.1. Skin inspection and skin-specific contraindications

In addition to the standard physical examination described in Chapter 5, the donor's skin must be inspected in a particular manner before skin procurement. Skin should be visually checked for mechanical damage, open wounds, multiple (> 100) or dysplastic naevi (see Appendix 16), dermatitis, local infections, scars and ectoparasites. The results must be recorded and taken into account.

The list of selection criteria for donors is based on a risk analysis related to the use of the tissue on patients, i.e. to minimise the risk of disease transmission to the recipient and to ensure the appropriate quality of skin for optimal functional results. The following conditions contraindicate skin donation:

a. autoimmune diseases and systemic connective tissue diseases affecting skin;
b. diseases affecting the dermis (e.g. dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria, lupus erythematosus);
c. toxicity of the skin as a result of the presence of toxic agents or poisons;
d. systemic use of corticosteroids or Cushing disease, inducing severe skin atrophy.

The following relative contraindications for skin donation should be considered case by case, and eventually require risk assessment:

a. extensive lacerations, haematoma or scars;
b. skin diseases with extensive involvement (e.g. psoriasis, eczema);
c. relevant skin ulcers, pressure ulcers, stoma, pyoderma or mycoses;
d. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos, jaundice);
e. diabetes mellitus with skin complications (e.g. ulcers, amputation, neuropathy);
f. pre-malignant conditions such as actinic keratoses and Bowen's disease;
g. mechanical, thermal or microbial damage where skin is to be procured;
h. extreme peripheral oedema, high body mass index (BMI) (≥ 40) or poor nutritional status affecting procurement or body reconstruction.

The common practice is not to procure skin from donors aged < 15 years but many tissue establishments (TEs) do not indicate any age limits, which are basically determined by the medical director of the TE, according to characteristics and quality of tissues. Some TEs define a minimum body weight criterion for procuring skin.

21.3. Skin procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure skin grafts safety. Risks are often multi-factorial and, to help quantify and map them, a dedicated chapter (Chapter 3) has been developed with the tailored EDQM Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk reducing measures, as needed.

Skin can be obtained from deceased donors after brain death (DBD) or circulatory death (DCD). It is recommended to procure the skin within a

period of 12 h, should the body not be refrigerated, or up to 24 h after death if the body has been cooled or refrigerated within 6 h of death. It is obvious that refrigeration of the DCD donor before procurement will reduce skin contamination and facilitate skin procurement due to the harder consistency of the subcutaneous tissue.

It may be permissible to extend procurement times to > 24h after death if skin processing has been validated to guarantee quality and microbiological safety; in these cases the blood samples for serological testing should be taken just prior to death or within 24 h after death (to avoid extensive haemolysis) [6]. See Chapter 6 for details of sample collection.

Skin can also be obtained from living donors if there is a shortage of *post mortem* donors, from patients having abdominoplasty or mammoplasty procedures who consent to tissue donation. Potential living donors are evaluated similarly to deceased donors to determine donor suitability according to standard exclusion criteria for skin donation and absence of adverse physical, psychological or emotional outcome before, during or following the donation (see Chapter 5). In these cases, the procurement area is prepared by depilation and disinfection and the tissue is processed to obtain full-thickness skin grafts.

21.3.1. Procurement teams and sequence

Skin-procurement teams should consist of at least two people operating under aseptic conditions and appropriately clothed for the type of procurement. In the case of multi-tissue procurements, the order in which the tissues are removed should be standardised and predefined and, in the case of multiple procurement teams, should be agreed between the teams beforehand so that risks of cross-contamination between tissues are minimised (see Chapter 7). Studies show that, whether the skin procurement is done before or after bone procurement, the contamination rate of skin is not different if the procurement process is controlled and standardised [7, 8]; therefore, skin is usually retrieved in aseptic technique prior to bone tissue due to the difficulty in obtaining grafts of consistent quality in particular after extensive bone procurement.

Notably, procurement of skin before ocular tissue is recommended to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes. Some tissue banks place the head at a higher level than the donor's back to minimise bleeding.

21.3.2. Skin-procurement procedure

Skin is procured under aseptic conditions after shaving of the donor areas and appropriate pre-operative scrubbing and disinfection of the donor skin to remove the transient, and reduce the resident, microbial flora. An effective and validated procedure for skin disinfection should be established by the TE and applied to all procurement sites.

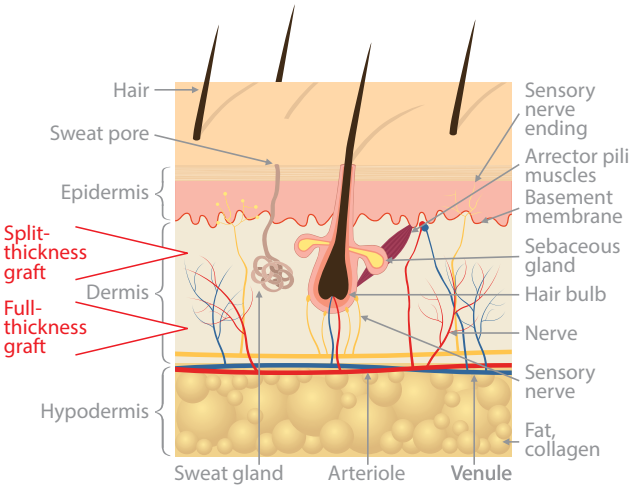
The procedure should aim to reduce the bioburden, which can significantly decrease the microbial positivity rate of processed skin samples. Therefore, suitable disinfectants, such as povidone-iodine or chlorhexidine, should be chosen. Their concentrations and the durations of exposure should also be evaluated and validated. Thorough cleaning of the skin with a surgical detergent prior to application of disinfectants will enhance their efficacy.

A local sterile field using sterile drapes must be used prior to procurement to effectively prevent microbial contamination. Skin grafts can be procured by manually, electrically, compressed-air or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs. Grafts should be obtained as homogeneously as possible.

According to graft thickness, skin grafts can be divided into split-thickness and full-thickness grafts. They consist of the entire epidermis and a dermal component of variable thickness. If the entire thickness of the dermis and skin adnexal structures is included, the appropriate term is full-thickness skin graft (FTSG). This type of skin graft can be procured using a scalpel instead of a dermatome. If less than the entire thickness of the dermis is included, this graft is referred to as a split-thickness skin graft (STSG). STSGs are categorised further as thin (0.2-0.3 mm), medium (0.3-0.45 mm) or thick (0.45-0.75 mm). The choice whether to procure STSG or FTSG can also be determined by the intended clinical use and downstream processing of the procured tissue (e.g. FTSG can be preferred in the production of acellular dermis to be used as a dermal-equivalent). The clinical use of full- or split-thickness grafting (see Figure 21.1) depends on wound conditions, location, thickness, size and aesthetic concerns [9, 10].

Staged separate procurement of particular body areas, with placement of procured material from each area into separate containers, is sometimes preferred in order to reduce eventual cross-contamination of the procured tissue. Containers and solutions for transportation of procured skin to processing TEs must be sterile and suitable for the intended use. Pre-labelling of the containers is important to prevent

Figure 21.1. **Differences between split- and full-thickness graft**



mix-up of tissues and to ensure their full traceability (see Chapter 15).

21.3.3. Reconstruction of the skin donor

For aesthetic reasons and with a view to a respectful reconstruction of the donor, it is not acceptable to take skin from the neck, face and other typically exposed areas of the body that might be visible when people pay their last respects to the donor, unless specific authorisations are obtained. Conditions which may affect body reconstruction after procurement such as extreme oedema or a high BMI (≥ 40) should be evaluated prior to procurement; body reconstruction should ensure that loss of fluid that accompanied skin procurement is not noticeable. Once the tissue has been procured, or at the end of recovery in the case of multi-tissue donors, appropriate dressing of the donation areas should be used to prevent leaking and oozing. This could include the use of sealing agents, padding and/or appropriate garments. Effective communication with all parties involved can help to meet expectations in regard to delays, as well as aesthetic considerations in case of unexpected leaking.

21.3.4. Temporary storage and transportation to the tissue establishment

Immediately after procurement, skin samples for initial microbiological control should be obtained and the recovered tissue must be stored in sterile, pre-labelled containers filled with a suitable refrigerated transport medium (e.g. PBS at pH 7.2-7.3). If samples are not taken immediately after procurement, or before the start of processing, the testing process should be validated to demonstrate that post-procurement transport/storage does not impact its accuracy. The containers must be sealed securely, refrigerated to 2-8 °C and transported to the processing facility or TE. Transportation at low temperatures prevents proliferation of most bacteria and fungi, and maintains skin viability (if viable grafts are required). Antibiotics (whose stability at the transport temperature should be defined) can be added to the transport medium, taking into account that the optimal operating temperature of most antibiotics is much closer to 37 °C than to 2-8 °C and, at refrigerated temperature, antibiotic cocktails can fail to decontaminate skin grafts [11-12]. If skin grafts are to be glycerolised (i.e. they are non-viable grafts), refrigerated transportation may not be required and the recovered skin can be stored and transported at room temperature in 50 % glycerol solution.

After procurement, skin grafts should be transferred to the processing TE as soon as possible; immediately in the case of cryopreserved skin grafts, and no longer than 72 hours after procurement for other types of graft.

Before processing, the recovered skin should be kept in a temperature-controlled refrigerator at 2-8 °C, without interruption throughout the refrigeration process. It is recommended that the cell nutrient medium used for viable grafts be changed shortly after receipt of skin grafts, or that the medium be validated for the storage time (i.e. adequate buffering capacity). All the manipulations where the transport containers are going to be exposed and the media changed must be performed in a controlled-air-environment (safety cabinet in a clean room of at least Grade D).

21.3.5. Procurement documentation

The organisation responsible for the skin procurement must produce a procurement report to be provided to the TE. In addition to the generic requirements defined in Chapter 7, it must contain a description and identification of the procured skin (including samples for testing) as well as any incident during procurement.

21.4. Skin processing

The recovered skin is processed to reduce microbial contamination and allow longer storage periods until transplantation. Skin pieces are trimmed, cut into defined pieces, and possible hair remnants are removed. Processing must not change the physical properties of the tissue, making it unacceptable for clinical use. The methods used must be in line with current state-of-the-art procedures and validated

procedures (see Chapter 2, Chapter 4 and Chapter 9). Different TEs apply specific preparation methods according to their own standard operating procedures (SOPs) and any applicable local authorisations. All processes must be validated in accordance with the guidance given in Chapter 2.

21.4.1. Skin processing methods

Depending on the intended clinical use and the quality requirements, skin grafts can be processed and preserved according to various methods (deep freezing, cryopreservation, glycerol preservation, lyophilisation), and potentially terminally sterilised with gamma-irradiation. These methods produce different grades of skin viability, integrity and immunogenicity. Skin grafts destined for cryopreservation should be processed as soon as possible after procurement in order to maintain cell viability and structural integrity. Skin allografts can also be processed into de-epidermised skin and acellular dermal matrix (ADM). Processing of skin grafts generally includes soaking in antibiotic and antimycotic cocktails; where maintenance of donor cell viability is desired, soaking in antibiotic cocktails selectively inactivates micro-organisms while affording some protection to cells. In accordance with the requirements stated in §21.3.4, the incubation temperature and the composition of decontamination cocktails should be defined, after validation, in written procedures by each TE, considering the initial tissue microbial contamination.

The usual width of the grafts procured depends on the width of the dermatome blades (usually 8 or 10 cm). The length of the grafts varies according to the size of the donor site and the final storage containers according to requirements of the end-user clinicians and final storage container capacity. Procured skin allografts can be cut into specific smaller sizes according to requirements of the end-user clinicians. The skin grafts may be provided as sheets or meshed (extended on a synthetic mesh to increase the surface area and to allow wound fluid drainage). If grafts are meshed by the TE, the appropriate mesh ratio should be agreed with clinical users. The graft's irregular edges should be trimmed and, typically, a rectangular shape should be obtained. The final graft sizes are measured with a ruler or calipers. The dimensions and area of each graft must be recorded and displayed on the label. The grafts should then be packaged in validated sterile packages and labelled appropriately (see Chapter 15).

21.4.2. Glycerol-preserved skin allografts

Glycerol-preserved skin allografts (GPA) were developed [13] to maintain skin allograft at 2-8 °C using an increasing series of glycerol concentrations (50 %, 70 %, 85 %) for preservation and storage without freezing. Glycerol preservation is an excellent preservation method to obtain de-vitalised skin grafts characterised by reduced immunogenicity and low antibacterial/antiviral properties, though it cannot be considered a sterilising agent [14-25]. If there are positive microbiology results from cryopreserved skin, the skin can be processed in 85 % glycerol as a recovery procedure [15-16]. The glycerol solutions used must be sterile and of high quality (e.g. see *European Pharmacopoeia* monograph 0497 – Glycerol 85 %). Most skin banks validate an expiry date of 5 years for GPA stored at 2-8 °C.

21.4.3. Unprocessed skin allografts

The use of unprocessed skin allografts ('fresh skin allografts') is not the preferred option because it may not allow for complete donor screening, autopsy reports and/or extensive microbiological testing. However, some TEs use unprocessed skin allografts as it is possible to maintain structural integrity and cell viability for short periods of time (maximum 7-8 days at 2-8 °C) [11]. These allografts were initially preferred in burn centres due to their high cell viability [26].

21.4.4. Cryopreserved and deep-frozen skin allografts

These preservation methods aim to maintain cell viability and structural integrity of skin allografts for extended periods of time. Biological and structural functions of skin tissue are preserved by cooling to subzero temperatures in a freezing medium with cryoprotectants, such as dimethylsulphoxide (DMSO) or glycerol [27, 28], to protect cells against freezing injury. Cryoprotectants can, however, adversely affect cell viability and graft efficacy. A controlled-rate freezing procedure is recommended to preserve cell viability. Cell viability is maintained in cryopreserved and, to a lesser degree, deep-frozen skin grafts. It facilitates the tissue engraftment to the wound bed, as a substrate for revascularisation and recolonisation by host cells [29-30, 12]. Skin-cell viability, referred to as the mean percentage of cell viability after 10-20 days of storage, is reported to be between 40 and 50 % that of the fresh skin [31-34]. When cell viability is required for clinical use, it must be validated and can be assessed by various methods,

including vital dye staining, oxygen consumption, and enzymatic and metabolic assays (described in §21.5.2).

Cryopreservation is considered to be the best method that allows the long-term preservation of skin [12]. After cryopreservation, skin allografts must be stored at a temperature of < −140 °C in liquid nitrogen tanks (vapour or liquid phase) or ultralow temperature mechanical freezers. Storage of grafts immersed in liquid nitrogen is not recommended. If stored below −140 °C, a storage period of up to 5 years is permissible; longer storage periods should be supported by validation studies. Storage at higher temperature (– 60 °C to – 80 °C) is a method applied for medium-term (maximum of 2 years) preservation of viable skin allografts [31-33]. Inappropriate storage compromises the potential to restore normal metabolic activity and, thus, physiological functioning after transplantation.

21.4.5. Lyophilised skin allografts

Processing of grafts by freeze-drying devitalises the grafts while maintaining their structure. A maximum limit for residual water content should be established and measured (ideally < 5 %). Lyophilised skin grafts can be stored at ambient temperature for 3-5 years.

21.4.6. De-epidermised skin

De-epidermising skin (frequently associated with de-cellularisation) is a method to lower the antigenicity of the skin graft. Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis. Various methods for separating the epidermis from dermis are reported such as chemical (sodium chloride, phosphate buffered saline, dispase), physical (heat, freezing and thawing) or mechanical (dermatome). These methods are frequently used in association to obtain optimal de-epidermisation. In cases of shortage of deceased skin donors, full-thickness skin can be obtained from living donors undergoing abdominoplasty or body-contouring procedures and is processed in a similar manner to produce thicker dermal allografts to be used in full-thickness skin loss if primary closure or donor-site availability of autografts is limited or suboptimal [35]. The result is an intact dermal matrix that can be cryopreserved, frozen, preserved in glycerol or lyophilised.

21.4.7. Skin tissue decellularisation

Tissue decellularisation is a technique that aims to remove all cell remnants from a tissue, maintaining an intact extracellular matrix (ECM). In recent years several innovative biological products based on decellularisation of donor-derived skin tissue have been developed using biotechnological sciences. Resulting tissues are acellular dermal allografts, as the decellularisation processes invariably remove the epidermis, with the possible exception of the basement membrane. The clinical applications of these grafts extend beyond the traditional role of skin allografts, including tendon repair, abdominal patches and breast reconstruction.

Three methods are actually used to achieve tissue decellularisation: chemical, physical and biological (enzymatic). Each of these methods has a different mode of action and effect on the ECM. A combination of methods is recommended to ensure effectiveness [36]. Chemical methods comprise alkaline/acid solutions, alcohols, hyperosmotic/hyposmotic solutions, ionic detergents, non-ionic, zwitterionic detergents, chelating agents; physical techniques are based on temperature (freeze–thaw cycles), hydrostatic pressure, mechanical agitation and sonication; biological methods include trypsin and endo/exonucleases (see Appendix 34). Decellularisation protocols generally start with cell lysis by physical/chemical treatments, detachment and solubilisation of the cell components using enzymatic treatments and detergents, and removal of the cell remnants from tissues. These processes may be associated with mechanical agitation/sonication to increase the treatment effectiveness. At the completion of the decellularisation process, it is important to thoroughly rinse the tissue matrix to minimise the residual amounts of any processing chemicals in the final graft, as these may be toxic or immunogenic, and impact its clinical efficacy.

Sterilisation techniques may be applied to provide a higher level of safety. There are significant advantages in combining decellularisation and sterilisation processes to ensure a clinically safe ECM, minimising the effect on its ultrastructure.

The common goal of all these methods is to obtain an acellular dermal matrix (ADM) characterised by an intact fibrous and collagenous architecture, able to be repopulated by autologous cells of the patient after its engraftment. The absence of immune response and graft rejection in patients is ensured by removing the cellular components (fibroblasts and endothelial cells) as well as the donor DNA and hair remnants. From a functional point of view, these dermal matrices act as cell-free scaffolds able to per-

manently reconstruct and regenerate damaged and/or pathological skin tissue.

The main biological characteristics of an optimal ADM are biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to induction of pro-inflammatory cytokines), integrity of the matrix (the maintenance of integrity of elastic fibres and collagen physiologically identified in the tissue), sterility (absence of Gram+/– bacteria, fungi and bacterial endotoxins), malleability and suturability (handling; mechanical resistance with or without stitches) and storage options (the ability to be stored by different methods e.g. cryostorage in nitrogen vapours, storage at – 80 °C, freeze-drying, dehydration, room-temperature storage, storage in high-percentage glycerol).

See Chapter 34 and Appendix 34 of this Guide for information on decellularisation of tissues as natural extracellular matrices.

Clinical indications of acellular dermal matrices are in the field of regenerative medicine and surgery and depend on wound thickness. Among them are:

- dermatology, plastic and reconstructive, general surgery and vascular surgery used for the treatment of acute (e.g. burns) and chronic (e.g. skin ulcers in various aetiology) skin wounds with extensive loss of substance: acellular dermal matrices with a thickness of 0.2-0.4 mm can be used in combination with a thin autologous split-skin graft to improve the scar quality of the wound; thicker acellular dermis is used for other indications such as reconstruction of the breast after mastectomy or hernia repair [37-47];
- orthopaedics for the repair of the rotator cuff of the shoulders as well as for the treatment of skin surgical wounds [48];
- maxillo-facial surgery, ENT (ear, nose and throat) surgery, dentistry for the sinus lift and implant dentistry for augmentation in gum reconstructions [49-50].

21.4.8. Processing facilities

In selecting an appropriate air-quality specification for processing skin grafts, and in accordance with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered. For EU countries, the background must be at least Grade D but, given the risks associated with the use of skin grafts which are not sterilised or treated with equivalent microbial reduction methods, more stringent requirements are recommended.

21.4.9. Sterilisation of skin allografts

When tissue viability is not required or when skin tests positive for microbiological contaminants, it can be sterilised by gamma or electron beam irradiation. Ionising radiation (in relation to its dose) can cause structural changes in the irradiated skin allografts, especially in the epidermis [51]. Research has shown that a maximum dose of 25 kGy irradiation of deep-frozen skin in radio-protective solutions sterilises tissue without relevant histomorphological or physical alterations (such as pliability) compared with normal cryopreserved skin [11, 52].

Sterilisation methods should be validated for the initial estimated level of bioburden prior to application of the sterilisation method to skin allografts. Sterilisation can be used to 'rescue' skin that has been initially processed as cryopreserved, viable skin but is not considered suitable for transplantation due to microbial contamination. Sterilisation with irradiation will destroy viable donor cells, but if performed appropriately does not compromise the structure and mechanical properties of the graft, meaning it can serve as a biological dressing. This type of graft is not expected to engraft to the wound bed, as cryopreserved skin grafts can. This can, however, result in the graft giving a better cosmetic outcome when applied to facial or hand burns, for example.

21.5. Quality control

21.5.1. Microbiological control

In addition to the standard microbiological controls described in Chapter 11, microbiological testing should be done before the start of processing and on post-processed samples of skin (after rinsing to remove as much disinfection and processing reagent as possible), before the skin is approved for clinical use. These approaches are based on the microbiological test methods of *Ph. Eur.* and cover the minimum standards to control the microbiological safety of preparations of human tissues. Deviations from such standards should be justified and the suitability of the intended alternative test methods must be demonstrated and validated. Specimens of a representative sample of finished product (e.g. a predefined number of pieces of skin allografts that have undergone all stages of production) should be sent for microbiological testing to check for aerobic and anaerobic bacteria and fungi using appropriate culture media [27]. Acceptance criteria for microbial

load and types of contaminant in processed tissues should be defined in advance and reported in written procedures.

If a positive microbiology result is obtained at the initial stage or at an intermediate stage of processing, a risk-assessment analysis should be conducted to assess the suitability of the skin tissue (and other tissues from the same donor), taking into account the micro-organism(s) detected. Basically, skin allografts may be accepted for clinical use, without sterilisation, when bacteriological and mycological assessment (refer to Chapter 11 for acceptable microbiological examination techniques) reveal only low bioburdens of inherent inhabitants of the residential skin flora. The surgeon must be informed of the skin-graft bioburden before the use of the graft (see §21.9 for further information). Bioburden can be determined using quantitative cultures, but it is permitted to use more pragmatic validated approaches in which bacterial density is measured in terms of bacterial lawn confluency or the appearance of turbidity in periodically inspected liquid cultures. The presence of micro-organisms in finished product samples results in a definite rejection of the donor tissue if no validated sterilisation or decontamination method is applied (see Figure 21.2). In exceptional cases, skin grafts could be released with low bioburdens of skin commensals "on board" [27], after appropriate risk assessment and consultation with surgeons (e.g. in urgent cases).

For terminally sterilised skin, an equivalent analysis should be carried out, taking into consideration the capacity of the sterilisation process as demonstrated through validation.

Specimens contaminated by (endo)spore-forming micro-organisms such as *Bacillus* (*anthracis* or *cereus*) and *Clostridium* spp. or any of the pathogens listed in Table 11.1 in Chapter 11, at any stage of the process (even if negative at the end of processing), unless otherwise validated should be discarded without corrective actions in order to remove potentially unsuitable tissue from the transplantation process, unless a terminal sterilisation process is to be applied.

21.5.2. Skin allograft performance and quality issues

Viable donor skin is still considered the gold standard for the temporary covering of burns. In cryopreserved skin allografts, the viability of skin is often considered as an essential requisite and should be tested. Different methodologies are used to assess cell viability in skin grafts before and after thawing:

- quantitative, e.g. tetrazolium salt assay (MTT), neutral red test (NRT), resazurin test, oxygen or lactate consumption assay [33-34];
- and /or qualitative, by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

Controversy exists in the literature data regarding the importance of cell viability in graft performance and quality. It is widely considered that viable skin allografts are superior to all other dressing materials, and the majority of physicians agree that higher viability is usually associated with better wound-bed preparation and graft take [33, 53-56]. Comparison of unprocessed, cryopreserved (viable) and glycerolised skin allografts by the use of animal studies (immuno-competent Balb/c mice) revealed a better performance (evaluated by histology) of unprocessed skin and, to a lesser degree, of deep-frozen (– 80 °C) and cryopreserved (in liquid nitrogen) skin [57]. These data demonstrated that graft performance of cryopreserved skin decreased with time.

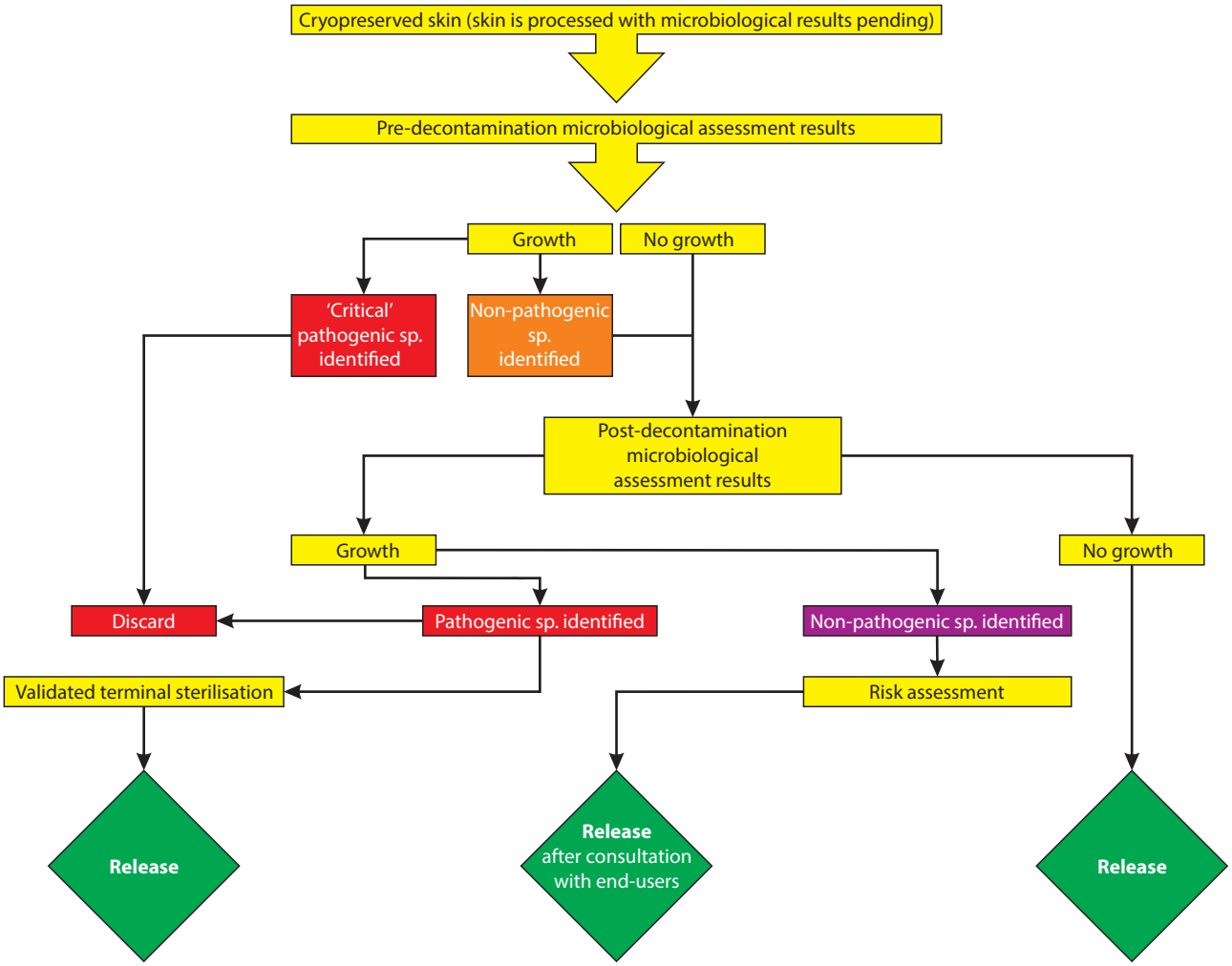
However, non-viable skin allografts can be successfully employed when viable cryopreserved skin allografts are not available or where cell viability is not required for wound treatment [4]. Literature studies [26] indicate that there is no evidence that viability of the graft influences healing outcomes. Thus, instead of viability, other aspects, such as structural integrity, clinical outcome and intrinsic anti-microbial safety of the preservation method and cost should be the primary criteria for the choice of preservation method to be used for allografts.

It is demonstrated that allografts' dermal component can easily take [55], whereas homologous epidermis is normally rejected within two weeks [58]. To obtain re-epithelialisation of full-thickness wounds/burns it is usually necessary to follow a two-step surgical procedure: reconstruction of the dermal compartment by skin or dermal allografts and then re-epithelialisation by autologous thin grafting. Recently, some authors [59] have reported a successful one-step procedure in the treatment of severe burns, by using mixed auto- and cryopreserved allograft skin grafting. This technique seems to shorten the wound healing time significantly, thus greatly raising the utilisation rate of autologous skin.

21.6. Packaging and labelling

The grafts are packaged in sterile containers (e.g. foil or polythene/polyethylene/EthylVinylAcetate bags) and coded, packaged and labelled in accordance with the guidance in Chapter 15. All packages must be labelled with the name of the processing in-

Figure 21.2. **Algorithm for acceptance or rejection of skin after bacteriological assessment**



Note: Pathogenic spp. identified during skin assessment should be understood as those defined in Chapter 11.

stitution, a unique identifier or serial number linking the tissue to the donor, the expiry date, size and type of skin graft (e.g., cryopreserved, glycerolised).

21.7. Storage

Processed skin grafts are stored in various conditions depending on processing method (see §21.4.1) summarised in Table 21.1.

21.7.1. Expiry date

In order to ensure the safety and quality of tissues and cells, the maximum shelf-life of tissue under each type of storage condition should be specified. The expiry or retest date should come from formal validation with stability studies, which should take into account, among other factors (e.g. expiry dates of reagents), possible deterioration of the required tissue and cell properties, integrity and stability of the packaging and labelling materials, according to the requirements of Chapter 9 and Chapter 10.

21.8. Skin allograft distribution

Skin allografts are considered life-saving therapeutic materials, so TEs should have a written procedure for allocation of grafts based on clinical

Table 21.1. **Storage conditions for processed skin grafts**

Type of skin grafts	Storage conditions
Glycerolised skin	Refrigerators (2 to 8 °C)
Lyophilised (freeze-dried) skin/dermis	Room temperature (15 to 25 °C)
Cryopreserved skin	Vapour phase of liquid nitrogen or mechanical freezer (< – 140 °C)
Deep-frozen skin	Mechanical freezers (– 80 to – 60 °C)

priority. Distribution of skin grafts for transplantation should be restricted to hospitals, TEs, physicians, dentists or other qualified medical professionals, in compliance with national regulations and the WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation (Chapter 1).

21.9. Acceptance criteria and exceptional release

The acceptance criteria should be based on validated protocols and reported in the TE's written procedures. The release criteria and specifications of skin/dermal allografts should be defined, validated, documented and approved. There should be a defined procedure for exceptional release of non-standard skin/dermal allografts under a planned non-conformity system. The decision to allow such release should be documented clearly, and traceability should be ensured.

21.10. Biovigilance

Adverse events and reactions as well as serious adverse events and reactions must be recorded, reported and investigated (in accordance with corresponding national regulations) to the Health Authorities for tissues and cells.

Non-exhaustive list of reportable SARs

- transmission of infective disease;
- transmission of malignant disease;
- allergic reaction (e.g. to antibiotics used for processing media);
- engraftment failure/delayed engraftment (related to the tissue graft);
- unexpected immunological reactions due to tissue;
- bleeding (wound bed preparation);
- aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied or delayed transport, discovered after patient is anaesthetised and the surgical procedure has begun) [59]. See also www.notifylibrary.org.

Non-exhaustive list of reportable SAEs

a. *Procurement*
- procurement without consent;

b. *Processing and labelling*
- use of non-sterile/expired materials for tissue processing;
- mistaken processing media (errors in media preparation);
- incorrect labelling;

c. *Storage*
- storage at inadequate temperature (e.g. in case of equipment failure; unattended alarm);

d. *Transport/distribution*
- loss of irreplaceable autologous tissues;
- delayed transport of cryopreserved skin (resulting in tissue discard);
- incorrect tissue type, i.e. a different type of tissue is supplied than was intended or requested;

e. *Testing*
- bacterial/viral/fungal contamination of tissues distributed for transplantation;
- loss of cell viability in cryopreserved viable tissue.

The Notify Library (www.notifylibrary.org) includes some well-documented cases of adverse occurrences in skin transplantation. Examples include:

- Contaminated skin graft that caused serious infection of a burn wound with *Acinetobacter* (Record Number 428).
- A case involving distribution of cryopreserved skin without review of the results of bacterial tests. Several allograft recovery cultures showed virulent pathogens ordinarily not accepted for use that prompted recall of >100 skin allografts, fortunately without any case of disease transmission (Record Number 128).
- Two cases describing incidents in which unsuitable skin grafts were released for clinical use. In one case, skin was torn upon thawing and implanting; in the second case, it was not measured appropriately, resulting in delay in patient treatment and graft loss in both cases (Record Numbers 126 and 127).

For further evaluated cases of adverse outcomes associated with skin banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

21.11. Developing applications

Wound healing is a major target in tissue-engineering research. In the past 40 years a large number of biological and synthetic skin/dermal substitutes have been produced. Products

such as bioengineered skin equivalents and synthetic/biosynthetic materials integrated with cultured epidermal cells have been developed for this purpose, and most of them would be classified in the EU as advanced therapy medicinal products (ATMP) (Chapter 34). However, the ideal skin substitute has not yet been established and human skin allografts remain a gold standard in the therapy of major burns and skin loss treatment. Thus, researchers in the field of tissue engineering are still working on the production of an ideal artificial skin able to act as a physiological skin.

21.11.1. Epidermal cell suspensions

Epidermal cell suspensions (non-cultured autologous epidermal cellular grafting) have been used in the surgical management of vitiligo since 1992, when Gauthier and Surlève-Bazeille developed a non-cultured cellular grafting technique [59]. With this technique an epidermal cell suspension is used without cell expansion to treat larger areas (8- to 10-fold size of donor skin) on an outpatient basis with simple laboratory procedures. Epidermal cell suspensions can be useful in a variety of epidermal defects, involving both keratinocytes and melanocytes, and several approaches to delivering autologous keratinocytes/epidermal cells to restore epithelialisation have been developed [60, 61].

A recent technique based on aerosol spraying of non-cultured epidermal cells suspensions represents an efficacious and rapid way to obtain re-epithelialisation. To prepare the epidermal suspension, a cutaneous biopsy is trypsinised and epidermal cells are obtained by scraping of the dermal side of the epidermis. A suspension of autologous keratinocytes, melanocytes and fibroblasts can be delivered onto the wound bed by a spray apparatus. Therefore, epidermal cell sprays can be considered as aerosolised skin grafts that can potentially treat a variety of epidermal defects for burns and traumatic injuries, but also in scar reconstruction, donor-sites repair and in skin resurfacing techniques [60]. In consideration of the presence of melanocytes, this technique is also effective in pigmentation defects, including vitiligo and post-burn leukoderma.

Special commercial devices or prefabricated cellular preparation kits have also been developed to isolate and apply non-cultured epidermal cells, dermal cells or adipocytes to wounds in a one-step surgical procedure.

21.11.2. Epidermal 3D cell cultures

It was in 1975 that Rheinwald and Green [62] first described the serial cultivation of human keratinocytes in monolayer culture obtained from primary keratinocytes seeded onto lethally irradiated murine fibroblast feeder layers. Since then numerous advances have been made in the cultivation of human keratinocytes, in both two-dimensional monolayer and three-dimensional organotypic culture. Cultured epidermis was originally used to re-epithelialise severe burns, but, because of the presence of melanocytes, it was also used in vitiligo and other skin pigmentation disorders and to treat scars, ulcers and skin-graft donor sites.

Three-dimensional (3D) bioprinting, a flexible automated platform for the fabrication of complex living architectures, is a novel approach to the design and engineering of human organs and tissues [60]. Natural decellularised extracellular matrices (ECM) represent emerging biomaterials to be printed and used in tissue engineering and regenerative medicine. These matrices provide a physiological environment acting as a bioscaffold which supports cell proliferation and differentiation. The 3D bioprinting process involves direct printing of the bio-ink (biomaterials + cells) by inkjet, micro-extrusion and laser-assisted bioprinters [63]. The most relevant challenges in skin bioprinting deal with the necessity of skin structures, such as epidermis–dermis interface, vascularisation and adnexal structures, and are still to be addressed [64].

All cell-culture methods are relevant in the field of tissue engineering and they comply, when considered for clinical applications, with the ATMP regulations. These tissues can be used to test pharmaceutical products, as well as cosmetics and consumer chemical products, *in vitro* where current regulations require testing that does not use animals.

21.11.3. Skin composite grafts – nipple preservation

Practically any human tissue can be procured and banked for clinical use. Patients with loss of the nipple and areola from cancer, excision, trauma or congenital absence can undergo nipple-sparing mastectomy in specific cases after histological examination of the tissue surrounding the nipple and areola to eliminate the possibility of eventual cancer invasion. If a patient's nipple–areola complex (NAC) is available for grafting after mastectomy, it is the best material to use for reconstruction. It can be cryopreserved as a composite graft to be autografted for reconstruction of the breast after mastectomy. According to

published literature and skin-bank protocols [65], a slow cooling procedure for cryopreservation is used by incubating the NAC in a cryoprotectant solution with 10 % DMSO or 15 % glycerol.

The timing of transfer usually ranges from 6 months to 1 year after breast reconstruction. At the time of transfer, the cryopreserved NAC is thawed in 37 °C water and grafted on a projection made by a denuded dermal flap on the reconstructed breast.

21.12. References

1. Wilson TC, Wilson JA, Crim B, Lowery NJ. The use of cryopreserved human skin allograft for the treatment of wounds with exposed muscle, tendon, and bone. *Wounds* 2016 Apr;28(4):119-25.
2. Kreis RW, Vloemans AF, Hoekstra MJ *et al.* The use of non-viable glycerol preserved cadaver skin combined with widely expanded autografts in the treatment of extensive third degree burns. *J Trauma* 1989 Jan;29(1): 51-4.
3. Kreis RW, Mackie DP, Vloemans AW *et al.* Widely expanded postage stamp grafts using a modified Meek technique in combination with an allograft overlay. *Burns* 1993 Apr;19(2):142-5.
4. Vloemans AF, Middelkoop E, Kreis RW. A historical appraisal of the use of cryopreserved and glycerol preserved in the treatment of partial thickness burns. *Burns* 2002 Oct;28(Suppl 1):S16-S20.
5. Cuono CB, Langdon R, Birchall N *et al.* Composite autologous-allogeneic skin replacement: development and clinical application. *J Plast Reconstr Surg* 1987 Oct;80(4):626-37.
6. Meyer T, Polywka S, Wulff B *et al.* Virus NAT for HIV, HBV, and HCV in post-mortal blood specimens over 48 h after death of infected patients – first results. *Transfus Med Hemother* 2012;39:376-80.
7. Pianigiani E, Ierardi F, Cuciti C *et al.* Processing efficacy in relation to microbial contamination of skin allografts from 723 donors. *Burns* 2010;36(3):347-51.
8. Fehily D, Brubaker SA, Kearney JN *et al.*, editors. *Tissue and cell processing: an essential guide*. Chichester: Wiley-Blackwell, July 2012.
9. Gupta DK. Thin and ultra thin split thickness skin grafts (STSG-UT, STSG-T). In: *Microskin grafting for vitiligo*. London: Springer, 2009.
10. Wax MK. Split thickness skin grafts. *Medscape*, updated 22 May 2017, available at https://emedicine.medscape.com/article/876290-overview#a2, accessed 13 April 2022.
11. Rooney P, Eagle M, Hogg P *et al.* Sterilization of skin allograft with gamma irradiation. *Burns* 2008;34(5): 664-73.
12. Kearney JN. Guidelines on processing and clinical use of skin allografts. *Clin Dermatol* 2005;23(4):357-64.
13. Mackie DP. The Euro Skin Bank: development and application of glycerol-preserved allografts. *J Burn Care Rehabil* 1997;18(1 Pt 2):S7-S9.
14. Van Baare J, Ligtvoet E, Middelkoop E. Microbiological evaluation of glycerolized cadaveric donor skin. *Transplantation* 1998;65(7):966-70.
15. Verbeken G, Verween G, De Vos D *et al.* Glycerol treatment as recovery procedure for cryopreserved human skin allografts positive for bacteria and fungi. *Cell Tissue Bank* 2012;13(1):1-7.
16. Saegeman VS, Ectors NL, Lismont D *et al.* Short- and long-term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. *Burns* 2008;34(2):205-11.
17. Richters CD, Hoekstra MJ, van Baare J *et al.* Morphology of glycerol-preserved human cadaver skin. *Burns* 1996;22:113-16.
18. de Backere ACJ. Euro Skin Bank; large scale skin-banking in Europe based on glycerol-preservation of donor skin. *Burns* 1994;20:S4-S9.
19. Van Baare J, Buitenwerf J, Hoekstra MJ, du Pont JS. Virucidal effect of glycerol as used in donor skin preservation. *Burns* 1994;20:S77-S80.
20. Marshall L, Gosh MM, Boyce SG *et al.* Effects of glycerol on intracellular virus survival: implications for the clinical use of glycerol-preserved cadaver skin. *Burns* 1995;21:356-61.
21. Cameron PU, Pagnon JC, Van Baare J *et al.* Efficacy and kinetics of glycerol inactivation of HIV-1 in split skin grafts. *J Med Virol* 2000;60:182-8.
22. Van Baare J, Ligtvoet EEJ, Middelkoop E. Microbiological evaluation of glycerolised cadaveric donor skin. *Transplantation* 1998;65:966-70.
23. Fluhr JW, Darlenksi R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. *Br J Dermatol* 2008;159:23-34.
24. Richters CD, Hoekstra MJ, du Pont JS *et al.* Immunology of skin transplantation. *Clin Dermatol* 2005; 23:338-42.
25. Astegiano S, Sidoti F, Costa C *et al.* Human cytomegalovirus load in fresh and glycerolized skin grafts. *New Microbiologica* 2010;33(3):253-6.
26. Greenleaf G, Hansbrough JF. Current trends in the use of allograft skin for patients with burns and reflections on the future of skin banking in the United States. *J Burn Care Rehabil* 1994;15(5):428-31.
27. Hermans MHE. Preservation methods of allografts and their (lack of) influence on clinical results in partial thickness burns. *Burns* 2011;37:873-81.
28. Pirnay JP, Verween G, Pascual B *et al.* Evaluation of a microbiological screening and acceptance procedure

for cryopreserved skin allografts based on 14 day cultures. *Cell Tissue Bank* 2012;13(2):287-95.

29. Aggarwal SJ, Baxter CR, Diller KR. Cryopreservation of skin: an assessment of current clinical applicability. *J Burn Care Rehabil* 1985 Nov-Dec;6(6):469-76.
30. Cinamon U, Eldad A, Chaouat M *et al.* A simplified testing system to evaluate performance after transplantation of human skin preserved in glycerol or in liquid nitrogen. *J Burn Care Rehabil* 1993 Jul-Aug; 14(4):435-9.
31. Franchini M, Zanini D, Bosinelli A *et al.* Evaluation of cryopreserved donor skin viability: the experience of the regional tissue bank of Verona. *Blood Transfus* 2009 Apr;7(2):100-5.
32. Pianigiani E, Tognetti L, Ierardi F *et al.* Assessment of cryopreserved donor skin viability: the experience of the regional tissue bank of Siena. *Cell Tissue Bank* 2016 Jun;17(2):241-53.
33. Castagnoli C, Alotto D, Cambieri I *et al.* Evaluation of donor skin viability: fresh and cryopreserved skin using tetrazolioum salt assay. *Burns* 2003 Dec;29(8): 759-67.
34. Landsman A, Rosines E, Houck A *et al.* Characterization of a cryopreserved split-thickness human skin allograft-TheraSkin. *Adv Skin Wound Care* 2016 Sep; 29(9):399-406.
35. Zidan SM, Eleowa SA. Banking and use of glycerol-preserved full-thickness skin allograft harvested from body contouring procedures. *Burns* 2014;40(4):641-7.
36. Hogg P, Rooney P, Ingham E, Kearney JN. Development of a decellularised dermis. *Cell Tissue Bank* 2013; 14(3):465-74.
37. Pirayesh A, Hoeksema H, Richters C *et al.* Glyaderm® dermal substitute: clinical application and long-term results in 55 patients. *Burns* 2015 Feb;41:132-44.
38. Patton JH Jr, Berry S, Kralovich KA. Use of human acellular dermal matrix in complex and contaminated abdominal wall reconstructions. *Am J Surg* 2007; 193(3):360-3.
39. Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns* 1995;21(4):243-8.
40. Guo X, Mu D, Gao F. Efficacy and safety of acellular dermal matrix in diabetic foot ulcer treatment: a systematic review and meta-analysis. *Int J Surg* 2017 Apr; 40:1-7.
41. Falanga V, Margolis D, Alvarez O *et al.* Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. *Arch Dermatol* 1998;134(3):293-300.
42. Jordan SW, Turin SY, Zielinski ER, Galiano RD. Matrices and dermal substitutes for wound treatment. In: Orgill D, editor, *Interventional treatment of wounds*. Cham: Springer, 2018.
43. Zelen CM, Orgill DP, Serena T *et al.* A prospective, randomised, controlled, multicentre clinical trial examining healing rates, safety and cost to closure of an acellular reticular allogenic human dermis versus standard of care in the treatment of chronic diabetic foot ulcers. *Int Wound J* 2017;14(2):307.
44. Valerio IL, Masters Z, Seavey JG *et al.* Use of a dermal regeneration template wound dressing in the treatment of combat-related upper extremity soft tissue injuries. *J Hand Surg* 2016;41(12):e453-e460.
45. Brigido SA. The use of an acellular dermal regenerative tissue matrix in the treatment of lower extremity wounds: a prospective 16-week pilot study. *Int Wound J* 2006;3(3):181-7.
46. Tognetti L, Pianigiani E, Ierardi F. Current insights into skin banking: storage, preservation and clinical importance of skin allografts. *Journal of Biorepository Science for Applied Medicine* 2017;5:41-56.
47. Macadam SA, Lennox PA. Acellular dermal matrices: Use in reconstructive and aesthetic breast surgery. *Can J Plast Surg* 2012;20(2):75-89.
48. Cooper J, Mirzayan R1. Acellular dermal matrix in rotator cuff surgery. *Am J Orthop* (Belle Mead NJ). 2016; 45(5):301-5.
49. Lee KC, Lee NH, Ban JH, Jin SM. Surgical treatment using an allograft dermal matrix for nasal septal perforation. *Yonsei Med J* 2008;49(2):244-8.
50. Sherris DA, Oriel BS. Human acellular dermal matrix grafts for rhinoplasty. *Aesthet Surg J* 2011;31(7 Suppl): 95S-100S.
51. Mrázová H, Koller J, Fujeríková G *et al.* Structural changes of skin and amnion grafts for transplantation purposes following different doses of irradiation. *Cell Tissue Bank* 2014 Sep;15(3):429-33.
52. Mahdavi-Mazdeh M, Nozary Heshmati B, Tavakoli SAH *et al.* Human split-thickness skin allograft: skin substitute in the treatment of burn. *Int J Org Transplant Med* 2013 Aug 1;4(3):96-101.
53. Kua EHJ, Goh CQ, Ting Y *et al.* Comparing the use of glycerol preserved and cryopreserved allogenic skin for the treatment of severe burns: differences in clinical outcomes and *in vitro* tissue viability. *Cell Tissue Bank* 2012;13(2):269-79.
54. Gaucher S, Elie C, Verola O, Jarraya M. Viability of cryopreserved human skin allografts: effects of transport media and cryoprotectants. *Cell Tissue Bank* 2012;13:147-55.
55. Cleland H, Wasiak J, Dobson H *et al.* Clinical application and viability of cryopreserved cadaveric skin allografts in severe burns: a retrospective analysis. *Burns* 2014;40:61-6.

56. Gaucher S, Jarraya M. Cryopreserved human skin allografts: Efficacy and viability. *Burns* 2014;40:526-7.
57. Ben-Bassat H, Chaouat M, Zumai E *et al.* The Israel national skin bank: quality assurance and graft performance of stored tissues. *Cell Tissue Bank* 2000;1(4): 303-12.
58. Agarwal P, Sharma D, Agarwal A, Patel LK. Can removal of epidermis delay rejection of cadaveric dermis-only allograft? A proof of concept study. *Indian J Burns* 2018;26:66-71.
59. Shizhao J, Yongjun Z, Lisen Z. Short- and long-term outcomes of small auto- and cryopreserved allograft skin grafting in those with >60%TBSA deep burn wounds. *Burns* 2017; 43:206-214.
60. Gauthier Y, Surleve-Bazeille JE. Autologous grafting with non-cultured melanocytes: a simplified method for treatment of depigmented lesions. *J Am Acad Dermatol* 1992;26:191-4.
61. Navarro FA, Stoner ML, Park CS *et al.* Sprayed keratinocyte suspensions accelerate epidermal coverage in a porcine microwound model. *J Burn Care Rehabil* 2000;21:513-18.
62. Mcheik NJ, Barrault C, Levard G *et al.* Epidermal healing in burns: autologous keratinocyte transplantation as a standard procedure: update and perspective. *Plast Reconstr Surg Glob Open* Sep 2014;2(9):e218.
63. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 1975;6: 331-44.
64. Taylor DA, Sampaio LC, Ferdous Z *et al.* Decellularized matrices in regenerative medicine. Acta Biomaterialia 2018 Jul 1;74:74-89.
65. Zhang B, Gao L, Ma L, *et al.* 3D Bioprinting: a novel avenue for manufacturing tissues and organs. Engineering 2019;5:777-94.
66. Nakagawa T, Yano K, Hosokawa K. Cryopreserved autologous nipple-areola complex transfer to the reconstructed breast. *Plast Reconstr Surg* Jan 2003;111(1): 141-7.

Related material
Appendix 16. Evaluation of pigmented skin lesions
Appendix 34. Decellularisation

Chapter 22. **Cardiovascular tissue**

22.1. Introduction

Cardiovascular tissues, i.e. cardiac and vascular (arterial or venous) tissues, can be procured from deceased donors (after brain death or after circulatory death) and living donors (e.g. heart valves from a patient undergoing a heart transplant).

Cardiac tissues, obtained from donated hearts, comprise aortic, pulmonary and mitral valves. In the majority of cases, after heart procurement, vascular segments (arteries and veins) are recovered as well. Procured vascular segments include ascending and descending abdominal aorta, aortoiliac bifurcation, iliac arteries and femoral arteries. Occasionally, procurement of veins is also considered (e.g. inferior cava, iliac and saphenous veins). In addition, in living donors, some surgeons recover saphenous veins during the procedure of stripping. After processing, the resulting cardiac and vascular tissues are referred to as allografts.

Pericardium, although considered a soft tissue graft, is also included. This is due to the anatomic relation with the heart and as a result this tissue is recovered together with the heart.

Heart valves are used mainly in paediatric cardiac surgery for treatment of congenital malformations, such as tetralogy of Fallot, valve atresia, bicuspid or monocuspid aortic valve, or transposition of great arteries. The most important reason for clinical use of heart-valve allografts in these indications is the identical morphology of the allografts and the native valve of the patient, which enables easy reconstruction of the left/right ventricular outflow tracts (RVOT/LVOT). Another advantage is that recipients, who are mainly children and neonates, do not need long-term anticoagulation because of the non-thrombogenicity of human tissue, thereby avoiding the side-effects of anticoagulation therapy in these patients. A second important indication is the reconstruction of the RVOT in the Ross operation, which is often used for the treatment of young female patients of child-bearing age and for athletes. This entails replacement of the diseased aortic valve with the autologous pulmonary valve (autograft) and reconstruction of the patient's RVOT with a pulmonary (or aortic) allograft.

A third important indication for use of allogeneic heart valves is the endocarditis of either the prosthetic or the native valves, as the allografts are believed to have a very high resistance against infection. So, it is very seldom that an allograft valve develops endocarditis after being implanted in the infected area. In recent years, allogeneic heart valves have also often been used to repair failed or infected biological valves implanted by endovascular procedure (TAVI, trans-arterial valve implantation). The most common indication for the use of vascular segments (arteries and veins) is infection of the prosthetic and/or native vascular tissue. Arteries may be used for peripheral re-vascularisation, vascular reconstruction in cases of malignant infiltration of the vascular wall (arterial, venous) [1] or reconstruction of the thoracic and/or abdominal aorta. Iliac vessels are sometimes removed from deceased donors at the time of organ donation, and they can be used to support organ transplantation. However, if they are not used during

the liver transplantation, they may be sent to the tissue establishment for further processing as vascular tissue allografts. From that moment the tissues need to comply with criteria set for tissue donation, and specific consent for tissue donation should be in place.

Respecting ischaemia time is very important, so as to be able to cryopreserve them at the appropriate moment. Following excellent collaboration between tissue establishments and organ procurement teams, an important number of iliac arteries have been procured as part of organ retrieval and subsequently sent to a tissue establishment, allowing their further use for other indications (e.g. abdominal oncology, vascular surgery).

In light of long-term follow-up studies, it has been identified that implanted cardiovascular allografts are the subject of premature deterioration, mainly in very young patients and children, perhaps because of immune-related deterioration [2, 3]. Therefore, methods for the reduction of immunogenicity in cardiovascular allografts have been the subject of much research during the past two decades. This has involved the development of new procedures to decellularise cardiovascular allografts. This may also facilitate recellularisation of the graft with cells from the recipient *in vitro* before implantation or repopulation of the graft *in vivo* after implantation.

The following generic chapters (Part A) of this Guide all apply to cardiovascular tissue banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the additional specific requirements for cardiovascular tissue.

22.2. Donor evaluation

22.2.1. Contraindications and exclusion criteria specific to cardiovascular tissue donation

The following exclusion criteria are specific to donation of cardiovascular tissue:

a. valvulopathy of both the aortic and pulmonary valves, with moderate-to-severe stenosis or incompetence (the vessels can still be acceptable);
b. aortic dissection (detachment of the intima and adventitia);
c. Marfan's syndrome and related diseases (e.g. Loeys–Dietz syndrome, Ehlers–Danlos syndrome);
d. bacterial or fungal endocarditis.

Other conditions to be evaluated as part of the donor-selection process are:

a. dilatation of ventricles and valve alteration such as dilated roots, wall alteration of aorta or pulmonary trunk;
b. untreated pneumonia in previous days due to highly pathogenic bacteria or fungi, such as *Staphylococcus aureus*, pneumococci or *Candida*;
c. Previous surgical interventions on the tissue to be procured.

Donor age limits vary between centres, with validations being performed to extend limits, based on the specific evaluation of the quality of the tissue. In general, the quality of cardiovascular tissue deteriorates with increasing age, and maximum age limits are a simple way to apply donor-selection criteria. However, it must be considered that other lifestyle factors, such as diet, lifestyle and history of smoking also impact on the quality of cardiovascular tissue, though these are more difficult to assess quantitatively. Where clinical demand for particular types or sizes of cardiovascular graft is not being met, tissue establishments (TEs) may choose to set higher age limits to increase the donor pool, in the knowledge that a significant proportion of the grafts donated may not be suitable for clinical application. Commonly applied age limits are shown in Table 22.1; higher age limits may be considered (see note below table).

Table 22.1. **Commonly applied age limits for cardiovascular donors**

Arteries	male 17-55 years of age female 17-60 years of age
Veins	up to 70 years of age
Aortic valves	newborn (3 kg) to 70 years of age
Pulmonary valves	newborn (3 kg) to 80 years of age

Note: The advice given on age limits is based on experience with respect to the quality of tissues procured from donors within these age limits. Aortic valves, in that respect, could also be procured from donors older than 60 years of age, but in most cases these valves may not be suitable for banking. Procuring of the pulmonary valves from the same heart, on the other hand, would show a much higher yield, explaining the acceptance of these hearts for donation.

22.3. Procurement

Minimising the risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure cardiovascular graft safety. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and can implement the most efficient mitigation/risk-reducing measures, as needed.

22.3.1. Procurement team

A cardiovascular procurement team should consist of at least two qualified people. They should work using aseptic techniques, and be scrubbed, gowned in sterile clothing and wearing sterile gloves, face shields and protective masks. Procurement should be carried out in an environment that is as clean and controlled as possible, ideally in the operating theatre, or in a suitable environment supported by risk assessment (e.g. in a specifically designed area in the Morgue for recovery of cardiovascular tissues; see Chapter 7).

22.3.2. *Post mortem* procurement time

It is recommended to procure cardiovascular tissue within 24 h after circulatory arrest, but only if the body has been cooled or refrigerated within 6 h of death. If the body was not cooled or refrigerated, then the tissues should be procured within 12 h after death. It may be possible to extend procurement times if subsequent processing has been validated to guarantee quality [4, 5] and microbiological safety; in these cases the blood samples for serological testing should be taken within 24 h after death (to avoid extensive haemolysis). See Chapter 6 for details of sample collection.

22.3.3. Procurement procedure

For heart valve procurement it is important to procure the ascending aorta and the pulmonary trunk with bifurcation (wherever possible) together with the heart. All efforts should be made to procure as much length of pulmonary artery distal to the pulmonary bifurcation as is practicable.

For vessel donors, the maximum possible length of the recovered vessel should be maintained, avoiding iatrogenic lesions during manipulation, and collateral branches should be cut 2-3 mm from the arterial wall to allow the surgeon to ligate or suture them during the surgical procedure to avoid unnecessary bleeding in the patient.

The recovered heart and/or blood vessels (arteries/veins) should be rinsed with saline solution to remove the blood before packaging. This is necessary to decrease the risk of bacterial growth in the recovered material.

22.3.4. Tissue transportation to the tissue establishment

Common practice is to place procured tissues in a crystalloid transport solution (e.g. physiological saline, Ringer's solution, Hanks balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin) or an antibiotic cocktail, and package them in at least two sterile packaging layers after procurement. Buffering the transport solution may be considered in order to ensure a physiological pH during transport.

In order to avoid contamination transmission, the heart and the vascular tissues from each body compartment (thoracic aorta, abdominal vessels or femoral vessels) should all be packaged separately, unless the objective is to obtain combined segments that are as long as possible.

Each package should then be placed in another container that ensures a temperature of 2-8 °C during transport, and protects the recovered tissues from mechanical damage during transit.

22.3.5. Procurement documentation

The organisation responsible for procurement must produce a procurement report to be provided to the TE; usually this accompanies the recovered tissue. In addition to the generic requirements defined in Chapter 7, this report must contain a description and identification of the recovered material (heart, arteries, veins, valves etc.).

22.4. Processing

Procured hearts are dissected to prepare (aortic, pulmonary and sometimes mitral) valves from them. Together with procured vascular tissues, they can be decontaminated and cryopreserved to reduce microbial contamination and facilitate longer storage periods. To ensure tissue quality, it is essential that the time between circulatory arrest and cryopreservation be as short as possible. Time from procurement of the heart to dissection and decontamination should not exceed 24 h. The total ischaemia time (cardiac arrest to cryopreservation and storage) should not exceed 72 h.

22.4.1. Cardiovascular tissue-processing methods

Processing of cardiovascular tissues includes dissection and evaluation of morphology and minimum functional requirements, as well as measurements of the diameters and lengths, to provide the required information to the implanting surgeons. Processing also includes a decontamination step, by soaking in antibiotic solution. The duration and temperature of antibiotic treatment and the composition of antibiotic cocktails should be defined by each TE, with prior evaluation of the initial tissue contamination (i.e. before the tissue comes into contact with an antibiotic solution) and following a validation of the effectiveness of the cocktail against the most common microbes likely to contaminate the tissues. In order to assess the effectiveness of antibiotic incubation, a bioburden test should be performed and repeated at regular intervals (see Chapter 11). TEs should establish a clear policy stating how pre- and post-processing microbiology results will be used to determine whether the grafts are suitable for clinical use. Following decontamination, cardiovascular grafts are immersed in a cryopreservation medium with a cryoprotectant, cryopreserved and stored (see §22.5). Note that post-processing testing should be performed immediately before cryopreservation.

The methods used must be in accordance with (long-term) developed procedures that should be validated according to internationally accepted best practice (see Chapter 2). Different TEs apply specific preparation processes according to their own standard operating procedures (SOPs) and in accordance with relevant recommendations of the national Health Authority.

As cardiovascular tissue is not visible for inspection once it has been cryopreserved it is recommended that the anatomical appearance, quality and other attributes noted during processing are documented to assist with the allocation of a suitable allograft for patient need. Appendix 35 provides an example of an evaluation form.

The annular diameter of valves and vessels should be measured using calibrated obturators. The length of the vessels should be recorded, as should the approximate position and size of any branching vessels. It is recommended that grafts be measured immediately prior to preservation, as measurements may alter following procurement [6].

22.4.2. Processing facilities

In selecting an appropriate air-quality specification for processing cardiovascular grafts, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered.

It is vital that the processing of cardiovascular allografts takes place in a microbiologically and physically controlled environment with temperature control, ventilation and air filtration, and with validated cleaning and disinfection. Cardiovascular tissue should be processed in optimal environments with air quality equivalent to Grade A in EU Good Manufacturing Practice (GMP) Guidelines, with an adequate background environment. For EU countries, the background must be at least Grade D but, given the risks associated with the processing, testing and implantation of cardiovascular tissues, it is recommended that, as a minimum, a Grade C background environment (EU GMP) be provided.

22.5. Cryopreservation and storage

Cryopreservation aims to maintain the biological and structural properties of cardiovascular grafts and is still considered to be the best preservation method for long-term storage. Cardiovascular tissues can be cryopreserved by using a controlled-rate freezer, following a validated protocol. During the cryopreservation process, the parameters of the freezing cycle must be recorded, as well as any

inconsistencies that might have occurred during the operation.

After cryopreservation, the frozen tissues can be transferred to a temperature-monitored storage tank (either a liquid or vapour phase nitrogen tank at a temperature below −140 °C). Cardiovascular tissue can be stored at < −140 °C for a storage period supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. Currently the generally accepted storage period is 5 years. However, a new study has been carried out by the team of the Cardiovascular Tissue Bank of Prague, showing the preservation of the heart valves in perfect state beyond 5 years (up to 10 years) [7].

Note that cryopreservation may not be applicable for decellularised cardiovascular tissues.

22.6. Decellularisation of cardiovascular tissues

The state of the art of tissue manipulation is in constant growth and evolution, due to the diversity of its clinical applications. Decellularisation of tissues is a reality, as this process is currently being applied in clinical practice with different types of tissue. Furthermore, decellularisation processes are continually improving and adapting in order to develop new types of tissue graft and/or new clinical applications.

22.6.1. Opportunities

Decellularised cardiovascular tissues are used to restore, replace or regenerate damaged tissues. The extracellular matrix (ECM) scaffold resulting from decellularisation is composed of ECM molecules – secreted by the resident cells of each tissue – which provide biological properties and are organised in a three-dimensional (3D) arrangement that confers mechanical and structural properties. The ECM is an interconnected network composed of proteins, lipids and proteoglycans. These scaffolds have several advantages for the effective treatment and restoration of unhealthy, missing or damaged tissue. In addition, the absence of constituent donor cells can help to create a micro-environment more conducive to recipient re-cellularisation and re-vascularisation *in vivo*. In most tissues, except tissues with an immune-privileged niche, such as articular cartilage or the eye, the removal of cells provides significant improvement in graft compatibility and enhances transplantation outcomes through the reduction of immunogenicity [8].

Heart valves and large vessels can be decellularised by employing different methods to eliminate cellular components [9-15]. Decellularisation protocols can employ physical methods (freezing, sonication), chemical methods (hyperosmotic solutions, ionic detergents, non-ionic detergents) and enzymatic methods (trypsin, endonucleases). The most robust and effective decellularisation protocols could include a combination of the three methods, and details related to technical procedures can be found in Chapter 9 and Appendix 34 [16, 17].

22.6.2. Challenges

Decellularisation of cardiovascular tissues must maintain an equilibrium between eliminating cellular content and maintaining the mechanical and biological properties of the extracellular matrix (ECM). It is challenging to develop a decellularisation process that fully retains the essential properties of the ECM for its final clinical applications.

Process validation and quality control should guarantee maintenance of the structure and the biomechanical properties of native valves and vessels, as well as demonstrating *in vivo* function. Decellularised heart valves, for example, must therefore maintain sufficient mechanical strength to function within the cardiovascular system while permitting infiltration and remodelling by recipient cells.

It should also be shown that residual quantities of any reagents used during the decellularisation process that are still present in the tissue do not provoke cytotoxic responses either *in vitro* or *in vivo*. Decellularisation protocols (especially enzymatic methods) should take into account that degraded collagen might have repercussions for *in vivo* re-endothelialisation of decellularised tissues.

Furthermore, the nature of decellularisation protocols, which may involve the use of bacterial-sourced enzymes and/or prolonged incubation periods in the processing protocol, may increase the risk of pyrogen, such as endotoxin, contamination of the finished graft. Endotoxins and other pyrogens, if present in the ECM, may induce strong biological responses in the patient, fever being the main manifestation. This risk should be evaluated and if necessary appropriate pyrogen elimination steps added to the protocol [18-20], bearing in mind that affecting the mechanical or biological properties of the ECM should be avoided. Alternatively to, or in conjunction with, this testing of the finished graft for pyrogens should also be considered (see Chapter 11 for further details).

During the set-up of a decellularisation process there are many different parameters to take into

account and analyse, with the aim of assuring the maintenance of the biological and mechanical characteristics of the tissue. As the aim of decellularisation is to preserve the native properties of the tissue while eliminating the cell remnants that could elicit a host immune response, it is necessary to evaluate such parameters as the removal of cells, the elimination of genetic material, quantification of ECM protein content and the mechanical properties in order to assess the quality of the decellularisation protocol [21, 22].

Furthermore, it is necessary to ensure that toxicity resulting from the implantation of decellularised tissues is not a risk for the host. Although complete decellularisation (with the elimination of 100 % of cell material) may be impossible to achieve, it is pragmatic to evaluate the effectiveness of the decellularisation protocols with some minimal criteria, such as cell removal and the accepted residual DNA [21-25].

In summary, the quality control of tissues after decellularisation, including cardiovascular tissues, should consider the following:

a. effective removal of cells and cellular components;
b. effective removal of microbial contamination (see Chapter 11) and any potentially toxic microbial products (e.g. endotoxins);
c. effective removal of undesirable and potentially toxic reagents;
d. maintenance of desired ECM structural characteristics.

Note that, depending on the protocol used, decellularised cardiovascular tissues may be stored at refrigerated (2-8 °C) temperatures, frozen or cryopreserved.

22.7. Quality control

Quality-control tests for heart valves should consider the following minimum quality criteria:

a. Functional competence. It should be noted that fenestrations within the margins of the lunulae are very often not a pathological finding. Provided the coaptation of the graft is ensured by adequate sizing, marginal fenestrations should not induce valve regurgitation either in the short or long term. Large fenestrations, particularly when they are in opposing cusps, should constitute a rejection criterion. Additionally, low-positioned fenestrations in the leaflets with moderate to severe leak should constitute a rejection criterion.
b. Good morphology (no fissures, no congenital defects, no/minimal calcification, or no other significant anatomical abnormality). Only small calcifications in the distal wall of the aorta or around the coronary ostia, where they are most likely not to interfere with graft functioning, can be accepted, although information on their size and location must be clearly reported to the clinical user.
c. Anatomical suitability (i.e. accurate length of conduit and diameter of annulus). Special attention should be paid to achieving an accurate measurement of the diameter of the annulus to avoid overstretching; this is particularly critical for the pulmonary valve.
d. Intact structure of the tissue matrix.

It is recommended that the quality-control tests on vascular grafts should consider the following minimum quality criteria to assess suitability for banking:

a. integrity of the vascular walls;
b. minimal presence of calcification, atheroma and fibrosis;
c. anatomical suitability – absence of aneurysm or stenosis;
d. pathological analysis on remnants of the heart to check for malignancy, endocarditis and myocarditis, and at least a piece of aortic vascular wall should be analysed to check for connective tissue diseases; at the same time conclusions can be drawn on the level of atherosclerosis.

Cardiovascular allografts must be microbiologically sampled and cultured for aerobic and anaerobic bacteria, as well as fungi and yeasts, according to *European Pharmacopoeia* criteria (see Chapter 11), before and after antibiotic incubation, to be carried out on:

a. the transport medium at the beginning of the processing procedure;
b. the sub-valvular (aortic and pulmonary) myocardial tissue and vessels before antibiotic incubation;
c. a final sample of each graft after antibiotic incubation and rinsing,
d. a sample of the cryoprotectant solution.

Post-processing results must be negative, but some contaminants can be accepted if only found pre-processing. If a positive microbiology result is obtained, depending on the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other valve and the rest of the

tissues, obtained from the same donor. In Chapter 11 some micro-organisms are listed that, if detected in any cardiovascular tissue culture (even if detected just before decontamination) require the tissue to be designated as unsuitable for clinical use. Hence, for example, detection of *Enterococcus* spp. in a pre-antibiotic sample of aortic myocardium with a negative result in all other samples (e.g. transport medium, post-processing aortic sample, before final packaging) should result in rejection of the aortic valve from this donor, and a risk assessment should be done for the remainder of the tissues.

It should be noted that the list in Chapter 11 is non-exhaustive. Individual TEs may have a different list of micro-organisms that result in tissue discard.

22.8. Cardiovascular allograft distribution

Transportation of cardiovascular tissues can be carried out using dry-shipping containers (vapour phase nitrogen < −140 °C). This allows re-storage of the tissues in the liquid or vapour phase of nitrogen without affecting the expiry date. If the tissue needs to be stored from −60 to −80 °C, for example following issue to an end user, expiry date must be reduced to a time period supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.

If dry ice is used for transportation of the vascular allograft, the tissue should not be returned to liquid or vapour phase nitrogen tanks unless the process has been validated not to adversely impact the critical properties of the graft, or is supported by an appropriate risk-based rationale.

Transport temperatures above −60 °C for cryopreserved cardiovascular tissues are to be strictly avoided to ensure the stability of the product and maximum safety for the recipient. The receiving TE must ensure that all packaging and distribution processes have been carried out under controlled conditions [26, 27].

Please note that transport protocols for decellularised cardiovascular tissues should be appropriate to their storage temperature.

22.9. Cardiovascular tissue thawing

Thawing, removal of the cryoprotective medium (dilution) and re-establishment of the isotonic state of the cardiovascular allograft are of critical importance in order to guarantee the integrity of the cryopreserved tissue. The record that accompanies the cryopreserved tissue must contain the detailed protocol to be used for thawing, dilution and tissue reconstitution, together with a comprehensive list of the materials required. Where cryopreserved grafts are thawed directly from vapour or liquid nitrogen, for example if they have been transported in a dry-shipper, care must be taken to ensure that thawing is not too rapid in order to avoid thermal shock, which can result in micro cracks (fractures) in the grafts [28, 29]. Once cardiovascular tissues have been thawed, they cannot be re-frozen and should be implanted as soon as possible. A maximum period between thawing and transplantation should be defined, based on validation data or a documented rationale.

22.10. Biovigilance

The Notify Library includes well-documented cases of adverse occurrences in the transplantation of cardiovascular tissue. Examples include:

- Donor-to-recipient transmission of hepatitis C virus (HCV) by transplantation of a saphenous vein after confirmation of transmission to a tendon recipient from the same donor. Imputability was confirmed by detection of identical HCV genotype 1a and phylogenetic nucleic acid arrangement (Record Number 564).
- Transmission of hepatitis B virus by aortic valve allograft resulting in asymptomatic seroconversion in the recipient (Record Number 424).
- Serious adverse events such as an incorrectly sized heart-valve package opened by mistake (Record Number 122) and the heart valve determined to be unusable due to excess tissue attachments (Record Number 123), both resulting in delay in treatment and graft loss.

For further evaluated cases of adverse outcomes associated with banking of cardiovascular tissue, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

Typical serious adverse reactions or events that may occur with cardiovascular grafts and that should be reported include:

- post-implantation infection,
- acute arterial allograft rejection [30],
- any factors suggesting rapid degeneration/deterioration/failure of the graft, e.g. regurgitation with heart valves, or stenosis/claudication with vessels,
- rupture of the allograft after implantation, possibly as a result of inappropriate thawing.

22.11. Developing clinical applications

22.11.1. Veins

In addition to the long-established vascular allografts, such as pulmonary valves, aortic valves or femoral arteries, over recent years there have been several publications on the use of veins, particularly saphenous veins [31, 32].

The suggested clinical indications for saphenous veins include:

- peripheral vascular disease,
- coronary artery bypass grafting (CABG),
- patients with infected fields or at risk of infection,
- arteriovenous access,
- reconstruction of vascular tree in liver transplantation,
- oncologic surgery (infiltration in the vascular tree).

The benefits of saphenous veins are broadly similar to benefits of other vascular allografts:

- morphologically similar to the native tissue,
- resistance to infection,
- alternative to autologous veins.

The processing and storage protocols for saphenous veins and other venous allografts are the same as those employed for other vascular allografts in that they have venous branches ligated, they are antibiotic-treated and cryopreserved or stored cold at 2-8 °C.

Other clinical indications for veins are currently being explored, as are other processing techniques such as decellularised veins (see Appendix 34).

22.12. References

1. Jashari R, Van Hoeck B, Ngakam R *et al.* Banking of cryopreserved arterial allografts in Europe: 20 years of operation in the European Homograft Bank (EHB) in Brussels. *Cell Tissue Bank* 2013 Dec;14(4):589-99. https://doi.org/10.1007/s10561-012-9359-4.
2. Meyns B, Jashari R, Gewillig M *et al.* Factors influencing the survival of cryopreserved homografts. The second homograft performs as well as the first. *Eur J Cardiothorac Surg* 2005 Aug;28(2):211-16; discussion 216.
3. Dekens E, Van Damme E, Jashari R *et al.* Durability of pulmonary homografts for reconstruction of the right ventricular outflow tract: how relevant are donor-related factors? *Interact Cardiovasc Thorac Surg* 2019; 28(4):503-9.
4. Bester D, Botes L, van den Heever JJ *et al.* Cadaver donation: structural integrity of pulmonary homografts harvested 48 h *post mortem* in the juvenile ovine model. *Cell Tissue Bank* 2018;19(4):743-54.
5. Smit FE, Bester D, van den Heever JJ *et al.* Does prolonged post-mortem cold ischemic harvesting time influence cryopreserved pulmonary homograft tissue integrity? *Cell Tissue Bank* 2015;16(4):531-44. https://doi.org/10.1007/s10561-015-9500-2.
6. Dexter F, Donnelly RJ, Deverall PB, Watson DA. Post-mortem shrinkage of homograft aortic valves. *Thorax* 1972 May;27(3):312-14.
7. Fiala R, Kochová P, Kubíková T *et al.* Mechanical and structural properties of human aortic and pulmonary allografts do not deteriorate in the first 10 years of cryopreservation and storage in nitrogen. *Cell Tissue Bank* 2019;20(2):221-41.
8. Langer R, Vacanti JP. Tissue engineering. *Science* 1993 May 14;(80-)n.s.260(5110):920-6. https://doi.org/10.1126/science.8493529.
9. Grauss RW, Hazekamp MG, Oppenhuizen F *et al.* Histological evaluation of decellularised porcine aortic valves: Matrix changes due to different decellularisation methods. *Eur J Cardiothoracic Surg* 2005; 27(4):566-71.
10. Rieder E, Kasimir MT, Silberhumer G *et al.* Decellularization protocols of porcine heart valves differ importantly in efficiency of cell removal and susceptibility of the matrix to recellularization with human vascular cells. *J Thorac Cardiovasc Surg* 2004;127(2): 399-405.
11. Schenke-Layland K, Vasilevski O, Opitz F *et al.* Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. *J Struct Biol* 2003;143(3):201-8.
12. Bader A, Schilling T, Teebken OE *et al.* Tissue engineering of heart valves – Human endothelial cell seeding of detergent acellularized porcine valves. *Eur J Cardiothoracic Surg* 1998;14(3):279-84.
13. Uchimura E, Sawa Y, Taketani S *et al.* Novel method of preparing acellular cardiovascular grafts by decellularization with poly (ethylene glycol). *J Biomed Mater Res* 2003;67(3):834-7.
14. Conklin BS, Richter ER, Kreutziger KL *et al.* Development and evaluation of a novel decellularized vascular xenograft. *Med Eng Phys* 2002;24:173-83.
15. Schmidt CE, Baier JM. Acellular vascular tissues: Natural biomaterials for tissue repair and tissue engineering. *Biomaterials* 2000;21:2215-31.
16. Cebotari S, Tudorache I, Ciubotaru A *et al.* Use of fresh decellularized allografts for pulmonary valve replacement may reduce the reoperation rate in children and young adults: early report. *Circulation* 2011 Sep 13; 124(11Suppl);S115-S123.
17. TudoracheI, Horke A, Cebotari S *et al.* Decellularized

aortic homografts for aortic valve and aorta ascendens replacement. *Eur J Cardiothorac Surg* 2016 Jul; 50(1):89-97.
18. Singh R, Singh D, Singh A. Radiation sterilization of tissue allografts: a review. *World J Radiol* 2016;8:355.
19. Dai Z, Ronholm J, Tian Y *et al.* Sterilization techniques for biodegradable scaffolds in tissue engineering applications. *J Tissue Eng* 2016;7:204173141664881.
20. De Paula C, Truncale K, Gertzman A *et al.* Effects of hydrogen peroxide cleaning procedures on bone graft osteoinductivity and mechanical properties. *Cell Tissue Bank* 2005;6:287-98.
21. Crapo PM, Gilbert TW, Badylak DVM. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011;32:3233-43.
22. Hrebikova H, Diaz D, Mokry J. Chemical decellularization: a promising approach for preparation of extracellular matrix. *Biomed Pap* 2015;159:12-17.
23. Zheng MH, Chen J, Kirilak Y *et al.* Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: possible implications in human implantation. *J Biomed Mater Res – Part B Appl Biomater* 2005;73(1):61-7.
24. Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. *Cell* 2010;140:619-30.
25. Ahn SJ, Costa J, Emanuel JR. PicoGreen quantitation of DNA: Effective evaluation of samples pre- or post-PCR. *Nucleic Acids Res* 1996;24:2623-5.
26. Pitt TL, Tidey K, Roy A *et al.* Activity of four antimicrobial cocktails for tissue allograft decontamination against bacteria and *Candida* spp. of known susceptibility at different temperatures. *Cell Tissue Bank* 2014; 15(1):119-25.
27. Jashari R, Goffin Y, Van Hoeck B *et al.* Belgian and European experience with the European Homograft Bank (EHB) cryopreserved allograft valves – assessment of a 20 year activity. *Acta Chir Belg* 2010 May-Jun; 110(3):280-90.
28. Wassenaar C, Wijsmuller EG, Van Herwerden LA *et al.* Cracks in cryopreserved aortic allografts and rapid thawing. *Ann Thorac Surg* 1995 Aug;60(2 Suppl):S165-7.
29. Pegg DE, Wusteman MC, Boylan S. Fractures in cryopreserved elastic arteries. *Cryobiology* 1997 Mar;34(2): 183-92.
30. Soquet J, Chambon JP, Goffin Y, Jashari R. Acute rejection of a cryopreserved arterial homograft. *Cell Tissue Bank* 2015 Sep;16(3):331-3.
31. Hartranft CA, Noland S, Kulwicki A *et al.* Cryopreserved saphenous vein graft in infrainguinal bypass. *J Vasc Surg* 2014 Nov;60(5):1291-6.
32. Harlander-Locke MP, Lawrence PF, Ali A *et al.* Cryopreserved venous allograft is an acceptable conduit in patient with current or prior angioaccess graft infection. *J Vasc Surg* 2017 Oct;66(4):1157-62.

Related material

Appendix 34. Decellularisation
Appendix 35. Sample forms for the evaluation of heart valves

Chapter 23. **Musculoskeletal tissue**

23.1. Introduction

Human bone and soft-tissue grafts are used widely in surgery to rebuild and replace musculoskeletal structures either for structural or filling purposes. Bone is the most commonly banked and transplanted musculoskeletal tissue.

The main indications for bone grafting are:

a. to induce osteoinduction – the process by which osteogenesis is induced, by stimulating primitive, undifferentiated and pluripotent cells to develop into the bone-forming cell lineage.

b. to facilitate osteoconduction – by providing a scaffold that facilitates the growth of new bone on a surface or into pores or channels.

Fresh autologous bone is considered to be the 'gold standard' in bone-grafting material because it combines all the properties advantageous for a bone graft material. However, use of autografts is limited by the amount that can be procured and the risk of donor-site morbidity; so, in most cases, allografts are used.

Allografting of bone and musculoskeletal soft tissues allows in most cases adequate and predictable restoration of structure and functionality, including mechanical properties, analogous to the original healthy tissue at the recipient site. In addition, bone allografts offer the benefit of osteoconductive properties or even, depending on the processing applied, different degrees of osteoinduction through growth factors originally present and preserved in the graft [1, 2]. Compared to synthetic materials, allografts offer a better capacity for osseointegration: i.e. direct anchorage of an implant by the formation of bony tissue around the implant, without the growth of fibrous tissue at the bone–implant interface.

Musculoskeletal tissues can be procured from deceased donors after brain death or after circulatory death and from living donors (e.g. in the case of a patient undergoing hip or knee surgery).

Musculoskeletal tissues include bones, ligaments, tendons, cartilage and other soft tissues. The current indications for the transplantation of musculoskeletal tissues include, but are not limited to, tumour surgery, prosthesis replacement, filling where there is bone loss, fractures, malunion, bone fusion (spine and limbs), and ligament and cartilage replacement.

Musculoskeletal tissues can be preserved *ad integrum* or shaped in many different ways, and stored at different temperatures depending on clinicians' needs and logistical capacities. Bone grafts can be frozen or dehydrated to facilitate their storage. In some cases, bone grafts are demineralised to enhance the osteoinductive properties; the result is demineralised bone matrix (DBM). Large osteochondral grafts and whole-bone segments are also provided, matched to the requirements of the recipient site [3].

This chapter defines the specific requirements for musculoskeletal tissue donation, donor evaluation and processing and preservation procedures that meet adequate quality and safety standards, and also addresses biovigilance, patient follow-up and new developments.

The following generic chapters (Part A) of this

Guide all apply to musculoskeletal tissue banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the additional specific requirements for musculoskeletal tissue.

23.2. Donor evaluation

Consideration should be given to the fact that critical quality attributes of musculoskeletal grafts may be affected by the donor's age and sex, and medical and lifestyle history. Donor selection and screening criteria should be established accordingly.

23.2.1. Donor age

Donor age limits differ for different types of musculoskeletal tissue. These limits may be revised, based on performance of a validation study. Some countries have national guidelines or requirements but, in their absence, the following age limits, for male or female donors, are recommended although not mandatory:

a. for bone, the minimum age for both sexes is 6 years;
  i. for non-structural grafts, no upper limit is applied;
  ii. for structural support, younger donors (age 15-55 years) are preferred;
b. for osteoarticular grafts, cartilage and menisci, the age range is 15-45 years;
c. for tendons and fascia lata, the recommended age range is 15-65 years.

23.2.2. Musculoskeletal tissue: specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 5, screening of donors of musculoskeletal tissue should be conducted for:

a. diffuse connective-tissue disease which may reduce tissue quality;
b. metabolic bone diseases (severe osteoporosis, osteopetrosis, Paget disease etc.);
c. corticosteroid and bisphosphonate treatment (depending on doses and treatment duration);
d. evidence that the donor has ingested, or been otherwise exposed to toxic substances that could be transmitted in donated material in dosages that could endanger the health of recipients (e.g. cyanide or heavy metals such as mercury or gold);
e. significant local infection on potential donor site;
f. prior high radiation exposure at the location of the tissue to be donated;
g. evidence of trauma (e.g. open fracture) at the procurement site, or presence of joint deformities (evaluate the possibility of contraindication for osteochondral, structural bone and/or cartilage);
h. iatrogenic or degenerative tears or lesions detected during procurement of cartilage, menisci, tendons and osteoarticular grafts;
i. lifestyle factors; poor nutritional status, diseases of the digestive system and excess alcohol consumption can impact on the quality of musculoskeletal grafts.

23.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) is crucial to ensure the safety of musculoskeletal grafts. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution,

and can implement the most efficient mitigation/risk-reducing measures, as needed.

23.3.1. Procurement from deceased donors

The musculoskeletal tissues most frequently procured from deceased donors are:

- long bones (femur, tibia, fibula, humerus, radius, ulna, rib);
- irregular bones (iliac crest, hemipelvis, vertebrae, sternum, clavicle, scapula, mandible);
- soft tissues:
  - tendon: Achilles, anterior and posterior tibialis, peroneus longus, gracilis, semitendinosus, quadriceps; and patellar ligament,
  - cartilage: meniscus, acetabular labrum, costal cartilage;
  - fascia lata.

23.3.1.1. *Procurement conditions*

The procurement of musculoskeletal tissue should occur as soon as possible after death or circulatory arrest. The general conditions applying on time limits are those described in Chapter 7.

23.3.1.2. *Procurement team*

Staff must have the experience, education and training necessary to procure tissues, including the necessary anatomical knowledge to accurately obtain not only the regular tissues procured (femur, patellar ligaments etc.), but also specially requested materials (e.g. whole elbow).

It is recommended that the musculoskeletal procurement team for deceased donation should be composed of at least two (but preferably three) people. The number of people involved in procurement should be determined in advance, depending on the amount of donated tissues per procurement procedure. To minimise the risk of contamination during procurement it is recommended to limit the maximum number of team members [4]. It is important to define in the SOP the functions of the individual members of the team for the different procurement processes (e.g. donor preparation, draping, procurement, microbiological sampling, packaging, reconstruction) and also to define the role of the team leader or person responsible for procurement.

Procurement team members, after surgical hand disinfection, should be gowned in sterile clothing and wear sterile gloves, face shields, glasses and protective masks.

23.3.1.3. *Procurement procedures*

The steps for musculoskeletal procurement are:

a. Donor preparation: it includes washing, shaving and pre-operative disinfection of skin to reduce transient and resident microbial flora;
b. Donor draping: a local sterile field using disposable sterile drapes must be established before procurement to effectively reduce risk of microbial contamination;
c. Tissue procurement: all musculoskeletal (MSK) tissues must be procured using an aseptic technique to decrease the risk of contamination. The following best practices can be applied:
   i. Personnel located at one side of the donor should not change to the other side until they have finished procuring all tissues from their own side, and surgical instruments should not be shared between personnel;
   ii. The packaging area should be isolated and separate from the operating field;
   iii. A fixed procurement sequence should be established, from 'cleanest' (e.g. lower limbs) to 'dirtiest' areas (e.g. abdominal cavity);
   iv. Gloves and surgical blade should be changed after the recovery of each tissue, or at least after the recovery of tissues in a specific area of the body (e.g. left leg) to avoid microbiological cross-contamination between tissue fragments of different zones.
d. Microbiological control: it is recommended to perform a microbiological control on each procured tissue. Such controls can be performed immediately after procurement or before commencement of processing to identify any initial microbiological growth. Sampling methods should be realised in conformity with Chapter 11 and defined in SOPs in conjunction with the microbiological laboratory in charge of analysis.
e. Tissue packaging: procured tissue must be inspected and identified appropriately before packaging and labelling to avoid mix-ups (see Chapter 15). Musculoskeletal tissue must be packaged in a manner that minimises contamination risk, using a validated packaging system, to assure its isolation from the external environment;
f. Donor reconstruction (see §23.3.1.4);
g. Procurement documentation (see §23.3.4).

23.3.1.4. *Reconstruction of the deceased donor's body*

Once tissues have been procured from a deceased donor, the body must be reconstructed to maintain its original anatomical appearance.

For aesthetic reasons and with a view to a re-

spectful reconstruction of the donor, a replica prosthesis approximating the size of the donated bone may be used to replace the procured bone. The subcutaneous tissue and skin should be sutured. The sutures should ensure no leakage can occur from the incision, and be cosmetically unobtrusive as possible. The use of materials and sutures suitable for cremation should be considered.

23.3.2. Procurement from living donors

Musculoskeletal tissues can also be procured from living donors:

- Allograft
  - Femoral head from hip replacement or bone fragments from knee arthroplasty can be collected as surgical residues and donated if the patient provides an informed consent.

- Autograft
  - Cranial flaps can be removed during neurosurgical procedures in case of brain oedema to avoid cerebral damage due to compression. The tissue is stored and replaced in the same patient once brain swelling has diminished.
  - Cartilage can be used for producing autologous chondrocyte cultures. See §34.6.2.2.

The same requirements for procurement of tissue from deceased donors also apply to procurement of tissue from living donors. However, musculoskeletal tissue from living donors is likely to be procured by healthcare professionals whose primary role is not tissue donation. Therefore, it is important that these individuals be trained in the requirement to handle and package the grafts aseptically, and label the grafts and document the procedure appropriately.

23.3.3. Temporary storage and transportation to the tissue establishment

Once procured, the tissues are individually placed in a sterile and airtight packaging until they are transported to the tissue establishment. Storage conditions, temperature and duration should consider whether maintenance of cell viability is crucial or not: tissues must be refrigerated between 2 and 8 °C for preferably not longer than 12 hours immediately after procurement to preserve viability, otherwise validated defined conditions (temperature and duration) should be used to ensure viability.

Depending on the further steps of the process, tissues can be temporarily stored in a dried state or placed in a transport solution buffered at a physiological pH with the possible addition of antibiotic cocktail, culture medium or preservation fluid (e.g. highly concentrated saline solution). Each individual segment of tissues should be individually packaged in at least two sterilised packaging layers after procurement and placed in a container able to ensure storage at the required temperature. However, in cases where multiple segments of tissue are to be processed in the same batch, different tissues may be packaged together. In either case, individual pre-processing microbiological evaluation of each segment is mandatory.

Temporary storage areas or units for tissues and cells must be monitored (and alarmed, if necessary) and checked to ensure that expected environmental requirements are being met.

Temperature and duration during temporary storage and transport must be validated.

Transportation to the tissue establishment should be done in accordance with Chapter 12.

To prevent mix-ups, physically separate and distinguishable areas, storage devices or secured segregation must be allocated and prominently labelled (including at least the minimum required information – see Chapter 15).

23.3.4. Procurement documentation

The organisation responsible for procurement must gather all relevant information associated with the procurement procedures and produce a report to be given to the TE. The generic requirements are defined in Chapter 7 but, as a minimum, this report should contain the following information:

a. description and identification of the procured material (specifying all procured tissues);
b. any relevant morphological detail of procured tissues;
c. presence of anatomical damage or abnormalities, including those introduced during procurement;
d. Additional information may also be included, for example:
   i. non-procured standard tissues and the reason;
   ii. traceability information about: equipment, reagents and disposal of material.

23.4. Processing

23.4.1. Processing facilities

In selecting an appropriate air-quality specification for processing musculoskeletal tissue, in

accordance with their special characteristics, the criteria and tools should be considered that were identified in Chapter 3 (such as the MiRCA tool), Chapter 8 and Chapter 9. All stages of tissue processing should take place within a controlled environment. Although classified cleanrooms are often not formally required for initial processing steps when validated sterilisation and virus-inactivation processes are applied subsequently, it is nevertheless necessary to control media quality (especially air and water) and to work with appropriately disinfected or sterilised equipment.

For terminally sterilised grafts, at a minimum, the packaging step after cleaning and/or disinfection, but before sterilisation, should be conducted in a qualified cleanroom; see European Union Good Manufacturing Practices (GMP) classification. The official requirements vary between jurisdictions, but EU GMP Grade C is usually specified (WHO TRS 823, 1992).

For non-terminally sterilised grafts, the requirements for the processing environment depend upon whether the national authority mandates conformity with the EU GMP guidelines, or whether different guidelines – e.g. the GTP (good tissue practice) guidelines – are applicable.

Tissues that are exposed to the environment without a subsequent microbial inactivation process must be processed in environments with an air quality equivalent to Grade A as defined in EU GMP. Many national requirements are more stringent, requiring Grade B (EU GMP) as a background, which may be more appropriate for the processing of bone and tendons that are not followed by a terminal sterilisation step. Bone that is destined for terminal sterilisation can be processed in a Grade C environment.

23.4.2. Cleansing (physical preparation/ defatting)

The initial processing of bone and other MSK tissue generally involves mechanical steps that remove extraneous tissue. In the case of bone, residual muscle tissue and periosteum are resected, and cartilage may be debrided. Although not recommended, this step can be carried out at procurement stage, as described in Chapter 7.

After procurement, MSK tissue allografts may undergo no processing prior to packaging and storage for transplantation, or be subjected to various types of physical and/or chemical downstream processing.

Residual bone marrow, lipids and blood components in and/or on MSK tissue grafts can have a negative effect on subsequent processing and final graft quality. Such residues may increase the viable microbiological load, may have a negative effect on sterilisation processes and might be a cause of immunogenic reactions or delayed incorporation in graft recipients. They may also contain infectious agents derived from the donor. Such residual components should therefore be removed or depleted where possible. For this purpose, various treatments may be applied, such as chemical treatment able to dissolve or break up lipids and cells with organic solvents (e.g. acetone, chloroform, ethanol) or such as modulating osmolarity of aqueous solutions. Residue elimination is then generally achieved with physical treatments including centrifugation, ultrasound or high-pressure waterjet [5-10]. Elevating the temperature can enhance the efficacy of chemical and physical blood- and marrow-depletion processes. Treatment with supercritical carbon dioxide is an extraction method allowing dissolution and removing of the lipid in a single step due to its low viscosity and high solvent power [11].

All processes should be validated. The TE should determine what properties of the allograft are essential for safe and effective clinical application, select appropriate tests to evaluate these properties and design a validation plan accordingly. The TE may choose to validate individual processes for individual graft types or may choose a worst-case validation plan to cover all graft types. The latter should be based on the results of a risk assessment. The types of graft that can be obtained include (but are not limited to):

- whole femoral heads;
- cancellous and corticocancellous (chips or cubes, blocks, rings and wedges) e.g. obtained from epiphyses of the long bones, vertebral bodies, or ilium, calcaneus or talus;
- cortical grafts (chips, struts, cylinders) e.g. obtained from diaphysis of long bone;
- whole bones (with or without tendon insertions);
- structural bone segments (whole or halved diaphyses, rings, struts or condyles);
- tendons (with or without bone blocks);
- menisci, either whole (with or without bone blocks) or sections;
- fascia lata patches;
- costal cartilage segments.

Appropriately processed bone grafts will provide 'osteoconduction' (i.e. they act as a scaffold and 'guide rail' for osteoclasts and osteoblasts) and therefore promote the incorporation of the graft and its remodelling [11]. Donor bone may contain residues of functional bone growth factors. These

so-called bone morphogenic proteins (BMP) are found in the organic part of the extracellular matrix and are embedded in the bone mineral. They can be exposed by bone demineralisation. This usually involves soaking bone in a dilute hydrochloric acid solution (e.g. 0.5 or 0.6 M HCl) to significantly reduce the mineral content. The type of graft that can be obtained from this process is referred to as demineralised bone matrix (DBM). Depending on other aspects of bone processing and the initial BMP content, the exposure of BMP may promote 'osteoinduction' (i.e. when bone healing is initiated and maintained via stem-cell recruitment) in environments lacking an adequate local population of osteoclasts and osteoblasts [12]. Due to its nature, DBM is often mixed with a carrier material to improve its handling properties and help retain the graft at the site of the transplantation/grafting/application. The safety of these carrier materials, and their effects on the essential properties of the graft, must be established.

Pooling of musculoskeletal tissue from two or more donors during processing is not recommended. The only exception is where it is supported by a comprehensive risk–benefit assessment and it has been demonstrated to be the only way of providing sufficient clinically effective tissues or cells. If performed, traceability must be fully ensured (see Chapter 9).

23.4.3. Removal of micro-organisms and virus inactivation

Sterilisation and disinfection processes for musculoskeletal grafts are applied to minimise the risk of transmission of microbiological agents. They may be applied before, during or after any processing procedure and must be validated. For a detailed discussion of the principles of sterilisation and disinfection, please see Chapter 9.

Sterilisation and disinfection protocols are, by their nature, aggressive processes that have the potential to inactivate micro-organisms as well as cause damage to the graft. It is vital therefore that, if they are to be applied, a protocol is selected that does not unacceptably impact the critical quality attributes of the graft. For example, sterilisation with gamma irradiation may damage the biomechanical properties of grafts, so should be employed with caution when applied to grafts which are expected to play a mechanical role, such as tendons or structural bone grafts. For osteochondral allografts, maintenance of donor-cell viability is crucial, so options for disinfection are limited to gentle surface decontamination.

It should also be considered that many of the routine processing techniques applied to musculoskeletal allografts, such as the use of elevated-temperature water washing, physical removal of adherent tissues and bone marrow, the use of washes with solvents, acids or oxidising agents and supercritical CO_2 alone or with co-solvent, can also reduce microbial load [8-10, 13-14].

Any claim of inactivation or elimination of micro-organisms (virus, bacteria, spores, fungi) should be validated using organisms typically found in the tissue, model organisms representative of their type or ideally a "worst case" organism known to be highly resistant to the method in question.

23.4.4. Terminal sterilisation

In order to ensure a sterile state for tissues in their final packaging, it is common to apply a terminal sterilisation step. The choice of the method should be made with regard to several parameters, notably: efficacy to achieve the desired sterility assurance level, ability to penetrate inside the tissue, potential damage of the tissue matrix or possibility of parametric release. The final choice should be based on a documented risk-assessment analysis. Product stability, safety and functionality should be validated to be retained throughout the graft's designated shelf life. In all cases, the process of terminal sterilisation should be validated and periodically monitored.

Achievement of sterility depends on various parameters including method efficacy but also initial microbial bioburden. Determination of the bioburden can be done on every batch or periodically in compliance with international standards requirements. Proof of sterility can be obtained by performing microbiological tests on tissue samples or by parametric release based on confirmed application of a minimum level of sterilisation (for example, achieving a certain core temperature for a defined period, or a defined dose of irradiation).

23.5. Quality control

Quality-control tests on musculoskeletal grafts should take at least the following quality criteria into account:

a. morphology and integrity of the musculoskeletal grafts;
b. shape and size of the graft, especially for certain types of graft, for example meniscal cartilage, which require close size matching between the donor and recipient; the relevant measurements should be made using calibrated instruments when all physical processing has been completed;

c. residual moisture or available water in lyophilised or dehydrated grafts (the minimum and the maximum level to be defined according to validation studies);
d. osteo-inductive activity (*in vivo* or *in vitro*) in demineralised bone;
e. sterilisation indicators;
f. no evidence of microbiological growth.

During procurement or before processing, microbiological samples should be collected to establish the initial contamination to assist in making a decision during quarantine regarding the release of procured material for further processing. The inactivation capacity of manufacturing processes (e.g. disinfection, sterilisation) should be taken into account.

Samples for microbiological testing should also be collected before or during packaging of the final product. Possible sampling techniques for microbiological testing include:

- swabs;
- destructive methods (e.g. biopsy or sacrificing a proportion of ground tissue);
- collection of the last portion of the fluid used for washing of the tissue graft for subsequent analysis, usually following filtration.

The result of the microbiological control before final release must be negative. If a positive microbiology result is obtained, the tissue should be discarded or sterilised with a validated method taking into account the nature and level of the microbial bioburden. Depending on the micro-organism found, a risk-assessment analysis should be done to assess the suitability of the other musculoskeletal tissues from the same donor.

Chapter 11 contains a list of micro-organisms that if identified in pre-processing tissue or cell samples should result in discard of tissues and cells (Table 11.1). A risk assessment including the potency of any sterilisation processes employed, and the clinical relevance of the micro-organism, should be done to analyse the suitability of the rest of the musculoskeletal tissue from same donor [15].

See Chapter 11 for more detailed guidance on the principles of microbiological testing.

23.6. Labelling and packaging

Generic requirements are detailed in Chapter 15. Processed musculoskeletal tissues must be packaged in a way that avoids contamination risk from external sources. It is recommended that musculoskeletal tissues be at least double-packed separately in pouches or in sterile sealed containers to avoid cross contamination and labelled immediately.

The material used for the packaging must be validated for use at the designated storage temperature, and shown to maintain its integrity during worst-case storage and transport conditions. Consideration should also be given to the risk that components of the packaging material, for example plasticisers, may contaminate the tissue. The absence of adverse effects of packaging material on tissue must be supported by a documented risk assessment or validation data.

The shelf life of the tissue when kept at the designated storage temperature, which is dependent upon the packaging system (to guarantee the integrity and sterility of the graft) and the storage methods used, must be specified on the final label, and supported by validation data or a documented risk assessment.

23.7. Preservation/storage

After processing, grafts are stored at a TE in quarantine and after release in final storage following instructions detailed in Chapter 10.

Different preservation methods have been developed to maintain the biological properties of tissues for long periods of time, from processing to distribution for transplant.

23.7.1. Methods of preservation/storage

Usual temperature limits applied to each method are reported in Table 23.1.

23.7.1.1. *Frozen and deep-frozen*

Preservation and storage of MSK tissues (including cancellous, corticocancellous and cortical bone, ligaments and tendons) by deep freezing without use of cryoprotectants is a common method. There is limited scientific evidence to justify particular temperature limits but, in general, it is accepted that freezing an allograft has little impact on the mechanical properties of the tissue, and for unprocessed allografts can diminish their immunogenicity.

23.7.1.2. *Cryopreservation*

Cryopreservation is a process whereby tissues are preserved by cooling to temperatures of < −140 °C at a controlled slow rate, and is generally used where maintenance of donor cell viability is required. It involves the use of cryoprotectants – e.g. glycerol, dimethyl sulphoxide (DMSO) – which are added to the tissue to protect cells against freezing injury during the controlled-rate freezing process. The role of cryopreservation for the storage of MSK tissues is unclear;

historically it has been used for storage of osteochondral grafts, where donor cell viability is essential for clinical performance. However, it is now known that this is not an appropriate storage method for this type of graft. Nevertheless, preservation of tissue at very low temperatures is an excellent method for assuring the long-term maintenance of their structural properties.

23.7.1.3. *Dehydration and freeze-drying*

Removal of water content is an excellent method to ensure stability of tissue by preventing degradative chemical reactions that require the presence of liquid water. As the processes required to remove water also kill donor cells, they also reduce the graft immunogenicity and permit graft storage and transport at ambient temperature, which may have operational advantages [16].

Elimination of water can be performed using different methods. Lyophilisation (freeze-drying) involves decreasing the water content of frozen tissue under vacuum through sublimation. Alternatively, solvent dehydration can be applied, usually using chemical substances such as acetone or ethanol, or more basically the tissue can be allowed to dehydrate in a stream of air, perhaps in the presence of hygroscopic chemicals. For bone transplants, a residual moisture between 1 and 6 % of the weight of the tissue is recommended.

23.7.1.4. *Fresh*

Storage of unprocessed tissues at hypothermic or near normothermic (≈ 33 °C) temperatures allows maintenance of cell viability (i.e. osteochondral grafts) for a short period (1-3 months) [17]. The selected storage temperature, length of time and storage solution must be validated to maintain the required level of donor cell viability. Consideration should be given to ensuring that the storage time is sufficient to ensure that essential microbiology tests can be completed before the shelf life expires. If grafts have to be released with preliminary results, systems should be put in place to review and take action when full results are available.

23.7.2. Storage temperatures

As mentioned in section 23.7.1, different preservation methods require different storage temperatures (see Chapter 10), as shown in Table 23.1.

Table 23.1. **Storage temperatures for different preservation methods**

Type of graft	°C minimum	°C maximum
Frozen	–40	–15
Deep frozen	–80	–60
Cryopreserved	–196	–140
Dehydrated and freeze-dried*	+ 4	+ 30
Fresh (hypothermic)	+ 2	+ 8
Organ culture	28	37

* At room temperature (15-25 °C) in normal conditions of humidity.

23.8. Distribution and transport conditions

Transportation of musculoskeletal tissues should guarantee the preservation of graft-storage conditions from TE to end user.

Transportation systems will vary, depending on the preservation method used:

a. Frozen and deep-frozen grafts can be carried using a container with dry ice or qualified cooling system. Once the graft has been thawed, it cannot be re-frozen;

b. Cryopreserved grafts can be carried using dry-shipping containers (vapour-phase nitrogen < –140 °C). If dry ice is used for transportation of the musculoskeletal allograft, the tissue should not be returned to liquid or vapour-phase nitrogen tanks unless validated or supported by a documented scientific rationale. Once the graft has been thawed, it cannot be re-frozen;

c. Dehydrated and freeze-dried grafts can be carried using a container sufficient to protect the integrity of the packaging system;

d. Fresh grafts can be carried using a container that ensures the defined storage temperature;

e. Storage containers with tamper-evident seals must be used (see Chapter 12).

f. Instructions for thawing frozen, deep frozen or cryopreserved grafts, or for rehydrating dehydrated grafts, are established and validated by the TE before use in the ORHA.

23.9. Biovigilance

The Notify Library includes many well-documented cases of adverse occurrences in the field of musculoskeletal tissue transplantation, such as:

- Bone

– A case of human T-cell lymphotrophic virus type-1 transmission by a deep-frozen bone allograft, resulting in asymptomatic seroconversion of the recipient, is described in Record Number 587.
– A case of human immunodeficiency virus (HIV) transmission, through frozen femoral head used in scoliosis surgery, is documented in Record Number 19. Both donor and recipient developed acquired immunodeficiency syndrome (AIDS) 40 months after transplant; in the same line, after the transplantation of lyophilised bone chip, HIV was transmitted to the recipient, Record Number 1431.
– Four cases of tuberculosis (*Mycobacterium tuberculosis*) transmitted by use of frozen rib allografts in scoliosis surgery. Ribs were obtained from patients with active tuberculosis during thoracoplasty and frozen in penicillin and streptomycin (Record Number 21).
– Several cases of serious adverse events resulting from unsuitable bone allograft release are reported in the database. Record numbers 139, 140, 141 and 142 describe bone allografts with chondrosarcoma, lymphocytic lymphoma, Paget's disease and rheumatoid arthritis respectively; all were diagnosed during histological examination of the femoral head and resulted in discarding of allografts.
– Transmission of a bacterial infection (*E. coli*) after bone filling during orthopaedic surgeries to the recipient which required antibiotics (Record Number 1102). Also transmission of *Enterobacter cloacae* Record Number 966.

• Tendon or ligament
– In Record Number 459, a donor-transmitted invasive group-A streptococcal infection, with the diagnosis confirmed by emm gene-sequence analysis of isolates from the blood and hemi-patellar tendon tissue of the donor and recipient; similar case was reported with the transmission of *Streptococcus pyogenes* group A, Record Number 641 and *Serratia marcescens* in 3 patients receiving bone from the same donor (Record Number 630).
– A case of donor-to-recipient hepatitis C virus (HCV) transmission by patellar tendon transplantation is described in Record Number 563 and confirmed by identical HCV genotype 1a and phylogenetic nucleic acid arrangement between donor and recipient.
– An HIV type-1 transmission from a seronegative organ-and-tissue donor confirmed by the recipient's seroconversion 3 weeks post-transplant (Record Number 581).

• Meniscus
– Records nos. 173 and 174 describe meniscus allografts with anatomic abnormality and fracture, respectively; both were discovered in the hospital and resulted in discarding of allografts, thereby delaying treatment. Record 608 shows the transmission of bacterial infection to two recipients from the same donor. One of the recipients died of *C. sordellii* sepsis after knee surgery. The recipient of meniscus was also infected but recovered with antibiotics.

For further evaluated cases of adverse outcomes associated with musculoskeletal tissue banking, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by record number.

23.10. Patients follow-up

Patients undergoing musculoskeletal transplantation need systematic and regular clinical follow-up in order to comply with international standards for patient care. Follow-up may take place at the hospital exclusively or as shared care between the transplant centre (or hospital) and primary care. Regular routine follow-up is needed to recognise complications, such as infection, suture-loosening or rejection episodes among others. If not noted and untreated, these events may result in failure of graft incorporation or loss of mobility.

The frequency of follow-up visits is specific to the type of musculoskeletal transplant procedure, the indication for surgery and presence of possible risk-factors in the recipient. Low-risk procedures include filling bone in dental procedures while high-risk procedures may include transplantation of structural bone, or tendons. High-risk procedures need closer follow-up.

Systematic longer-term follow-up at 1 and 2 years after high-risk musculoskeletal transplantation is typically performed in many transplant centres, either as part of an internal quality programme and/or as part of participation in national or international recommended practices. After 2 years, lifelong annual or biannual clinical follow-up visits are often recommended.

Systematic follow-up includes registration of some basic clinical and quality criteria. Most physicians record the main clinical indications to surgery;

patient-reported outcomes measure the time for complete osteointegration, range of motion recovered, etc.

One of differences from other tissues is that musculoskeletal tissues can be used in different presentations (lyophilised, frozen demineralised, etc.) in many different situations, so it is not easy to establish comparison between different procedures and recipient diagnosis.

Usually, musculoskeletal is not the unique treatment applied; in general, is used in conjunction with other procedures, such us new prosthesis in revision surgery when bone filling is necessary; there are many registries but many of them are more focused on the prosthesis aspects than on the use of grafts. Many countries have national registries for arthroplasty as the Network of Orthopaedic Registries of Europe, but the use of bone is not analysed [18, 19, 20, 21].

The same occurs with ligament substitution; there are national registries related to the primary diagnosis, and some of them take into consideration the graft used, but not as the primary item as in the case of cornea transplant registries [22, 23].

In another situation, bone is used as a structural element, as in case of tumour when the mechanical strength is a crucial actor together with graft incorporation as a complementary treatment procedure [24]. Some countries, such as Denmark, have national registries but not all of them collect which kind of graft was used [25].

Actually, there are no specific national or international registries on musculoskeletal transplantation; the current registries are focused either on the primary diagnosis or if a prosthesis was used. This situation makes it difficult to record the level of risk of an SAR and the level of success in tissue integration of the different methods of preservation.

Systematic patient follow-up and participation in registries would be an important benefit for musculoskeletal transplant centres, not only by providing the best treatment of individual patients at the level of international standards, but also by comparing our own results to international standards.

Quality management systems are aimed at preventing errors and maintaining a consistent standard of agreed specification for tissues and cells released for clinical application; however, adverse events or reactions can occur at any moment from donor selection to clinical application. Serious adverse events and reactions must be recorded, reported and investigated according to relevant national regulations to Health Authorities for tissues and cells, as described in Chapter 17.

23.11. Developing and niche applications

Developments in musculoskeletal tissue banking focus mainly on the following areas:

- developing new preservation methods that maintain the mechanical and biological properties of the grafts;
- developing new procedures such as decellularisation or specific cell seeding to improve graft incorporation in recipients. In recent years, several innovative biological products based on decellularisation of MSK tissue (especially tendons) have been developed using biotechnological sciences, based on the experience of skin-derived and cardiovascular grafts (heart valves and vessels). More information about decellularisation processes can be found in Chapter 9 and Appendix 34.
- improving the safety of grafts through the development of better decontamination processes [26].

Bone grafts (predominantly cancellous and corticocancellous chips, cubes, etc.) can be used as carriers for antibiotics. The idea is that impregnated bone grafts release antibiotics at high concentrations and for a prolonged time at the site of implantation. This finds an application in the prevention or treatment of post-operative infections especially in hip revision surgery, which is among the most common procedures where bone transplants are being used [27, 28].

23.12. References

1. Lavernia CJ, Malinin TI, Temple HT, Moreyra CE. Bone and tissue allograft use by orthopaedic surgeons. *J Arthroplasty* 2004;19(4):430-5.
2. Galea G, ed. *Essentials of tissue banking.* 1st edition. New York: Springer; 2010.
3. Blokhuis TJ, Lindner T. Allograft and bone morphogenetic proteins: an overview. *Injury* 2008;39(Suppl 2):S33-S36.
4. Segur JM, Suso S, Garcia S *et al.* The procurement team as a factor of bone allograft contamination. *Cell Tissue Bank* 2000;1(2):117-19.
5. Fages J, Marty A, Delga C *et al.* Use of supercritical CO2 for bone delipidation. *Biomaterials* 1994 Jul;15(9):650-6.
6. Lomas R, Drummond O, Kearney JN. Processing of whole femoral head allografts: a method for

improving clinical efficacy and safety. *Cell Tissue Bank* 2000;1(3):193-200.
7. Yates P, Thomson J, Galea G. Processing of whole femoral head allografts: validation methodology for the reliable removal of nucleated cells, lipid and soluble proteins using a multi-step washing procedure. *Cell Tissue Bank* 2005;6(4):277-85.
8. Eagle MJ, Man J, Rooney P *et al.* Assessment of an improved bone washing protocol for deceased donor human bone. *Cell Tissue Bank* 2015;16(1):83-90.
9. Eagle MJ, Man J, Rooney P *et al.* Assessment of a closed wash system developed for processing living donor femoral heads. *Cell Tissue Bank* 2017;18(4):547-54.
10. Eagle MJ, Rooney P, Kearney JN. Development of an improved bone washing and demineralisation process to produce large demineralised human cancellous bone sponges. *Cell Tissue Bank* 2015 Dec;16(4):569-78.
11. Putzier M, Strube P, Funk JF *et al.* Allogenic versus autologous cancellous bone in lumbar segmental spondylodesis: a randomized prospective study. *Eur Spine J* 2009 May;18(5):687-95.
12. Reddi AH. Bone morphogenetic proteins, bone marrow stromal cells, and mesenchymal stemcells. *Clin Orthop Relat Res* 1995 Apr;313:115-19.
13. Fages J, Poirier B, Barbier Y *et al.* Viral inactivation of human bone tissue using supercritical fluid extraction. *ASAIO J* Jul-Aug 1998;44(4):289-93. https://doi.org/10.1097/00002480-199807000-00009.
14. White A, Burns D, Christensen TW. Effective terminal sterilization using supercritical carbon dioxide. *J Biotechnol* 2006 Jun 10;123(4):504-15. Epub 2006 Feb 21. https://doi.org/10.1016/j.jbiotec.2005.12.033.
15. Domanovic D, Cassini A, Bekeredjian-Ding I *et al.* Prioritizing of bacterial infections transmitted through substances of human origin in Europe. *Transfusion* 2017 May;57(5):1311-17.
16. Mahirogullari M, Ferguson M, Whitlock PW *et al.* Freeze-dried allografts for anterior cruciate ligament reconstruction. *Clin Sport Med* 2007;26(1):625-37.
17. Goodfriend B, Essilfie AA, Jones IA *et al.* Fresh osteochondral grafting in the United States: the current status of tissue banking processing. *Cell Tissue Bank* 2019 Sep;20(3):331-7.
18. Network of Orthopaedic Registries of Europe, available at www.efort.org/about-us/nore/research/, accessed 14 April 2022.
19. Lübbeke A, Silman AJ, Barea C *et al.* Mapping existing hip and knee replacement registries in Europe. *Health Policy.* 2018 May;122(5):548-57.
20. Lübbeke A, Silman AJ, Prieto-Alhambra D *et al.* The role of national registries in improving patient safety for hip and knee replacements. *BMC Musculoskelet Disord.* 2017 Oct 16;18(1):414.
21. Lacny S, Bohm E, Hawker G *et al.* Assessing the comparability of hip arthroplasty registries in order to improve the recording and monitoring of outcome. *Bone Joint J.* 2016 Apr;98-B (4):442-51.
22. Engebretsen L, Forssblad M, Lind M. Why registries analysing cruciate ligament surgery are important. *Br J Sports Med* 2015 May;49(10):636-8. https://doi.org/10.1136/bjsports-2014-094484.
23. Yang XG, Wang F, He X *et al.* Network meta-analysis of knee outcomes following anterior cruciate ligament reconstruction with various types of tendon grafts. *Int Orthop* 2020 Feb;44(2):365-80.
24. Rose P. What's new in musculoskeletal tumor surgery. *J Bone Joint Surg Am.* 2020 Dec 16;102(24):2111-19.
25. Rahr-Wagner L, Lind M. The Danish Knee Ligament Reconstruction Registry. *Clin Epidemiol* 2016 Oct 25:8:531-5.
26. Wang S, Wang Y, Song L *et al.* Decellularized tendon as a protective scaffold for tendon repair. *Mater Sci Eng C Mater Biol Appl*; 2017 Aug 1;77:1290-1301. https://doi.org/10.1016/j.msec.2017.03.279.
27. Peeters A, Putzeys G, Thorrez L. Current insights in the application of bone grafts for local antibiotic delivery in bone reconstruction surgery. *J Bone Joint Infect.* 2019 Oct 15;4(5):245-53; https://doi.org/10.7150/jbji.38373.
28. Erivan R, Lopez-Chicon P, Fariñas O *et al.* Which type of bone releases the most vancomycin? Comparison of spongious bone, cortical powder and cortico-spongious bone. *Cell Tissue Bank* 2020 Mar;21(1):131-7. EPub 2019 Dec 21. https://doi.org/10.1007/s10561-019-09806-2.

Related material
Appendix 34. Decellularisation

Chapter 24. Haematopoietic progenitor cells from bone marrow and peripheral blood

24.1. Introduction

Haematopoietic progenitor cells (HPC) transplantation represents one of the most widely used forms of cell therapy, in part because haematopoiesis represents the best-known biological model of somatic stem cell and tissue differentiation. Following the first case reports more than sixty years ago, the procedure rapidly established itself as a life-saving treatment for adult and paediatric patients with a variety of malignant and non-malignant diseases. The latter is the case when the haematopoietic tissue is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies, haemoglobinopathies, metabolic diseases or bone marrow failure. More recently, the use of autologous HPC transplantation in combination with immuno-suppressive agents has been evaluated for patients with certain autoimmune diseases.

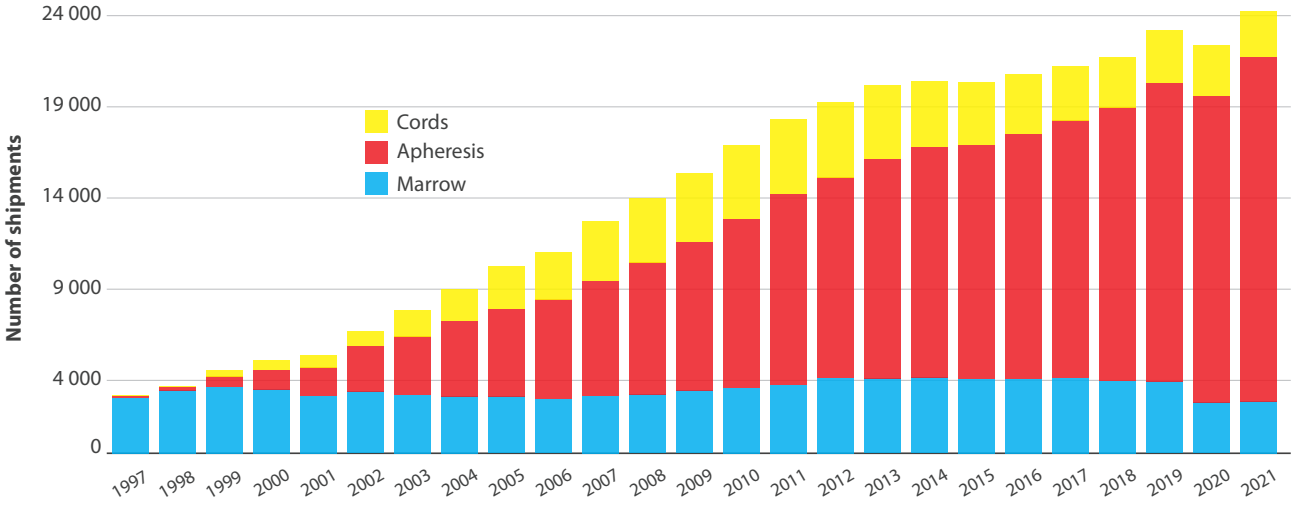
In its main field of application, i.e. as a component of the treatment of patients with poor-risk or advanced haematological malignancies, it is now well established that allogeneic HPC transplantation exerts its beneficial effects through the recognition of residual tumour cells in the recipient by donor-derived immune effectors (graft-*versus*-tumour effect, or GvT). Thus, allogeneic HPC transplantation represents a clinically useful, immune cellular therapy. Limits to the use of these therapeutic procedures are their intrinsic toxicity, dominated by (though not limited to) graft-*versus*-host disease (GvHD), an immune disorder in which donor-derived immune effectors recognise and harm the host's normal tissues such as skin, gut and liver, lungs and eyes. In addition, the availability of donor cells might be a limitation.

The field has tremendously developed in the past half-century, and integrated medicinal and technical innovations, including the use of new immunosuppressive agents, the use of different sources of HPC, such as bone marrow, mobilised stem cells from peripheral blood and cord blood, the procurement of cells from unrelated donors and much improved supportive care for patients. During recent years, the use of volunteers as unrelated donors has dramatically increased due to the extensive improvements in HLA-typing and matching algorithms and the growth of donor registries throughout the world, which are associated in the World Marrow Donor Association (WMDA) (Figure 24.1).

Several other biotechnological advances, including stem cell selection, lymphocyte depletion and immune effector cells activation, have entered clinical practice and made special types of transplants such as haplo-identical transplantation a valid clinical choice with rapidly increasing numbers (Figure 24.2).

However, despite these advances, procurement of HPC remains relatively unchanged. Hospitals that care for recipients often obtain autologous or family donor allogeneic HPC from procurement and processing facilities at hospitals or blood establishments in their immediate vicinity. Each of the procurement

Figure 24.1. **Unrelated HPC marrow, HPC apheresis and HPC cords shipped, 1997-2019 (WMDA Annual report 2019)**



and processing facilities works on a typically small to medium scale. In more than 50 % of allogeneic HPC transplantations, grafts from unrelated donors are used, which very often have to be imported from other countries or continents. Given the high rate of international exchange of donated HPC material, harmonisation of the practices in this field is of great benefit.

This chapter defines the additional specific requirements for procurement, processing, storage and transplantation of HPC derived from bone marrow – HPC, Marrow, known as HPC(M) – or from peripheral blood – HPC, Apheresis, known as HPC(A) – and the requirements for mononuclear cells (MNC) concentrates procured by apheresis – MNC, Apheresis, known as MNC(A) – either for immediate use or for further development of immunocompetent cells used after or instead of HPC transplantation.

The cells discussed in this chapter are regulated in the European Union (EU) under the Tissues and Cells Directive 2004/23/EC and its associated Commission directives as well as in country-specific directives and regulations. It should be noted, however, that if these cells are subjected to substantial manipulation (such as expansion or genetic modification), or are used in the recipient for an essential function that is different from the original function in the donor, in the EU they are then regulated as medicinal products. This means that their processing, storage, distribution and use in patients must respect the requirements of Regulation 1394/2007 on advanced therapy medicinal products ('the ATMP Regulation') as well as all other relevant provisions of the EU and country-specific medicines rules.

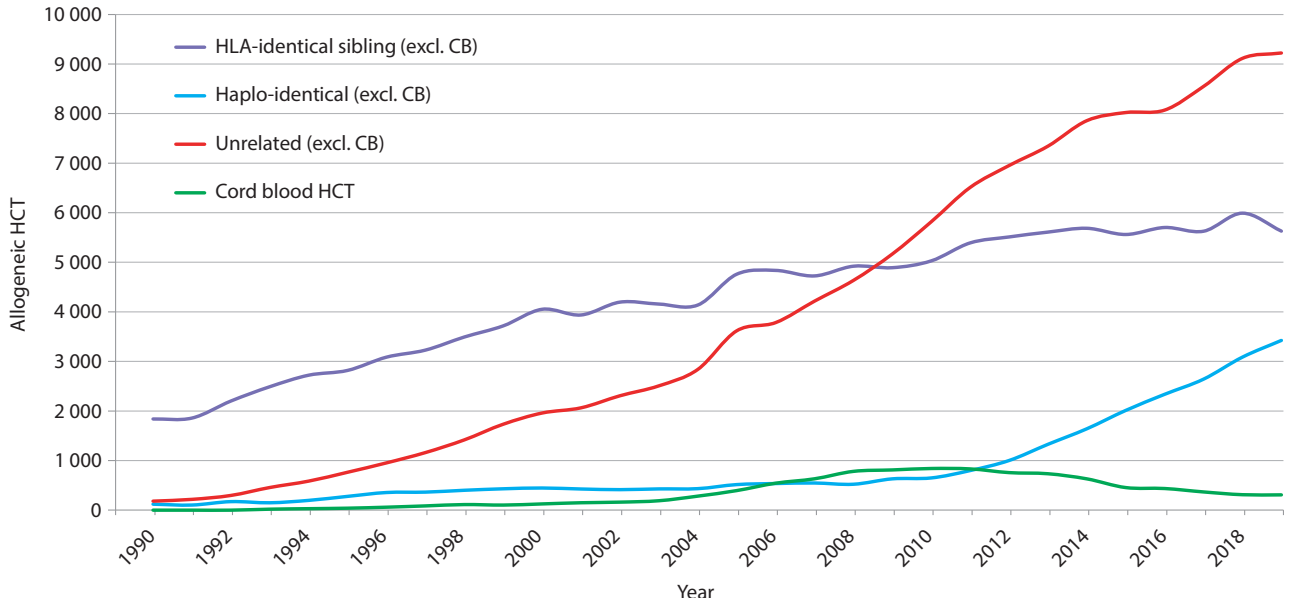
The following generic chapters (Part A) of this Guide all apply to HPC transplantation and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

24.2. Recruitment of potential donors, identification and consent

Most of the patients who could benefit from HPC transplantation do not have an HLA genotypically identical sibling donor. The chance of having a fully HLA-identical sibling donor is 25-30 %, depending on the number of siblings. In

Figure 24.2. **HPC transplantation reported to the European Bone Marrow Transplantation (EBMT) registry by donor type, 1990-2018 – patient numbers per year with first transplant**

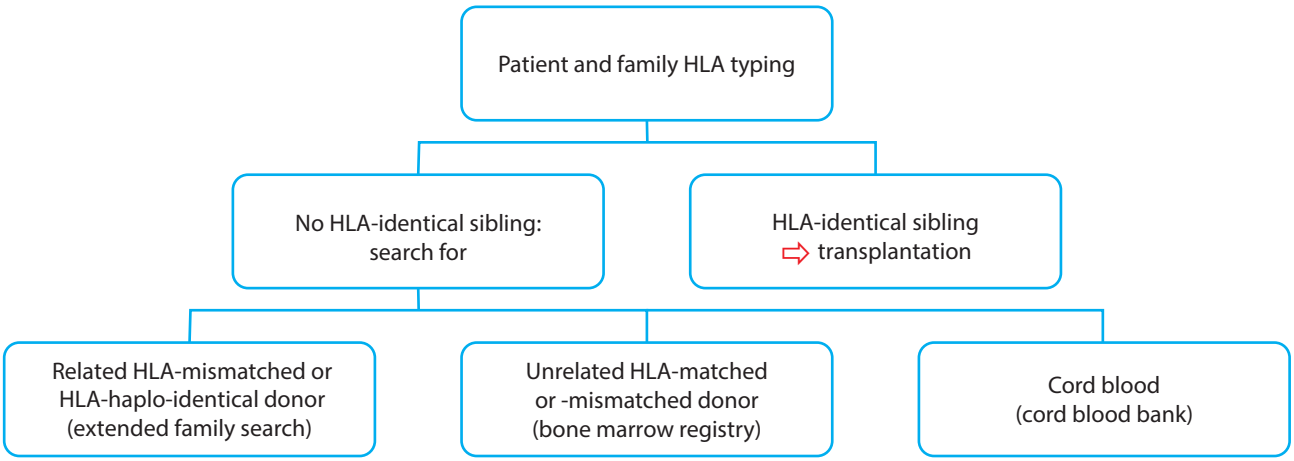


some cases, an extended family search can provide an HLA phenotypically identical donor. This can happen in cases of consanguinity due to cultural or geographical reasons, or in cases of common haplotypes, and can increase the likelihood of finding a matched or partially matched family donor [1]. If no HLA-matched suitable donor is found in the patient's family, an alternative HPC graft can be considered. This would include searching for an unrelated donor through bone marrow or umbilical cord blood registries, and extending the search to a mismatched unrelated source or an HLA genotypically haplo-identical relative (e.g. parents, siblings, children). The most common donor-selection algorithm is described in the European Bone Marrow Transplantation (EBMT) handbook (see Figure 24.3) [2]. For haplo-identical transplantation using HPC(M) or HPC(A), promising protocols have been developed recently [3] and EBMT have published recommendations on haplo-identical donor selection criteria [4].

The relevance of HLA matching is dependent on a variety of factors, including but not limited to GvHD prophylaxis, transplant conditioning, graft manipulation and clinical donor characteristics such as age, sex and *Cytomegalovirus* serostatus. Biologically, mismatched HLA can be recognised by alloreactive donor T-cells, leading both to toxic GvHD and to beneficial graft-*versus*-leukaemia (GvL) as counterbalancing effects [5]. Moreover, missing self-HLA on patient cells or mismatches between certain groups of HLA-C alleles in donor and recipient [6] can also lead to alloreactivity by natural killer (NK) cells, a phenomenon associated with GvL but not GvHD [7].

In unrelated stem-cell transplantation, it is

Figure 24.3. **Donor selection algorithm**



Source: adapted from the EBMT Handbook [2]

generally accepted that the best donor is matched for at least 8/8 HLA-A, B, C, DRB1 alleles, with every mismatch leading to an approximate 10 % reduction in survival probability [8]. Mismatches at HLA-DQB1 and DPB1 are more controversial. For HLA-DPB1, the concept of permissive, clinically well-tolerated mismatches was pioneered on the basis of experimentally defined T-cell epitope groups (TCE). This led to the increasingly accepted notion that avoidance of non-permissive HLA-DPB1 TCE mismatches improves outcome and should be favoured when several 8/8 matched unrelated donors are available [9]. A mismatch at HLA-DQB1 seems to be unfavourable in the presence of other mismatches. In apparent contradiction to the dramatic effect of subtle HLA mismatches in unrelated transplantation, transplants across several mismatches or an entire HLA haplotype are possible in cord blood and haplo-identical family donor transplantation, respectively [10, 11]. This is probably due to graft composition in the former and GvHD prophylaxis in the latter, with a higher proportion of regulatory and naïve T-cells in cord blood compared to adult stem-cell sources, and an important attenuating effect of agents such as post-transplant cyclophosphamide or high-dosage anti-thymocyte globulin on GvHD in the haplo-identical setting. The important question of whether and to what extent these specific characteristics will change the landscape also of unrelated stem-cell transplantation in the future will have to be answered in prospective clinical trials under way.

The first bone marrow donor registry was established in 1974 by the Anthony Nolan Bone Marrow charity in London. In 1988 the EBMT group with the Europdonor Foundation set up Bone Marrow Donors Worldwide (BMDW), based in Leiden, Netherlands. In 2017 the WMDA took over the activities of BMDW and the NetCord Foundation, and now co-ordinates the collection and listing of the HLA phenotypes and other important data of volunteer HPC donors and cord blood units. As of July 2022 the WMDA database includes more than 40 million HPC donors and over 800 000 cord blood units. Since ethnic minorities are under-represented in HPC donor registries, it is very important that donor centres and cord blood banks work with these communities to explain the need to increase the ethnic diversity of the registry and to recruit new potential donors. In the case of unrelated adult donors, once the HLA-typing and personal data are entered onto the donor registry, further blood samples may be requested, leading to possible haematopoietic stem cell donation at any time in the future.

The WMDA, an organisation of representatives of HPC donor registries, cord blood banks, other organisations and individuals with an interest in HPC transplantation (www.wmda.info) plays an important role in the field of HPC transplantation from unrelated donors, providing a forum for discussion and guidelines on the more critical aspects of the procurement and clinical use of HPC. Moreover, the WMDA offers to donor registries an accreditation programme according to internationally accepted standards.

The main role of the registries is to facilitate interactions between HPC transplant centres and donor centres. The majority of registries and transplant centres use the Search & Match Service, a web application provided by WMDA to search worldwide for matched adult and cord blood donors. The following search processes – including HLA typing requests and donor selection – are operated in most of the countries using EMDIS (the European Marrow Donor Information System), an international computer network which allows fast and direct communication between registries (see Appendix 36). The worldwide registries share their donors and cord blood units data in the Search, Match (& Connect) global database operated by WMDA, in order to facilitate the worldwide unrelated donor (UD) search. The Connect functionalities are under development.

Emergency rescue procedures should exist to limit consequences related to unforeseen unavailability of a donor (e.g. acute illness, accident, failed mobilisation). These procedures could be: identifying a backup donor, searching for a cord blood unit, a haplo-identical donor or an autologous transplantation with previously cryopreserved autologous HPC.

24.2.1. Donor evaluation

24.2.1.1. *Allogeneic donor*

One of the fundamental principles of allogeneic stem cell donation is the right of the potential donor to proceed to donation with a minimum of extraneous influences and no pressures. Protection of the unrelated donor's identity has to be guaranteed. Therefore, all the activities related to the donor's physical examination and collection of personal data must be performed in a dedicated and restricted area, as the access to all the donor information (medical and personal) must be protected and limited to authorised individuals [12]. Recommendations on assessing the medical suitability of adult stem cell donors are shown in Table 24.1.

For the evaluation of allogeneic donors, written criteria in the form of standard operating procedures (SOPs) should exist. Criteria must take into consid-

Table 24.1. **Recommendations on assessing the medical suitability of adult progenitor cell donors [19]**

Assessment stage	Method	Topics to consider	Specific for this stage
Recruitment/ registration (unrelated)	History/questionnaire	Malignancy Autoimmune disease Cardiovascular disease (or a combination of risk factors) Chronic disease (pulmonary/neurologic/ haematologic/serious allergies) Relevant medical history (malignancy, thrombo-embolic disease etc.) Risks of infectious diseases (behaviour) Inherited/genetic disease	Look for permanent diseases or behaviour that have a clear donor risk or unacceptable recipient risk and that are relatively easy to assess
During selection stage (unrelated)	History/questionnaire Blood tests for infectious disease markers (HIV, hepatitis B, hepatitis C, HTLV, syphilis, *Cytomegalovirus*)	Update history of topics above, and also: Risks of infectious diseases – behaviour, (medical) invasive procedures, (planned) travel (Planned) medical procedures (including blood transfusion, dentist, vaccination, tattoo etc.) Serious psychosocial or psychiatric disease with impact on availability/ capacity to go through donation procedure Medication Non-prescription drug use Height and weight Blood pressure Pregnancy, pregnancy planning, breastfeeding Back problems, chronic pain	Identify contraindications for one of the two collection methods Provide information about (possible) transmissible disease to the transplant centre Provide information to the transplant centre about any availability issues
Prior to HLA-typing (related)	History/questionnaire	As above	Identify contraindications before concluding that the relative donor is the best match; may save time and disappointment
During work-up/PE (related and unrelated)	Complete medical history; Complete physical examination; Laboratory tests: • Infectious disease markers: HIV-1,2 antibody, p24 antigen, HIV NAT, hepatitis B surface antigen and core antibody, hepatitis B NAT, hepatitis C antibody, hepatitis C NAT, validated serological testing algorithm for syphilis, on indication/per request transplant centre: Hepatitis E, HTLV I+II, Chagas, Zika, malaria, West Nile Virus, etc.; • Full blood count; ESR; if indicated: coagulation screen; blood film; haemoglobin electrophoresis; • ABO and Rh typing, screening for red blood cell and HLA-antibodies in case of HLA mismatch; • Biochemistry: Urea, creatinine, electrolytes, liver enzymes, LDH, ferritin; random glucose; β-HCG (for females of child-bearing age), protein electrophoresis; • Chest X-ray; • Electrocardiogram	As during selection stage, in addition any signs of undiagnosed disease.	Emerging infectious disease: check latest infectious disease epidemiology maps (CDC, ECDC)

eration not only the recipient's safety but also the donor's safety. The Donor Medical Suitability Wiki from WMDA provides minimum standards for unrelated donors which can be used for donor assessment [13]. Specific recommendations should be in place, especially for related donors who would not be eligible as unrelated donors due to age (e.g. young donors < 18 years, elderly donors > 60 years) or specific health issues [14-17]. The risk of donation should be evaluated and documented. To avoid a conflict of interest, the physician who evaluates the donor should be independent of the transplant team. In any case, donor

evaluation must be completed before the patient starts with the preparative regimen and, in cases involving HPC from the peripheral blood, before the donor receives the first dose of the mobilisation agent. To ensure the security of donor and recipient, a physician involved in the HPC(A) procurement procedure must be available for consultation during the procurement period, from the beginning of G-CSF (granulocyte-colony stimulating factor) injections to the post-procurement period.

Criteria for donors should include, in addition to general requirements, at least:

a. suitability for anaesthesia (for procurement of bone marrow);
b. assurance of adequate venous access;
c. additional tests if appropriate (e.g. in cases of family history or elderly donor);
d. exclusion criteria (see Chapter 5);
e. policy for making decisions in cases of 'only one' donor but who does not meet eligibility criteria (e.g. only one suitable donor but with risky behaviour).

Donors with a history of malignant disease (excluding haematological malignancies) after a minimum disease-free period of 5 years can be considered as suitable for sibling donation after careful assessment of the risk for malignant disease transmission [14].

It is up to the discretion of the transplant physician whether to accept donors with previous or existing infectious diseases (e.g. hepatitis B or others) if no other donor is available, based on careful risk evaluation. The specificity of allogeneic HPC transplantation lies in the fact that, for the vast majority of patients, the HPC graft is infused immediately after procurement. Thus, safety is reliant mostly on stringent evaluation of donors, which can be performed appropriately only if all needs are fully anticipated. In cases where there is a risk to patient or product safety, a formal acceptance of that risk should be signed by the transplant physician and the patient or their legal representative (urgent medical need).

The increasing age of recipients of allogeneic HPC transplants is related not only to the increasing age of the population but also to the introduction of less toxic conditioning regimens, allowing elderly patients and patients with co-morbidities to undergo HPC transplantation. Even if the age limit to donate is well defined for unrelated donors, and is over 18 and under 55-60 years for most international registries, these limits do not strictly apply for related donors. Any decision to collect from young (< 18 years) and elderly donors (> 60 years) must include an accurate risk assessment and appropriate informed consent of the donor stating that risk [14-16].

Decisions with regard to donor safety should be the responsibility of a physician who is not directly involved in recipient care. Some donors will present with co-morbidities (discovered or not) during evaluation. If these co-morbidities result in contraindication of the person for HPC donation, the physician who decided to contraindicate this person must ensure that a correct medical follow-up will be initiated in accordance with the medical condition of the rejected donor.

If the co-morbidities found during evaluation allow HPC donation, the physician in charge must manage these co-morbidities during the entire donation process, including specialist consulting as needed.

There should be a written plan to care for paediatric donors, donors with co-morbidities and elderly donors during selection [14-17].

24.2.1.2. *Autologous donors*

For autologous donors, suitability criteria for HPC donation are less strict than for allogeneic donors. For evaluation, written criteria – i.e. SOPs – should exist. Criteria must take into consideration the patient's safety. Criteria should include threshold values of the complete blood count before starting bone marrow procurement or leukapheresis. The risk of donation should be evaluated and documented. A donor advocate should be available to represent autologous donors who are mentally incapacitated or not capable of full consent at the time of HPC mobilisation and donation (e.g. for patients with primary CNS lymphoma).

Infectious disease markers in autologous donors should be tested as required by applicable laws and regulations (see Chapter 6). Autologous donors can donate even if results of the required tests are reactive or positive, or if other risk factors have been identified in the patient's medical history, as long as procedures for prevention of potential cross-contamination during HPC procurement, processing and storage are implemented.

24.2.1.3. *Specific considerations for paediatric donors and other persons not capable of full consent*

If minors are being considered as allogeneic HPC donors, in addition to the criteria shown in Chapter 3, national regulations must be followed.

The use of haematopoietic growth factors and insertion of a central venous line are not recommended. Procurement methods for paediatric donors

should employ appropriate adjustments due to age and size to the procedure.

Children should become donors only in very specific circumstances, as family donors only and never through public registries (see Chapter 4).

There are special circumstances, such as in minor donors and individuals not capable of full consent, where it is of utmost importance to protect the donor and to be sure that the donation fulfils the ethical and legal requirements. As stated in the Oviedo Convention [18], the authorisation of a representative, authority or person or body provided for by law should be given specifically and in writing and with the approval of the competent body. In the situation of an allogeneic related donor who is mentally incapacitated or not capable of full consent, the figure of a donor advocate should be available to represent and protect the individual as recognised in Recommendation CM/Rec (2020) 6 of the Committee of Ministers to member states on establishing harmonised measures for the protection of haematopoietic progenitor cell donors [36].

24.2.2. Informed consent

Informed consent in free decision-making process is required for an allogeneic and also for an autologous donation. The informed consent should include terms and conditions for the HPC storage, disposal and possible use for research purposes of products not needed for HPC transplantation any more. Volunteer HPC donors joining a donor registry express their commitment to donate, but they must nevertheless sign a formal consent before the HPC procurement. General considerations are shown in Chapter 4. Discard of allogeneic grafts from related and unrelated donors should comply with written agreements of collection centres and donor registries, respectively.

24.3. Procurement

Minimising the risk of grafts acquiring contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure graft safety. This risk is multi-factorial and, to help quantify and map this risk, the MiRCA tool (Chapter 3) has been developed. It is recommended that TEs use this tool to better understand the overall risk from their protocols, and how this risk is distributed along the tissue/cell supply chain, from procurement to distribution. This enables factors which contribute highly to the overall risk to be identified and mitigated.

24.3.1. Haematopoietic progenitor cell graft sources

HPC(M) and HPC(A) are obtained from living donors only, either from the patient themself (in the case of autologous transplantation) or from a fully or partly HLA-matched allogeneic related or unrelated donor. The choice of the donor is based on the best HLA matching, and other factors like age, gender, *Cytomegalovirus* status, ABO compatibility and NK alloreactivity.

Table 24.2. **Advantages and disadvantages of methods of haematopoietic progenitor cells procurement**

Procurement method	Advantages	Disadvantages
Bone marrow procurement	Donor: • Single procurement; • Use of cytokines (mobilisation agents) not necessary Recipient: • Less chronic GvHD	Donor: • General or epidural anaesthesia • Invasive procedure • Considerable risk of morbidity (associated with anaesthesia, procurement method, mobilisation agents if used) • Potential tissue damage/infection at procurement site • Possible need for blood transfusion Recipient: • Slower engraftment of neutrophils and platelets • Potential graft contamination with skin contaminants • Possible contamination with tumour cells in autologous HPC procurements
Peripheral blood apheresis	Donor: • No anaesthesia Recipient: • Faster engraftment of neutrophils and platelets • Potentially less contamination of autologous product by tumour cells	Donor: • Procurement may take more than one day (i.e. several procedures may be needed) • May require placement of a central venous catheter for procurement (risk of haemorrhage, embolism, pneumothorax/haematothorax and infection) • Potential loss of platelets • Considerable risk of morbidity (associated with mobilisation agents and apheresis technique including anticoagulation) Recipient: • Increased risk of chronic GvHD

Source: EBMT [17].

For autologous purposes, nowadays HPC are obtained almost exclusively from peripheral blood stem cells HPC(A) and used to accelerate haematopoietic recovery after high doses of chemotherapy. In the allogeneic setting, the HPC graft source depends on the age and size of the donor and recipient – i.e. paediatric or adult donor, since some countries do not support G-CSF administration and apheresis in paediatric sibling donors – and the kind of disease (malignant or non-malignant), as well as the transplant protocol (myeloablative, reduced intensity, T-cell replete or deplete haplo-identical transplantation).

Currently HPC(A) represents 80 % of the stem cell source in allogeneic transplantation. However, HPC(M) is still the preferred source in allogeneic paediatric transplantation from HLA-matched related or unrelated donors and for patients with non-malignant diseases (e.g. bone marrow failures).

Indications for HLA-mismatch transplantation and selection of 'alternative' donors – in the sense of donors without a 10/10 HLA match with the recipient (considered as 'standard' donors) – are constantly implemented as they are evaluated in the context of biomedical research or registry studies. To date, prospective clinical studies comparing all sources of alternative HPC donors in different clinical settings are still ongoing. Hence, transplantation programmes should carefully follow their own local algorithms defining the 'best donor' for each patient according to the different situations, and use these algorithms to guide the donor choice.

Particular attention should be paid in HLA-mismatched donor selection (parents, brothers/sisters, adult children, or other family members). The criteria of choice should be explained in advance to the patient and potential family donors.

24.3.2. Procurement procedures

The majority of HPC are provided using one of two technologies: either procurement of bone marrow or apheresis. The advantages and disadvantages of these technologies are shown in Table 24.2.

For HPC procurement and associated procedures – e.g. central venous line placement – written procedures must be established and reviewed regularly, with evidence of continued training of the staff. The International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration were developed by two organisations, JACIE (the Joint Accreditation Committee–ISCT & EBMT) and FACT (Foundation for the Accreditation of Cellular Therapy), and provide minimum guidelines for transplant, procurement and processing facilities. These standards can serve as guidance [20].

Severe adverse reactions can occur in donors with the administration of the mobilisation agents, and during and after allogeneic HPC donations. Hence, careful and documented training of clinicians and involved healthcare professionals caring for donors is needed, as is appropriate follow-up of donors. In all cases, safety of the donor is the major concern, meaning that the pre-donation work-up should be particularly meticulous [12, 14, 21-24].

24.3.2.1. *HPC from bone marrow*

Procurement of bone marrow is an aseptic process that should be undertaken in an operating theatre by appropriately trained personnel. Special attention should be paid to the training of clinicians, to written criteria for required competence and experience in bone marrow procurement and aftercare of the donor, and to vigilance and surveillance of donors as well as of recipients. There must be provision for counselling of donors and for their routine post-donation follow-up.

Bone marrow for therapeutic use is obtained through multiple punctures, usually from the posterior iliac crests. When absolutely necessary, the anterior iliac crests can also be used. The sternum is not considered an appropriate method of bone marrow procurement. Punctures are usually undertaken under general anaesthesia. Epidural anaesthesia may be considered. A pre-anaesthesia visit of the donor is mandatory before procurement of bone marrow.

For procurement of bone marrow, written procedure(s) should be established, including at least:

a. disinfection technique;
b. preparation of media and materials used to obtain bone marrow;
c. puncture technique;
d. provision of containers for procured bone marrow;
e. monitoring of the volume of the procured bone marrow;
f. irrigation of procurement syringes;
g. bone marrow filtration;
h. quality controls (e.g. TNC counting, sterility, etc.);
i. labelling.

Bone marrow grafts contain bony spicules, fat and clots that should be filtered during procurement or processing. The bone marrow total nucleated cell number (TNC) is used to determine the adequacy of the procurement. The recipient's body weight and type of post-procurement manipulation determine

the target TNC and volume of bone marrow to be procured. The procurement and anaesthesia times should not exceed 120 and 150 minutes, respectively. A maximum volume of 20 mL of bone marrow/kg donor weight should be procured. Procurement teams should not aspirate a volume more than 5 mL at each aspiration to avoid dilution of the bone marrow with blood [2]. The minimum target for autologous transplantation without graft manipulation is 2×10^8 TNC/kg recipient body weight. The target dose for most allogeneic transplantations is $2\text{-}3.5 \times 10^8$ TNC/kg recipient body weight.

Adverse reactions related to HPC marrow procurement are associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection.

Blood cultures should be taken from bone marrow donors if there is presence of fever immediately after donation, to investigate a possible microbial contamination of the procured HPC graft.

A 24-hour blood component donor-support protocol, including the provision of *Cytomegalovirus* antibody-negative (or equivalent), irradiated and leukocyte-depleted blood components, should be available. However, all efforts should be made to manage allogeneic donors in such a way as to minimise the need for blood transfusions. Autologous red blood cell (RBC) donation before bone marrow procurement can be considered, but potentially induces iron deficiency, because the time from final selection of donor to procurement can be short. Therefore, a patient blood management programme taking care of iron supplementation if necessary should be considered. Procurement procedures in paediatric donors should be adjusted according to donor age and size [20].

24.3.2.2. *Haematopoietic progenitor cells from peripheral blood*

In both autologous and allogeneic settings, HPC from peripheral blood should be procured in an apheresis facility by health professionals who have appropriate experience in care for patients with haematological or oncological diseases, HPC mobilisation and therapeutic apheresis. Special attention should be paid to paediatric patients and the specific circumstances pertaining to apheresis in young patients, whose weight (often < 20 kg) places them at risk of haemodynamic changes, both on commencement and during the procedure. Priming of the apheresis device with matched, irradiated red blood cell units has to be considered in patients weighing less than 20 kg. Expertise to carry out apheresis is of particular importance for small children (< 20 kg) for autologous procurement (which is usually indicated in solid tumours or haematologic malignancies); the transplantation programme must maintain trained and experienced personnel for apheresis in paediatric units.

Before each apheresis procedure, the donor (autologous and allogeneic) should be evaluated. At least the following studies should be carried out:

a. complete blood count (including platelet count) ≤ 24 h before procurement;
b. vital signs and temperature;
c. blood cultures in presence of fever to investigate a possible microbial contamination of the procured product;
d. update of medical history.

24.3.2.2.1. Allogeneic donors

Mobilisation of HPC to peripheral blood before allogeneic procurement is ensured by recombinant human granulocyte-colony stimulating factor (rhG-CSF), which is administered to healthy adult donors in order to mobilise HPC from bone marrow into peripheral blood. The side-effects and risks associated with the procedures must be discussed with the donors. In particular, the donors must be informed about all aspects of rhG-CSF administration, including known short- and long-term effects, and given the opportunity to ask questions. Immediate and severe side-effects associated with rhG-CSF administration are rare (see Table 24.3) and raise the issue of inpatient *versus* outpatient administration. Donors who live far away from the transplant centre will require administration at home. However, because of a potential risk of allergic reaction, the first dose of rhG-CSF should be given under the supervision of trained healthcare professionals, and the donor should be followed up for at least 30-60 minutes. The transplantation programme, or the physicians in charge of mobilisation and procure-

Table 24.3. **Very common adverse reactions associated with agents for haematopoietic progenitor cells mobilisation (> 10 %)**

Agent	Adverse reaction
Rh-Granulocyte-colony stimulating factor (rhG-CSF)	bone pain musculoskeletal pain thrombocytopaenia hyperleukocytosis transitory elevation of levels of liver enzymes elevation of levels of lactate dehydrogenase headache asthenia
Haematopoietic progenitor cell binding inhibitors (Plerixafor)	diarrhoea nausea reaction at injection site

Source: EBMT [17].

ment of HPC from the donor, should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SARs) occur, especially for anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

Routinely, HPC(A) procurement takes place on day 5 after 4 days of rhG-CSF administration. In cases of very low $CD34^+$ cell numbers, rescue strategies should be established (e.g. 'immediate' bone marrow procurement). Up to now the administration of HPC binding inhibitors in allogeneic donors is off-label use and must be done with appropriate ethical permission.

Approximately 5-10 % of donors may be asked to provide a subsequent donation of HPC or MNC concentrates to the same patient. The frequency of second donations seems to be higher for HPC(M) donors, and it may increase with the application of new therapeutic strategies. The interval between donations, for the same or for a different recipient, should be established by individual registries or transplant centres on the basis of the risks to the donor and the patient. However, these limits do not strictly apply to related donors.

24.3.2.2.2. Autologous donors

Cell mobilisation before autologous procurement is ensured by administration of various types of mobilisation regimens. RhG-CSF is the usual haematopoietic growth factor used to mobilise progenitors and can be administered alone or in combination with chemotherapy or other agents (e.g. HPC binding inhibitors such as Plerixafor, immunostimulants).

Circulating levels of $CD34^+$ cells guide commencement of apheresis. The number of cells required varies with the size of the patient and number of transplantations indicated (double grafting is indicated for some diagnoses). Procurement centres should have protocols that can determine the optimal number of cells to be procured, considering the patients' well-being during and after procurement, as well as their needs as future recipients.

24.3.2.2.3. Apheresis procurement yield

Processed blood volumes vary, depending on the procurement protocol and cell separator. Weight discrepancies between donor and recipient have to be considered as this can affect the $CD34^+$ cell yield per kg recipient weight. The target number of $CD34^+$ cells should be set before starting apheresis according to institutional protocols. The target will vary for autologous and allogeneic donations, and will depend on clinical need and regulations (as well as best available professional practices). Target dose of $CD34^+$ cells for a single autologous transplantation ranges from a minimum of 2×10^6 $CD34^+$ cells/kg recipient weight to a more preferable 5×10^6 $CD34^+$ cells/kg recipient weight. The ability to achieve this goal is dependent on the underlying disease of the patient, therapy and mobilisation protocol.

The target for allogeneic donations is higher, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation; HPC(A) doses above 4×10^6 $CD34^+$ cells/kg might be needed, especially when $CD34^+$ enrichment (a loss of $CD34^+$ cells is expected) or T-cell depletion methods is used. In addition to optimising HPC procurement, apheresis should ensure that procured cells have minimal contamination with other cells that could compromise subsequent processing steps or contribute to side-effects in recipients. HPC from apheresis contain small volumes of RBC (haematocrit < 5-10 %) so the risk of donation-related anaemia is very low [16]. Depending on the device used for procurement of HPC(A), the donor may experience a marked loss of platelets, in some cases resulting in post-donation values < 100 G/L (10^9/L). Under such circumstances a consecutive donation has to be carefully considered. Monitoring the collection efficiency of the apheresis device can help to identify technical problems (e.g. inaccurate camera setting) if the expected apheresis yield according to the $CD34^+$ pre-cell count in the peripheral blood is not reached during procurement.

Some Health Authorities do not permit the use of G-CSF in paediatric donors and so bone marrow procurement might be employed.

24.3.2.3. *Mononuclear cells from peripheral blood*

To enhance immune responsiveness after HPC transplantation, specific mononuclear cells are being used and/or evaluated in clinical trials. However, these immunocompetent cells are also used in patients who had not undergone an allogeneic or autologous HPC transplantation previously. As starting material for further processing (e.g. CAR-T cells, chimeric antigen-receptor T-cells), MNC concentrates are procured from an unstimulated donor.

An MNC donor can be a former HPC donor (i.e. donor lymphocytes infusion), a third party donor or the patient (autologous donor). In addition, MNCs could be collected for off-line extracorporeal photopheresis (ECP) for the treatment of T-cell mediated diseases.

Donor lymphocytes infusions (DLI) can be administered to the selected patient after allogeneic HPC transplantation, either prophylactically to augment the anti-tumour immune response (fol-

lowing reduced-intensity conditioning protocols) or in cases of mixed chimerism or of relapse of an underlying disease (mostly myeloid malignancies). The goal of this therapy is either to induce complete donor chimerism or a remission of the patient's malignancy by a process called graft-*versus*-tumour (GvT) effect.

The following cells are being used and/or evaluated in clinical trials:

a. DLI to enhance immune surveillance against infections in patients with poor immune recovery experiencing relapsing/resistant viral infections;
b. T regulatory cells (Treg) for the prevention and control of GvHD;
c. natural killer (NK) cells as GvT effectors by alloreactivity of killer Ig-like receptors (KIRs) in donor–recipient direction;
d. viral- and fungal-specific T-cells for the treatment of several infections (e.g. *Cytomegalovirus*, Epstein–Barr virus, *Adenovirus*, *Aspergillus*);
e. vaccination with peptide-loaded dendritic cells (DC) for induction of tumour-specific T-cell responses for treatment of metastatic disease, or for treating GvHD;
f. mesenchymal stem cells to enhance engraftment in allogeneic and autologous HPC transplantations, or in treatment of GvHD;
g. autologous or allogeneic chimeric antigen-receptor (CAR) T-cells;
h. MNC for off-line ECP.

24.3.3. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, HPC(A) and HPC(M) are stored in non-frozen conditions before processing and infusion or during transportation. Survival of HPC stored in a non-frozen state is dependent on the concentration of leukocytes (TNC), buffering capacity of the HPC and anti-coagulant in the graft, product volume and storage temperature. Therefore, storage conditions (such as maximum TNC concentration, storage temperature and time) must be validated. Cell viability decreases and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators (see Chapter 10, also Table 8.2). Therefore, maximum storage in the non-frozen state should be ≤ 72 h. In cases where HPC have to be cryopreserved, this should be done as early as possible to avoid the cell loss and reduced viability during processing observed in this situation. The facility should undertake a validation study of the storage and transport conditions.

The same applies to MNC(A) concentrates procured for DLI.

24.4. Processing of haematopoietic progenitor cells

Processing of minimally manipulated HPC is intended to provide appropriate conditions for preservation and storage or to improve the risk–benefit ratio of autologous or allogeneic HPC transplantation [2, 20]. It does not affect the main biological property of the procured cells, which is to support the marrow re-populating ability (MRA) and the establishment of haematopoietic chimerism in myelo-ablated or immunosuppressed recipients in allogeneic transplant.

Generic requirements for processing facilities, together with standards, are described in Chapter 8 and Chapter 9. In selecting an appropriate air-quality specification for processing HPC, the criteria identified in Chapter 7, Chapter 9 and the output from the MiRCA tool should be considered.

While HPC are exposed to the environment, processing should be performed in a laminar-flow cabinet of GMP Grade A with background environment at least equivalent to GMP Grade D as required by Directive 2006/86/EC. It is appropriate that HPC processing takes place in a microbiologically and climate-controlled environment (control of temperature, ventilation, air filtration) with validated cleaning and disinfection. The same requirements apply for autologous or allogeneic donations.

For all processing steps, written procedures must be established and reviewed on a regular basis with evidence of continued training of the staff. The current FACT–JACIE International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration also apply to processing facilities.

24.4.1. Volume reduction

Volume reduction is either a preparatory step to further processing (including cryopreservation and storage) or a means to reduce the volume of the infused cells and, thus, prevent recipient side-effects relating to volume overload in the transplanted patient. Various centrifugation-based techniques can be used that are validated at the site. Cell loss associated with volume reduction must be evaluated and expected recoveries defined.

24.4.2. Red blood cell depletion

HLA identity does not preclude the existence of major or minor ABO incompatibility between HPC donor and recipient. In case of major ABO incompatibility (i.e. isohaemagglutinins of the recipient blood group directed against red blood cells of the donor blood group) acute haemolysis can occur during infusion of the stem cell graft. Red blood cell depletion is almost exclusively performed if bone marrow is used as the HPC graft source, whereas HPC(A) are usually not red blood cell depleted due to the low content of red blood cells. Various techniques for blood cell depletion are available, including buffy-coat centrifugation or apheresis cell separation. The efficiency of the technique must be monitored by measuring the residual content of red cells, which should be as low as possible. Similarly, the cell loss associated with such procedures must be evaluated and the expected recoveries and amount of acceptable residual red blood cells must be defined.

24.4.3. Plasma removal

Plasma removal represents a critical step in cases with minor ABO incompatibility between a donor and a recipient in the allogeneic setting (HLA identity does not preclude the existence of major or minor ABO incompatibility). The necessity of plasma removal in case of minor ABO incompatibility can be judged using a titration of anti-A and anti-B antibodies in the donor blood during the period that precedes the donation. In minor ABO incompatibility, where anti-recipient-RBC antibodies are 1:256 or greater, plasma should be removed, especially from bone marrow grafts [2]. Plasma removal is usually done by centrifugation of the procured cells. The cell loss associated with such procedures must be evaluated and expected recoveries must be defined.

24.4.4. Cryopreservation, thawing and infusion

24.4.4.1. *Cryopreservation of haematopoietic progenitor cells*

The purpose of cryopreservation is to preserve HPC in such a way as to ensure their viability and potency. To minimise the volume infused, peripheral blood and bone marrow may be concentrated before cryopreservation. The volume of cell suspension per bag should be adjusted by the body weight of the patient. A maximal daily dose of dimethyl sulphoxide (DMSO) of 1 g/kg body weight, which is equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution, should not be exceeded. Special attention should be paid if the recipient is a small child and if a patient has impaired renal or liver function.

Cryopreservation is used systematically in the autologous setting. In the allogeneic setting, cell procurement from the donor is usually synchronised with administration of a conditioning regimen to the recipient and direct infusion of the HPC product (within ≤ 48-72 h after procurement) without freezing. However, sometimes allogeneic HPC are being cryopreserved for logistical reasons, such as unavailability of the donor at the scheduled date of transplantation (procurement in advance), professional constraints, unforeseen changes in transplantation schedules or over-collection of stem cells. In a case of cryopreservation of HPC from an unrelated donor, registries can request an additional consent from the respective donor as this approach can lead to the risk that collected cells are not used.

HPC should be cryopreserved as soon as possible. Shelf life of HPC without cryopreservation is acceptable up to 72 h. However, cell viability decreases if cells are frozen at the end of their shelf life.

The cryopreservation method for HPC(A) or HPC(M), once RBC and plasma are depleted from the latter, is the same. The method involves addition of 5-10 % DMSO to a suspension of HPC and protein-rich medium, with or without dextran or hydroxyethyl starch (HES). Immediately after DMSO addition, HPC are cooled at −1 °C to −2 °C per minute. For most therapeutic cells, the cooling rate is controlled by a controlled-rate freezer in which vapour-phase liquid nitrogen is pumped into the freezing chamber facilitating a sudden temporary drop of the temperature in order to compensate for the thermal release caused by the solidification of the suspension.

Although this is the recommended method for cryopreservation of therapeutic cells, other methods may be used (e.g. freezing in a mechanical freezer) as long as they result in acceptable post-thaw viability and potency. After cryopreservation of HPC in a mechanical freezer at – 80 °C, cells should be transferred for long-term storage in storage devices maintaining a temperature of <−140 °C as soon as possible. The final phase of cooling in a controlled-rate freezer is usually quicker, with the temperature drop adjusted to 5 °C/min. When the mixture has reached approximately −100 °C to −120 °C, it is transferred to a storage container. Methods to minimise the risk of contamination or cross-contamination must be in place (e.g. secondary bag, liquid nitrogen vapour phase). Temperature fluctuations may result in a loss of viability. The validation of cryopreservation proce-

dure must include evidence that the storage temperature is adequate to preserve the grafts.

Once frozen, HPC should be stored at < – 140 °C (either in vapour phase or in liquid nitrogen). Variations in cryopreservation methods include the concentration of frozen cells, the amount and source of plasma protein and the cooling rate. The method chosen must be validated.

Maximal shelf life has not been defined for cryopreserved HPC. HPC(M) or HPC(A) have been transplanted successfully even more than 11 years after cryopreservation. A stability programme for cryopreserved grafts should be implemented in order to evaluate viability and potency at different storage durations. For cellular products a definition of the storage duration as well as the way of disposal or transfer to research should be defined.

24.4.4.2. *Cryopreservation of mononuclear cells*

Processing of MNC(A) mainly involves adjustment of volume and cell number according to the clinical protocol used. The number of MNC and specifically the number of $CD3^+$ T-cells is determined by flow cytometry analysis, and further characterisation of T-cell subpopulations may be undertaken according to special requirements and needs.

For DLI, frequently a T-cell dose of 1×10^6 $CD3^+$/kg body weight of the recipient is the starting dose, and then further treatments with escalating doses may be used. This amount can be even lower in haplo-identical transplantation. Some protocols also include cell-selected preparation. The tissue establishment (TE) should validate the freezing technique in order to establish the expected level of viable T-cells after thawing.

24.4.4.3. *Thawing and infusion*

HPC(M) and HPC(A) can be thawed at the bedside or in a processing facility, and time to infusion must be minimised to avoid decrease of cell viability. If cells are not infused immediately after thawing or have to be transported to the clinical facility, the maximum possible time between thawing and infusion should be validated. Several studies have demonstrated that the occurrence of adverse reactions during HPC infusion is related to the amount of DMSO and/or cell debris in the product, and some centres remove DMSO after thawing prior to infusion. This procedure is performed in the processing facility by manual centrifugation or by automated washing in closed systems using specific equipment. Despite the progress that has been achieved in the development of new washing methods, such as membrane filtration and new devices, automated washing is still performed only in few transplant centres because of the associated risks: cell clumping, cell loss, osmotic injury, contamination and the high cost.

Hence, washing of HPC(A) and HPC(M) must be reserved only for patients at a high risk of adverse reactions. Good practice recommends (if possible) not exposing all cells to the risk of washing procedures at once unless there is a validation that demonstrates the maintenance of morphological and functional characteristics of the cells.

HPC should be infused immediately after thawing and as fast as possible, at approximately 5-20 mL/min using standard transfusion sets or equivalent, although this interval may be longer if the HPC are washed. Leukoreduction filters must not be used.

24.4.5. Cell-selected preparations

Specific Conformité Européenne (CE)-marked devices are available to select $CD34^+$ cells or deplete other lymphocyte subpopulations from bone marrow or peripheral blood on the large scales needed for clinical transplantation. The use of such medical devices requires adequate training for personnel involved in these procedures. There must be written criteria – i.e. SOPs – for cell-selection and cell-depletion preparations and the criteria must be reviewed on a regular basis with evidence of continued training of the staff. The method chosen must be validated and ensure a sufficient purity and viability for a safe transplant and sustained engraftment.

24.4.5.1. *T-cell depletion and depletion of alloreactive immune effectors*

T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immunosuppression) consequences that prevent its adoption in routine clinical practice, and it is rarely used in HLA-identical or HLA-matched transplantation. This is because the advantages of reducing GvHD are offset by associated increases in relapse rates and graft failures. Indications for T-cell depletion depend on the clinical protocol, such as use of haplotype-mismatch donors and transplantation for non-malignant diseases. In these situations, it is important that T-cell depletion is as extensive as possible.

Accurate determination of the residual T- and B-cell content is mandatory. The highest acceptable dose of residual T- and B-cells must be defined in advance by the medical team in charge of the recipient. $CD34^+$-positive immunoselection can also be considered a T-cell depletion method because, as in standard $CD3^+$ depletion, almost all T-cells are elim-

inated, including the T-cell receptor (TCR) gamma/delta-positive T-cells not involved in GvHD and exerting anti-leukaemic activity, as demonstrated by several authors. A specific depletion of TCR alpha/beta-positive cells spares the gamma/delta T-cells and is more beneficial over the depletion of all T-cell subsets. Combining this with a $CD19^+$ B-cell depletion for preventing transmission of Epstein–Barr virus (EBV) is a very encouraging strategy, especially in haplo-identical transplantation settings [26].

Other specific procedures evaluated by clinical trials include depletion of activated and alloreactive T-cells (i.e. those that can be identified by the expression of the CD25 T-cell receptor subunit). Removal of $CD25^+$ T-cells can be done using immuno-selection devices similar to those routinely used for $CD34^+$ positive cell selection or T-cell depletion. Accurate determination of the residual T- and B-cell content is critical. The highest acceptable dose of residual alloreactive T-cells must be defined in advance by the medical team in charge of the recipient and their guidance sought by the procurement team if this objective cannot be met.

24.4.5.2. *Tumour cell depletion in the autologous setting*

Autologous tumour cells procured with normal HPC may contribute to post-transplant relapse, but this has not been firmly established on the basis of clinical and biological observations. A definitive advantage for tumour-purging of autologous grafts has not been demonstrated by clinical trials. The use of $CD34^+$ cell-selection devices for this purpose is only applicable in a few clinical protocols (e.g. neuroblastoma) but, if a transplant team decides to use such a procedure, then detection of residual tumour cells should be as accurate as possible, using either immuno-histochemical techniques or flow cytometry analysis, or molecular biology techniques.

For all processing steps, written procedures must be established and reviewed on a regular basis with evidence of continued training of the staff.

24.5. Quality control

24.5.1. Biological information needed to confirm donor suitability and recruitment

All clinical and biological information pertaining to donor identification, screening and recruitment must be kept, along with all information pertaining to processing and distribution. This information must remain as a permanent part of the preparation and release file.

Details on the nature of such information and the procedure to obtain it are provided in chapters 4 and 5 of this Guide.

24.5.2. Safety controls

Detection of transmissible infections is undertaken through donor screening (using microbiological and other testing, as required by national, European and international guidance and regulations) and through microbiological testing of samples obtained at the different stages of cell procurement, processing and distribution (see also chapters 6, 9 and 11).

Detection of donor-transmissible diseases other than occult pre-neoplastic or neoplastic diseases or other disorders is through donor screening, using medical questionnaires, physical examination and biological testing, as necessary.

The proportion of the various subpopulations of leukocytes in the procured cell preparations must be measured. High numbers of mature blood cells such as granulocytes or contamination with red cells may negatively affect several subsequent processing steps and may contribute to recipient side-effects at re-infusion.

Removal of red blood cells through specific processing procedures must be documented, as must the removal of T-cells or other immune effectors.

The removal of tumour cells from autologous cell preparations using specific processing procedures must also be documented where applicable.

24.5.3. Immunophenotyping and colony-forming unit assay

The number of total nucleated cells (TNC) in combination with the number of viable $CD34^+$ cells is a widely used measure for evaluating the quality of procured bone marrow. The cell dose for recipients is usually expressed in TNC and $CD34^+$/kg of recipient weight. In addition, nucleated cell counts are largely used as in-process controls to document that technical procedures have been appropriately conducted in the processing facilities (i.e. recovery of TNC following plasma removal, volume reduction, red blood cell depletion).

$CD34^+$ cell counts are used as a marker for HPC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the procured cells, whether from apheresis following mobilisation or from bone marrow donation. $CD34^+$ cell counts are usually measured by flow cytometry, using monoclonal antibodies that recognise one or several epitopes on the human CD34 membrane

antigen. Use of a single platform, rather than a dual platform, minimises errors in calculating cell counts. The ISHAGE (International Society for Hemotherapy and Graft Engineering) algorithm provides a robust and reproducible gating strategy to measure $CD34^+$ cells [27, 28].

Evaluation of $CD34^+$ cell recovery and total viability after storage and cryopreservation are an acceptable way to measure the potency of an HPC graft when the detection of colony-forming units (CFU) in clonogenic assays is not feasible. These functional tests are hampered by the delay required to produce results (usually two weeks); thus, the results are usually only available long after a non-cryopreserved cell preparation has been transplanted in an allogeneic recipient. Clonogenic assays are also hampered by poor intra- and inter-laboratory reproducibility. This particular issue could be improved by using commercially available and standardised culture media and by participation in proficiency testing and external quality-assessment schemes. The frequency of $CD34^+$ cells that form colonies differs among the sources of HPC and is higher in HPC(A) than in HPC(M). A clonogenic assay can provide additional information about the functionality of the graft; in particular, it is recommended after a long storage period. It can be used as a qualitative (e.g. growth or no growth) or a quantitative potency test. In both cases, a policy should be defined to deal with grafts where $CD34^+$ cells are at a low frequency.

Colonies are enumerated and classified on the basis of their morphologic characteristics: CFU-GM (granulocytes and macrophages), CFU-GEMM (granulocyte-, erythrocyte-, macrophage-, megakaryocyte) and BFU-E (burst-forming units – erythroblast).

24.5.4. Release criteria

The cell-processing facility, along with its clinical counterparts, must define which safety and quality controls serve as release criteria. It must also define which criteria must be strictly met and which ones may lead to documented waivers. Specific instructions should be established in the TE on how to deal with the recipient, donor and stem cells throughout the donation, through the processing and issue stages and all the way through to transplantation. Acceptance and release criteria may differ between autologous and allogeneic grafts. In autologous grafts, infectious disease-marker (IDM) test results may be positive (this is normally not the case in allogeneic grafts). In allogeneic grafts, which are mainly used directly after procurement, microbiological test results are pending at the time of administration and cannot serve as release criteria (in contrast to autologous grafts).

Processing and transplant facilities should agree on the cell dose (nucleated cell count, mononuclear cell count, $CD34^+$ cell count or clonogenic assays as appropriate for the source of HPC) required to achieve reliable and sustainable engraftment.

If cells are required for administration to a patient, a prescription for infusion is required. This prescription should list the type of cell preparation that is suitable for that patient and provide specific information on dosing. If necessary, the cells may be further processed before infusion (e.g. washing, dilution) and this should be recorded.

24.5.5. Quality control for mononuclear cells

In addition to the safety controls (listed in §24.5.2) and definition of release criteria (§24.5.4), the specific requirements include establishing the absolute number and the frequency of T-cells ($CD3^+$ and/or subpopulations) and cellular viability by flow cytometry analysis. The anti-tumour effect should be evaluated at intervals as defined in the clinical protocol.

24.6. Labelling and packaging

Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect personnel and the environment. The primary packaging must be made of a biologically compatible material. Cryopreservation requires the use of low-temperature-resistant packaging, which can also withstand contact with liquid nitrogen.

Labelling must unambiguously identify the donor, the intended recipient, the cell preparation and its nature, the additives used and the conditions under which the cells are to be stored and distributed. Following procurement, the donor identifier should be always on the 'transit' label when cells are delivered to the processing facility. The recipient must be identified (but not the donor) when cells are distributed for administration. In all cases there must be an audit trail to the donor.

International standards for labelling now exist (e.g. ISBT 128, Eurocode-IBLS) and must be used to promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. For tissues and cells procured and distributed in the EU, the Single European Code for Tissue and Cells (SEC) must be used. In addition, due to the growing number of cell donors worldwide, the

WMDA has developed a unique global donor identifier to guarantee safe and unequivocal donor identification: the Global Registration Identifier for Donors (GRID) [29]. See also Chapter 15.

24.7. Storage

Storage must be defined in SOPs and done in conditions that minimise the risk of contamination, cross-contamination and mix-up. A process for quarantine storage should be in place to avoid the possibility that grafts with incomplete or positive IDM test results are accidentally transferred to caring physicians for application.

Conditions for temporary storage must be defined for each type of cell preparation and for each stage of the process, from procurement to release for administration (including pre-processing and post-thawing, etc.). There should be a stability protocol that evaluates the viability and potency of long-term stored cryopreserved cellular therapy grafts, on a regular basis.

The cryogenic system used for long-term storage must be continuously monitored, and processes must be in place to detect failures in the system, such as temperature rises and changes in the level of liquid nitrogen. An emergency plan should be in place describing the actions to be taken in case a storage device fails, and a procedure should specify how to maintain the cryopreserved grafts at the defined storage temperature using a backup device of appropriate temperature available either in the same or a different facility.

24.8. Distribution and transport conditions

Internal and external transport must be controlled. Transportation within the same institution (e.g. from the procurement facility to the processing facility, or from the processing facility to the transplant ward) must be defined by SOPs. Periodic container validation and courier qualification should be performed. When service providers are used for transport or shipment of unprocessed or cryopreserved cell preparations, the conditions by which the service is delivered must be established and regularly audited by the facility, which remains responsible for the delivery of cell preparations. Appropriate training of the personnel in charge of transportation should be documented. The transport containers should conform to the applicable regulations and should be secured where applicable. Records of transports must be maintained to allow tracing of the product. For cross-border transport of HPC from unrelated donors, international standards and guidelines are available [30].

For cryopreserved cellular therapy grafts, a dry-shipper should be used. During shipment of HPC grafts, the temperature should be monitored, and records must be maintained by the shipping facility and shared with the receiving facility. See also Chapter 12.

24.9. Contingency plan for HPC collections

A contingency plan should be implemented for HPC collections in case of any emerging issues negatively impacting this activity, and this plan should be part of the quality management system. Natural hazards such as earthquakes or hurricanes, pandemics or other related moments of global crisis can be challenging for the collection of HPC, their transportation and timely infusion.

Recently, during the Covid-19 pandemic and the lockdown of countries, significant problems for HPC transplant programmes emerged. Transplantation societies and donor registries recommended cryopreservation of unrelated donor HPC products before the recipient commenced conditioning therapy, to mitigate the donor and travel risks associated with the pandemic. Therefore, HPC programmes started to cryopreserve HPCs as soon as they arrived at the tissue establishment and started with the conditioning regimen thereafter. Registries can request an additional consent from the respective donor for cryopreservation because this approach can lead to the risk that collected products will not be used, either due to the possibility of a clinical deterioration of the patient (who is then no longer eligible for transplantation) or due to poor post-thaw graft quality.

Data on post-thaw quality of allogeneic products with respect to pre-cryopreservation conditions are lacking. A recent Australian study reported a median post-thaw $CD34^+$ cell recovery of 76 % (range, 6 % to 122 %) [31]. In this study a longer transit time before cryopreservation, higher leukocyte count during storage and complex product manipulation before cryopreservation were independently associated with inferior post-thaw $CD34^+$ cell recovery and therefore poor product quality. This may put the recipient at risk of delayed or incomplete engraftment [31].

24.10. Biovigilance

As an effective vigilance and surveillance (V&S) system for tissues and cells used in transplantation and assisted reproduction, the EU project SoHO V&S was developed in 2013. The Guidelines on vigilance and surveillance of human tissues and cells [32] were published for healthcare professionals responsible for all types of HPC (bone marrow, peripheral blood stem cells, cord blood) for human application. In EU member states, the requirements for traceability, notification of serious adverse reactions and events, and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

TEs and healthcare professionals should use SoHO V&S guidelines because they provide essential information for the detection, reporting/confirmation and investigation of serious adverse reactions and events (SAREs). There is general guidance on implementation of good V&S practice, as well as definitions of SAREs, in Chapter 17.

TEs should have written procedures for managing SAREs. They should also provide centres carrying out clinical applications with accurate and updated information on various SAREs in the area of HPC transplantation. Moreover, WMDA has set up a central global reporting system for its member organisations to report serious events and adverse reactions related to HPC grafts: Serious (Product) Event and Adverse Reactions or S(P)EAR [33]. The scope of this system is to collect information on serious events and adverse reactions that occurred during procurement or processing of HPC from unrelated donors.

24.10.1. Follow-up of haematopoietic progenitor cell donors

Chapter 17 on biovigilance also applies to HPC transplantation and must be read in conjunction with this chapter. The donor, whether related (paediatric and adult donors) or unrelated, should be followed up by the donation centre in the short, mid and long term according to the policy suggested by scientific organisations or as requested by national regulations. In particular, a short-term follow-up to document SAREs, and a long-term follow-up on a regular basis to document late effects of the donation or the mobilising agent, should be performed [19, 34]. The EBMT (for related donors) and the WMDA (for unrelated donors) recommend the long-term follow-up and care of donors for conditions related to the HPC donation for a minimum of ten years after donation. For unrelated donors this includes a mechanism for donors to contact the registry to report related medical concerns.

In addition, there are other European initiatives as ARTHIQS [35], which presented guidelines on the set-up and regulation of haematopoietic stem-cell donor follow-up registries and banking of cord blood, and Recommendation CM/Rec (2020)6 on measures for the protection of haematopoietic progenitor cell donors [36] from the Council of Europe, which develops recommendations to member states to establish harmonised HPC donor protection measures. Among others, they recommend ensuring that donors (related and unrelated, adults and minors) have been offered both appropriate psychological support in the event of post-donation difficulties and medical care.

24.10.2. Serious adverse reactions in the recipient

24.10.2.1. *Complications related to haematopoietic stem cell infusion*

Infusion of HPC is, in general, well tolerated. Complications are consequences of immunological incompatibility, iatrogenic toxicities, microbiological contamination and manufacturing/administrative errors. Some complications are similar to the complications caused by transfusion of standard blood products, which is particularly true for allogeneic HPC preparations if they contain ABO-incompatible RBC or plasma. Other complications are specific to HPC infusion, and are related to allogeneic and autologous HPC.

Adverse reactions can be immunological and non-immunological, acute and delayed.

24.10.2.1.1. Haemolysis of red blood cells

Donor–recipient mismatching in erythrocyte antigens is not a contraindication for HPC transplantation, but haemolytic reactions due to ABO and non-ABO antibodies may occur. Reactions can be acute and delayed. The risk of haemolysis is also dependent upon the type of HPC preparation because the content of RBC and plasma is different.

Acute haemolytic reactions are severe complications of HPC infusion. They are caused by ABO incompatibility between the donor and recipient. Usually, the risk of acute haemolysis is greater if the RBCs of the donor are incompatible with the recipient's plasma (major ABO incompatibility). However, haemolysis can also occur if the donor's plasma is incompatible with the recipient's RBC (minor ABO incompatibility). In general, low titres < 1:64 are associated with mild or no reactions, whereas high titres

(e.g. 1:1024) are associated with acute haemolytic reactions. The volume of incompatible RBC infused also determines reaction severity. Bone marrow contains a high amount of RBC and can cause acute haemolysis. The risk of haemolysis can be reduced by removal of antibodies from the patient's circulation utilising extracorporeal apheresis procedures or by removal of RBC from the bone-marrow preparations of the donor. In contrast, apheresis preparations usually have < 10–15 mL RBC, which is not enough for significant reactions.

Signs and symptoms of acute haemolytic reactions can be: chills, fever, dyspnoea, chest pain, back pain, headache, hypotension, oliguria, anuria, bleeding, shock and pain along veins. If an acute haemolytic reaction is suspected, cardiovascular and renal function must be maintained and development of disseminated intravascular coagulation prevented.

Delayed haemolytic reactions may occur if the recipient is allo-immunised on the infused RBC antigens of the donor, or if the recipient receives the donor's B lymphocytes within an HPC preparation, which can produce antibodies against the recipient's RBC ('passenger lymphocytes syndrome'). If the recipient is allo-immunised on the donor's RBC antigens, infusion of RBC can stimulate an anamnestic immune response of the residual B lymphocytes of the recipient. The antibody will reach a clinically significant level within 2-14 days after HPC infusion, whereas the infused RBC will remain in circulation. The haemolysis is limited by the amount of infused RBC.

If the recipient receives the donor's B lymphocytes in an HPC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, haemolysis can be more serious because of passenger lymphocyte syndrome. At greater risk are recipients who receive ABO minor-incompatible HPC. Typically, haemolysis will occur within 1-3 weeks after HPC infusion. Occasionally, life-threatening haemolysis can occur. Apheresis HPC preparations contain higher numbers of lymphocytes and carry a greater risk of delayed haemolysis.

Signs and symptoms of delayed haemolytic reactions are the same as for acute haemolytic reactions, but the severity differs.

In autologous settings and if cryopreserved HPC are used, passive haemolysis is more common.

24.10.2.1.2. Complications within the respiratory tract

HPC infusion frequently induces complications within the respiratory tract. During administration, patients often start coughing. Coughing is related primarily to application of cryopreserved autologous HPC, and is usually accompanied by nausea and vomiting. Dyspnoea, with mild decreases in the vital capacity of the lungs, is noted quite often.

Severe respiratory complications, such as acute respiratory failure, are rare. Transfusion-related acute lung injury (TRALI) can occur if granulocyte activation in the pulmonary vasculature is caused by neutrophil antibodies or bioactive mediators, which increase microcirculation permeability and allow massive leakage of fluids and proteins into the alveolar space and interstitium. Signs and symptoms of TRALI usually occur < 6 h after HPC infusion, and include acute respiratory distress, low-grade fever, hypoxaemia (oxygen saturation < 90 % on room air) and bilateral pulmonary infiltrates on frontal radiographs of the chest. If hypoxia is observed during HPC infusion, the infusion should be stopped immediately. Respiratory support should be as intensive as dictated by the clinical picture. Supplementation is necessary in almost all cases. Corticosteroids and diuretic drugs are not useful. In severe cases, transfer to an intensive care unit (ICU) may be necessary.

24.10.2.1.3. Febrile non-haemolytic reactions

During HPC infusion, patients may experience febrile non-haemolytic transfusion reactions (FNHTR). These reactions may be observed in allogeneic and autologous transplantation. FNHTR are manifested by a low-grade fever during, shortly after or ≤ 2 h after infusion of cells. FNHTR can be accompanied by chills, rigor and mild dyspnoea without evidence of haemolysis. This phenomenon may reflect the action of antibodies against leukocytes or the action of cytokines (present in infused preparations or generated by the recipient) after cell infusion.

No laboratory tests are helpful in predicting and preventing FNHTR. Any patient with fever, rigor and chills during HPC infusion should be evaluated, clinically and by laboratory tests, for haemolytic, septic or TRALI reactions. An underlying infection must also be excluded. FNHTR are short-lived complications, and anti-pyretic agents usually provide effective symptomatic relief.

24.10.2.1.4. Dimethyl sulphoxide toxicity

Dimethyl sulphoxide (DMSO) is the most widely used cryoprotectant, but it can detrimentally affect cell viability and is the cause of many side-effects observed during infusion. DMSO toxicity is the most common complication of infusion of cryopreserved HPC.

Within minutes of starting the infusion, a metabolite of DMSO is excreted through the lungs and

causes a garlic-like odour that can lead to a foul taste in the mouth. Infusion of DMSO can induce a wide range of other symptoms: pruritus; sedation; headache; nausea; vomiting; abdominal cramps; diarrhoea; flushing; low-grade fever; chills; dizziness; garlic-like odour; haemoglobinaemia with red-coloured urine; elevation of levels of hepatic enzymes; elevation of levels of creatinine kinase. DMSO toxicity has been linked to cardiovascular side-effects such as bradycardia or tachycardia, hypotension and, in rare cases, myocardial infarction.

DMSO toxicity is dose-dependent. The maximum daily intravenous dose of DMSO is 1 g/kg, which is equivalent to an infusion of 10 mL/kg of cells in 10 % DMSO solution. Premedication with anti-histamines, slowing the infusion rate, increasing the resting time between multiple infusion aliquots, dilution of thawed HPC preparations by albumin–dextran-40 solution at a ratio of 1:2 or 1:3, or removal of DMSO by washing can prevent symptoms and reduce the risk of DMSO-related toxic effects.

24.10.2.1.5. Neurological complications

Neurological symptoms during HPC infusion vary widely. Headache is common and can be related to increased intravascular volume. Occasionally, patients experience more severe side-effects such as muscle spasms and seizures. Cerebral infarcts and acute encephalopathy are rare. Simple muscle spasms often resolve spontaneously. For patients with acute mental changes, loss of consciousness or seizures, urgent intervention is necessary. HPC infusions should be stopped; rapid neurological assessment should be done as well as basic laboratory tests, including electrolytes and glucose. If seizures persist, anti-epileptic drugs are indicated. Patients should be transferred to an ICU.

Neurological complications are probably linked to a large number of non-mononuclear cells and/or caused by DMSO, but this suspicion has not been clearly demonstrated.

24.10.2.1.6. Cardiac toxicity

Cardiac toxicity is common and manifests as bradycardia and other disorders of cardiac rhythm. It is usually mild (though severe cases of arrhythmias have been reported). Severe bradycardia occurs more often in recipients of cryopreserved bone marrow, and may require aggressive supportive care.

Aetiology may be because of hypervolaemia due to extensive hydration before infusion, large volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells or underlying cardiac conditions.

24.10.2.1.7. Allergic reactions

Allergic reactions usually manifest as urticaria and pruritus. Most occur in patients receiving allogeneic transplants. Anaphylactic-type reactions are rare. Allergic reactions present as bronchospasm and/or laryngospasm, hypotension, severe dyspnoea, pulmonary and/or laryngeal oedema, facial burning and flushing, abdominal pain, diaphoresis, diarrhoea and dizziness.

Causes of allergic reactions are not clear. They may be related to the substances used during cell procurement, cell processing or cryopreservation, such as HES, dextran or DMSO, that can react with antibodies in the donor or recipient plasma, or with anti-immunoglobulin (Ig)A antibodies in IgA-deficient recipients. No laboratory tests can help to predict or prevent allergic reactions. Therapy is dependent upon symptoms. For mild reactions, administration of anti-histamines will be helpful or, in severe cases, corticosteroids, epinephrine and cardiorespiratory support.

24.10.2.1.8. Anticoagulation effects

Patients receiving non-cryopreserved and non-manipulated bone marrow have a greater risk of haemorrhage because of high concentrations of unfractionated heparin in bone-marrow grafts.

Risk of bleeding is increased in thrombocytopaenic recipients.

24.10.2.1.9. Hypertension/hypotension

Hypertension is more common in cryopreserved HPC or unmanipulated bone marrow HPC, because such grafts have a higher volume. Hypertension is a result of acute volume overload due to rapid infusion, prophylactic hydration and the hyperosmolality of the infused preparation.

Hypotension is also more common in cryopreserved HPC. It is linked with vasodilatation due to histamine generation. Premedication by anti-histamines decreases the incidence and severity of hypotension.

24.10.2.1.10. Acute renal failure

Acute renal failure is more common in application of a high volume of cryopreserved HPC. It is caused by a large amount of DMSO and cellular debris.

24.10.2.1.11. Bacterial contamination

Bacterial contamination of an HPC product is possible. Bone marrow, which is procured into an open system, has a higher rate of contamination than HPC collected from peripheral blood. Contam-

ination may occur at several steps in the process. It can be due to occult asymptomatic bacteraemia in the donor. For autologous donation, because of the particular nature of the graft and the recipient's condition because of the treatment, it is vital that HPC are procured irrespective of the possible febrile status of the patient, even if sepsis may be present. Although it is a rare occurrence, it should be kept in mind as a potential cause of bacterial contamination of grafts.

Contamination of HPC can also occur during procurement, processing, storage, thawing or sampling, due to breach of sterility.

After transfusion of contaminated HPC, symptoms of a septic reaction usually develop rapidly. High fever, tachycardia and hypotension, nausea and vomiting, and a 'shock-like' clinical picture should arouse suspicion of bacterial septicaemia. If such symptoms occur during HPC administration, the infusion should be stopped immediately and all infusion bags and equipment examined.

Known bacterial contamination of an HPC unit is not an absolute contraindication for HPC infusion. Patients receiving culture-positive preparations require antibiotic therapy, which can be antibiogram-specific or cover a broad spectrum of bacteria, starting optimally 2 days before transplantation.

There must be criteria for administration of preparations with positive microbial culture results. A contingency plan is expected to be in place in case of urgent medical need.

24.10.2.1.12. Transmission of infectious and genetic diseases

HPC preparations should be tested for transfusion of transmissible diseases according to national requirements. However, the potential risk of transmission of infectious agents by infected donors or cross-contamination during storage cannot be removed completely. A viral infection < 6 months after transplantation must be suspected to be due to transmission of hepatitis B or C virus (HBV, HCV) or human immunodeficiency virus (HIV). For other viral infections the period will differ, depending on the incubation period.

Screening of HPC volunteer donors for genetic disease is mainly based on their medical history and on the results of laboratory tests. The risk of transmission of a genetic disease is higher with cord blood than with the other sources of HPC (bone marrow and PBSC), since some diseases might not be evident at birth or even some months later.

According to the Notify Library, very few cases of genetic diseases transmission have been described after bone-marrow transplants (cyclic neutropaenia, Gaucher's disease). Autoimmune diseases transmission has also been reported (thyroiditis, type 1 diabetes, myasthenia gravis, vitiligo, etc.) [18]. All cases of suspected post-transplantation infection or genetic disease transmission related to HPC infusion must be reported immediately to the procurement site and/or donor registry, who have to follow the requirements of the national vigilance system.

24.10.2.1.13. Engraftment failure

After HPC transplantation, recovery must occur in populations of myeloid, erythroid and immune cells. The earliest sign of haematopoietic recovery is an increase in numbers of granulocytes and platelets in peripheral blood within days and weeks after graft infusion. Engraftment of erythroid lines and immune reconstruction occurs within weeks or months. Engraftment is dependent on the dose and source of progenitor cells, method of cell preparation, function of bone-marrow stroma, intensity of the preparative regimen, donor–recipient relationship and ABO compatibility.

Measurement of granulocyte and platelet engraftments provides essential information about the success of clinical protocols as well as the quality of procurement and processing of HPC.

Primary graft failure for HPC transplantation from bone marrow or peripheral blood is defined as a lack of neutrophil engraftment 28 days after transplantation and up to 42 days in the case of cord blood transplantations. Leukocyte recovery is designated as the first of three consecutive days in which the absolute neutrophil count is $> 500 \times 10^6$/L. Platelet engraftment is designated as the first day on which the platelet count is $> 20 \times 10^9$/L in an untransfused patient. The sign of erythroid recovery is $> 30 \times 10^9$/L reticulocytes or > 1 % reticulocytes in peripheral blood in an untransfused patient. T-cell engraftment is proof of mixed donor–host chimerism (5-95 % donor T-cells). Reasons for failure can be graft composition, graft source, HLA mismatch, ABO incompatibility or other reasons that can be attributed to the patient.

The transplant unit should report graft failure to the TE to enable thorough investigation of the quality and handling of grafts.

24.10.2.1.14. Graft-*versus*-host disease

GvHD is a serious and potentially lethal complication of allogeneic HPC transplantation. GvHD occurs if infused T-lymphocytes engraft in the recipient and react against the recipient's tissues. Any allogeneic HPC preparations can cause GvHD. Classic acute GvHD occurs < 100 days after transplanta-

tion, whereas chronic GvHD occurs > 100 days after transplantation, and the overlap GvH syndrome may occur without limit of time. Risk factors for the development of GvHD are: donor–recipient relationship (HLA disparity, gender matching, donor parity, donor age, ABO group mismatching), stem cell graft factors (source and graft composition, cell processing) and transplantation factors (condition and post-transplant immunosuppression regimens).

Clinical manifestations of GvHD typically involve the skin, liver and gastrointestinal tract in the acute setting, but can affect (among others) the eyes, oral mucosa, vagina, lungs, joints and neurological system.

To predict the outcome of acute GvHD, scoring based on organ involvement is important. Chronic GvHD is more likely in recipients of peripheral HPC than in recipients of bone-marrow HPC. It can be localised, affect only skin areas, and manifest as progressive systemic sclerosis, Sjögren's syndrome or primary biliary cirrhosis.

Treatment of GvHD includes high-dose corticosteroids, T-cell-suppression drugs, monoclonal antibodies targeting T-cells, extracorporeal photopheresis or mesenchymal stem cells (MSC).

24.10.3. Serious adverse reactions and events related to the graft

SAREs related to the graft – also referred to here as S(P)EAR (serious product events and adverse reactions) as defined by the WMDA – can be: inappropriate transportation, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital or infusion of a unit into the wrong recipient. The transplantation centre must report these incidences immediately to the TE, to the registries if appropriate and, according to national legislation, to Health Authorities responsible for tissues and cells.

24.10.4. Serious adverse reactions in haematopoietic progenitor cell donors

Deaths in unrelated HPC donors are very rare, and few cases have been reported to WMDA. A small number of deaths have been reported also in related donors, from causes such as subarachnoid haemorrhage, sickle cell crisis, myocardial infarction and pulmonary embolism. In some of these donors, pre-existing medical conditions were identified *post mortem*, highlighting the need for stringent medical suitability criteria and assessment of all HPC donors [14, 15, 34].

Bearing in mind that HPC donations are voluntary and altruistic acts of assumedly perfectly healthy individuals, it is the ethical and professional obligation of medical professionals and also good practice to notify, document, investigate and report SARs in the living donor, and not only those influencing the quality and safety of tissues and cells. SARs are uncommon in healthy donors and rare types of SAR or emerging trends are likely not to be noticed at the national level. SARs in stem cell registry donors are followed at the international level by the WMDA through an online web application to report in a standardised way any incident occurring to a donor or donated product [37]. Unfortunately, no consistent follow-up exists at present for related donors. The European Bone Marrow Transplantation Group (EBMT) has established a donor follow-up, which is included in the EBMT database [34].

24.10.4.1. *Complications in haematopoietic progenitor cell (bone marrow) donors*

Donation of bone marrow is, in general, a safe and well-tolerated procedure, but some mild symptoms related to induction of general anaesthesia are common. In most cases, donors recover fully within 2 weeks.

Reactions in bone-marrow donors include constitutional symptoms such as nausea, vomiting, anorexia, insomnia and fatigue (most common).

Complications related to puncture of bone marrow, such as pain upon procurement, walking, sitting and climbing stairs, as well as minor infection, are rare. Bone and soft-tissue trauma at the harvest site may cause pain, bleeding, oedema or nerve compression. Damage to a lumbosacral nerve root or penetration into the pelvic cavity or internal iliac vessels may cause severe morbidity. Anaesthesia carries an unavoidable (albeit very small) risk of life-threatening cardiac or respiratory events, as well as the possibility of allergic or idiosyncratic reactions to anaesthetic agents. Removal of large volumes of blood may cause symptoms of hypovolaemia or anaemia [19, 21, 24].

Cytopaenias (anaemia, thrombocytopaenia) and more serious reactions such as deep-vein thrombosis (DVT), thromboembolism, cerebrovascular accident and subdural bleeding have been documented. Post-donation septicaemia and anaesthesia-related complications have also been described, as well as respiratory complications such as pulmonary alveolitis and oedema.

24.10.4.2. *Complications in haematopoietic progenitor cell (apheresis) donors*

Complications are related to apheresis and administration of granulocyte-colony stimulating factor (rhG-CSF) or other mobilising agents used. Symptoms related to citrate infusion are the most common.

Additional complications related to apheresis include haematoma, arterial punctures, delayed bleeding, pain from injury to nerves or tendons, thrombophlebitis, local allergy, generalised allergic reactions, vasovagal reactions, haemolysis and air embolism. All severe conditions requiring hospitalisation or intervention, or resulting in death < 24 h after procurement, should be reported immediately.

Use of rhG-CSF in mobilisation is, in general, safe. Common short-term reactions related to rhG-CSF are bone pain, headache, myalgia, nausea, vomiting, diarrhoea, fatigue, fever and irritation at injection site. Most of these effects are reversible after discontinuation of rhG-CSF administration. Other rare reactions are splenic rupture, anaphylaxis, thrombosis, gout, iritis, keratitis, autoimmune hyperthyroidism, acute lung injury, capillary leak syndrome, exacerbation of rheumatoid arthritis, insomnia and reduced numbers of thrombocytes.

Short-term reactions due to the use of HPC binding inhibitors such as plerixafor are reported to occur in approximately 30 % of cases and consist of a local reaction at the injection site (erythema) and gastrointestinal side effects (diarrhoea, abdominal distention).

Reports from long-term follow-up studies in unrelated and related apheresis HPC donors mobilised with rhG-CSF demonstrate a similar incidence of leukaemia and other malignancies to those seen in the general population. All malignant diseases in all donors treated with rhG-CSF should be reported, regardless of the time of occurrence.

24.10.5. Biovigilance of mononuclear cells

The same requirements as for HPC(A) and HPC(M) apply also to MNC(A) (see above). The processing of MNC involves several steps where unexpected events may occur that have to be documented and reported (see §24.9 and Chapter 17), for instance, lower viability of frozen and thawed MNC than expected or human errors in calculating the dose of T-cells in DLI. In cases of low viability, DLI may still be used but this has to be documented and a risk analysis carried out. Possible adverse events associated with DLI are the development of acute and/or chronic GvHD and low blood counts. These complications may appear after 1 or more weeks after the administration of the $CD3^+$ cells.

As noted in section 24.9, similar complications, such as DMSO toxicity, transmission of infectious diseases or GvHD, may occur. In rare circumstances, serious product adverse events/reactions or complications in the donor are possible. Follow-up of the donor as stated in section 24.9 and Chapter 17 is recommended.

24.11. References

1. Schipper RF, D'Amaro J, Oudshoorn M. The probability of finding a suitable related donor for bone marrow transplant in extended families. *Blood* 1996; 87:800-4.
2. Carreras E, Dufour C, Mohty M, Kröger N. European Group for Blood and Marrow Transplantation (EBMT). *Haematopoietic stem cell transplantation and cellular therapies* [EBMT handbook. ISBN 978-3-030-02277-8]; 2019.
3. Farhadfar N, Hogan, WJ. Overview of the progress on haploidentical hematopoietic transplantation. *World J Transplant* 2016;6:665-74.
4. Ciurea SO, Al Malki MM, Kongtim P *et al.* The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. *Bone Marrow Transplant* 2020;55:12-24.
5. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med* 2006;354:1813-26.
6. Ruggeri L, Capanni M, Urbani E *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002 Mar 15; 295(5562):2097-100.
7. Shaffer BC, Hsu KC. How important is NK alloreactivity and KIR in allogeneic transplantation? *Best Pract Res Clin Haematol* 2016;29:351-8.
8. Petersdorf EW. Mismatched unrelated donor transplantation. *Semin Hematol* 2016;53:230-6.
9. Fleischhauer K, Shaw BE. HLA-DP in unrelated hematopoietic cell transplantation revisited: challenges and opportunities. *Blood* 2017;130:1089-96.
10. McCurdy SR, Fuchs EJ. Selecting the best haploidentical donor. *Semin Hematol* 2016;53:246-51.
11. Stavropoulos-Giokas C, Dinou A, Papassavas A. The role of HLA in cord blood transplantation. *Bone Marrow Res* 2012;2012;485160. https://doi.org/10.1155/2012/485160.
12. Sacchi N, Costeas P, Hartwell L *et al.* Quality assurance and clinical working groups of the World Marrow Donor Association. Haematopoietic stem cell donor registries: World Marrow Donor Association

recommendations for evaluation of donor health. *Bone Marrow Transpl* 2008;42(1):9-14.

13. WMDA (World Marrow Donor Association). Donor Medical Suitability Wiki, available at https://wmda.info/professionals/promoting-donor-care/donor-suitability/, accessed 14 April 2022.
14. Worel N, Buser A, Greinix HT *et al.* Suitability criteria for adult related donors: a consensus statement from the Worldwide Network for Blood and Marrow Transplantation Standing Committee on Donor Issues. *Biol Blood Marrow Trans* 2015;21(12):2052-60.
15. Bitan M, van Walraven SM, Worel N *et al.* Determination of eligibility in related pediatric hematopoietic cell donors: ethical and clinical considerations. Recommendations from a working group of the Worldwide Network for Blood and Marrow Transplantation Association. *Biol Blood Marrow Trans* 2015 Aug;22(1):96-103, pii S1083-8791(15) 00546-7. https://doi.org/10.1016/j.bbmt.2015.08.017.
16. American Academy of Pediatrics, Committee on Bio-Ethics. *Children as hematopoietic stem cell donors*, 2010.
17. Kenyon M and Babic A (eds). *The European Blood and Marrow Transplantation textbook for nurses*. Cham: Springer (under the auspices of EBMT), available at https://doi.org/10.1007/978-3-319-50026-3, accessed 22 April 2022.
18. Oviedo Convention and its protocols, available at www.coe.int/en/web/bioethics/oviedo-convention, accessed 14 April 2022.
19. Lown RN, Philippe J, Nawarro W *et al.* Unrelated adult stem cell donor medical suitability: recommendations from the Clinical working group committee of the World Marrow Donor Association. *Bone Marrow Transpl* 2014;49:880-6.
20. FACT–JACIE international standards for hematopoietic cellular therapy product collection, processing, and administration, 7th edition. Barcelona: European Society for Blood and Marrow Transfusion; 2018, available at www.ebmt.org/jacie-standards/7th-edition-effective-june-1st-2018, accessed 14 April 2022.
21. Halter J, Kodera Y, Ispizua AU *et al.* Severe events in donors after allogeneic hematopoietic stem cell donations. *Haematologica* 2009;94(1):94-101.
22. Foeken LM, Green A, Hurley CK *et al.* Donor registries working group of the World Marrow Donor Association. Monitoring the international use of unrelated donors for transplantation: the WMDA annual reports. *Bone Marrow Transpl* 2010;45(5):811-18.
23. Hölig K, Kramer M, Kroschinsky F *et al.* Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single center experience in 3928 donors. *Blood* 2009;114(18):3757-63.
24. Pulsipher MA, Chitphakdithai P, Miller JP *et al.* Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the National Marrow Donor Program. *Blood* 2009;113(15):3604-11.
25. WMDA (World Marrow Donor Association). *Recommendation for recombinant human G-CSF (G-CSF) that stem cell donor registries can use – the use of biosimilar G-CSF.* Document 20170905, available at www.wmda.info/wp-content/uploads/2017/06/20170905-WGME-Recommendation-GCSF1.pdf, accessed 13 April 2022.
26. Schumm M, Lang P, Bethge W *et al.* Depletion of T-Cell receptor alpha/beta and CD19 positive cells from apheresis products with CliniMACS device. *Cytotherapy* Oct 2013;15(10):1253-8. https://doi.org/10.1016/j.jcyt.2013.05.014.
27. Sutherland DR, Anderson L, Keeney M *et al.* The ISHAGE [International Society of Hematotherapy and Graft Engineering] guidelines for $CD34^+$ determination by flow cytometry. *Hematotherapy* Jun 1996; 5(3):213-26.
28. Keeney M, Chin-Yee I, Weir K *et al.* Single platform flow cytometric absolute $CD34^+$ cell counts based on the ISHAGE [International Society of Hematotherapy and Graft Engineering] guidelines. *Cytometry* 15 Apr 1998;34(2):61-70.
29. WMDA (World Marrow Donor Association). Global registration identifier for donors, available at https://wmda.info/professionals/optimising-search-match-connect/why-global-identifier/, accessed 14 April 2022.
30. WMDA (World Marrow Donor Association). International standards and guidelines for cross-border transport of HPC, available at https://wmda.info/wp-content/uploads/2021/05/GTR-2020-Summary-slides.pdf, accessed 22 April 2022.
31. Purtill D, Antonenas V, Chiappini P *et al.* Variable CD34+ recovery of cryopreserved allogeneic HPC products: transplant implications during the COVID-19 pandemic. *Blood Adv* 2020;4:4147-50. https://doi.org/10.1182/bloodadvances.2020002431.
32. SoHO V&S. *Guidelines for healthcare professionals on vigilance and surveillance of human tissues and cells*, Part 2 – Haematopoietic stem cells, Deliverable 10. Strasbourg, France: Council of Europe, available at www.notifylibrary.org/sites/default/files/SOHO%20V%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%202%20HPCs_0.pdf, accessed 14 April 2022.
33. WMDA (World Marrow Donor Association). Adverse events (SPEAR), available at https://wmda.info/professionals/promoting-donor-care/adverse-events-searspear/, accessed 8 July 2022.
34. Halter JP, van Walraven SM, Worel N *et al.* Allogeneic

hematopoietic stem cell donation – standardized assessment of donor outcome data: a consensus statement from the Worldwide Network for Blood and Marrow Transplantation (WBMT). *Bone Marrow Transpl* 2013;48:220-5.

35. ARTHIQS, *Guidance for establishing a hematopoietic progenitor cells donor follow-up registry*, Deliverable No. 8, available at www.arthiqs.eu/assets/user/Guidance%20WP5.pdf, accessed 14 April 2022.
36. Council of Europe, Recommendation CM/Rec (2020) 6 of the Council of Ministers to member states on establishing harmonised measures for the protection of haematopoietic progenitor cell donors, available at https://rm.coe.int/0900001680 9fdd5b, accessed 14 April 2022.
37. WMDA (World Marrow Donor Association). User Guide to S (P)EAR: Reporting serious (product) events and adverse reactions via an online reporting tool, available at https://spear.wmda.info/login?returnUrl=%2Freports, accessed 8 July 2022.

Related material

Appendix 36. Donor search through registries for haematopoietic progenitor cell transplantation

Chapter 25. **Umbilical cord blood progenitors**

25.1. Introduction

Umbilical cord blood (UCB) is a source of haematopoietic progenitor cells (HPC) that can reconstitute the haematopoietic and immune systems. These cells bear unique properties, including a high progenitor cell proliferation ability and immune naïvety. Usually UCB is discarded after delivery, but the blood remaining in the placenta after clamping can be collected, processed and stored safely.

Since the first transplant procedure performed in Paris by Professor Gluckman and her team in 1988 in a child with Fanconi anaemia, more than 55 000 patients have received transplants. According to the World Marrow Donor Association (WMDA), more than 800 000 units are registered at the WMDA Search & Match Service[1-2]. The database is searchable for any patient in need.

The use of UCB cells offers several advantages, including no risk for the donor, prompt availability as an off-the-shelf medicinal product and clinical benefits like low incidence of graft-*versus*-host disease, even in partially matched transplants, which increases the patient's chance of finding a suitable donor. UCB banks have therefore facilitated universal access to the therapy, in particular to ethnic minorities. However, there are also some disadvantages: the number of stem cells in UCB is relatively low and often associated with slow engraftment, and it is not possible to use a donor lymphocyte infusion after transplantation. There are interesting approaches to improve outcomes, including the use of very high cellular units, double cord and intrabone transplantation, and promising protocols for progenitor cell *ex vivo* expansion. The most applied protocol in older patients uses reduced intensity conditioning and a double UCB graft approach. Recently, immune active properties like an enhanced graft-*versus*-leukaemia effect have been proposed, and reconstitution of the immune system is the current research area to achieve an improvement of UCB transplant methods [3].

Table 25.1. **Advantages and limitations of the use of umbilical cord blood transplantation *versus* bone marrow transplantation and peripheral blood transplantation**

Advantages	Limitations
Expanded access to transplant • Higher availability of donor • Faster search and shorter time to transplant • Greater human leukocyte antigen (HLA) disparity allowed with low incidence of acute graft-*versus*-host disease Lower risk of transmission of viral infections More versatile transplant planning No risk of donor refusal	Slower engraftment Higher risk of non-immunological rejection (graft failure) Remote possibility of transmission of a genetic disease Greater delay in immune reconstitution No possibility of donor lymphocyte infusion

Source: *The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies* [4].

Thus, UCB transplantation is an alternative option in the absence of a fully matched related or unrelated donor available at an appropriate time. Advantages and limitations of its use over adult stem cell sources are summarised in Table 25.1.

The regulatory framework of UCB collection and processing to make it available to patients in need has evolved considerably over the past two and a half decades. Accreditation and regulation have instilled confidence in clinicians, allowing them to select a UCB unit from across a wide range of banks in many countries.

A UCB bank is a multidisciplinary structure that is responsible for the recruitment and subsequent management of maternal donors as well as the collection, processing, testing, cryopreservation, storage, listing, reservation, release and distribution of units for administration. In addition to the legal requirements of Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004, some scientific societies have developed high quality standards. Professional societies like the FACT and AABB have developed international standards [5-6] that have a worldwide perspective to improve the quality of the products available for transplantation. The accreditation status of the UCB bank is one of the selection criteria for many transplant centres during the search for an UCB unit. Table 25.2 shows the components of a UCB bank, facilities needed and their characteristics.

The following generic chapters (Part A) of this Guide all apply to UCB banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance

Table 25.2. **Structural components of an umbilical cord blood bank**

Facility	Characteristics
Procurement	Collection must take place in a maternity unit and be performed by trained health professionals using a validated technique without any interference in labour care. Besides the general requirements described in Chapter 7, a collection site must ensure secure storage of the UCB unit, associated samples, maternal samples and related documentation until they are transported to the UCB processing facility. Transportation method must be validated and time minimised.
Processing	All general requirements described in Chapter 9 apply. Processing must be secure and have adequate space to perform all activities in a safe and sanitary manner. Relevant environmental conditions that could affect the safety and potency of the UCB unit need to be defined and monitored.
Cryostorage	UCB units must be stored in either liquid or vapour-phase liquid nitrogen below – 140 °C. All refrigerators, freezers and cryostorage tanks used for storage of UCB units, associated reference samples and maternal samples must have a system to continuously monitor and regularly record the temperature. There must be an alarm system in place 24 h a day. Additional storage devices of appropriate temperature must be available in the event that a primary storage device fails.
Testing	Agreements must be in place with laboratories performing cell counts, flow cytometry and potency assay. Other laboratories needed are: • accredited human leukocyte antigen (HLA) laboratory, • immuno-haematology lab, • a certified laboratory for infectious disease marker (IDM) testing, • laboratories for haemoglobin screening. Testing should be undertaken in accordance with national and international regulations (see also Chapter 6).
Administrative area	UCB banks need to communicate with maternal donors, registries and transplant centres. A quality-assurance system must ensure that UCB units become available for international registry search only after processing, with medical and quality review, has been completed. Documentation related to requests for UCB units or for samples, results of testing and records of transportation and shipping between facilities must be retained in accordance with applicable national laws and regulations.

r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines the additional specific requirements for UCB banking and transplantation.

25.2. Recruitment of potential donors, identification and consent

25.2.1. Donor recruitment

The therapeutic properties of UCB-derived cells and potentially of their components require the establishment of a donation programme that enables the safe collection of residual blood contained in the placenta and umbilical cord after giving appropriate information to the maternal donors.

Public initiatives promote donation of UCB units, usually to a not-for-profit organisation, with the aim of providing UCB units for transplantation or other approved clinical applications to any patient in need. Many public UCB banks offer also a service for family UCB banking in case of illness in one of the siblings or other members of the donor's family [7]. Public UCB banks are generally committed to networking in order to increase access to UCBs through worldwide donor registries, associated in WMDA.

The Council of Europe has been studying the issue of UCB donation for several years and has always been concerned about the proliferation of UCB banks dedicated to the procurement and storage of UCB for autologous or family use. This concern resulted in adoption of Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous UCB banks, and its explanatory memorandum [8], which recommends that member states allow establishment of UCB banks only for altruistic and voluntary donations of UCB. In the case of autologous (or family-use) UCB banks, those organisations must clearly inform parents about the differences between the various medical objectives of autologous and allogeneic donations and about the uncertainties relating to the medical applications of autologous UCB preservation. In any case, autologous UCB banks must meet the same quality and safety standards as for allogeneic UCB donation and banking.

The Council of Europe has published the booklet *Umbilical cord blood banking: a guide for parents* to provide clear, accurate and balanced information about the use of UCB in medical treatment and to guide parents through their blood-storage options [9]. If a family member with a potential transplantable condition exists, related UCB units can be collected prospectively and stored for later use.

Donor recruitment (see also Chapter 4) usually starts during pregnancy, with information given by the woman's healthcare provider, but it may also occur as late as at admission into the maternity unit, as soon as parents are informed about this possibility and the mother is in good condition for signing an informed consent.

Information leaflets or brochures to inform mothers about UCB banking are an important part of the recruitment plan. This, supported by the right information, may clearly bring parents to decide the final application of their donation. Information should include at a minimum:

a. donor exclusion criteria;
b. the potential benefits and risks of UCB donation;
c. testing to be performed;
d. potential use (transplantation or other proven clinical use);
e. UCB unit exclusion criteria;
f. data protection.

Any claims made in recruitment material should be supported by scientific evidence.

It is during pregnancy that expectant mothers are encouraged to gather information and ask questions about the UCB collection procedure. Motivated personnel at collection sites are an important way to approach potential maternal donors. Training of physicians and health professionals on this step is essential to make sure that information provided to parents is accurate and that all their questions can be answered.

25.2.2. Informed consent

Informed consent has to be signed by women who agree to donate the UCB of their offspring to a UCB bank. General considerations are shown in Chapter 4. The consent that mothers must sign must cover in writing all aspects related to the donation. Normally, there are questions about performing infectious disease marker (IDM) tests, contacting the maternal donor in case an IDM test is positive, using units for research, checking medical notes, etc. Asking for informed consent and providing information about it is not recommended during active labour in order to avoid distraction linked to physical and emotional stress. Consent can be obtained in a single step prior to collection, when the donation process is initiated early in the pregnancy, or in two steps: a pre-consent followed by a full consent before

the UCB unit is placed into a clinical inventory. As an example, Appendix 37 shows a model of informed consent from a European CB bank initiative.

25.2.3. Donor evaluation

After maternal donor recruitment, trained personnel must determine the donor's eligibility. It is important to ensure that the donation is safe for future recipients. Maternal and infant donor eligibility must be determined on the basis of the results of screening and testing in accordance with national regulations. To assess donor eligibility, a donor medical history interview, which includes assessment for high-risk behaviours, must be conducted to identify risk factors for transmissible and genetic diseases (see Chapter 5 for further details). The mother will be asked to provide personal and family medical details. There must be written criteria for maternal and infant donor evaluation and management.

In general, transmissible disease, either infectious or genetic, and certain risk behaviours exclude the possibility of donation, and those factors should be taken into account when evaluating a potential donor. In addition to these considerations, there are absolute contraindications that should be taken into account when evaluating a potential donor [10]; they can be summarised in the following non-exhaustive list:

a. autoimmune diseases if the mother received treatment in the last 12 months;
b. malignancy (except basal cell carcinoma and *in situ* cancer treated and cured);
c. inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis);
d. if the mother has received donated eggs or embryos since 1980;
e. evidence of active or chronic infection;
f. live immunisation (vaccination) during this pregnancy;
g. myasthenia gravis;
h. myelodysplastic or myeloproliferative syndrome;
i. unexplained night sweats;
j. animal bite;
k. organ recipient.

Appendix 38 shows an example of a maternal health questionnaire to evaluate donor eligibility as used in a European CB bank and Appendix 39 shows an example of a Covid-19 infection risk assessment.

The medical history evaluation must be obtained while the mother is able to concentrate on the answers to the questionnaire and is not distracted by aspects of labour. The language used must be understood by her. It is not recommended that either family or friends serve as interpreters or translators. Confidentiality must be preserved. The results of this evaluation must be documented in the clinical history and reviewed by trained personnel.

If responses generate medical concerns, the collection should be rejected or cancelled. The mother's travel history to endemic areas must be obtained and documented, and eligibility determined according to national regulations. Screening for human transmissible spongiform encephalopathy, including Creutzfeldt–Jakob disease, must be documented. If history of communicable disease risk was obtained in advance of the maternal donor's presentation for delivery, the history must be updated to include information up to the time of delivery. In the case of a woman who gives birth to an infant donor not genetically hers, her communicable disease risk history must be obtained. The questionnaire must include questions to obtain at a minimum genetic history, malignant disease and inherited disorders that may be transmissible to the recipient.

In addition, IDM tests on maternal blood samples must be performed within seven days before or after collection of the UCB unit. These samples will be tested for evidence of infection of HIV1, HIV2, hepatitis B, hepatitis C, syphilis and any additional markers according to local regulations. Assays used for testing must be validated for use in volunteer blood or tissue donations. According to the EU regulation, if initial IDM tests do not include nucleic acid tests for HIV, HBV and HCV, then a second IDM test 180 days after donation must be performed. (See Chapter 6 for further details.)

25.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) is crucial to ensure umbilical cord blood safety. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk-reducing measures, as needed.

25.3.1. Procurement procedures

Procurement typically involves the following steps:

a. The umbilical cord is clamped as distal from the placenta as possible. No interference with labour and delivery must occur in order to protect mother and newborn safety. Nowadays, many obstetrical medical associations recommend delayed clamping. Evidence suggests that an acceptable time of 1 minute is compatible with public UCB banking [11];
b. A section of the cord is cleaned with a suitable disinfectant;
c. A needle that is attached to the collection bag is inserted into the umbilical cord vein;
d. The collection bag is filled by gravity until the cord looks 'white' and all the blood from the placenta and umbilical cord is drained into the bag;
e. The collection bag must be appropriately labelled.

There are two main techniques to collect blood from the cord vein: before the placenta is delivered *(in utero)* or after the placenta is delivered *(ex utero)*. Both procurement techniques give similar results and their use will depend on the ability of the maternity unit to collect the UCB.

In any case, the individuals performing the collection must be adequately trained. In both cases the collection bag must contain an adequate volume of anticoagulant (i.e. CPD) to prevent clotting.

After procurement, the healthcare provider in charge completes a report describing the labour phase, listing the events to be evaluated for acceptance of the unit, such as presence of fever, complications, type of delivery, etc. Before temporary storage, there is an opportunity to check the units and decide whether to transport them to the lab for processing and storage. If for example the TNC count is too low, it could be a criterion for not shipping a UCB from a collection site to the banking site [12]. This decision must be documented.

Within the biovigilance process, severe adverse events and reactions need to be notified to the Health Authority in accordance with established laws and regulations (see also Chapter 17).

25.3.2. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC viability occurs during non-frozen storage. Nevertheless, UCB should be temporarily stored in non-frozen conditions after collection, during transportation to the processing facility. Cell viability decreases, and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators. The distance from the collection and the processing facilities may be considerable, and therefore the maximum time between collection and the start of processing should be established. It is recommended that cryopreservation of unrelated UCB units should be initiated as soon as possible within 48 h and cryopreservation of related units within 72 h [5]. The UCB bank should validate the storage and transport conditions of the UCB units.

The UCB procurement bag will be identified with the following labels and paperwork:

a. unique UCB and maternal codes;
b. product name;
c. procurement site name or identifier;
d. date/time of collection;
e. name and volume/concentration of anticoagulants;
f. recommended storage temperature;
g. biohazard sign and/or other warning labels, following national regulations;
h. statement 'Related donor' where applicable;
i. donor name for related UCB units;
j. recipient family or individual name if known.

Transportation should be done using the same criteria as for other progenitor cell products, especially in the requirements for containers, temperature monitoring and labelling (see Chapter 7). Storage containers with tamper-evident seals must be used (see Chapter 12). Shipping method must be validated and must meet transport regulations for this type of product. Upon receipt, the integrity of the UCB units and their containers will be checked, and any deviation will be recorded within the processing records.

25.4. Processing of umbilical cord blood progenitor cells

A UCB bank must have appropriate facilities and personnel for the reception, processing, testing and storage of UCB and maternal blood. All processes should be performed in compliance with national and EU regulations (in EU countries). In selecting an appropriate air-quality specification for processing UCB, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered.

Where aspects of processing, testing or storage are performed by an external party, there must be a

written agreement in place between the UCB bank and the external party providing the service. Factors influencing the air-quality specification for processing HPC from umbilical cord also apply (see Table 25.5 and Chapter 8).

A UCB bank structure needs to co-ordinate different lab facilities, including a processing laboratory, a cryogenic storage area and associated testing laboratories for quality control of individual batches.

Setting up a high-level processing lab is very important since there is a clear correlation between good practice, quality certification and the outcome of UCB transplantation [13].

25.4.1. Reception at processing facility

On receipt of a UCB unit, a series of checks needs to be performed on the unit, on the associated samples and on the accompanying documentation, to verify and determine whether specific acceptance criteria are met. These include parameters such as volume, total nucleated cell (TNC) content, correct documentation and labelling, signed maternal donor consent, appropriate transport temperature, absence of large/multiple clots, transport conditions and acceptable time in transit from procurement centre to processing laboratory. Once a UCB unit meets the initial acceptance criteria it will continue on to be processed.

25.4.2. Volume/red blood cell reduction

Despite some loss of cells, volume reduction including red blood cell (RBC) and plasma depletion has practical and clinical benefits: it allows efficient storage of UCB in terms of space and cost, and, most importantly, it eliminates the following when infused after thawing:

- potentially ABO-incompatible plasma,
- free haemoglobin,
- RBC stroma.

The eliminated RBC and plasma components can be used for immediate or future testing, if validated test systems for this application are available, thereby minimising the loss of the actual UCB product for testing purposes [14].

The final product volume and cellular characteristics are dependent on the starting product as well as the processing/separation technique. Over the past decade three major methods have been used in large-scale banking which produce reproducible results that could be standardised. These include the manual method using hydroxyethyl starch for RBC sedimentation or other proprietary reagents, the semi-automated bottom-and-top method, and newer fully automated and programmable closed systems.

Whichever platform is employed, it is essential that the equipment and reagents used do not adversely affect the viability of the cells, that the process does not allow the introduction of adventitious agents or the transmission of communicable disease, and that the method be validated to allow optimal recovery of the fraction of interest.

25.4.3. Cryopreservation, thawing and infusion

25.4.3.1. *Cryopreservation*

The selection of a suitable protocol for cryopreservation of UCB is critical to optimise the recovery of functionally viable HPC [15]. Potential causes of cell damage include type and concentration of cryoprotectant, cell concentration, and cooling and warming rates, as well as level of control of storage conditions (see also Chapter 10).

Standard operational procedures (SOPs) related to cryopreservation should specify that the following information is recorded for each unit:

a. TNC concentration within a defined range;
b. the type of cryoprotectant, its final concentration and the duration of the cell exposure prior to freezing;
c. method of freezing and end-point temperature of cooling;
d. cooling rate within a defined range;
e. freezing curve parameter within a defined range;
f. storage temperature.

UCB units must be stored in freezing bags designed and approved for the cryopreservation of human cells and placed into metal cassettes to provide protection during freezing, storage, transportation and shipping. It is important that, after filling, each freezing bag is visually examined for possible leaks and breakage of seals. In this regard storage temperature must be maintained at −140 °C or colder. Both vapour or liquid phases can be used, but the use of an overwrap bag for prevention of cross-contamination is required if the units are immersed in liquid nitrogen. As reference samples each freezing bag must have integrally attached, at minimum, two segments of adequate volume to assess identity and potency of the UCB cells prior to release.

UCB units should be cryopreserved using a controlled-rate freezer with a validated freezing programme. The majority of UCB banks use cooling rates of 1-5 °C/min in order to allow the cells to slowly

dehydrate as the ice phase progresses and the extracellular solute concentration increases. Cryoprotectants used for UCB are those generally established for other HPC sources. In general, a concentration of 10 % DMSO is considered optimal for UCB. When used in conjunction with DMSO, other cryoprotectants, like Dextran-40, enhance the cryoprotective effect by allowing stabilisation of the cell membrane. While alternatives have been proposed, it is generally considered that a combination of 10 % DMSO and 1 % Dextran-40 results in the best recovery rates for TNC, $CD34^+$ and colony-forming units (CFU). Prolonged exposure of cells to DMSO can result in damage to cells. It is therefore essential that the duration from addition of cryoprotectant to initiation of freezing is minimised and the maximum time allowed should be validated by the bank.

In addition to the two contiguous segments, for each banked unit it is necessary to store several samples. Netcord-FACT International Cord Blood Standards require, from each UCB unit, at least 2×10^6 TNC divided in two vials, suitable material for preparation of genomic DNA, and plasma; and, from the maternal donor, serum and/or plasma and suitable material for preparation of genomic DNA. All the samples must be stored at −70 °C or colder. Representative samples intended for viability or potency analysis must be stored under the same conditions as the UCB unit.

25.4.3.2. *Thawing and infusion*

It is important to ensure that the transplant centre receives information on how to handle and use the UCB unit. Handling includes thawing, dilution and washing of the UCB unit. Providing information about indications, contraindications and cautions is the responsibility of the UCB bank. A jointly prepared document, *Circular of information for the use of cellular therapy products*, is available online [4, 16]. Along with this circular, UCB banks should be able to provide instructions for a validated thawing method of their UCB unit. Units that have not been red cell reduced prior to cryopreservation should be washed, as recommended by FACT-JACIE, while a buffy coat enriched UCB unit can be simply diluted [17] (see also §24.4.4.3).

25.5. Quality control

25.5.1. Biological information needed to confirm donor suitability and recruitment

All clinical and biological information pertaining to donor identification, screening and recruitment must be kept, along with all information pertaining to processing and distribution. This information must remain as a permanent part of the preparation and release file; see Chapter 2 and Chapter 16 for more details.

Details on the nature of such information and the procedure to obtain it are provided in Chapter 4 and Chapter 5 of this Guide.

25.5.2. Safety controls

In order to provide a safe UCB product for release, it is essential that UCB units are screened and tested for communicable diseases (see also Chapter 6). Maternal blood obtained within 7 days before or after the collection of the unit is used as a surrogate test for IDMs, and is strongly reflective of the infectious status of the UCB units due to the shared circulation during gestation. Testing the UCB unit for IDM provides an additional degree of safety.

At a minimum, prior to release for administration, the maternal donor of each UCB unit must be tested for evidence of infection by at least the following communicable disease agents:

a. human immunodeficiency virus, type 1;
b. human immunodeficiency virus, type 2;
c. hepatitis B virus;
d. hepatitis C virus;
e. *Treponema pallidum* (syphilis);
f. any additional agents required by national regulations or locally endemic disease.

A medical and genetic history of the infant donor's family must also be obtained to prevent the transmission of malignant diseases and inherited disorders.

UCB units for unrelated use must be shown to be free of microbial contamination. Microbial testing must be performed using a system validated for the growth of aerobic and anaerobic bacteria and fungi (see §11.3). For related UCB units, the results of positive microbial tests must include identity and antibiogram(s) of the organism(s), and these results must be reported to the prospective clinical programme.

Prior to release for administration, each UCB unit must have undergone haemoglobinopathy screening, regardless of the family's ethnic background or history.

Mechanisms for donor counselling should be in place if there is a positive test result for any IDM (other than *Cytomegalovirus*), an abnormal haemoglobinopathy screening or any other abnormal test finding. Every effort should be made to notify the

mother, and/or her physician. The UCB bank must have policies for handling specific cases.

25.5.3. Quality specifications

In order to characterise a UCB unit, identity, purity and potency assays must be performed and evaluated. Table 25.3 shows a list of reference values suggested by the 7th edition of the Netcord–FACT standards to determine the quality of a UCB unit stored for clinical administration.

Meeting UCB quality specifications and having very good banking practice will ensure a successful UCB transplantation [18].

25.5.4. Release criteria

The UCB bank must receive a formal request from the transplant centre before the work-up starts. Return of unrelated UCB units is generally not permitted.

The three tests to be performed by the bank before a UCB unit can leave storage premises are: verification of donor identity; potency assessment; and safety evaluation. These tests are summed up below.

25.5.4.1. *Verification of donor identity*

UCB unit identity can be verified by performing HLA-typing using a contiguous segment physically attached to the freezing bag containing cryopreserved UCB cells. The UCB bank must have a policy in place for the cases where there are no remaining attached segments. Verifying the maternal HLA haplotype would add additional safety requirements to validate HLA typing and to ensure maternal testing and assessment corresponds to the product selected [19].

25.5.4.2. *Potency assessment*

It is a requirement to assess the functional capacity of the UCB unit prior to release to the transplant centre. CFU assay is the surrogate potency usually used and its ability to predict engraftment should be validated. CFUs are grown from functionally viable cells and the results of this assay increase confidence in UCB unit quality and ability to engraft. It is recommended to perform CFU assay from a frozen contiguous segment prior to release for administration since 8-12 days are needed for the read-out of cellular growth.

25.5.4.3. *Safety evaluation*

IDM testing of the maternal samples is understood to be a surrogate test, and strongly reflective of the infectious status of the UCB unit. Prior to release for administration the results of maternal donor screening must be available. Because of differing national regulations, testing for additional infectious agents by IDM test may be required by the transplant centres.

Table 25.3. **Specification requirements for unrelated UCB units**

Test	**Unrelated Specification**		**Related Specification**	
	Prior to cryopreservation	**Thawed representative sample**	**Prior to cryopreservation**	**Thawed representative sample**
Total nucleated cell count	$\geq 5.0 \times 10^8$		Enumerated	
Total nucleated cell recovery	Should be ≥ 60 %		Should be ≥ 60 %	
Viability of TNC	≥ 85 %		≥ 70 %	
Viable CD34 count	$\geq 1.25 \times 10^6$			
Viability of CD34 cells		≥ 70 %		
CFU (or other potency assays)		Growth (or positive result for potency)		Growth (or positive result for potency)
Microbial screen	Negative for aerobes, anaerobes, fungus		Negative for aerobic and anaerobic bacteria and fungi – OR – identify and provide results of antibiotic sensitivities	
Donor screening and testing	Acceptable as defined by applicable law		Acceptable as defined by applicable law	
Identity		Verified		Verified

Source: Modified from 7th edition of Netcord-FACT standards [5].

25.6. Labelling and packaging

Packaging is designed at all steps with two objectives: to protect the cell preparation and to protect personnel and the environment. The primary packaging must be sterile and made of a biologically compatible material. Cryopreservation requires the use of liquid-nitrogen-resistant bags and labels.

From procurement to distribution, labelling must unambiguously identify the UCB unit. Each label must include at least the unique identifier, the proper name of product, the intended recipient (if known), the type of manipulation, the anticoagulants and additives used and the conditions under which the cells are to be stored and distributed. The recipient must be identified (but not the donor) when cells are distributed for administration. Labelling must allow the UCB bank to ensure the link between the UCB unit and its samples and records. Because UCB bags are normally too small for a standard-size label, a partial label at distribution is acceptable and must include at least a unique numeric or alphanumeric identifier, the proper name of the product and the product code. Additional information can be included in a tie tag and/or in the accompanying documentation.

International standards for labelling cellular therapy products are now available (e.g. ISBT128, Eurocode) and their implementation is required by specific accreditation bodies. Global labelling systems promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. For tissues and cells procured and/or distributed in the EU, the Single European Code (SEC) must be used. See also Chapter 14 and the EU coding platform [20].

25.7. Storage

In addition to the general requirements described in Chapter 10, including the long-term storage required, UCB banks must have an inventory management system to ensure that each UCB unit and its associated reference samples, maternal samples and records can be located in a timely manner. This inventory management system should prevent mix-ups or contamination of the UCB units during storage, and address the duration of the storage for cryopreserved UCB units. The UCB banks need to establish and validate the duration and conditions of storage; the effects of long-term storage on the viability, potency and sterility of the UCB cells should be evaluated in a stability protocol. A procedure for quarantine to minimise the risk of microbial cross-contamination of UCB units must be in place. Release of a UCB unit from quarantine should be based upon the evaluation of the testing and screening results pertinent to that UCB unit, in accordance with applicable national laws and regulations.

Refrigerators and freezers used for the storage of UCB units and all associated reference products should not be used for any other purpose, in order to minimise the risk of cross-contamination.

UCB units are intended for long-term storage and must be stored at −140 °C or colder. Each facility should assess the potential risk of transient warming events during processing and/or storage. Examples of these events include transfer of UCB units from the controlled-rate freezer to the cryostorage tank, removal of segments for HLA verification testing or other tests required and storage of UCB units in vapour vessels that may exhibit unstable temperatures when open. Each step should be validated to show that the viability and potency of the UCB unit have not been compromised.

25.8. Distribution and transport conditions

Internal and external transport or shipping must be controlled, and records must allow tracking and tracing of the UCB unit from UCB bank to the transplant centre. Methods of transportation and shipping must be described in operating procedures. Container validation and courier qualification should be performed periodically. Transport containers must be appropriately labelled and secured with tamper-evident seals, and must conform to applicable regulations. For shipment of the cryopreserved UCB units, a dry-shipper must be used, and the temperature monitored and recorded to detect temperature excursions. A plan for alternative transportation or shipping in an emergency should be in place. Transportation records must be maintained by the shipping facility and shared with the receiving facility. Appropriate training of the personnel in charge of transportation should be documented. See also Chapter 12.

25.9. Biovigilance

Adverse events and reactions (serious and non-serious) must be recorded, reported and investigated according to corresponding national regulations for tissues and cells as described in Chapter 17.

In EU member states, the requirements for traceability, notification of serious adverse reactions and events and certain technical requirements for the

coding, processing, preservation, storage and distribution of human tissues and cells are detailed in Directive 2006/86/EC.

Tissue establishments must have standard operational procedures (SOPs) for managing serious adverse reactions and events (SAREs). They should also provide centres carrying out clinical applications with accurate and updated information and training on SAREs in the area of HPC transplantation.

Tissue establishments and healthcare professionals may use EU SoHO V&S guidelines as they provide essential information for the detection, reporting/confirmation and investigation of SAREs. There is general guidance on implementation of good vigilance and surveillance practice, alongside definitions of SAREs, in Chapter 17.

25.9.1. Serious adverse reactions and events in the recipient

25.9.1.1. *Complications related to UCB stem cell infusion*

This topic is also discussed in Chapter 24 (see §24.10.2). In the use of UCB the most frequent reactions are those related to effects derived from infusing incompatible RBC, cryoprotectant or other adventitious substances used for volume reduction. The most common infusion reactions are hypertension, nausea and vomiting, bradycardia and chest pain. Table 25.4 summarises the most common reactions.

25.9.1.2. *Patient follow-up*

It is recommended to request information on defined time periods for every CB unit released for administration of haematopoietic reconstitution including post-thaw cellular data to evaluate graft purity and potency, and additionally clinical follow-up including, for example, time to neutrophil and platelet engraftment, chimerism in case of allogeneic transplantation, graft-*versus*-host disease incidences and survival results.

This information is valuable to assess overall performance of the CB bank procedures and additionally confirm products' quality and their stability over time.

Table 25.4. **Common reactions after UCB infusion**

Immune reactions	• haemolysis of red blood cells • febrile non-haemolytic reactions • allergic reactions
Systemic complications	• complications within the respiratory tract • neurological complications • cardiac toxicity • acute renal failure • anticoagulation effects
Biological	• engraftment failure • graft-*versus*-host disease
Transmissible	• bacterial contamination • transmission of infectious and genetic diseases

25.9.2. Serious adverse reactions and events related to the product

Despite following JACIE guidelines on UCB, there may occur what are called serious product events and adverse reactions, such as inappropriate transportation, receipt of a wrong unit, receipt of a damaged unit package, incorrect/non-labelled unit, non-receipt of a transplant, inappropriate storage in hospital or infusion of a unit into the wrong recipient. In all such cases the transplantation centre must immediately report this matter to the tissue establishment and, according to national legislation, to the Health Authorities. In addition, SARs related to the product are followed at the international level by the WMDA through an online web application to report in a standardised way any incident on unrelated donated product [21].

If clinically relevant, it is recommended that a policy to inform the maternal and/or UCB donor is adopted if there are donor consequences of (genetic) findings in donor cells in the recipient.

25.9.3. Serious adverse reactions and events in umbilical cord blood progenitor cell donors and donor follow-up

There is almost no risk in CB collection. But, in any case CB collection should respect the recommended policy of cord clamping that, according to WHO guidelines on intrapartum care for a positive childbirth experience [22], requires the practice of delayed clamping (waiting at least 1 minute before clamping according to recommendations). In addition, CB collection must avoid any interference with labour.

The UCB bank must have a policy for the follow-up of both maternal and infant donors and for the management of donation-associated adverse events. The policy must define the time period within which to contact donors (see Chapter 3 for further details).

25.10. References

1. World Marrow Donor Association (WMDA), website, available at www.wmda.info/ accessed 15 April 2022.
2. Querol S, Rubinstein P, Madrigal A. The wider perspective: cord blood banks and their future prospects. *Br J Haematol* 2021 Nov;195(4):507-17. https://doi.org/10.1111/bjh.17468. Epub Apr 20 ahead of print. PMID: 33877692.
3. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood* 2013 Jul 25;122(4):491-8.
4. Querol S, Rocha V. Procurement and management of cord blood. In: *The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies*, ed. Carreras E, Dufour C, Mohty M, Kroger N. Cham, Switzerland: Springer 2019. https://doi.org/10.1007/978-3-030-02278-5.
5. Netcord–FACT. *Standards*, 7th edition. Foundation for the accreditation of cellular therapy at the University of Nebraska medical center, available at www.factwebsite.org, accessed 15 April 2022.
6. AABB UCB banking standards. Advancing Transfusion and Cellular Therapies Worldwide, available at www.aabb.org/standards-accreditation/standards, accessed 15 April 2022.
7. Gluckman E, Ruggeri A, Rocha V *et al.;* Eurocord, Netcord, World Marrow Donor Association and National Marrow Donor Program. Family-directed umbilical cord blood banking. *Haematologica* 2011 Nov;96(11):1700-7.
8. Recommendation Rec (2004) 8 of the Committee of Ministers to member states on autologous cord blood banks and its explanatory memorandum, available at https://search.coe.int/cm/Pages/result_details.aspx?ObjectId=0900001680 5dcf9f, accessed 15 April 2022.
9. Committee on Organ Transplantation (CD-P-TO) of the Council of Europe. Umbilical cord blood banking – a guide for parents, available at https://freepub.edqm.eu/publications/PUBSD-94/detail, accessed 29 November 2022.
10. United Kingdom Blood Transfusion Services (UKBTS). Cord blood donor selection guidelines (TDSG-CB), February 2016, available at www.transfusionguidelines.org/export/dsg/dsg-cb-pdf/203/22/01/tdsg-cb_203_22_01.pdf, accessed 15 April 2022.
11. Ciubotariu R, Scaradavou A, Ciubotariu I *et al.* Impact of delayed umbilical cord clamping on public cord blood donations: can we help future patients and benefit infant donors? *Transfusion* 2018 Jun;58(6):1427-33.
12. Wagner AM, Krenger W, Stettler S *et al.* A novel, bedside technique to rapidly identify umbilical cord blood units with high total nucleated cell numbers. *Transfusion* 2016 Jun;56(6):1406-12.
13. Saccardi R, Tucunduva L, Ruggeri A *et al.* Impact of cord blood banking technologies on clinical outcome: a Eurocord/Cord Blood Committee (CTIWP), European Society for Blood and Marrow Transplantation and NetCord retrospective analysis. *Transfusion* 2016 Aug;56(8):2021-9.
14. Solves P, Mirabet V, Roig R. Volume reduction in routine cord blood banking. *Curr Stem Cell Res Ther* 2010 Dec;5(4):362-6.
15. Elmoazzen H, Holovati JL. Cord blood clinical processing, cryopreservation, and storage. *Methods Mol Biol* 2015;1257:369-79.
16. Circular of Information Cellular Therapy Task Force. Circular of information for the use of cellular therapy products, available on the website of the International Society for Cell & Gene Therapy at https://community.isctglobal.org/blogs/isct-head-office1/2018/10/11/circular-of-information-for-the-use-of-cellular-th, accessed 15 April 2022.
17. Akel S, Regan D, Wall D *et al.* Current thawing and infusion practice of cryopreserved cord blood: the impact on graft quality, recipient safety, and transplantation outcomes. *Transfusion* 2014 Nov;54(11):2997-3009.
18. Purtill D, Smith K, Devlin S *et al.* Dominant unit $CD34^+$ cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice. *Blood* 2014 Nov 6;124(19):2905-12.
19. Querol S. A case of mistaken identity. *Blood* 2009 Aug 20;114(8):1459-60.
20. EU Coding platform, available at https://webgate.ec.europa.eu/eucoding/, accessed 15 April 2022.
21. WMDA online application to report in a standardised way any incident on unrelated donated product, available at https://wmda.info/professionals/promoting-donor-care/adverse-events-searspear/, accessed 15 April 2022.
22. WHO recommendations: Intrapartum care for a positive childbirth experience, available at https://apps.who.int/iris/bitstream/handle/10665/260178/9789241550215-eng.pdf, accessed 15 April 2022.

Related material

Appendix 37. Informed consent for cord blood donation
Appendix 38. Cord blood donor medical history questionnaire
Appendix 39. Covid-19 infection risk assessment.

Chapter 26. **Pancreatic islets**

26.1. Introduction

Diabetes affects 463 million people throughout the world (2019) and this number will rise to 700 million by 2045 (IDF Diabetes Atlas, 9th edition, 2019). Many cases of diabetes are successfully treated with lifelong multiple daily injections of exogenous insulin and monitoring of blood glucose levels. In recent decades significant improvements in insulin therapy, thanks to new preparations (i.e. ultrafast and long-lasting insulin analogues) and the adoption of intensive diabetes management (infusion pumps and continuous glucose monitoring system), have resulted in an overall improvement of patients' glycaemic control and a decreased incidence of chronic complications of diabetes [1, 2]. However, exogenous insulin administration cannot attain the desirable tight control in the majority of diabetics [3-5] and cannot avoid the long-term complications of diabetes in all patients, while the life expectancy of patients with diabetes is still shorter than that of the general population [6-8].

Diabetes is one of the leading causes of end-stage renal disease, blindness and amputation [9]. In principle, the treatment for type 1 diabetes (T1DM), type 3c diabetes (T13cDM) and many cases of type 2 diabetes (T2DM) lies in the possibility of replacing destroyed or exhausted beta cell mass in order to restore two essential functions: sensing blood sugar levels and secreting appropriate amounts of insulin in the vascular bed, ideally into the portal system. Currently, the only available clinical approach of restoring beta cell mass in patients with diabetes is the allogeneic/autologous transplantation of beta cells (i.e. pancreas or islet transplantation). Clinical trials performed in the last three decades have shown that restoration of beta-cell function via transplantation of isolated islet cells or vascularised pancreas allows reproducibly achievement of a more physiological release of endocrine hormones than exogenous insulin in subjects with diabetes [10]. Transplanting islets of Langerhans consists of implantation in the recipient's hepatic portal system of endocrine pancreatic tissue, with a variable degree of purification [11]. Isolated islets are transplanted using minimally invasive techniques with lower morbidity than vascularised pancreas transplantation, which requires major surgery.

The field of islet transplantation has evolved significantly since the initial attempts by doctors Minkowski and von Mering in 1882 [12], with remarkable acceleration over the last three decades, thanks to the incredible efforts of the research community worldwide, with continual improvements in cell processing and transplantation techniques, patient management and development of specific immunotherapy protocols. In addition, islet transplantation represents an excellent platform for the development of cellular therapies aimed at the restoration of beta-cell function using stem cells in the near future. Europe is currently the most active region in the field of clinical islet transplantation and many of the leading groups are actually achieving similar good or beneficial outcomes. Islet isolation and clinical management are key elements in any islet transplant programme. Over the past three decades, the field

of islet processing has progressively introduced the automation of islet isolation [13, 14] and purification [15], the use of purified enzyme blends [16-18] and new systems for pancreas perfusion [19, 20], the analysis of different factors able to influence islet isolation outcome [21], and the development of standard operating procedures for guaranteeing the quality and safety of the processing [12, 22].

The following generic chapters (Part A) of this Guide all apply to pancreatic islet transplantation and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

26.2. When to consider islet transplantation

Transplantation of pancreatic islets may be considered as a therapeutic option in several conditions associated with loss of beta-cell function (Table 26.1). The procedure may be performed as islet transplant alone (ITA) in non-uraemic subjects, an option generally indicated for the treatment of iatrogenic (surgery-induced) diabetes and for non-uraemic patients with type 1 diabetes. Subjects with end-stage renal disease (ESRD) may be considered for simultaneous islet–kidney (SIK) or, if they have already undergone renal transplantation, islet after kidney (IAK) transplantation, respectively. In special situations, transplantation of islets may be considered in combination with other organs (i.e. in the context of multi-visceral transplantation following exenteration comprising the pancreas) [23]. The source of the islets for transplantation may be the patient's own pancreas (autologous or auto-transplant), mainly when surgical removal of the gland is required due to different conditions. After total pancreatectomy, the subject develops surgery-induced (iatrogenic) insulin-requiring diabetes. Introduced in the early 1970s [24], islet auto-transplantation allows the achievement of optimal metabolic control without the need for exogenous insulin in approximately 70 % of the cases when adequate islet numbers can be recovered from the pancreas (generally > 250 000 islet equivalents).

More than 500 auto-transplants in patients with near-total or total pancreatectomy have been performed to date [25]. The largest series were published by the University of Minnesota [26-29], the University of Cincinnati [30, 31] and Leicester [32-35]. Even when an inadequate islet mass to attain insulin-independence has been recovered, stable metabolic control and excellent management can be achieved in most subjects undergoing autologous islet transplantation [28, 36-41]. In the past auto-transplant has been performed almost exclusively in patients undergoing pancreatectomy because of chronic pancreatitis, successfully preserving β-cell mass and preventing diabetes after major pancreatic resections [25-26, 42-43]. Additional indications for auto-transplant other than chronic pancreatitis are still controversial [44], and have been limited to the procedure performed only in small case series [45-50] of benign enucleable tumours or pancreatic trauma. Recently, broader selection criteria for auto-transplant have been published [49, 51], exploring the possibility of extending auto-transplant to patients with known malignancy, either having completion pancreatectomy as treatment for severe pancreatic fistulae or extensive distal pancreatectomy for neoplasms of the pancreatic neck or pancreatoduodenectomy because at high risk of pancreatic fistula (Table 26.1).

In the case of subjects who lost islet function (mainly patients with type 1 diabetes or, more rarely, previous total pancreatectomy) the only option currently available for transplantable islet cells is allogeneic donor pancreata. These are generally obtained through multi-organ donation after cerebral death, following conventional donor–recipient ABO blood type matching. The use of a segment of the pancreas from living related donors is technically feasible [52-53], but at the present time not preferred for islet transplantation due to the limited duration of graft function after transplantation of suboptimal islet numbers under standard immunosuppressive proto-

Table 26.1. **Indication for islet transplantation**

Condition	Procedure	Type of transplant
Diabetes mellitus		
Type 1	ITA, SIK, IAK	Allogeneic
Type 2	ITA, SIK, IAK	Allogeneic
Surgery-induced diabetes (iatrogenic)		
Chronic pancreatitis	ITA	Autologous/Allogeneic
Trauma	ITA	Autologous/Allogeneic
Multi-visceral transplantation	Different combinations: liver-islet transplant, bowel-liver-islet transplant etc.	Allogeneic
Cystic fibrosis	ITA Lung-islet transplantation	Autologous/Allogeneic Allogeneic
Benign enucleable tumours	ITA	Autologous
Borderline/malignant pancreatic neoplasms	ITA	Autologous/Allogeneic
Grade C pancreatic fistula requiring completion pancreatectomy	ITA	Autologous/Allogeneic

ITA = islet transplant alone; SIK = simultaneous islet–kidney; IAK = islet after kidney.

cols, as well as the intrinsic risks for the donor (i.e. morbidity and risk of developing diabetes) [54].

The current main indication for an allogeneic islet transplant is type 1 diabetes, which is characterised by the selective destruction of islet beta cells due to an autoimmune process. Ongoing clinical trials of allogeneic islet transplantation are recruiting subjects with unstable type 1 diabetes 18-65 years of age, either sex, with frequent metabolic instability requiring medical treatment (hypo- or hyperglycaemia, ketoacidosis) despite intensive insulin therapy; hypoglycaemia unawareness (< 54 mg/dL); severe metabolic lability (mean amplitude of glycaemic excursion > 11.1 mmol/L or 200 mg/dL). The inadequate efficacy of medical therapy to attain the desirable metabolic control in this specific patient population with unstable diabetes justifies the use of transplantation of pancreatic islets, either isolated cellular graft or vascularised whole pancreas [55]. The main objective of the transplant is to correct the high susceptibility to severe hypoglycaemia and glycaemic imbalance that are associated with high mortality (8 % in nonuraemic subjects in the waiting list for 4 years to receive pancreas transplantation).

Three successful large-scale Phase 3 clinical trials in islet transplantation have been published recently: CIT-07 (multicentre, single-arm) and TRIMECO (multicentre, open-label, randomised) and REP0211 [56-58]. All these studies demonstrate that human islets transplanted in T1D subjects with impaired awareness of hypoglycaemia and severe hypoglycaemic events can safely and efficaciously maintain glycaemic balance and eliminate one of the most severe complications associated with insulin therapy. Further indications for an islet transplant are the presence of progressive complications of diabetes and psychological problems with insulin therapy that may compromise adherence to the therapeutic regimen. Islet transplant is indicated also for cases of subcutaneous insulin resistance requiring intraperitoneal or intravenous infusions, which are associated with substantial management hurdles and morbidity.

26.3. Islet isolation and transplantation

Islets are highly vascularised cell clusters ranging from < 50 μm to ≈ 800 μm in diameter that constitute the endocrine component of the pancreas. It has been estimated that a healthy pancreas may contain approximately 3.2 million islets scattered throughout the gland [59], and accounting for only ≈ 1 % of total pancreatic tissue. Each cluster comprises several thousands of endocrine cell subsets that are closely in touch with capillaries and with each other. Complex cell–cell interactions between different cell subsets, innervation, incretins and metabolites (sugar and amino acids, among others) in the blood and interstitial space all contribute to the proper control of glucose homeostasis [60]. Preservation of the integrity of islet cell cluster is a prerequisite for their optimal function. The procedure currently used to extract islets from human pancreas is the so-called automated method for isolation of the islets of Langerhans, established in 1987 by Ricordi and colleagues [13].

26.3.1. Donor evaluation

Donor criteria for pancreatic islet transplantation are similar to, but not identical to, those generally

applied for pancreatic transplantation. All suitable deceased donor pancreases that have not been placed for vascularised whole-organ transplantation should be allocated for pancreatic islet transplantation according to a prioritised (inter)national waiting list. ABO compatibility is required. A negative cross-match against T and B cells is also required. Donors suffering from diabetes mellitus type 1, or type 2 diabetes with evidence of beta cell deficiency, are excluded from donation for this clinical use. However, additional criteria for donation of tissues and cells (see Chapter 4 and Chapter 5) should be applied.

Donor characteristics – such as body surface area, body mass index and number of vasopressor types used – are predictors of successful pancreatic islet isolation. Other characteristics such as age, cold ischaemia time, and blood chemistry levels of glycated haemoglobin HbA1c, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen, amylase, lipase, sodium and glucose could influence pancreatic islet isolation yield. However, there is no specific threshold for these parameters that can be considered an absolute criterion to exclude the donor. Donor age until 80 years old and BMI higher than 30 can be acceptable for islet preparation. The maximum acceptable cold ischaemia time is 24 h. However, other parameter thresholds for acceptance vary widely among centres, based on their experiences, and there is no general agreement [61]. For example, the maximum acceptable intensive care unit (ICU) length of stay varies from 5 to 10 days, or it is evaluated as part of a combination of factors. The maximum acceptable value of donor amylase varies from 600 U/L to 110 U/L, or is defined as 3 times the normal range or as part of a combination of factors. Values from 10 to 60 min are indicated as the maximum acceptable for cardiac arrest duration. Donation after circulatory death (DCD) is acceptable and, generally, more stringent criteria are suggested for DCD than for donation after brain death (lower age and shorter cold ischaemia time).

26.3.2. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) is crucial to ensure pancreatic islet safety. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk reducing measures, as needed.

The consistency of pancreatic islets manufacturing is highly dependent on the quality of the procured organ. Pancreas procurement should be conducted in ice to ensure organ viability, immediately after liver and before kidneys, using similar procedures as for whole pancreas transplantation, even if vascular access is not required. If a distant team has procured donor pancreases, the TE should have agreements with the procurement centre(s) on organ procurement, warm and cold ischaemia time, organ preservation methods, cold preservation fluid and ice shipping conditions. Accurate packaging with properly donor identification and enclosed procurement documentation have to be assured, as detailed in Chapter 15.

26.3.3. Processing and storage

Once the organ has arrived at the isolation facility, the transport conditions and the accuracy of the documentation are checked before proceeding further.

When selecting an appropriate air-quality specification for processing pancreatic islets, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9, should be considered.

Before the beginning of the isolation procedure, the spleen and the duodenum are removed from the pancreas and an accurate dissection and removal of the peripancreatic fat, lymph nodes and vessels is performed. After pancreas cleaning, the pancreas is divided at the neck and two 16-20 gauge angiocatheters are inserted into the main pancreatic ducts. The organ is then perfused at a pressure of 140-180 mmHg with cooled perfusion solution containing collagenase/protease mix. After 10 minutes of cold perfusion, the distended pancreas is further cut into smaller sections, and placed into the Ricordi chamber [12]. This chamber is composed of a superior and an inferior part, separated by a filter that has pores of about 700 µm. Seven to nine stainless steel balls and the fragments of the pancreas are placed into the inferior part of the chamber, which is then filled with the digestion solution and closed together with the superior part of the chamber. A peristaltic pump connected to the system is activated creating a flow of 40 (35-45) mL/min.

The digestion runs in a closed circuit where warm isotonic salt solution is pumped in the inferior chamber and the tissue released in the solution passes

in the superior chamber through the filter. The collagenase is re-circulated at a temperature not exceeding 37 °C and the chamber is agitated. When most of the islets are free of the surrounding acinar tissue, and intact islets are observed, the heating circuit is bypassed. The temperature is progressively decreased to 4-12 °C and the collagenase diluted with cold cell medium. The free islets are then collected in containers, washed several times, re-suspended in cold organ preservation solution and purified with a continuous density gradient using a cell separator. Alternatively discontinuous gradient can be used. At the end of the procedure, samples of the islet preparation are collected and evaluated through staining with dithizone (DTZ), which marks zinc in the insulin granules, resulting in a characteristic red stain. Adding a few drops of DTZ solution to a sample allows easy evaluation of the morphology and the number of isolated islets through computerised digital analysis or manually under microscope.

Purified islets are usually maintained in temporary storage before use in media that do not alter the physiological properties of insulin-producing islets, at 22-37 °C and 3-10 % CO_2 depending on the sodium bicarbonate concentration of your medium. Storage of pancreatic islets in media under stringent conditions before implantation has logistical benefits: it enables additional quality-control tests, and allows time to prepare the patient for transplant or to ship pancreatic islets to a distant transplant centre [62-63]. Islets are usually transplanted within 72 h culture and a daily change of storage media is recommended. Storage time longer than 3 days before release for transplant has to be validated through quality control tests on islets [64].

26.3.4. Quality controls/release criteria

The islet isolation processes must be validated, characterised and monitored for sterility, pyrogenicity, identity (insulin content, optional), cell number (amount of tissue, counting of islets, beta cells count), purity (percentage of islet *v.* other cells e.g. ductal, acinar cells), viability (PI/FDA staining, islet nucleotide content), potency (insulin secretory response, optional) and finally stability (recovery after culture). Pancreatic islet cells exhibit a wide variety of functions that should be tested during quality control procedures. The TE should define – alongside the general tissue-and-cell release criteria – additional criteria for pancreatic islet transplantation, including:

a. quantification of the pancreatic islet cell mass (total islet number and the islet equivalent, known as IEQ), or of the number of insulin-positive cells;
b. cell viability (e.g. qualitative determination by Hoechst/propidium iodide, fluorescein diacetate/ethidium bromide or functional assessments);
c. microbiological testing;
d. bacterial endotoxin testing (see §11.3.4);
e. beta-cell function (e.g. glucose-stimulated insulin secretion or insulin synthesis); but this information is not available prior to transplantation in all programmes.

Many of the currently utilised biological assays measuring islet functions and sterility are not always rapid enough for use in routine release testing because of the short period between pancreatic islet isolation and transplantation, varying from several hours to a few days. The TE should define how it will deal with incomplete test results. Specific features of the final islet preparation are required for islet preparations used in islet transplantation, in particular purity (> 20 % of the preparation being islets), adequate number of islets (suggested islet mass ≥ 5 000 islet equivalents (IEQ)/kg recipient body weight for the first infusion, > 3 000 IEQ/kg for further infusions or equivalent beta cell number). Total tissue volume (< 5 mL) and purity less than 20 % (e.g. in transplantation) but should be accompanied with intraportal pressure monitoring when this is the case.

26.3.5. Packaging and distribution

Transport temperature is usually maintained at 12-25 °C. Pancreatic islet cells are transported in liquid media, so special notice might be necessary; depending on airline transport regulations (see Chapter 12 and Chapter 15).

26.3.6. Traceability

The attached documentation for the clinical transplantation centre should include, for example, details of the donor, organ transport/ischaemic times, pancreas quality, quantification of the pancreatic islet cell mass or of the number of insulin-positive cells, sterility, viability and function. Records covering the complete process from donor to recipient should be kept at the TE, and it should be possible to trace also other organ recipients from the same donor, and vice versa.

26.3.7. Islet infusion

The infusion of the islets can be performed a few hours after the end of the isolation process or up to 72 h thereafter. Storage time longer than 3 days is possible without loss of function [64]. However, extension of the time has to be validated through quality control tests on islets. The implantation site is usually the hepatic parenchyma through the portal system of the recipient. Recently other implantation sites have been proposed [65] in the clinical setting, like the bone marrow [66] [67], the subcutaneous site [68], the gastric submucosa [69], the omentum [70-71] or striated muscle [72-73], which in the future may prove to be valid alternative sites for islet transplantation. The adequate amount of islets obtained is calculated with respect to the body weight of the recipient and re-suspended immediately before intrahepatic transplantation in 40-60 mL of a solution suitable for injection.

Percutaneous transhepatic catheterisation is the most common access route, as well as a minilaparotomy and cannulation of an omental or mesenteric vein, or recanalisation of the umbilical vein. Access to the portal vein is usually provided by interventional radiologists. If the portal pressure is documented to be below 20 mmHg, the islet infusion bag is connected with the portal vein catheter and infused over a period of 15 to 60 minutes. Islet infusion is halted if the portal pressure exceeds 22 mmHg. After completion of the islet infusion, the catheter is withdrawn; coils and gelatin-sponges are deployed in the puncture tract to prevent bleeding.

26.4. Biovigilance

Any unforeseen events influencing islet isolation and storage conditions are to be considered as adverse events that should be recorded and reported to the Health Authority. Some examples are:

- loss of pancreatic islets during isolation or temporary storage, due to failure of equipment and monitoring systems (e.g. overheating, carbon dioxide concentration, cooling),
- loss of pancreatic islets due to incorrect use of media (e.g. pH problems, sterility of media, concentration of additives, shelf-life).

The procedure of islet transplantation has proved to be very safe, especially when compared with whole pancreas transplant [74-75].

For allogeneic islet transplantation, bleeding is the most common procedure-related complication, occurring with an incidence as high as 13 %. The use of fibrin tissue sealant and embolisation coils in the hepatic catheter tract seems to effectively minimise the bleeding risk [76-77]. Partial portal vein thrombosis complicates fewer than 5 % of islet infusion procedures [78], and complete portal venous thrombosis is rare. The use of purer islet preparations, greater expertise in portal vein catheterisation and new radiological devices (catheters medicated with anticoagulation) will continue reducing the risk of portal vein thrombosis, although the risk is unlikely be eliminated.

Other complications of islet cell transplantation include transient liver enzyme elevation (50 % incidence) [79], abdominal pain (50 % incidence), focal hepatic steatosis (20 % incidence) [80-81] and severe hypoglycaemia (< 3 % incidence). Another complication related to the intrahepatic islet transplantation procedure is portal hypertension that can occur acutely during the islet infusion, especially in the case of infusions other than the first one [82]. Iatrogenic hypoglycaemia in the immediate post-transplant period is a rare event. Frequent blood glucose monitoring immediately following islet transplantation is recommended to avoid severe unrecognised hypoglycaemia in the early post-transplant period. As with any allogeneic transplant, islet transplant recipients may become sensitised to islet donor histocompatibility antigens (HLA), leading to the development of panel reactive alloantibodies (PRA). Data on the development of cytotoxic antibodies against donor HLA in islet allotransplant recipients with failing grafts have been reported from several islet transplant centres [83-85]. A potential consequence of high PRA levels in recipients of a failed islet transplant is that, if these individuals develop diabetic nephropathy in the future, a high PRA may increase their time on a transplant list for a suitable kidney graft. The need to implement anti-rejection therapy exposes transplant recipients to an increased risk of untoward side effects expected in any immunosuppressed subjects (Table 26.2) [86].

Direct organ toxicity of immunosuppressive drugs has been recognised. Symptoms associated with neuro- and/or nephro-toxicity are relatively frequent in subjects receiving chronic immunosuppressive agents currently in use in the clinical arena. Nephrotoxicity from sirolimus and/or tacrolimus has been described in patients with T1D undergoing islet transplantation, particularly when kidney function is already impaired because of pre-existing diabetic nephropathy [87-88]. In these cases, modification of the anti-rejection regimen is indicated, with dose reduction or conversion to a different combination of drugs. In the majority of cases, these changes resolve the symptoms without compromising graft survival

Table 26.2. **Non-exhaustive list of possible reportable SARs in islet transplant recipients**

Infusion procedure-related	
	• Haemorrhage • Portal thrombosis • Transient transaminitis
Immunosuppression-related	
Haematological	• anaemia • leukopaenia • neutropaenia
Metabolic	• dyslipidaemia
Gastro-intestinal	• oral ulcers (sirolimus) • diarrhoea (mycophenolic acid) • CMV colitis
Respiratory tract	• upper respiratory infections • interstitial pneumonitis (sirolimus)
Neurological	• neurotoxicity (tacrolimus)
Genito-urinary	• urinary infections • ovarian cysts • dysmenorrhoea • nephropathy • proteinuria
Cutaneous	• infections • cancer

[89-90]. Opportunistic infections of urinary tract, upper respiratory tract and skin are frequent, along with myelosuppressive and gastrointestinal effects of the immunosuppressive drugs. In the majority of cases, these effects are not severe and resolve without sequel with medical treatment. The risk of transmission of *Cytomegalovirus* (CMV) disease from donor to recipient has been surprisingly low in recipients of islet allografts, particularly in the most recent period with routine use of purified islet preparations [79]. Elevation of viraemic titres for CMV or Epstein–Barr virus in the presence of overt clinical symptoms (i.e. *de novo* infection or reactivation in seropositive subjects) imposes the implementation of anti-viral therapy and reduction of immunosuppressive drug dose [91]. Timely intervention may result in faster resolution of the symptoms without compromising graft survival.

The Tenth Annual Report of the Collaborative Islet Transplantation Registry (CITR) reported 1 854 adverse events (AEs) on 877 ITA, 364 AEs on 183 IAK, and 53 AEs on 24 SIK. In the first 30 days following islet transplantation, about 26 % of recipients experienced a reportable adverse event. The majority (70 %) were adjudicated by the local investigator as possibly or definitely related to either the infusion procedure or the immunosuppression. The majority were not unexpected, such as abnormal lymphocyte count and increased liver function. Very few AEs were infections. About 14 % of allo-islet recipients experienced a serious adverse event in the first 30 days, which occurred about equally in IAK as in ITA, and have declined somewhat over the era. In the first year after islet transplantation, which includes a majority of the re-infusions that were performed, about 43 % of all recipients experienced a reportable adverse event. About one-third have experienced a serious adverse event within the first year, with again a significant decline in the most recent era, a pattern seen for all adverse events in all follow-up after islet transplantation.

The outcomes of the reported adverse events have improved over the decade, with fewer patients experiencing long-term sequelae of their adverse events in the most recent era. Many adverse events seen in this population are unrelated to islet transplantation but not unexpected in a cohort of older T1D with significant co-morbidity. Overall, 17 % of all recipients failed to recover completely from an adverse event. This is the worst outcome of all adverse events, including those not related to the islet infusion or immunosuppression. Among related adverse events, only 11 % failed to recover completely. Life-threatening events have occurred in 13.5 % of islet-alone, in 18.0 % of IAK recipients and in 29.2 % of SIK recipients ($p < 0.0001$). Recent eras have seen a substantial decline in the incidence of life-threatening events. Most involved neutropenia and abnormal liver function. The vast majority recovered, 3 % died, 3 % did not recover, and 8 % recovered with sequelae.

An assessment of the surgical complication of islet auto transplantation was recently reported for the entire Minnesota series ($n = 413$) [26]. Surgical complications requiring reoperation during the initial admission occurred in 15.9 % of the patients. The most common reason for reoperation was bleeding, occurring in 9.5 % of the procedures. Anastomotic leaks occurred in 4.2 % of the patients, biliary in 1.4 % and enteric in 2.8 %. Intra-abdominal infection requiring reoperation occurred in 1.9 % of patients, wound infections requiring operative debridement in 2.2 %. Gastrointestinal issues, such as bowel obstruction, omental infarction, bowel ischaemia, delayed reconstruction because of bowel oedema or tube perforation, required reoperation in 4.7 % of the patients. Two patients (< 1 %) required reoperation to remove an ischaemic or bleeding spleen after spleen sparing pancreatectomy (done in 30 % of patients). For further guidance on biovigilance, please refer to Chapter 17.

26.5. Developing applications for patients

The field of cellular therapies for the treatment of diabetes is rapidly evolving. Several new developments emerging in recent years may push this interesting technology to a new, broader dimension. While a wide range of improvements may be implemented in the donor selection and organ allocation scheme to increase pancreas utilisation for transplantation, there is increasing excitement about the use of unlimited alternative sources of transplantable islets, such as xenogeneic (i.e. obtained from other species, such as porcine islets, which are reviewed in [92]; see also §5.3.4.f in Chapter 5) or derived from human stem cells [93-98]. Pig islets may be available in plentiful amounts. Importantly, the ability to obtain genetically modified pigs that lack or overexpress specific molecules may be of assistance in developing cellular products with reduced immunogenicity for transplantation into humans.

In turn, this technology may allow the achievement of long-term function under immunosuppressive regimens that are used for allogeneic cells or may facilitate the induction of long-term acceptance of xenogeneic islet cells. Another area reporting great progress is that of regenerative medicine using human stem cells from embryonic or adult sources. Encouraging experimental data suggest that insulin-producing cells can be obtained from human multipotent stem cells, and great efforts are currently concentrated on developing cellular products with consistent potency and safety profile (ability to generate tumours) for future clinical application [93-96, 99-100]. A current limitation on islet transplantation is the inability to use non-invasive or minimally invasive predictive tests as well as biomarkers of early graft dysfunction to guide timely interventions aimed at preserving functional islet cell mass.

26.6. References

1. Gregg EW, Sattar N, Ali MK. The changing face of diabetes complications. *Lancet Diabetes Endocrinol* 2016; 4(6):537-47.
2. Harding JL, Pavkov ME, Magliano DJ *et al.* Global trends in diabetes complications: a review of current evidence. *Diabetologia* 2019;62(1):3-16.
3. Mannucci E, Monami M, Dicembrini I *et al.* Achieving HbA1c targets in clinical trials and in the real world: a systematic review and meta-analysis. *J Endocrinol Invest* 2014;37(5):477-95.
4. Edelman SV, Polonsky WH. Type 2 diabetes in the real world: the elusive nature of glycemic control. *Diabetes Care* 2017;40(11):1425-32.
5. Rodbard D. State of type 1 diabetes care in the United States in 2016-2018 from T1D Exchange Registry Data. *Diabetes Technol Ther* 2019;21(2):62-5.
6. Livingstone SJ, Levin D, Looker HC *et al.* Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. *JAMA* 2015;313(1):37-44.
7. Lind M, Svensson AM, Kosiborod M *et al.* Glycemic control and excess mortality in type 1 diabetes. *N Engl J Med* 2014;371(21):1972-82.
8. Alva ML, Hoerger TJ, Zhang P, Cheng YJ. State-level diabetes-attributable mortality and years of life lost in the United States. *Ann Epidemiol* 2018;28(11):790-5.
9. Gregg EW, Li Y, Wang J *et al.* Changes in diabetes-related complications in the United States, 1990-2010. *N Engl J Med* 2014;370(16):1514-23.
10. Marzorati S, Pileggi A, Ricordi C. Allogeneic islet transplantation. *Expert Opin Biol Ther* 2007;7(11): 1627-45.
11. Piemonti L. *Islet Transplantation*. 2000, revd 2019, available at www.ncbi.nlm.nih.gov/books/NBK278966/.
12. Piemonti L, Pileggi A. 25 Years of the Ricordi automated method for islet isolation. *CellR4* 2013;1(1):e128.
13. Ricordi C, Lacy PE, Finke EH *et al.* Automated method for isolation of human pancreatic islets. *Diabetes* 1988; 37(4):413-20.
14. Goto M, Eich TM, Felldin M *et al.* Refinement of the automated method for human islet isolation and presentation of a closed system for *in vitro* islet culture. *Transplantation* 2004;78(9):1367-75.
15. Robertson GS, Chadwick DR, Contractor H *et al.* The optimization of large-scale density gradient isolation of human islets. *Acta Diabetol* 1993;30(2):93-8.
16. Bucher P, Mathe Z, Morel P *et al.* Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. *Transplantation* 2005;79(1):91-7.
17. Linetsky E, Bottino R, Lehmann R *et al.* Improved human islet isolation using a new enzyme blend, liberase. *Diabetes* 1997;46(7):1120-3.
18. O'Gorman D, Kin T, Imes S *et al.* Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1. *Transplantation* 2010;90(3):255-9.
19. Min CG, Papas KK. Recent developments in persufflation for organ preservation. *Curr Opin Organ Transplant* 2018;23(3):330-5.
20. Cross SE, Hughes SJ, Partridge CJ *et al.* Collagenase penetrates human pancreatic islets following standard intraductal administration. *Transplantation* 2008;86(7):907-11.
21. Hilling DE, Bouwman E, Terpstra OT, Marang-van de Mheen PJ. Effects of donor-, pancreas-, and

isolation-related variables on human islet isolation outcome: a systematic review. *Cell Transplant* 2014; 23(8):921-8.

22. Ricordi C, Goldstein JS, Balamurugan AN *et al.* National Institutes of Health-sponsored Clinical Islet Transplantation Consortium Phase 3 trial: manufacture of a complex cellular product at eight processing facilities. *Diabetes* 2016;65(11):3418-28.
23. Tzakis AG, Ricordi C, Alejandro R *et al.* Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 1990;336(8712): 402-5.
24. Najarian JS, Sutherland DE, Matas AJ, Goetz FC. Human islet autotransplantation following pancreatectomy. *Transplant Proc* 1979;11(1):336-40.
25. Bramis K, Gordon-Weeks AN, Friend PJ *et al.* Systematic review of total pancreatectomy and islet autotransplantation for chronic pancreatitis. *Br J Surg* 2012;99(6):761-6.
26. Sutherland DE, Radosevich DM, Bellin MD *et al.* Total pancreatectomy and islet autotransplantation for chronic pancreatitis. *J Am Coll Surg* 2012;214(4): 409-424; discussion 424-6.
27. Bellin MD, Beilman GJ, Dunn TB *et al.* Islet autotransplantation to preserve beta cell mass in selected patients with chronic pancreatitis and diabetes mellitus undergoing total pancreatectomy. *Pancreas* 2013; 42(2):317-21.
28. Bellin MD, Freeman ML, Schwarzenberg SJ *et al.* Quality of life improves for pediatric patients after total pancreatectomy and islet autotransplant for chronic pancreatitis. *Clin Gastroenterol Hepatol* 2011; 9(9):793-9.
29. Bellin MD, Carlson AM, Kobayashi T *et al.* Outcome after pancreatectomy and islet autotransplantation in a pediatric population. *J Pediatr Gastroenterol Nutr* 2008;47(1):37-44.
30. Wilson GC, Sutton JM, Salehi M *et al.* Surgical outcomes after total pancreatectomy and islet cell autotransplantation in pediatric patients. *Surgery* 2013; 154(4):777-83; discussion 783-4.
31. Sutton JM, Schmulewitz N, Sussman JJ *et al.* Total pancreatectomy and islet cell autotransplantation as a means of treating patients with genetically linked pancreatitis. *Surgery* 2010;148(4):676-85; discussion 685-6.
32. Webb MA, Illouz SC, Pollard CA *et al.* Islet auto transplantation following total pancreatectomy: a long-term assessment of graft function. *Pancreas* 2008;37(3):282-7.
33. Clayton HA, Davies JE, Pollard CA *et al.* Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the Leicester General Hospital. *Transplantation* 2003; 76(1):92-8.
34. White SA, Davies JE, Pollard C *et al.* Pancreas resection and islet autotransplantation for end-stage chronic pancreatitis. *Ann Surg* 2001;233(3):423-31.
35. Garcea G, Weaver J, Phillips J *et al.* Total pancreatectomy with and without islet cell transplantation for chronic pancreatitis: a series of 85 consecutive patients. *Pancreas* 2009;38(1):1-7.
36. Teuscher AU, Kendall DM, Smets YF *et al.* Successful islet autotransplantation in humans: functional insulin secretory reserve as an estimate of surviving islet cell mass. *Diabetes* 1998;47(3):324-30.
37. Robertson RP, Lanz KJ, Sutherland DE, Kendall DM. Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. *Diabetes* 2001;50(1):47-50.
38. Blondet JJ, Carlson AM, Kobayashi T *et al.* The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis. *Surg Clin North Am* 2007 Dec;87(6):1477-1501, x. https://doi.org/10.1016/j.suc.2007.08.014.
39. Bellin MD, Sutherland DE. Pediatric islet autotransplantation: indication, technique, and outcome. *Curr Diab Rep* 2010;10(5):326-31.
40. Bellin MD, Sutherland DE, Beilman GJ *et al.* Similar islet function in islet allotransplant and autotransplant recipients, despite lower islet mass in autotransplants. *Transplantation* 2011;91(3):367-72.
41. Robertson RP. Consequences on beta-cell function and reserve after long-term pancreas transplantation. *Diabetes* 2004;53(3):633-44.
42. Chinnakotla S, Bellin MD, Schwarzenberg SJ *et al.* Total pancreatectomy and islet autotransplantation in children for chronic pancreatitis: indication, surgical techniques, postoperative management, and long-term outcomes. *Ann Surg* 2014;260(1):56-64.
43. Dong M, Parsaik AK, Erwin PJ *et al.* Systematic review and meta-analysis: islet autotransplantation after pancreatectomy for minimizing diabetes. *Clin Endocrinol (Oxf)* 2011;75(6):771-9.
44. Dudeja V, Beilman GJ, Vickers SM. Total pancreatectomy with islet autotransplantation in patients with malignancy: are we there yet? *Ann Surg* 2013;258(2): 219-20.
45. Ris F, Niclauss N, Morel P *et al.* Islet autotransplantation after extended pancreatectomy for focal benign disease of the pancreas. *Transplantation* 2011 Apr 27; 91(8):895-901.
46. Jin SM, Oh SH, Kim SK *et al.* Diabetes-free survival in patients who underwent islet autotransplantation after 50% to 60% distal partial pancreatectomy for benign pancreatic tumors. *Transplantation* 2013;95(11): 1396-1403.

47. Balzano G, Nano R, Maffi P *et al.* Salvage islet auto transplantation after relaparatomy. *Transplantation* 2017;101(10):2492-2500.
48. Balzano G, Maffi P, Nano R *et al.* Autologous islet transplantation in patients requiring pancreatectomy: a broader spectrum of indications beyond chronic pancreatitis. *Am J Transplant* 2016;16(6):1812-26.
49. Balzano G, Maffi P, Nano R *et al.* Extending indications for islet autotransplantation in pancreatic surgery. *Ann Surg* 2013;258(2):210-18.
50. Balzano G, Maffi P, Nano R *et al.* Diabetes-free survival after extended distal pancreatectomy and islet auto transplantation for benign or borderline/malignant lesions of the pancreas. *Am J Transplant* 2019; 19(3):920-8.
51. Balzano G, Piemonti L. Autologous islet transplantation in patients requiring pancreatectomy for neoplasm. *Curr Diab Rep* 2014;14(8):512.
52. Matsumoto S, Okitsu T, Iwanaga Y *et al.* Insulin independence of unstable diabetic patient after single living donor islet transplantation. *Transplant Proc* 2005;37(8):3427-9.
53. Matsumoto S, Tanaka K, Strong DM, Reems JA. Efficacy of human islet isolation from the tail section of the pancreas for the possibility of living donor islet transplantation. *Transplantation* 2004;78(6):839-43.
54. Matsumoto I, Shinzeki M, Asari S *et al.* Evaluation of glucose metabolism after distal pancreatectomy according to the donor criteria of the living donor pancreas transplantation guidelines proposed by the Japanese Pancreas and Islet Transplantation Association. *Transplant Proc* 2014;46(3):958-62.
55. Choudhary P, Rickels MR, Senior PA *et al.* Evidence-informed clinical practice recommendations for treatment of type 1 diabetes complicated by problematic hypoglycemia. *Diabetes Care* 2015;38(6):1016-29.
56. Hering BJ, Clarke WR, Bridges ND *et al.* Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe hypoglycemia. *Diabetes Care* 2016;39(7):1230-40.
57. Lablanche S, Vantyghem MC, Kessler L *et al.* Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO): a multicentre, randomised controlled trial. *Lancet Diabetes Endocrinol* 2018;6(7):527-37.
58. Maffi P, Lundgren T, Tufveson G *et al.* Targeting CXCR1/2 does not improve insulin secretion after pancreatic islet transplantation: a phase 3, double-blind, randomized, placebo-controlled trial in type 1 diabetes. *Diabetes Care* 2020;43(4):710-18.
59. Ionescu-Tirgoviste C, Gagniuc PA, Gubceac E *et al.* A 3D map of the islet routes throughout the healthy human pancreas. *Sci Rep* 2015 Sep 29;5:14634. https://doi.org/10.1038/srep14634.
60. Cabrera O, Berman DM, Kenyon NS *et al.* The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* 2006 Feb 14;103(7):2334-9. https://doi.org/10.1073/pnas.0510790103.
61. Nano R, Kerr-Conte JA, Scholz H *et al.* Heterogeneity of human pancreatic islet isolation around Europe: results of a survey study. *Transplantation* 2020;104(1): 190-6.
62. Noguchi H, Miyagi-Shiohira C, Kurima K *et al.* Islet culture/preservation before islet transplantation. *Cell Med* 2015;8(1-2):25-9.
63. Ichii H, Sakuma Y, Pileggi A *et al.* Shipment of human islets for transplantation. *Am J Transplant* 2007;7(4): 1010-20.
64. Lee D, Gillard P, Hilbrands R *et al.* Use of culture to reach metabolically adequate beta-cell dose by combining donor islet cell isolates for transplantation in Type 1 diabetes patients. *Transplantation* 2020 Oct;104(10):e295-e302. https://doi.org/10.1097/TP.0000000000003321.
65. Cantarelli E, Piemonti L. Alternative transplantation sites for pancreatic islet grafts. *Curr Diab Rep* 2011; 11(5):364-74.
66. Maffi P, Balzano G, Ponzoni M *et al.* Autologous pancreatic islet transplantation in human bone marrow. *Diabetes* 2013;62(10):3523-31.
67. Maffi P, Nano R, Monti P *et al.* Islet allotransplantation in the bone marrow of patients with type 1 diabetes: a pilot randomized trial. *Transplantation* 2019;103(4):839-51.
68. Yasunami Y, Nakafusa Y, Nitta N *et al.* A novel subcutaneous site of islet transplantation superior to the liver. *Transplantation* 2018;102(6):945-52.
69. Wszola M, Berman A, Ostaszewska A *et al.* Islets allotransplantation into gastric submucosa in a patient with portal hypertension: 4-year follow-up. *Transplant Proc* 2018;50(6):1910-13.
70. Berman DM, Molano RD, Fotino C *et al.* Bioengineering the endocrine pancreas: intraomental islet transplantation within a biologic resorbable scaffold. *Diabetes* 2016;65(5):1350-61. https://doi.org/10.2337/db15-1525.
71. Baidal DA, Ricordi C, Berman DM *et al.* Bioengineering of an intraabdominal endocrine pancreas. *N Engl J Med* 2017;376(19):1887-9.
72. Christoffersson G, Henriksnas J, Johansson L *et al.* Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. *Diabetes* 2010; 59(10):2569-78.
73. Bertuzzi F, Colussi G, Lauterio A, De Carlis L.

Intramuscular islet allotransplantation in type 1 diabetes mellitus. *Eur Rev Med Pharmacol Sci* 2018;22(6): 1731-6.

74. Gaba RC, Garcia-Roca R, Oberholzer J. Pancreatic islet cell transplantation: an update for interventional radiologists. *J Vasc Interv Radiol* 2012;23(5):583-94; quiz 594.
75. Maffi P, Scavini M, Socci C *et al.* Risks and benefits of transplantation in the cure of type 1 diabetes: whole pancreas versus islet transplantation. A single center study. *Rev Diabet Stud* 2011;8(1):44-50.
76. Villiger P, Ryan EA, Owen R *et al.* Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution. *Am J Transplant* 2005;5(12):2992-8.
77. Froud T, Yrizarry JM, Alejandro R, Ricordi C. Use of D-STAT to prevent bleeding following percutaneous transhepatic intraportal islet transplantation. *Cell Transplant* 2004;13(1):55-9.
78. Ryan EA, Paty BW, Senior PA *et al.* Five-year follow-up after clinical islet transplantation. *Diabetes* 2005;54(7):2060-9.
79. Barshes NR, Lee TC, Goodpastor SE *et al.* Transaminitis after pancreatic islet transplantation. *J Am Coll Surg* 2005;200(3):353-61.
80. Bhargava R, Senior PA, Ackerman TE *et al.* Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes* 2004;53(5):1311-17.
81. Maffi P, Angeli E, Bertuzzi F *et al.* Minimal focal steatosis of liver after islet transplantation in humans: a long-term study. *Cell Transplant* 2005;14(10):727-33.
82. Casey JJ, Lakey JR, Ryan EA *et al.* Portal venous pressure changes after sequential clinical islet transplantation. *Transplantation* 2002;74(7):913-15.
83. Campbell PM, Senior PA, Salam A *et al.* High risk of sensitization after failed islet transplantation. *Am J Transplant* 2007;7(10):2311-17.
84. Hilbrands R, Huurman VA, Gillard P *et al.* Differences in baseline lymphocyte counts and autoreactivity are associated with differences in outcome of islet cell transplantation in type 1 diabetic patients. *Diabetes* 2009;58(10):2267-76.
85. Piemonti L, Everly MJ, Maffi P *et al.* Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes* 2013; 62(5):1656-64.
86. Hafiz MM, Faradji RN, Froud T *et al.* Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. *Transplantation* 2005; 80(12):1718-28.
87. Andres A, Toso C, Morel P *et al.* Impairment of renal function after islet transplant alone or islet-after-kidney transplantation using a sirolimus/tacrolimus-based immunosuppressive regimen. *Transpl Int* 2005;18(11):1226-30.
88. Gala-Lopez BL, Senior PA, Koh A *et al.* Late cytomegalovirus transmission and impact of T-depletion in clinical islet transplantation. *Am J Transplant* 2011; 11(12):2708-14.
89. Molinari M, Al-Saif F, Ryan EA *et al.* Sirolimus-induced ulceration of the small bowel in islet transplant recipients: report of two cases. *Am J Transplant* 2005; 5(11):2799-2804.
90. Ponte GM, Baidal DA, Romanelli P *et al.* Resolution of severe atopic dermatitis after tacrolimus withdrawal. *Cell Transplantation* 2007;16(1):23-30.
91. Cure P, Froud T, Leitao CB *et al.* Late Epstein Barr virus reactivation in islet after kidney transplantation. *Transplantation* 2008;86(9):1324-5.
92. Marigliano M, Bertera S, Grupillo M *et al.* Pig-to-non-human primates pancreatic islet xenotransplantation: an overview. *Curr Diab Rep* 2011;11(5):402-12.
93. Pagliuca FW, Millman JR, Gurtler M *et al.* Generation of functional human pancreatic beta cells *in vitro*. *Cell* 2014;159(2):428-39.
94. Rezania A, Bruin JE, Arora P *et al.* Reversal of diabetes with insulin-producing cells derived *in vitro* from human pluripotent stem cells. *Nat Biotechnol* 2014;32(11):1121-33.
95. Kroon E, Martinson LA, Kadoya K *et al.* Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 2008;26(4):443-52.
96. Schulz TC, Young HY, Agulnick AD *et al.* A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. *PLoS One* 2012;7(5):e37004.
97. Pellegrini S, Piemonti L, Sordi V. Pluripotent stem cell replacement approaches to treat type 1 diabetes. *Curr Opin Pharmacol* 2018;43:20-6.
98. Odorico J, Markmann J, Melton D *et al.* Report of the key opinion leaders meeting on stem cell-derived beta cells. *Transplantation* 2018;102(8):1223-9.
99. Pellegrini S, Ungaro F, Mercalli A *et al.* Human induced pluripotent stem cells differentiate into insulin-producing cells able to engraft *in vivo*. *Acta Diabetol* 2015 Dec;52(6):1025-35. https://doi.org/10.1007/s00592-015-0726-z.
100. Sordi V, Pellegrini S, Krampera M *et al.* Stem cells to restore insulin production and cure diabetes. *Nutr Metab Cardiovasc Dis* 2017;27(7):583-600. https://doi.org/10.1016/j.numecd.2017.02.004.

Chapter 27. **Hepatocytes**

27.1. Introduction

Hepatocyte transplantation is an alternative treatment to liver transplantation for patients with metabolic liver diseases or acute liver failure, or as a temporary support for patients with liver failure while waiting for an organ transplant [1, 2]. Patients with metabolic liver diseases are characterised by deficiency of one particular enzyme or protein, giving rise to hepatic and/or extrahepatic disease while all other liver functions are unimpaired. Thus, replacement of the whole liver by liver transplantation may not be required, and selective replacement of a fraction of the liver cell mass should be therapeutic. There is evidence that replacement of 5 %-10 % of the liver with healthy donor hepatocytes can correct a wide range of inherited metabolic liver diseases [3, 4]. In patients with acute and chronic liver failure, hepatocyte transplantation could provide temporary liver support until the native liver has recovered or a whole liver is available for transplantation.

Hepatocyte transplantation has potential advantages over whole-organ transplantation: the procedure is a less invasive approach, resulting in lower morbidity and mortality; it can be repeated several times; and it is reversible. Functional hepatocytes can be isolated from unused segments of donor livers that had been retrieved for whole-organ transplantation; and, in contrast to whole organs, cells can be cryopreserved and stored until needed.

This chapter defines the additional specific requirements for liver tissue and hepatocyte isolation for transplantation only. The following generic chapters (Part A) of this Guide all apply to hepatocyte isolation and transplantation and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

27.2. Donor evaluation

Liver tissue for hepatocyte isolation can be procured from donors after brain death (DBD) and

from donors after circulatory death (DCD). Hepatocyte isolation does not require the whole organ; using segments or a lobe from a size-reduced donor organ is ideal for hepatocyte isolation [5].

Liver tissue can also be procured from healthy living donors. In theory a healthy living donor could donate a part of their liver. However, this procedure has been rarely performed so far because of the risk of morbidity and mortality for the living donor when balanced against the results obtained in clinical hepatocyte transplantation [6]. The liver from a living donor can also be the explanted liver in a so-called domino procedure where a patient is undergoing a liver transplantation [7], provided that the indication for the transplant (for example, maple syrup urine disease) [8] is not considered to be a contraindication for the hepatocyte recipient. However, explanted livers of patients with familial amyloidotic polyneuropathy (FAP) are usually used for transplantation in another recipient rather than for the preparation of hepatocytes.

Steatotic donor livers, which are becoming more common with the increasing incidence of obesity in European populations, are currently not considered to be a viable source of cells for hepatocyte transplantation, as steatotic hepatocytes display impaired metabolic function and lower engraftment [9]. The average hepatocyte yield after perfusion varies from approximately 3×10^6 to 2×10^7 hepatocytes per gram of tissue, with variable viability yields reported (20-85 %) [9]; several billion cells are generally infused into one patient. Primary human hepatocytes do not proliferate *in vitro* and therefore cannot be expanded. Cryopreservation may have harmful effects on the viability and metabolic function of the cells [10].

All these limiting factors have prompted researchers and clinical teams to investigate the use of hepatocytes produced by the differentiation of pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) [11], which can both be indefinitely amplified and have the potential to become a permanent source of quality-controlled hepatocytes. Clinical-grade hepatocytes derived from these cells are now being produced by small companies and should be tested for clinical use in the coming years.

Donor criteria for hepatocyte donation are the same as those generally applied for organ donation for liver transplantation and with applicable criteria for tissue and cell donation. Donors positive for HIV, HBV or HCV, as well as for malignant tumours, are excluded.

Conditions to be evaluated as part of the donor-selection process are:

a. liver-originated disease of the donor that could be transferred to the recipient and might cause disease, e.g. hyperoxalosis, familiar amyloidotic polyneuropathy;
b. alterations to the liver vessels that could complicate perfusion and isolation of hepatocytes (though this is uncommon);
c. donor liver characteristics that might affect hepatocyte quality, such as the size of liver tissue, the degree of steatosis and the length of both warm and cold ischaemia and hypoxia [12, 13].

Neonatal livers are not generally considered for organ transplantation in view of the increased incidence of thrombosis and due to size limitations. Neonatal livers may, however, be a valuable source of hepatocytes and their function is comparable to (may even be superior to) hepatocytes derived from adult donors. Post-thaw viability of cryopreserved neonatal hepatocytes is significantly higher when compared to adult hepatocytes [14, 15].

27.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) is crucial to ensure graft safety. Risks are often multi-factorial, and to help quantify and map them a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and can implement the most efficient mitigation/risk-reducing measures, as needed.

Liver tissue is usually procured from deceased donors by the surgical liver-retrieval team of the transplant unit. Staff performing the procurement must be adequately trained in liver retrieval. Liver tissue should be flushed either *in situ* or *ex vivo* with an appropriate organ-perfusion solution through the hepatic artery and/or portal vein.

The procured liver is then placed in an appropriate organ-storage solution and triple-packaged in sterile packaging. This package should then be placed in another container that ensures a temperature of 2-8 °C and protects the recovered tissues during transport. Organ-perfusion machines are currently being evaluated for storage and transportation of liver tissue for organ transplantation and could potentially lead to a change in practice.

27.4. Processing and storage

Organs are transported to the designated isolation facility for processing. In selecting an appropriate air-quality specification for processing hepatocytes, the criteria identified in Chapter 7, Chapter 9 and the output from the MiRCA tool should be considered.

Depending on the size of the organ, the liver may be divided and perfused in parts. It has been reported that liver tissue preserves liver function better than isolated hepatocytes, so for repeated infusions of fresh hepatocytes (i.e. not cryopreserved) it may be better to isolate hepatocytes from different segments at different times to assure good perfusion and to minimise the time of isolated cells in suspension [16]. Vessels are cannulated to ensure perfusion of the liver tissue.

The liver tissue is perfused in a 2- or 3-step procedure at 37-38 °C. First, buffer containing ethylene glycol tetra-acetic acid (EGTA) is pumped through the tissue to remove divalent ions, thereby disrupting cell–cell connections, and then the EGTA is washed away by perfusion with buffer only. Finally, the tissue is perfused with collagenase/protease to digest extracellular matrix [8]. In some protocols, the second (wash-out) step may be omitted. Addition of the antioxidant N-acetylcysteine to the perfusion solution when isolating hepatocytes from fatty liver has demonstrated significant improvement in cell viability and metabolic function, and may be added for isolation of hepatocytes for clinical use. Isolated hepatocytes are purified by low-speed centrifugation. Cells that meet the release criteria after quality assessment (see §27.5) can be transplanted immediately after isolation, or cryopreserved and stored. Cells for transplantation are suspended in transplant medium (Plasmalyte or Eagle's minimum essential medium) containing 300 mM glucose heparin and human serum albumin (4 % final concentration) at a concentration of approximately 1-2 × 10^7 cells/mL [17].

27.5. Quality controls/release criteria

Hepatocytes exhibit a wide variety of functions that can be individually tested. Indeed, quality testing could be made appropriate to the recipient's disease, e.g. measurement of urea synthesis for recipients with urea cycle defects, or phase II conjugation activity for patients with Crigler–Najjar syndrome [18]. However, when fresh hepatocytes are used, there is limited time for functional assessment before infusion. The most important quality-control tests are viability (should be > 50 %), as assessed by trypan blue exclusion, and number of cells. It should be remembered, however, that the trypan blue exclusion test detects only cell-membrane damage; it cannot detect apoptotic cells nor determine metabolic or physiological function. Functional tests should, however, be performed on aliquots of hepatocytes from the same batch used for transplantation, either in parallel or afterwards, for the evaluation of hepatocyte function for each batch/donor.

Cryopreserved hepatocytes have the advantage that more extensive quality and genetic testing can be performed, which is not possible when using fresh hepatocytes due to time constraints. However, current cryopreservation protocols induce severe hepatocyte damage [19, 20], which decreases both viability and function [9]. Testing should therefore be repeated after thawing of aliquots.

The following tests could also be considered as quality-control tests:

a. plating efficiency on coated plates (collagen, laminin, fibronectin or EHS matrigel), ability to attach to each other (spheroid formation);
b. enzyme activities (cytochrome P450 activities, conjugation of bile acids, metabolism of molecular probes such as EROD, PROD, CDFDA);
c. synthesis (albumin, A1AT, bile acids, lipoproteins);
d. urea cycle activity, metabolism of ammonia into urea;
e. markers of apoptosis.

Since none of these endpoints have specifically been demonstrated to correlate with engraftment or *in vivo* proliferation of hepatocytes, no specific assays can be mandated at this time. If such assays are conducted, the data from any individual assay should not be considered sufficient cause to exclude the use of the cells for a transplant. These assays will provide additional information on hepatic function that can be used in conjunction with additional data, including trypan blue exclusion, to help in the decision whether to use or not use cells for a transplant or to evaluate outcome.

Limited testing (viability tested on trypan blue and sterility on Gram staining) is used when fresh cells are transplanted; however, subsequent analysis allows for retrospective data on sterility and function of the cells.

27.6. Packaging and distribution

Hepatocytes can either be transported under hypothermic conditions (2-8 °C) or cryopreserved.

Hepatocytes transported under hypothermic conditions should be stored in an appropriate preservation solution. Transportation time under hypothermic conditions should be kept as short as possible, because hepatocytes decrease in viability and function over time [16].

27.7. Administration of hepatocytes

Although a few cases of intraperitoneal and intra-splenic administration have been described, the most common route of infusion is into the portal vein. Intra-splenic infusion has been used in cases of liver cirrhosis, where intra-portal infusion is not possible or too risky. Intraperitoneal infusions are used when temporary liver support is needed for bridging. Intra-portal infusion is the main cell delivery route for clinical hepatocyte transplantation.

Intra-portal infusion is the main cell delivery route for clinical hepatocyte transplantation. The portal vein may be accessed by percutaneous transhepatic catheterisation of a portal vein branch, through catherisation of an ileal vein by a mini-surgical approach, or by repermeabilisation of the umbilical vein [21].

The transhepatic approach entails a risk of intraperitoneal bleeding and of portal thrombosis. Although both risks are low in patients with normal liver function (most hereditary metabolic liver diseases), they may be much higher in patients with liver failure and coagulation disorders (acute liver failure and acute-on-chronic liver failure). A complete study of coagulation disorders before administration and potential corrections of the coagulation disorders by intravenous infusion of fresh frozen plasma and platelets might be necessary. Clinical (intensive care unit) and radiological (ultrasonography) surveillance are required after the administration in order to detect intrahepatic haematoma, peritoneal bleeding or portal vessels thrombosis.

The ileal vein approach requires an abdominal incision. Catheterisation of a small ileal vein may be difficult in patients with unstable haemodynamic status.

Another risk of portal infusion of hepatocytes is obstruction of sinusoids by clumps of cells, resulting in an increase of the portal pressure [22]. A continuous monitoring of the portal pressure during the infusion should be performed. Repeated portography aids in evaluating possible portal thrombosis and portal flow. Infusion rate must be slow: 1-2 mL/min [17]. In allogeneic hepatocyte transplantation, immunosuppressive treatment is given before and after hepatocyte infusion. Immunosuppressive protocols resemble protocols for whole organ transplantation.

Table 27.1. **Liver disorders**

Inborn errors of metabolism
• Crigler–Najjar syndrome Type 1
• Familial hypercholesterolaemia
• FVII deficiency Glycogen storage disease type 1
• Infantile Refsum's disease
• Progressive familial intrahepatic cholestasis type 2 (PFIC 2)
• Urea cycle
• Ornithine transcarbamylase deficiency
– Argininosuccinate lyase deficiency
– Carbamoylphosphate synthase type 1 deficiency
– Citrullinaemia
Acute liver failure
• Drug
• Viral
• Idiopathic
• Mushroom poisoning
• Acute fatty liver of pregnancy
Acute-on-chronic liver failure
• Alpha 1 antitrypsin deficiency
• Viral
• Alcohol

Sources: based on [1-2, 21-22].

27.8. Traceability

Records covering the complete procedure – from donor selection to recipient transplantation – should be kept at the tissue establishment (see Chapter 2 and Chapter 15). If the donor also donated other organs, special care should be taken to ensure traceability from the organ donor to all other organ and tissue recipients, and vice versa.

27.9. Biovigilance

For all relatively new clinical applications of human cells, documentation of all adverse events and reactions is of particular importance because we can learn from them (see also Chapter 17). For example, the above-mentioned lack of *in vitro* endpoints that correlate with engraftment or proliferation of hepatocytes *in vivo* will only be clarified after collecting sufficient data as well as monitoring adverse events during procurement and processing of hepatocytes.

27.10. Developing clinical applications

Hepatocyte transplant has in the past been used as a 'bridge' to liver transplantation in patients

with the liver-based metabolic disorders shown in Table 27.1.

The literature shows that the outcome has not been satisfactory, partly due to a massive early cell loss after infusion and that long-term function is currently limited as the function of the transplanted hepatocytes declines and ceases to function past 300-500 days [23].

There are other options for alternative cell sources, such as neonatal derived hepatocytes or pluripotent stem cells, and strategies like encapsulation are being explored to inform the future as an alternative to hepatocyte transplant.

27.11. References

1. Dhawan A, Puppi J, Hughes RD, Mitry RR. Human hepatocyte transplantation: current experience and future *challenges*. *Nature Rev Gastroenterol Hepatol* 2010;7(5):288-98.
2. Hughes RD, Mitry RR, Dhawan A. Current status of hepatocyte transplantation. *Transplantation* 2012; 93(4):342-7.
3. Jorns C, Ellis EC, Nowak G *et al.* Hepatocyte transplantation for inherited metabolic diseases of the liver. *J Intern Med* 2012;272(3):201-23.
4. Horslen SP, McCowan TC, Goertzen TC *et al.* Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics* 2003;111(6 Pt 1):1262-7.
5. Mitry RR, Dhawan A, Hughes RD *et al.* One liver, three recipients: segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. *Transplantation* 2004 May 27;77(10):1614-16. https://doi.org/10.1097/01.tp.0000122224.98318.19. PMID: 15239631.
6. Enosawa S, Horikawa R, Yamamoto A *et al.* Hepatocyte transplantation using a living donor reduced graft in a baby with ornithine transcarbamylase deficiency: a novel source of hepatocytes. *Liver Transpl* 2014 Mar;20(3):391-3.
7. Gramignoli R, Tahan V, Skvorak K *et al.* New potential cell source for hepatocyte transplantation: discarded livers from metabolic disease liver transplants. *Stem Cell Res* 2013;11(1):563-73.
8. Soltys KA, Setoyama K, Tafaleng EN *et al.* Host conditioning and rejection monitoring in hepatocyte transplantation in humans. *J Hepatol* 2017;66(5): 987-1000.
9. Ibars EP, Cortes M, Tolosa L *et al.* Hepatocyte transplantation program: lessons learned and future strategies. *World J Gastroenterol* 2016;22(2):874-86.
10. Terry C, Dhawan A, Mitry RR *et al.* Optimization of the cryopreservation and thawing protocol for human hepatocytes for use in cell transplantation. *Liver Transpl* 2010;16(2):229-37.
11. Hannoun Z, Steicheny C, Dianat N *et al.* The potential of induced pluripotent stem cell derived hepatocytes. *J Hepatol* 2016;65(1):182-99.
12. Kawahara T, Toso C, Douglas DN *et al.* Factors affecting hepatocyte isolation, engraftment and replication in an *in vivo* model. *Liver Transpl* 2010;16(8): 974-82.
13. Sagias FG, Mitry RR, Hughes RD *et al.* N-acetylcysteine improves the viability of human hepatocytes isolated from severely steatotic donor liver tissue. *Cell Transplant* 2010;19(11):1487-92. https://doi.org/10.3727/096368910X514620.
14. Lee CA, Dhawan A, Iansante V *et al.* Cryopreserved neonatal hepatocytes may be a source for transplantation: evaluation of functionality towards clinical use. *Liver Transpl* 2018:24(3):394-406.
15. Tolosa L, Pareja-Ibars E, Donato MT *et al.* Neonatal livers: a source for the isolation of good-performing hepatocytes for cell transplantation. *Cell Transplant* 2014;23(10):1229-42.
16. Jorns C, Gramignoli R, Saliem M *et al.* Strategies for short-term storage of hepatocytes for repeated clinical infusions. *Cell Transplant* 2014;23(8):1009-18.
17. Puppi J, Tan, N, Mitry RR *et al.* Hepatocyte transplantation followed by auxiliary liver transplantation – a novel treatment for ornithine transcarbamylase deficiency. *Am J Transplant* 2008;8(2):452-7.
18. Bonora-Centelles A, Donato MT, Lahoz A *et al.* Functional characterization of hepatocytes for cell transplantation: customized cell preparation for each receptor. *Cell Transplant* 2010;19(1):21-8.
19. Stéphenne X, Najimi M, Ngoc DK *et al.* Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex 1. *Cell Transplantation* 2007; 16:409-19.
20. Stéphenne X, Najimi M, Sokal EM. Hepatocyte cryopreservation: Is it time to change the strategy? *World J Gastroenterol* 2010; 16(1):1-14. PMID: 20039443.
21. Dhawan A. Clinical human hepatocyte transplantation: current status and challenges. *Liver Transpl* 2015; 21(Suppl 1):S39-S44.
22. Jorns C, Nowak G, Nemeth A *et al.* De novo donor-specific HLA antibody formation in two patients with Crigler-Najjar Syndrome Type I following human hepatocyte transplantation with partial hepatectomy preconditioning. *Am J Transplant* 2016;16(3):1021-30.
23. Fox IJ, Chowdhury JR, Kaufman SS *et al.* Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med*. 1998 May 14;338(20): 1422-6. https://doi.org/10.1056/NEJM199805143382004.

Chapter 28. **Adipose tissue**

28.1. Introduction

28.1.1. Adipose tissue properties and background

Adipose tissue is a complex and specialised connective tissue consisting of a stromal vascular fraction (SVF) and adipocytes. Functions of adipose tissue are energy handling in the form of lipids, hormone production and local tissue architecture maintenance. The purpose of adipose tissue transplantation is reconstructive and aesthetic: to repair, to contour deformities or to augment.

The main advantage of autologous fat transplantation is that the adipose tissue is abundantly and easily available, and does not induce immune response in the recipient. The majority of the studies and clinical data on adipose tissue transplantation derive from patients receiving autologous adipose tissue, which is procured, processed and grafted in a one-step surgical procedure.

The first historical reference to adipose tissue transplant was reported by Neuber in 1893, transferring autologous adipose tissue from the forearm to the face; afterwards fat was used for facial atrophy and malar augmentation and, later on, the first fat injection through needle was reported [1].

Initially, the outcomes of fat transplantation were unclear because of the variability of tissue resorption on patients, and in 1950 the fat transplanted retention rate per year was around 40-50 % [2]. Nowadays long-term results at one year remain unpredictable, due to the high rate of resorption in the grafted site (ranging between 20 and 80 %) [3, 4]. The consequence of resorption is the need for additional procurement and grafting procedures with the increase of inherent surgical risk, costs and discomfort for the patient.

Some key points to improve adipose tissue transplantation outcomes are based on various studies. The importance of atraumatic procurement and processing to maximise fat viability/survival and adipose tissue transplantation success was first described in a 1993 study [5], where the viability of various fat cylinders was compared, resulting in a greater survival for those with less than 3 mm diameter/depth, observing that the central part of grafts of more than 3.5 mm was necrotised [6].

Adipose tissue banking eliminates the need for repeated liposuction and, thereby, reduces cost and the risk of morbidity. Discrepancies in adipose tissue storage efficiency have been reported [7, 8]. In summary, several studies [9-12] observed diverse effectiveness in the improvement of pathological conditions when using cryopreserved or fresh adipose tissue despite the length of the follow-up (5-120 months). Nevertheless, the existing scientific evidence is insufficient to permit the standardisation of procedures.

All the components of adipose tissue contribute to correct engraftment, regulating adipogenesis and angiogenesis and preventing adipocyte necrosis [13, 14, 15].

Finally, the lipofilling technique, reinjecting fat aspirated by liposuction, has become one of the most popular procedures performed by plastic and aes-

thetic surgeons [16, 17]. Nowadays, the most common method of adipose tissue procurement and transplantation is Coleman's technique [18].

28.1.2. Indications for adipose tissue transplantation

Indications in favour of fat transfer for reconstructive and aesthetic purposes are multiple, and include any area needing volume and/or regeneration. Among reconstructive indications it has been used for breast reconstruction [19], depressed scars, eyelid depression, pitting acne, post-traumatic defects, conditions such as Romberg's disease, facial microsomia and facial hemiatrophy, improvement of function and appearance of irradiated tissues, correction of asymmetry in Poland's syndrome, soft-tissue defect correction after trauma, oncologic treatments and lipodystrophies. In aesthetic surgery, to enhance volume in any part of the body (mainly face, breast and buttocks) and correct aesthetic defects (sequelae of operations, combined with implants).

The following generic chapters (Part A) of this Guide all apply to adipose tissue banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

28.2. Donor evaluation

The criteria for donor selection to be applied are the criteria for autologous donation. The patient must be provided with sufficient information on the process (including the planned storage period and tests performed) and must sign an informed consent form.

Additionally, it should be ascertained that donors do not have any major systemic diseases or lipid disorders, and that they are not underweight. As adipose tissue is to be stored and not only used in the same surgical procedure, infectious disease testing must be performed for all autologous adipose tissue patients, as described in Chapter 6. Patients known to have HIV or hepatitis B/C can be accepted for autologous use. In this case, the tissues and cells must be labelled accordingly (e.g. CAUTION: BIOLOGICAL HAZARD) and stored separately or under special conditions. (For further details, see §10.2.8 and §10.2.9.)

28.3. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) is crucial to ensure adipose tissue safety. Risks are often multi-factorial, and to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, tissue establishments can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and can implement the most efficient mitigation or risk-reducing measures, as needed.

Particular attention should be paid to procurement conditions, because they support the initial quality and low bioburden of the adipose tissue.

28.3.1. Donor site

There are various subcutaneous adipose areas of the body examined to identify natural fat deposits. The most common donor site is abdominal fat because it is one of the largest fat deposits. The second most common sites are the greater trochanteric region and the inside of the thighs and knee [20, 21]. Age, but not body mass index, seems to influence adipocytes' viability in relation to the choice of donor's site for procurement [22], and there is no significant difference between different donor sources in cell viability or volume retention. The superficial layer of subcutaneous adipose tissue should be recommended instead

of the deep layer to obtain the greatest stromal component, which ensures higher multipotency and stemness features [23], but the main inconvenience of using this layer is the potential morbidity associated with it, such as irregularities and visible deformities.

Local anaesthesia (lidocaine) and tumescent solutions, applied before procurement, seem to adversely affect the metabolism of adipocytes, with reduced glucose transport, lipolysis, viability and differentiation of pre-adipocytes or adipose-derived stromal/stem cells (ASC) [24, 25]. That effect, however, seems to disappear when the anaesthetic is removed [22] so, after washing, the tissue is able to fully regain its function.

28.3.2. Procurement techniques

Various procurement and preparation techniques have been introduced to obtain better and more reliable survival of adipose tissue. Tissue collection technique should guarantee minimal manipulation of the tissue before transplantation, allowing a soft mechanical disruption and giving the advantage of keeping all the structural elements of the tissue, including extracellular matrix and perivascular structures. The smaller the adipose tissue fragments, the better the oxygen and nutrient supply can be retained, favouring a higher rate of engraftment. Even if several strategies have been used, one of the major issues is still standardisation of the procedures because there are no data supporting the choice of a best technique for the procurement [26, 27].

The fat tissue is usually procured with a specific cannula with negative pressure from abdomen, thighs and hip with Coleman technique [28, 29]. The technique uses a series of cannulas aiming to obtain atraumatic fat and its safe infiltration, reducing the possibility of intravascular injection. The use of a small cannula size for the liposuction also negatively influences tissue viability and finally fat engraftment, affected by flow speed and the shear stress [30, 31]. Conventional liposuction techniques, hand-held syringes and new devices show no differences in fat outcomes [32-35]. Nonetheless, it has been observed that laser liposuctioning provides higher cell viability and enhances sample quality in comparison to mechanical liposuctioning [36].

28.3.3. Transfer to tissue establishment

Adipose aspirates are collected with a syringe in a specific container, refrigerated at a transport temperature of 4 °C and should be transferred immediately to the tissue establishment for processing and long-term storage [37].

28.4. Processing

There are several published protocols for processing adipose tissue, but there is no evidence to prefer one technique above another. During fat processing, the retrieved fat undergoes a process of eliminating fluid, blood, cell fragments and oil. In selecting an appropriate air-quality specification for processing adipose tissue, the criteria identified in Chapter 7 and Chapter 9 and the output from the MiRCA tool should be considered. Appropriate measures should be taken to minimise the risk of microbiological contamination, including an antibiotic decontamination step to the lipoaspirate.

Tissue processing includes washing (e.g. 0.9 % NaCl) [39], and centrifugation (e.g. 300-3400 rpm for 3 min) [39] or decanting, or procedures that allow increased concentration of the tissue, draining and separating the floating adipose tissue from the excess of fluids, blood and debris. New protocols recently suggest that the best results are obtained by washing and gentle centrifugation [40]. Centrifugation and decantation remove 50-60 % of red cells and leukocytes, and filtering and washing removes 90 % of erythrocytes and leukocytes [40]. The presence of blood reduces the viability of fat, causes inflammation and impairs engraftment. The utilisation of filtration bags for collection simplifies processing of the tissue because several steps can be done directly in the collection bag, allowing a rapid elimination of fluids and free lipids and the microfragmentation of the tissue with the preservation of the native architecture.

Adipose tissue is filled by lipids that make it very sensitive to external treatments (centrifuge, processing methods and temperature) so the storage at room temperature and high centrifugation speed should be avoided to reduce the loss of tissue viability [40, 41]. Centrifugation at 400 g is sufficient to remove red fraction and excess of fluids and greater forces produce higher oil fraction.

The better survival rates (similar to fresh specimens) were found with tissue samples frozen at a slow controlled rate (1-2 °C/min) in the presence of cryoprotectants. For long-term storage a temperature of < −140 °C (in vapour phase of liquid nitrogen) ensures preservation of maximum tissue viability [41-43].

In particular, as well as the requirements for cryoprotectants and controlled freezing/storage, the following factors can affect the viability of transplanted adipose tissue [44]:

- procurement → procurement method, source location, donor age;
- processing → wash solutions, centrifugation, disaggregation;
- storing → media, cryoprotectants, storage temperature;
- recipient bed → infusion solutions, growth factors;
- implantation → method, location, flexibility.

There must be written protocols for all procedures related to liposuction and tissue transfer to tissue-processing facilities. To cryopreserve adipose tissue, quality control is an essential issue. Adipose tissues must be processed under sterile conditions and in an aseptic manner. All biological tests should be performed as described in Chapter 6 and Chapter 11, if the tissue is processed (regardless of the location where this is done).

Quality control must include microbiological testing of each batch. Microbiological analyses of procured tissue, rinsing solutions and tissue after possible decontamination, must be carried out according to the *European Pharmacopoeia* (*Ph. Eur.*). In cases of positive test results after decontamination, the adipose tissue should be discarded.

To test the morphology and viability of tissue, histological analyses, cell-viability and/or functional assays are highly recommended. Histology gives only general information, mostly not linked to tissue viability given that the most popular staining protocols, such as haematoxylin and eosin, require tissue fixation [44].

The overall cell viability can be assayed using different stainings, based on cell-permeant/non-permeant [45] dyes that, when combined, mark live and dead cells at the same time. In addition, stromal vascular fraction and adipocytes can be isolated using enzymatic digestion from the same sample, allowing the assessment of live and dead cells of both components of adipose tissue.

28.5. Preservation/storage

Cryopreservation is one way to indirectly overcome the problem of absorption of the autologous fat graft results in repeated procurement procedures (with increasing cost and risks for the patient), as the patient only needs to undergo one procurement procedure. Unfortunately, an optimal technique for long-term preservation of adipose tissues is not available, and outcomes following implantation are diverse. Nevertheless, the literature clearly recommends the use of cryoprotectants when long-term storage of adipose tissue is desired [46-52].

The selection of cryoprotective agent is one of the key issues for obtaining optimal viability of mature adipocytes and their precursors. The most common cryoprotective agents used for adipose tissues are dimethyl sulphoxide (DMSO), trehalose [53, 54] and glycerol, or their use in combination. Published data report high cellular viability in the presence of DMSO [55] as cryopreserving when cooling is controlled and thawing is done by fast gentle warming [56].

Studies comparing different storage conditions have shown that the banking of adipose tissue at −20 °C [57] is not efficient because of the loss of adipocytes' viability, and for long-term storage processed adipose tissue should be kept in the vapour phase of liquid nitrogen (< −140 °C) [42].

When autologous adipose tissue is procured and stored, and serological test results become positive, this will not necessarily lead to discarding the tissue. For such tissues, isolated storage possibilities should be considered in order to exclude the risk of cross-contamination or mix-ups. Given that the best results of defect correction are achieved by multiple tissue injections, the storage of small aliquots is preferable [23, 58].

After cryopreservation, tissue can be thawed and after the removal of cryoprotective agent it can be shipped to clinicians, refrigerated in a temperature-controlled container using a transport buffer consisting of lactated Ringer's solution with 1 % human serum albumen [59]. To avoid loss of vital cells before lipoinjection, thawing at tissue establishments should be restricted to short-term transport. Preferably, cryopreserved fat should be shipped at −80 °C to the organisations for human application (OHRA) with written thawing procedures for clinicians.

Establishments must specify the shelf life of adipose tissue at defined temperature(s), to the best of their knowledge. This shelf life could be based on a recent paper [60] that reported that the time of cryostorage and cryoptotective agent does not influence the tissue recovered, even for long-term storage (>1 year).

28.6. Biovigilance

Any adverse reaction or event occurring during procurement, processing, thawing or reinjection of tissue must be notified, as described in Chapter 17.

Serious adverse reactions for adipose tissue transplantation include:

- infection or sepsis after cryopreserved fat grafting;
- graft failure (e.g. excessive volume loss, calcification);
- malignancy possibly attributable to the transplanted tissue (mainly due to cancer stem cells in the autologous transplant);
- fat embolism.

Serious adverse events include:

- wrong tissue supplied for the intended surgical procedure;
- tissue supplied was damaged or transported at wrong temperature;
- tissue supplied is beyond its expiry date.

There are two entries in the Notify Library (www.notifylibrary.org) related to infection from a cryopreserved autologous fat graft (Record ID 1689 and 1690):

- ID 1689 – Infection from a cryopreserved autologous fat graft 4 months after transplantation. The culture growth showed *Aspergillus*, and *Mycobacterium fortuitum* was also identified by PCR. The root cause was not identified but it is not likely to be donor derived since the autologous donor had no infections at the time of procurement. Suspicion of graft contamination was during cryopreservation steps, when it was stored for 2 months, during the thawing procedure or during implantation.
- ID 1690 – This study focused on the safety of cryopreserved fat injection. The samples studied showed that *Staphylococcus epidermidis* was the leading cause of cryopreserved fat contamination, and the resistance to methicillin is common. Based on the results, aseptic handling of fat during procurement and preservation appeared to be most important.

28.7. Developing applications

Currently, there is no consensus on adipose tissue cryopreservation and banking, neither regarding the cryopreservation agents nor the duration of the storage. The feasibility and efficacy of these procedures should also be investigated in clinical studies involving many patients. The answer to these questions will give certainty to the clinical translation of this approach, to achieve the optimal outcome for patients needing reconstructive surgeries.

Additionally, adipose tissue can be used not only as a structural support, but it may also be a source of SVF or stem cells that can be cryopreserved before or after their isolation [57, 61]. Cells can be used to improve fat grafting, as in cell-assisted lipotransfer [62], or for the immunomodulatory property of SVF, which is actually under extensive investigation for its possible use in treating sclerosis and autoimmune, inflammatory and neurologic conditions, taking into consideration that in some countries, following national regulations, it may fall under the scope of ATMPs. A search for clinical trials using SVF in www.clinicaltrials.gov reveals that 14 studies are currently actively recruiting for patients. Adipose-derived stem cells isolated from SVF and expanded *in vitro* are under investigation for a whole range of diseases [63, 64] (see Chapter 34).

28.8. References

1. Butterwick KJ. Autologous fat transfer: evolving concepts and techniques. *Surg Skin Proc Dermatol* 2014: 464.
2. Peer LA. Loss of weight and volume in human fat grafts. *Plast Reconstr Surg* 1950;5:217-30.
3. Niechajev I, Sevcuk O. Long term results of fat transplantation: clinical and histologic results. *Plast Reconstr Surg* 1994, 94:496-506.
4. Gallego S, Ramirez F, Echeverri A. Magnetic resonance imaging assessment of gluteal fat grafts. *Aesth Plast Surg* 2006;30:460-8.
5. Carpaneda CA, Ribeiro MT. Study of the histologic alterations and viability of the adipose graft in humans. *Aesth Plast Surg* 1993;17:43-7.
6. Khouri RKJ, Khouri RK. Current clinical applications of fat grafting. *Plast Reconstr Surg* 2017;140:466e-486e.
7. Shoshani O, Ullmann Y, Shupak A *et al.* The role of frozen storage in preserving adipose tissue obtained by suction-assisted lipectomy for repeated fat injection procedures. *Dermatol Surg* 2001;27(7):645-7.
8. Lidagoster MI, Cinelli PB, Levee EM *et al.* Comparison of autologous fat transfer in fresh, refrigerated and frozen specimens: an animal model. *Ann Plast Surg* 2000;44(5):512-15.
9. Butterwick KJ, Bevi AA, Lyer S. Fat transplantation using fresh versus frozen fat: a side-by-side two-hand comparison pilot study. *Dermatol Surg* 2006 May; 32(5):640-4.
10. Moscatiello F, Aznar-Benitah S, Grella R, Jover JH. Gluteal augmentation with cryopreserved fat. *Aesthet Surg J* 2010 Mar;30(2):211-16.
11. Ma H, Fang Y-H, Lin C-H. Facial recontouring with autologous cryopreserved fat graft. *Formosan Journal of Surgery* 2018;51:58-62.
12. Ohasi M, Chiba A, Nakai H *et al.* Serial injections of cryopreserved fat at -196° for tissue rejuvenation, sacar

treatment and volume augmentation. *Plast Reconstr Surg Glob Open* 2018 May;6(5):e1742.

13. Eto H, Kato H, Suga H *et al.* The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. *Plast Reconstr Surg* 2012;129:1081-92.
14. Fu S, Luan J, Xin M *et al.* Fate of adipose-derived stromal vascular fraction cells after co-implantation with fat grafts: evidence of cell survival and differentiation in ischemic adipose tissue. *Plast Reconstr Surg* 2013;132:363-73.
15. Dong Z, Peng Z, Chang Q *et al.* The angiogenic and adipogenic modes of adipose tissue after free fat grafting. *Plast Reconstr Surg* 2015:135:556e-567e.
16. Illouz YG. The fat cell graft: a new technique to fill depressions. *Plast Reconstr Surg* 1986;78:122-3.
17. Fournier PF. Facial recontouring with fat grafting. *Dermatol Clin* 1990;8:523-37.
18. Coleman SR. Structural fat grafts: the ideal filler? *Clin Plast Surg* 2001;28:111-19.
19. Bircoll M. Cosmetic breast augmentation utilizing autologous fat and liposuction techniques. *Plast Reconstr Surg* 1987;79:267-71.
20. Hamza A, Lohsiriwat V, Rietjens M. Lipofilling in breast cancer surgery. *Gland Surg* 2013;2(1):7-14.
21. Simonacci F, Bertozzi N, Grieco MP *et al.* Autologous fat transplantation for breast reconstruction: a literature review. *Ann Med Surg (Lond)* 2016 Nov 23;12: 94-100. eCollection 2016 Dec.
22. Geissler PJ, Davis K, Roostaeian J *et al.* Proving fat transfer viability: the role of aging, body mass index, and harvest site. *Plast Reconstr Surg*, 2014 Aug 1;134(2): 227-32.
23. Ohashi M. Cryopreserved fat: our clinical experience and applications. *Plast Aesthet Res* 2020;7:26. https://doi.org/10.20517/2347-9264.2020.15.
24. Kirkham JC, Lee JH, Austen WG. Fat graft survival: physics matters [invited commentary to "The impact of liposuction cannula size on adipocyte viability"]. *Ann Plast Surg* September 2014;73(3):359.
25. Keck M, Zeyda M, Gollinger K *et al.* Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes. *Plast Reconstr Surg* 2010;126:1500-5.
26. Pu LL, Cui X, Fink BF *et al.* The viability of fatty tissues within adipose aspirates after conventional liposuction: a comprehensive study. *Ann Plast Surg* 2005;54:288-92.
27. Svalgaard JD,·Juul S,·Vester-Glovinski PV *et al.* Lipoaspirate storage time and temperature: effects on stromal vascular fraction quality and cell composition. *Cells Tissues Organs* 2020;209:54-63.
28. Coleman SR. Lipoinfiltration in the upper lip white roll. *Aesth Surg* 1994;14:231-4.
29. Coleman SR. Structural fat grafts: the ideal filler? *Clin Plast Surg* 2001;28:111-19.
30. Kirkham JC, Lee JH, Medina MA *et al.* The impact of liposuction cannula size on adipocyte viability. *Ann Plast Surg* 2012 Oct;69(4):4.
31. Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study. *J Plast Reconstr Aesthet Surg* 2009; 62:1210-14.
32. Smith P, Adams WP Jr, Lipschitz AH *et al.* Autologous human fat grafting: Effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg* 2006;117:1836-44.
33. Keck M, Kober J, Riedl O *et al.* Power assisted liposuction to obtain adipose-derived stem cells: impact on viability and differentiation to adipocytes in comparison to manual aspiration. *J Plast Reconstr Aesthet Surg* 2014;67:e1-e8.
34. Lee JH, Kirkham JC, McCormack MC *et al.* The effect of pressure and shear on autologous fat grafting. *Plast Reconstr Surg* 2013;131:1125-36.
35. Fisher C, Grahovac TL, Schafer ME *et al.* Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation. *Plast Reconstr Surg* 2013;132:351-61.
36. Levenberg A, Scheinowitz M, Sharabani-Yosef O. Higher cell viability and enhanced sample quality following laser-assisted liposuction versus mechanical liposuction. *J Cosm Derm Sci App* 2015;5(03), 238-45. https://doi.org/10.4236/jcdsa.2015.53029.
37. Trivisonno A, Alexander RW, Baldari S *et al.* Intraoperative strategies for minimal manipulation of autologous adipose tissue for cell- and tissue-based therapies: concise review. *Stem Cells Transl Med* 2019 Dec;8(12):1265-71.
38. Fraser JK, Wulur I, Alfonso Z *et al.* Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots. *Cytotherapy* 2007;9:459-67.
39. Hoareau L, Bencharif K, Girard AC *et al.* Effect of centrifugation and washing on adipose graft viability: a new method to improve graft efficiency. *J Plast Reconstr Aesthet Surg* 2013;66:712-19.
40. Kurita M, Matsumoto D, Shigeura T *et al.* Influences of centrifugation on cells and tissues in liposuction aspirates: optimized centrifugation for lipotransfer and cell isolation. *Plast Reconstr Surg* 2008 Mar;121(3): 1033-41.
41. Lidagoster MI, Cinelli PB, Leveé EM, Sian CS. Comparison of autologous fat transfer in fresh, refrigerated, and frozen specimens: an animal model. *Ann Plast Surg* 2000 May;44(5):512-15.
42. Moscatello DK, Dougherty M, Narins RS, Lawrence N. Cryopreservation of human fat for soft tissue

augmentation: viability requires use of cryoprotectant and controlled freezing and storage. *Dermatol Surg* 2005 Nov;31(11 Pt 2):1506-10.

43. Moscatiello F, Aznar-Benitah S, Grella R *et al.* Gluteal augmentation with cryopreserved fat. *Aesthet Surg J* 2010 Mar;30(2):211-16.
44. Berry R, Church CD, Gericke MT *et al.* Imaging of adipose tissue. *Methods Enzymol* 2014;537:47-73.
45. Zubillaga V, Alonso-Varona A, Fernandes SCM *et al.* Adipose-derived mesenchymal stem cell chondrospheroids cultured in hypoxia and a 3D porous chitosan/chitin nanocrystal scaffold as a platform for cartilage tissue engineering. *Int J Mol Sci* 2020 Feb 3; 21(3):1004. https://doi.org/10.3390/ijms21031004.
46. Li BW, Liao WC, Wu SH *et al.* Cryopreservation of fat tissue and application in autologous fat graft: *in vitro* and *in vivo* study. *Aesthetic Plast Surg* 2012;36(3):714-22.
47. Pu LL, Coleman SR, Cui X *et al.* Cryopreservation of autologous fat grafts harvested with the Coleman technique. *Ann Plast Surg* 2010;64:333-7.
48. Cui XD, Gao DY, Fink BF *et al.* Cryopreservation of human adipose tissues. *Cryobiology* 2007;55(3):269-78.
49. Atik B, Ozturk G, Erdogan E *et al.* Comparison of techniques for long-term storage of fat grafts: an experimental study. *Plast Reconstr Surg* 2006;118:1533-7.
50. Pu LL, Cui X, Li J *et al.* The fate of cryopreserved adipose aspirates after *in vivo* transplantation. *Aesthet Surg J* 2006;26(6):653-61.
51. Wolter TP, von Heimburg D, Stoffels I *et al.* Cryopreservation of mature human adiposcytes: *in vitro* measurement of viability. *Ann Plast Surg* 2005;55: 408-13.
52. MacRae JW, Tholpady SS, Ogle RC *et al.* Ex vivo fat graft preservation: effects and implications of cryopreservation. *Ann Plast Surg* 2004;52(3):281-2.
53. Cui X, Pu LL. The search for a useful method for the optimal cryopreservation of adipose aspirates: part I. In vitro study. *Aesthet Surg J* 2009;29(3):248-52.
54. Cui X, Pu LL. The search for a useful method for the optimal cryopreservation of adipose aspirates: part II. In vivo study. *Aesthet Surg J* 2010;30(3):451-6.
55. Yong KW, Pingguan-Murphy B, Xu F *et al.* Phenotypic and functional characterization of long-term cryopreserved human adipose-derived stem cells. *Sci Rep* 2015 Apr 15;5:9596. https://doi.org/10.1038/srep09596.
56. Pu LL. Cryopreservation of adipose tissue. *Organogenesis* 2009;5(3):138-42.
57. Hwang SM, Lee JS, Kim HD *et al.* Comparison of the viability of cryopreserved fat tissue in accordance with the thawing temperature. *Arch Plast Surg* 2015;42: 143-9.
58. Kakagia D, Pallua N. Autologous fat grafting: in search of the optimal technique. *Surg Innov* 2014 Jun; 21(3):327-36.
59. Badowski MS, Muise A, Harris DT. Patient use of autologous cryopreserved intact adipose tissue from lipoaspirate. *AIMS Cell and Tissue Engineering* 2017; 1(3):224-35. https://doi.org/10.3934/celltissue.2017.3.224.
60. Badowski MS, Muise A, Harris DT. Long-term biobanking of intact tissue from lipoaspirate. *J Clin Med* 2019 Mar;8(3):327.
61. Choudhery MS, Badowski M, Muise A *et al.* Cryopreservation of whole adipose tissue for future use in regenerative medicine. *J Surg Res* 2014; 187(1):24-35.
62. Laloze J, Varin A, Bertheuil N *et al.* Cell-assisted lipotransfer: Current concepts. Transfert graisseux enrichi en cellules souches: concepts actuels. *Annales de Chirurgie Plastique Esthétique* 2017 Dec;62(6):609-16.
63. Roato I, Alotto D, Belisario DC *et al.* Adipose derived-mesenchymal stem cells viability and differentiating features for orthopaedic reparative applications: banking of adipose tissue. *Stem Cells Int* 2016; 2016:4968724. https://doi.org/10.1155/2016/4968724.
64. Feisst V, Meidinger S, Locke MB. From bench to bedside: use of human adipose-derived stem cells. *Stem Cells Cloning* 2015;8:149-62.

Chapter 29. **Medically assisted reproduction**

29.1. Introduction

This chapter refers to the medical procedures used to achieve pregnancies and live births involving the identification, procurement (collection), processing and/or storage as well as distribution of at least one of the following reproductive cells and tissues: oocytes, sperm, zygotes, embryos, ovarian and testicular tissue. These procedures may be carried out using freshly collected and/or cryopreserved gametes, zygotes or embryos originating from the couple being treated ('partner donation') and also from gamete or embryo donors ('non-partner' or 'third party donation'). For the procurement, processing and/or storage of ovarian and testicular tissue, we refer to Chapter 30 – Fertility preservation. These contexts are addressed separately because of the different risks involved. Ovarian stimulation, or any other clinical procedure that does not involve gamete procurement, is not addressed in this chapter.

Medically Assisted Reproduction (MAR) is also referred to as Assisted Reproductive Technology (ART). However, MAR is a broader term, which includes ART, but also includes ovarian stimulation and intra-uterine insemination of sperm, whereas the term ART refers only to procedures that include *in vitro* handling and culture of gametes or embryos.

MAR is carried out in centres specialised in treating patients with fertility problems. These centres usually consist of a tissue establishment (TE) and an organisation responsible for human application, bringing together a laboratory team and a clinical team in a multidisciplinary unit.

Procurement, processing and/or storage – as well as release and distribution of reproductive cells – may also be performed by gamete cryobanks that are not part of a fertility clinic. The recommendations in this chapter concern all institutions where reproductive cells are handled.

MAR comprises various procedures, among which are the following.

- Processing of sperm for the purpose of intra-uterine insemination. Sperm is processed and transferred into the uterus close to the estimated time of ovulation [1].
- *In vitro* fertilisation (IVF), either conventional, whereby collected and prepared sperm and oocytes are co-incubated (so-called routine or standard IVF), or intracytoplasmic sperm injection (ICSI), whereby a single spermatozoon is injected into a mature oocyte. IVF involves procurement and processing of gametes, fertilisation, embryo culture and transfer of embryos into the uterus.
- *In vitro* maturation (IVM) refers to the maturation of oocytes recovered from non-preovulatory follicles that have been primed or not with exogenous gonadotrophins.
- Cryopreservation and storage of gametes, zygotes, embryos and/or gonadal tissue.
- Pre-implantation genetic testing (PGT), which uses genetic identification methods to diagnose or screen oocytes or embryos *in vitro* to ascertain if they are affected by known inherited diseases or chromosomal rearrangements incompatible with the birth of a healthy child.

This approach comprises different forms of testing, such as PGT-M for monogenic/single gene defects and mitochondrial disorders, PGT-SR for chromosomal structural rearrangements and PGT-A for aneuploidy testing.

Cryopreservation of gametes or gonadal tissue can be used in patients affected by certain diseases (e.g. cancer, some chronic pathologies) whose treatment may be potentially harmful to their fertility. In those cases, long-term storage of cryopreserved reproductive cells and tissues may be proposed to children, adolescents and adults. This approach, called 'fertility preservation' (addressed in Chapter 30), also represents a treatment option for non-medical cases, so-called 'social freezing'. MAR treatments can also be proposed to partners at risk of transmitting a serious transmissible disease – e.g. human immunodeficiency virus (HIV) or hepatitis B and C viruses (HBV and HCV) – to one another (horizontal transmission) and/or to their future child (vertical transmission). These practices are applied only after risk assessment of vertical and horizontal disease transmission and taking into account the individuals' health condition [2]. In some countries, MAR can be offered to single women or homosexual female couples. In a few countries in Europe, under stringent conditions, surrogate motherhood is allowed for women without a uterus or with a non-functional uterus, or for male homosexual couples. Through insemination or embryo transfer, the gestational carrier (formerly known as 'surrogate mother') [1] carries and gives birth to a child for the intended parents.

MAR is performed in most countries in Europe. Each year, the European Society of Human Reproduction and Embryology (ESHRE) publishes a report of MAR activity in European countries, based on voluntary declarations. The latest published ESHRE data (from 2017) include data from 1 382 clinics in 39 countries and report 940 503 treatment cycles, including 165 379 IVF/ICSI, 271 476 of frozen embryo replacement (FER), 37 303 with preimplantation genetic testing, 69 378 with egg donation (ED), 378 of *in vitro* maturation (IVM) of oocytes and 5 210 cycles with frozen oocyte replacement (FOR). European data on intra-uterine insemination using husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 1 352 institutions in 25 countries and 21 countries, respectively. A total of 139 050 IUI-H and 49 001 IUI-D cycles were included. Thirteen countries reported 18 888 interventions for fertility preservation, including oocyte, ovarian tissue, semen and testicular tissue banking and pre- and post-pubertal patients [3]. This chapter aims to provide guidelines that can help to conceive singletons resulting in a healthy liveborn, which is the ultimate goal of MAR. The relevant medical activities may in some countries be considered ethically sensitive. The procedures described here are intended to achieve clinical efficiency in terms of delivery rates and also address the safety of patients, donors and children born.

In addition, the following generic chapters (Part A) of this Guide all apply to MAR and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

This chapter defines additional specific requirements for MAR. Procedures may vary from country to country as determined by national legislation.

29.2. Quality management, risk management and validation

The implementation of a quality management system is mandatory and will contribute to compliance as well as to the success of an MAR programme. This section should be read in conjunction with Chapter 2 and Chapter 3; however, certain MAR-specific matters concerning quality management are addressed below.

29.2.1. Risk-assessment analysis for laboratory activities

Risk management will help in assessing and prioritising the possible existing hazards for monitoring and control purposes, so that the probability of an adverse event occurring is kept to a minimum. The most commonly applied methods of risk assessment are: Failure mode and effects analysis (FMEA) [4], Failure mode, effects and criticality analysis (FMECA) and Hazard analysis and critical control points (HACCP). The process of risk assessment is described in Chapter 3 at §3.1.

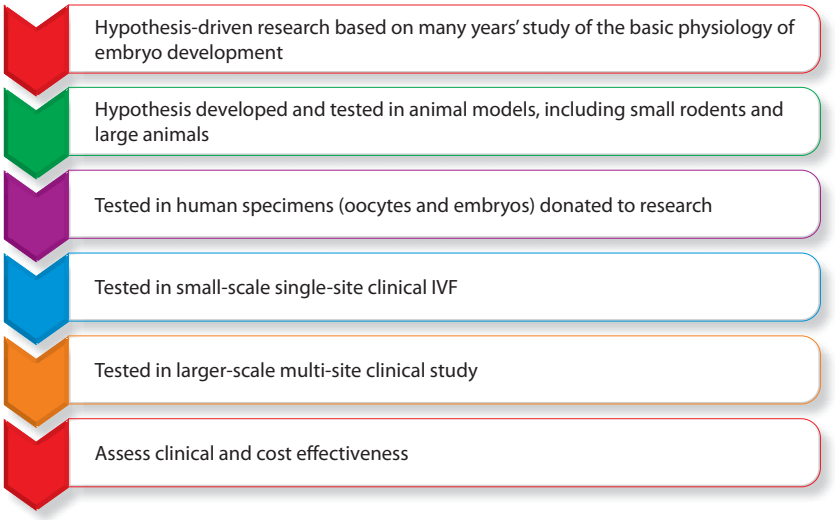
29.2.2. Validation

For MAR procedures, currently no test system with the necessary biological sensitivity exists, apart from an assessment of the actual clinical performance. New equipment should be qualified and methods should be validated before entering clinical use. Such validation should include installation, operational (technical) qualification and performance qualification, by comparison with laboratory key performance indicators (KPI) for similar types of equipment or methods already existing in the laboratory. The Special Interest Group Embryology of ESHRE, in collaboration with Alpha Scientists in Reproductive Medicine, have established minimum performance (competence) levels and aspirational (benchmark) values for the IVF laboratory. Based on the information presented, each laboratory should select its own set of KPIs founded on laboratory organisation and processes or on any available and useful data provided by national registries [5]. Recently, performance indicators for clinical practice in MAR were published [6].

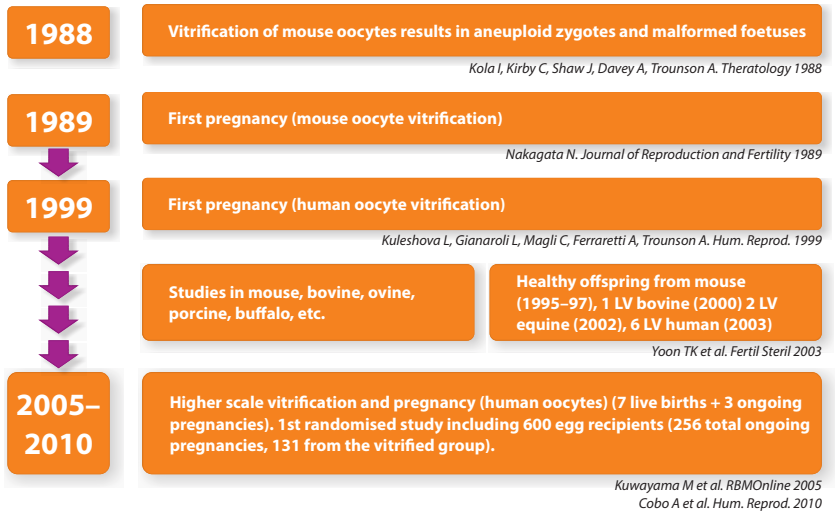
Likewise, it is desirable to ensure that the necessary research and development has been conducted before bringing new techniques into clinical practice, preferably by means of well-designed randomised

Figure 29.1. **Validation of new technologies in medically assisted reproduction**

1. Theoretical steps to be followed when introducing a new technology into clinical use in medically assisted reproduction



2. Example of a validation process in medically assisted reproduction



Source: Adapted from Harper J *et al.* When and how should new technology be introduced into the IVF laboratory? *Hum Reprod* 2012;27(2):303-13.

control trials with a follow-up of all children born from the procedure. The steps needed to validate a new technology before its introduction into clinical practice have been described (see Figure 29.1) [7, 8]. In some countries, a formal authorisation by Health Authorities is required before introducing a new technology into clinical practice.

29.2.3. Materials, consumables and reagents

Critical consumables and media should be quality-controlled, fit for their purpose, of embryo-culture grade and, when available for the intended use and providing at least equal results as qualified alternatives, be CE-marked.

Specific culture media for gametes and embryos that fulfil these requirements are needed during all processing activities in MAR, for instance fertilisation, culture and cryopreservation.

Patient or donor serum or follicular fluid should not be used as a protein supplement. Commercial suppliers of human serum albumin or media containing a serum-derived protein source should provide evidence of screening for transmissible viral diseases according to European and/or national regulations.

29.3. Recruitment of potential donors, identification and consent

29.3.1. Donor recruitment – non-partner donation

As with any tissues and cells, the donation of reproductive material must follow the principles of voluntary and unpaid donation, as described in Chapter 4 (see §4.2.1.3, which specifically relates to MAR). However, expenses related to the donation can be reimbursed, and loss of earnings may be compensated for.

National regulations will need to dedicate special attention to the existence of advertising and false or misleading promotion. In addition, the activities of TEs related to donation must have a non-profit character, which means that only the actual costs of the additional services (those required to allow the donation to be performed) should be charged.

29.3.2. Donor consent – partner and non-partner donors

MAR treatments can be carried out with partner gametes or non-partner-donated gametes (i.e. 'partner donation' or 'non-partner' donation). Chapter 4 describes consent-giving procedures for donation by living donors, and this also applies to gamete and embryo donors in the case of non-partner donation. Fully informed written consent is mandatory also for partner-donated gametes. This section describes additional aspects of relevant specific consent forms that should be addressed. In MAR, consent forms must be separated for female and male patients, although for certain treatments – and especially in partner donation – they may be combined in one document. It is important to emphasise that both partners are required to sign these combined documents on partner donation. Examples of separate consent forms for treatment and storage are given in Appendix 11 and Appendix 12 for the female patient and in Appendix 13 for the male patient.

The couple (or individual) subjected to MAR treatment should receive written and oral information (during medical consultation with the physician or paramedical personnel, through information sessions, leaflets, website etc.) on the following matters [9]:

a. national legislation governing MAR and its implications for those who have access to such treatment;
b. in cases of non-partner donation and embryo donation, the implications of current national legislation for the possible anonymity of the donor and for the possible right of the offspring to know their biological origins;
c. possibility of withdrawal of consent to treatment;
d. chances of success based on their medical history, the degree of invasiveness and the possible risks associated with MAR (including multiple pregnancies). In treatments involving hormone administration, special reference must be made to ovarian hyperstimulation syndrome (OHSS) and the risks linked to oocyte retrieval (e.g. bleeding, infection or perforation of bladder or bowel);
e. testing for genetic and infectious diseases, including relevant assessments carried out in gamete donors in non-partner donation;
f. full description of treatment at each stage of its implementation;
g. option to cryopreserve and store gametes and supernumerary embryos for future use according to national legislation;
h. total cost of a treatment cycle, and existing reimbursement policies, if applicable;
i. possibility that the physician may not proceed

with the entire treatment (or some of its parts) due to medical or deontological reasons;

j. possible ethical issues regarding MAR;

k. possible undesired psychological effects imposed by MAR treatment;

l. possible risks to the offspring resulting from MAR treatment (particularly in multiple pregnancies) and the limited follow-up data on the long-term health of the offspring;

m. when applicable, the information that their personal data will be included in a national registry.

In addition, psychological counselling should be offered to the couple/patient.

MAR care normally comprises a series of separate treatments. Therefore, consent forms should be signed for each treatment or else be valid for consecutive treatments until the treatment is successful, until a predefined date or until relevant circumstances change.

If the treatment is undertaken with cryopreserved gametes, consent for thawing must be given for each treatment. In the case of cryopreserved embryos originating from partner donation, both partners must give consent before each treatment. This policy would prevent a treatment from being initiated by either of the partners without the knowledge or consent of the other.

In the consent form, the couple should state whether embryos or gametes may be cryopreserved or not. Depending on possible options, the couple should receive information on different success rates and on the implications of national legislation regarding the final fate of cryopreserved gametes or embryos. The destiny of cryopreserved gametes or embryos could be: to keep for own reproduction, donate to another couple, donate for scientific research, donate for laboratory validation if allowed (depending on local regulation) or destroy. The consent form could also include aspects on the duration of storage.

There should be specific consent whenever additional methods to IVF, ICSI and cryopreservation are used. In some countries, a very specific case in MAR is the option to consent for the use of cryopreserved gametes or embryos left over from previous treatment(s) after one of the partners has died ('posthumous donation'). This needs to be clearly specified in the consent form.

A woman who undertakes ART treatment could decide not to use all of her oocytes for her own treatment, but to donate some of them to other couples/individuals for reproductive purposes. This procedure is called 'oocyte sharing' and implies that this woman should be considered both a patient and a non-partner donor. Screening should therefore be conducted as described in Chapter 5 and Chapter 6, and specifically for ART as in sections 29.5.1 and 29.5.2 below.

29.4. Donor evaluation

29.4.1. Evaluation of partner donors

29.4.1.1. *Interview*

Couples who experience inability to conceive should be evaluated together because infertility constitutes a mutual medical condition for partners. Counselling before, during and after treatment is widely practised and is recommended because infertility and its investigation and treatment can cause psychological stress [10].

29.4.1.2. *Taking of medical history and physical examination*

Full medical history – including surgical, sexual, contraceptive, genetic, family and pregnancy history, as well as travel history for the assessment of certain viral diseases – should be taken from both partners. Both partners should also undergo a physical examination.

29.4.1.3. *Screening of the female*

Screening of the female should include:

a. assessment of ovulation, with a complete menstrual history;

b. assessment of ovarian reserve, including biochemical tests and/or ultrasound imaging of the ovaries;

c. assessment of tubal patency;

d. assessment of uterine abnormalities, such as submucous fibroids, polyps, adhesions or Müllerian malformations (septae, bicornuate uterus);

e. testing for immunity to rubella should be carried out before treatment; vaccination should be offered to seronegative women before they commence any MAR treatment.

29.4.1.4. *Screening of the male*

a. At least one diagnostic semen analysis should be carried out before starting treatment. If the seminal parameters are altered, a second semen evaluation can be repeated after 2 to 3 months; procedures and reference values are described in the *WHO laboratory manual for the examination and processing of human semen* [11];

b. Men with azoospermia or severe oligozoo-

spermia should be screened for genetic abnormalities (e.g. Klinefelter syndrome or Y-chromosome deletions) and, if a chromosomal abnormality is detected, appropriate genetic counselling should be offered; in the presence of obstructive azoospermia, cystic fibrosis or renal-tract abnormalities should be screened for; in addition to genetic testing, hormonal testing and a scrotal ultrasound should be performed in order to formulate a diagnosis of testicular failure.

29.4.1.5. *Inclusion/exclusion criteria for treatment*

A full medical evaluation will help to determine if a couple is suitable for MAR treatment. The risk–benefit analysis should be estimated case by case.

The number of repeat cycles should be assessed on the individually estimated probability of a live birth. Irrespective of such an assessment, patients have the right of self-determination.

29.4.2. Evaluation of non-partner donors

The purpose of evaluation in non-partner donors is to ensure that donors whose gametes may cause a health risk in the recipient or to the offspring (e.g. infectious disease, genetic disease) can be excluded [10]. In addition, ensuring that the donation process does not cause harm to the health of the donor is equally important. Evaluation tests and the exclusion criteria may depend on national legislation.

In order to donate his/her sperm/oocytes, the potential donor must undergo:

a. consultation and counselling with a healthcare professional;
b. completion of a health/medical/donor family history questionnaire;
c. psychological assessment;
d. medical examination: gynaecological examination and ultrasound for female donors, and genital examination in males;
e. laboratory testing (including screening for infectious diseases);
f. blood group typing;
g. genetic testing as indicated by family history and prevalence of carrier status in specific populations; karyotype testing is strongly recommended; extensive genetic screening for common recessive genetic mutations – such as carrier testing for cystic fibrosis and spinal muscular atrophy (SMA) – is now available and should be considered in order to reduce the risk of transmitting genetic disease to the future child. Genetic testing will be performed according to the prevalence of the diseases in the origin of the donor;
h. semen analyses for sperm donors; freeze–thaw test may also be recommended to assess sperm quality after freezing and thawing;
i. assessment of ovulation and ovarian reserve (including endocrine work-up) in oocyte donors;
j. informed consent before any procedure.

29.4.2.1. *Exclusion criteria for oocyte donors*

a. age < 18 years or > 36 years;
b. diminished ovarian reserve
c. positive results in tests for dominant genetic disease and/or infectious disease;
d. any risk factor to her own health;
e. unsuitability for donation based on interview.

29.4.2.2. *Exclusion criteria for sperm donors*

a. age < 18 years and > 45 years;
b. positive results in tests for dominant genetic disease and/or infectious disease;
c. poor sperm quality;
d. poor survival rate after thawing;
e. unsuitability for donation based on interview.

In cases in which embryos are donated, the partners of donors from whom the gametes originated must also be considered as non-partner donors and must comply with the general criteria for non-partner donation in this section and in Chapter 4 and Chapter 5.

29.4.2.3. *Psychological examination (non-partner donation)*

In MAR, psychological evaluation of non-partner donors is highly recommended and should focus on a psychological anamnesis, including but not limited to: motivation, pattern of personal stability, discussing psychological downstream implications of being a gamete donor, giving psychological guidance in the preparations for becoming a gamete donor. Personality and/or psychological diagnostic tests could also be considered [10].

29.4.2.4. *Welfare of non-partner gamete donors*

To secure the welfare of gamete donors is very important. Although the minimum age limit is 18 years, it would be advisable according to good clinical practice not to include very young gamete donors in a donation programme, but to recruit an older donor group, possibly with proven fertility or fulfilled child-wish. For example, oocyte donors should preferably be accepted after having achieved a successful

pregnancy of their own. It is important to counsel male and female non-partner gamete donors that the donors' DNA will be transmitted to any future children. Therefore, donating gametes might have an impact on the donor and his/her partner and family, including their own future children and their offspring. Although the donor can be unknown to the recipient, in some countries it is possible that – upon request – the identity of the donor might be disclosed to the child. It is therefore also important, where applicable, to address the possibility of contact between future donor children and their gamete donors and to make sure that existing regulations about future contact are clearly described in the consent for donation.

Regarding female donors, the risk of OHSS, bleeding, infection and ovarian torsion should be minimised, as it should be in all women submitted to MAR treatment. For both male and female donors the number of times he/she may donate may be determined by several factors, such as the number of children and/or families achieved with this donor's gametes, the medical and psychological risks to the donor and the relevant legislation in the country of donation. Preferably, any gamete donation by non-partner donors should be limited to a single TE; such a restriction allows control over the number of donations and the number of recipients (sometimes determined by national legislation). Implementation of national registries for gamete/embryo donors as well as for recipients should be encouraged.

29.5. Donor testing

The purpose of testing gamete donors is to prevent transmission of severe infectious and genetic diseases from the donor to the recipient and their offspring, and to protect the staff while handling the patients and their gametes.

Testing of gamete donors is discussed here separately for each type of donation:

a. partner donation
b. non-partner donation.

Less stringent biological testing is justifiable in the donation of reproductive cells between partners who have an intimate physical relationship (i.e. for partner donation).

29.5.1. Testing in partner donation

The following tests must be carried out:

a. anti-HIV-1 and anti-HIV-2;
b. HBsAg (HBV surface antigen) and anti-HBc (HBV core antigen);
c. anti-HCV.

Beyond these tests, TEs should, based on analyses of risk or depending on stricter national legislation or recommendations, also carry out additional tests:

a. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);
b. testing for human T-lymphotropic virus (HTLV)-1 antibody for donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originated from those areas;
c. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure as well as the characteristics of the tissue or cells donated, e.g. RhD (D antigen), diagnostic tests for malaria, Zika virus, *Cytomegalovirus*, *Chlamydia* and *Trypanosoma cruzi* (infectious agent for Chagas disease). Likewise, specific tests may be performed if procurement occurs at times when the incidence/prevalence of certain infectious diseases is high.

Blood samples for serology testing must be obtained before the first donation. In European Union (EU) member states, this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

Positive serology test results do not exclude donation between partners. Nonetheless, robust procedures should be in place to prevent the risk of contamination, to partner or to personnel, and of cross-contamination. If results for tests of HIV-1 and -2, HBV or HCV are positive, or if the donor is known to be a source of infection risk, a system of separate handling and storage must be put in place. In the case of positive serological tests, partner donation should be performed after consultation with a specialist on viral infections.

If the TE can demonstrate that the risk of cross-contamination and exposure to personnel has been addressed through validated processes, biological testing may not be required in the case of sperm processed for IUI and not intended for storage.

29.5.2. Testing in non-partner donation

The following serological tests must be carried out for a single donation (*see note below):

a. anti-HIV-1 and anti-HIV-2;
b. HBsAg and anti-HBc;
c. anti-HCV;
d. syphilis (a treponemal-specific test or a non-specific treponemal test can be used);
e. Sperm donors also need to test negative for *Chlamydia trachomatis*. In the EU, this must be done from a urine sample by a nucleic acid test (NAT).

In some cases, further tests may be required:

f. if required by stricter national legislation (in some countries), testing for HTLV-1/2 is mandatory;
g. testing for HTLV-1 antibodies must be done in donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or where the donor's parents originated from those areas;
h. additional testing may be required in certain circumstances, depending on the donor's history of travel/exposure and the characteristics of the tissue or cells donated, e.g. RhD – D antigen, diagnostic tests for malaria, antibodies to *Cytomegalovirus*, antibody to *Trypanosoma cruzi*, Zika virus infection. Latest epidemiological updates can be found at the European Centre for Disease Prevention and Control (www.ecdc.europa.eu/en).

All serological samples must be obtained at the time of donation. Sperm donations must be quarantined for ≥ 180 days after the last collection, after which repeat testing is required. If, at each donation, serology testing is combined with NAT for HIV, HBV and HCV, quarantine is not necessary unless further tests are required as mentioned in points f, g, h above.

Since sperm donation can also take place regularly every week, or several times a week for a longer coherent period of time, alternative periodic screening protocols have been proposed [12] and described by the ECDC technical report on laboratory testing of non-partner sperm donors [13]. As in any screening procedure a balance has to be drawn between the potential benefits and the harm of a particular screening algorithm to the donor, also taking into account the cost of the screening.

For oocyte donors, the same serological tests must be performed. For oocyte donation, the time of donation could be considered as the starting date of stimulation. For the safe use of fresh oocytes, additional NAT testing at the time of donation is needed. It is desirable to have the results before insemination.

29.6. Procurement

Minimising risks of contamination during procurement (see Chapter 7) and processing (see Chapter 9) activities is crucial to ensure gametes and embryos safety. Risks are often multi-factorial and, to help quantify and map them, a dedicated chapter (Chapter 3) has been developed as well as EDQM's tailored Microbiological Risk of Contamination Assessment tool (MiRCA). By using this tool, TEs can better understand the overall risk of their protocols, and how risk factors are distributed along the tissue/cell supply chain, from procurement to distribution, and implement the most efficient mitigation/risk reducing measures, as needed.

29.6.1. Sperm

29.6.1.1. *Collection by masturbation*

Semen is usually obtained through manual stimulation or penile vibratory stimulation, or in rare cases through intercourse using a specially designed condom free of spermicidal substances. Patients should be given clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.).

After thorough cleaning of the hands and genital area, semen is collected into a sterile collection container. The circumstances under which a semen sample is collected and delivered to the laboratory can influence the results of semen analyses. Since the time that spermatozoa are kept in contact with the seminal plasma can affect their survival, motility and fertilising ability, the start of diagnostic/therapeutic interventions must be standardised. If the sample can be collected in a special room adjacent to the laboratory, the risk of delays during transportation and cooling of the sample is virtually abolished. This situation calls for appropriate design and equipping of the laboratory and semen-collection room. However, with respect to contamination during semen collection, considering the short duration of exposure to the environment and the reduction of bioburden with validated methods during processing, the risk of contamination is very low and therefore strict environmental measures are not required. If necessary, the semen sample can thus be collected at home as well, provided that the patient is well informed on how to transport the sample to the laboratory.

In general, patients are asked to collect a semen sample after 2-7 days of abstinence from ejaculation

[11]. Both too long and too short period of abstinence may influence the quality of the sample.

Semen samples should be collected into sterile, plastic containers that should be tested for sperm toxicity. The use of spermicidal condoms, creams or lubricants must be avoided. The container should be clearly labelled, and correct identification should be confirmed by the patient. After collection, the sample should be delivered to the laboratory as soon as possible, avoiding extreme temperatures (< 20 °C and > 37 °C). Analysis should start within one hour of collection.

For traceability of samples to be used for treatment, records should be kept of the type of container used, the time of collection and the time interval between collection and analysis/preparation. The use of medication, fever during the previous months and completeness of the ejaculate collection should be documented.

29.6.1.2. *Surgically retrieved sperm*

In patients diagnosed with non-obstructive or obstructive azoospermia, sperm can be retrieved by surgical means from the testis or epididymis in an operating room. The method used is dependent upon the nature of the cause. This method may also be used for patients who cannot produce an ejaculate by masturbation.

29.6.1.2.1. Collection of sperm from the epididymis

Percutaneous epididymal sperm aspiration (PESA) is a method for sperm collection if the vasa deferentia are blocked. It involves the use of a sterile needle to aspirate sperm from the epididymis without a surgical incision. Sperm can also be collected from the epididymis by microsurgical sperm aspiration (MESA).

29.6.1.2.2. Collection of sperm from the testis

An alternative to sperm collection from the epididymis is collection of sperm from the testis. This can be performed by testicular sperm extraction (TESE or micro TESE), and possibly by tissue removal (testis biopsy), and could be accompanied by a histopathology study for diagnosis. TESE can also be undertaken *via* a percutaneous approach – testicular sperm aspiration (TESA) – using a sterile fine needle or a biopsy needle. This is a less invasive procedure but usually results in less material than when TESE is undertaken.

29.6.1.3. *Retrograde ejaculation*

In cases of retrograde ejaculation, the sperm ends up in the urinary bladder after ejaculation. In these cases sperm can be collected from the urine after voiding, where the urine pH has been increased by applying alkaline bicarbonates via either oral intake or urinary catheterisation. Should this method yield very low-quality sperm, epididymal or testicular biopsies could be a better option for these patients.

29.6.1.4. *Collection by electro-ejaculation*

In some patients (e.g. in case of injury to the spinal cord, pelvic surgery, multiple sclerosis, diabetes mellitus with nerve involvement, unexplained anejaculation), ejaculation by masturbation is not possible. In these cases, ejaculation can be stimulated using a rectal probe with electrodes. This low-voltage stimulation is usually sufficient to produce a semen ejaculate. However, the quality of the ejaculate is often not as good as that obtained by masturbation. Also in this case, epididymal or testicular biopsies could represent a better option.

29.6.2. **Oocytes**

Before oocyte collection from the ovaries, also known as oocyte retrieval, the patient will be given hormonal treatment to stimulate the growth and maturation of ovarian follicles and associated oocytes (referred to as controlled ovarian hyperstimulation or COH). During treatment, the patient is monitored closely to follow the response to the hormonal treatment and to assess the risk of OHSS.

Oocytes are collected through transvaginal ultrasound-guided ovarian puncture and aspiration of follicular fluid. The procedure can be carried out under local anaesthesia (paracervical block), sedation or general anaesthesia [14].

29.7. Processing

The following section is based largely on *Revised guidelines for good practice in IVF laboratories* by ESHRE [15]. These guidelines were drawn up by the Special Interest Group (SIG) Embryology and constitute the minimum requirements for any laboratory performing MAR procedures.

In selecting an appropriate air-quality specification for processing gametes and embryos, and according with their special characteristics, the criteria and tools identified in Chapter 3 (such as the MiRCA tool), Chapter 7 and Chapter 9 should be considered.

According to the EU Tissues and Cells Directive, tissues and cell processing must be performed in a Good Manufacturing Practice (GMP) Grade A environment with a background of at least GMP Grade D. However, if it is detrimental or not feasible

to carry out a specific procedure in a Grade A environment, or if a validated microbial inactivation process is applied, a less stringent environment may be acceptable. If so, an environment must be specified and it must be demonstrated and documented that the chosen environment achieves the quality and safety required.

29.7.1. Premises for processing of gametes and embryos

29.7.1.1. *Laboratory design*

The laboratory handling gametes and embryos must have adequate space and should be as close as possible to the operating room in which clinical procedures are carried out. Laboratory construction must ensure aseptic and optimal handling of gametes and embryos during all phases of treatment. To achieve this, high-efficiency filtration of particulates and volatile organic compounds in the air supplied to the laboratory and rooms in which clinical procedures are carried out should be considered [16]. In addition, a number of protective measurements should be implemented to minimise the risk of contamination as indicated in Chapter 3 and Chapter 9.

29.7.1.2. *Laboratory equipment*

All critical equipment must be qualified as fit for its purpose, and – where applicable – its performance must be verified by calibrated instruments. If the equipment is intended to be used, alone or in combination, for a purpose as specified in the definition of a "medical device", in the EU, it must fulfil the medical devices regulation (EU 2017/745) and/or national regulations.

The laboratory must contain all essential/critical equipment required for IVF, clearly identified and in numbers appropriate to workload. Incubators in which gametes and embryos are cultured should be organised to facilitate their identification. The number of incubators is critical and should be based on the number of cycles and embryo-culture duration. Gametes and embryos should be conveniently distributed across incubators to minimise door openings and to maintain stable culture conditions.

Devices for maintenance of a constant temperature during manipulation of gametes and embryos outside the incubators must be in place (i.e. warm stages, heating blocks). Regular checks – or continuous monitoring – of critical parameters such as temperature, pH related to CO_2 and O_2 levels must be carried out.

A sufficient number of cryostorage units should be available and be continuously monitored and equipped with alarm systems, detecting any out-of-range temperature and/or levels of liquid nitrogen (LN_2).

29.7.2. Processing of gametes and embryos

As indicated in Chapter 2, approved SOPs for all activities influencing the quality or safety of tissues and cells, including SOPs for handling of gametes and embryos, should be developed and maintained.

Handling of biological material should be performed in laminar-flow hoods (Grade A environment, if possible) and these should be equipped with heating stages and pre-warmed heating blocks, using aseptic techniques at all times. Certain processes, such as ICSI and embryo biopsy, can be performed outside the laminar hood since they need to be undertaken using an inverted microscope. Class-II hoods should be used for documented contaminated samples (e.g. HIV, HCV) since they provide protection to the operator.

Measures must be taken to ensure that oocytes and embryos are always maintained at the appropriate temperature, pH and osmolality during culture and handling. Exposure to volatile or toxic substances, or harmful radiation, should be minimised.

Pipetting devices must be used for one type of procedure only and must never be used for more than one patient. If possible, unit-dose sterile disposable pipettes are preferred. Each sample must be handled individually and its processing should be completed before moving to the next sample in order to prevent cross-contamination or mix-up of samples (see §29.13 on biovigilance).

29.7.2.1. *Oocyte processing*

Oocyte retrieval is a particularly sensitive procedure, and special attention should be given to temperature and pH, as well as efficient and rapid handling [15]. A patient identity check before oocyte retrieval is mandatory. The time between oocyte retrieval and culture should be minimal. Prolonged oocyte exposure to follicular fluid should be avoided. Appropriate equipment must be in place to maintain oocytes at temperature as close as possible to 37 °C. Flushing medium, collection tubes and dishes should be pre-warmed. Follicular aspirates should be searched for the presence of oocytes using a stereomicroscope equipped with a heated stage, usually at 8-60× magnification. Exposure of oocytes to high-energy light should be minimised. Timing of retrieval, number of collected oocytes and identity of the operator should be documented.

29.7.2.2. *Sperm processing*

A pre-clinical sperm preparation test may be advisable in order to propose the most suitable insemination technique; a frozen back-up sample should be requested if difficulty in sperm collection on the day of oocyte retrieval is anticipated. Before starting sperm processing, a patient identity check is mandatory. In the case of ejaculated sperm, sample preparation aims at:

- eliminating seminal plasma, debris and contaminants;
- concentrating progressively motile sperm;
- selecting against morphologically abnormal and immotile sperm.

On the day of oocyte retrieval, an appropriate sperm-preparation method should be chosen, according to the characteristics and origin of the individual samples. The swim-up technique and discontinuous density-gradient centrifugation are most frequently used and widely accepted.

In case of azoospermia on the day of oocyte retrieval, a second semen sample should be requested before considering alternative sperm-retrieval procedures or oocyte cryopreservation.

For surgically retrieved sperm, several techniques are available to maximise sperm recovery and to select viable sperm among immotile testicular sperm cells [17]. In case of epididymal recovery, the aspirate is generally processed by swim-up or discontinuous density-gradient centrifugation, depending on sperm number. For testicular sperm, mechanical procedures to harvest the sperm from the tissue may be combined with enzymatic treatment (e.g. collagenase) in order to facilitate release of sperm from tissue and increase recovery rates [11].

29.7.2.2.1. Specific treatments

Although less often used, phosphodiesterase inhibitors (pentoxifylline, theophylline) or the hypo-osmotic swelling test are sometimes used in absence of motile sperm.

29.7.3. Insemination of oocytes

Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time should be decided on the basis of the number of hours elapsed from ovulation trigger and/or oocyte retrieval, also keeping in mind that fertilisation will need to be checked 16 to 18 hours later.

29.7.3.1. *Conventional in vitro fertilisation (IVF)*

The number of progressively motile sperm used for insemination must be sufficient to optimise the chance of normal fertilisation. Typically, a progressively motile sperm concentration in the fertilisation dish between 0.1 and 0.5 × 10^6/mL per oocyte is used.

The final sperm suspension should be prepared in the smallest possible volume using a medium compatible with oocyte culture.

Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient.

29.7.3.2. *Intracytoplasmic sperm injection (ICSI) procedure*

29.7.3.2.1. Preparation of oocytes for intracytoplasmic sperm injection

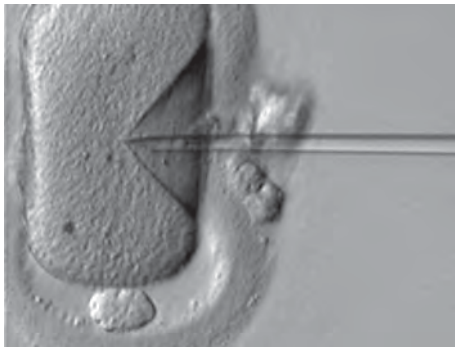
When removing cumulus cells from oocytes, hyaluronidase concentration and exposure should be kept to a minimum. In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting avoided. After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase. The maturation stage of the oocytes should be recorded.

29.7.3.2.2. The injection procedure

See Figure 29.2. It is recommended to keep records of the injection time (start and end of the procedure) and the performing operator. The duration of sperm identification and immobilisation followed by injection should be minimised. In order to minimise the time out of the incubator the number

Figure 29.2. **Intracytoplasmic sperm injection**

Source: Image provided by Thomas Ebner (Austria).



of oocytes transferred to the injection dish should relate to the operator's skills and the sperm quality. Appropriate temperature and pH should be maintained during injection. Viscous substances such as polyvinylpyrrolidone (PVP) or hyaluronic acid/hyaluronan based alternatives can be used to facilitate sperm manipulation. In case of only immotile sperm cells, a non-invasive vitality test can be used to select viable sperm for injection. Only mature metaphase II oocytes should be injected.

29.7.4. Assessment of fertilisation

All inseminated or injected oocytes should be examined for the presence of pronuclei (PN) and polar bodies at 16 to 18 hours post-insemination. A normally fertilised oocyte (zygote) contains 2 PN and 2 polar bodies (Figure 29.3). For conventional IVF, cumulus cells must be removed and 2 PN oocytes transferred into new dishes containing pre-equilibrated culture medium.

Fertilisation assessment should be performed under high magnification (at least 200 ×), using an inverted microscope equipped with Hoffman or equivalent optics to allow verification of the number and morphology of pronuclei.

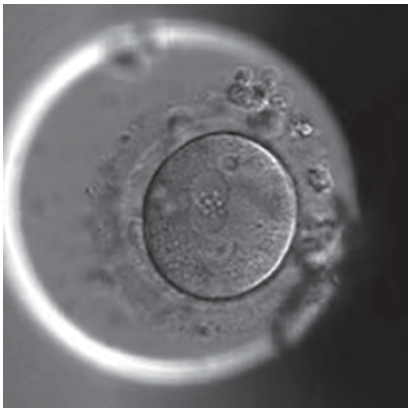
29.7.5. Embryo culture and transfer

In order to optimise embryo development, fluctuations in culture conditions should be minimised. Precautions must be taken to maintain adequate conditions of pH, temperature and osmolality, to protect embryo homeostasis during culture and handling.

Embryo scoring should be performed at high magnification (at least 200 ×, preferably 400 ×) using an inverted microscope with Hoffman or equivalent optics. Evaluation of cleavage-stage embryos should include cell number, relative size and symmetry, percentage of fragmentation, granulation, vacuoles and nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade (associated with size of blastocoel cavity) and morphology of both cell lineages, the inner cell mass and the trophectoderm. Assessment should be performed at standardised times post-insemination. Embryo development can also be assessed using time-lapse imaging, allowing a dynamic evaluation of the timing of consecutive events during embryo culture. These systems also allow more stable culture conditions that may be of benefit. For an overview of embryo morphology scoring and annotation of morphokinetics see [18, 19].

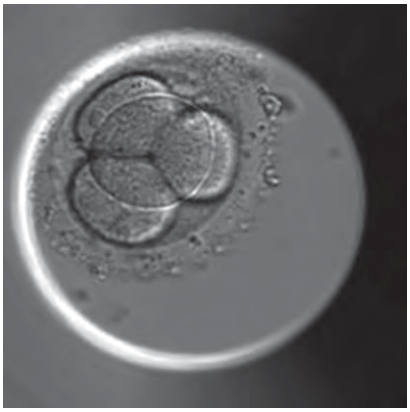
Embryo quality assessment records should include the operator(s), date and time of assessment,

Figure 29.3. **Zygote with 2 pronuclei and 2 polar bodies**



Source: Image provided by María José De los Santos Molina (Spain).

Figure 29.4. **Embryo at the 4-cell stage**

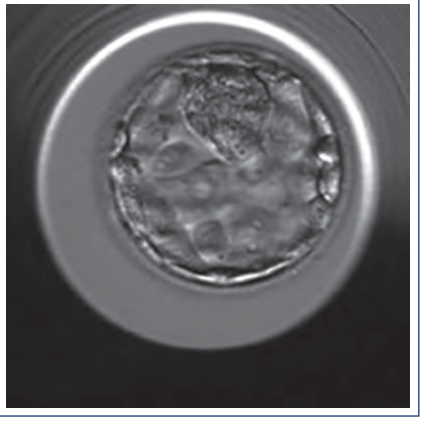


Source: Image provided by María José De los Santos Molina (Spain).

Figure 29.5. **Embryo at the 8-cell stage**

Source: Image provided by María José De los Santos Molina (Spain).

Figure 29.6. **Embryo at the blastocyst stage**



Source: Image provided by María José De los Santos Molina (Spain).

and embryo morphological characteristics, which should be noted with the developmental stage (see Figure 29.4 and following).

Embryo selection for transfer is primarily based on developmental stage and morphological aspects. Other deselection criteria like abnormal morphokinetics and irregular cleavages may be considered [19, 20].

Single embryo transfer is recommended to avoid multiple pregnancies. The decision on the number of embryos to transfer should be based on embryo quality and stage of development, female age, ovarian response and rank of treatment, or the number can be determined by national regulations. It is good practice to limit the maximum number of cleavage stage embryos transferred to 2 and the number of blastocyst stage embryos transferred to 1 in all embryo transfer procedures to limit the number of multiple pregnancies.

Supernumerary embryos may be cryopreserved, donated for research or to the laboratory for validation, or discarded, according to their quality, patient's informed consent and national legislation.

If the laboratory is at some distance from the embryo transfer room, arrangements should be made to maintain temperature and pH while transporting embryos.

A double identity check of patient, patient's files and culture dish(es) is mandatory immediately prior to the transfer.

29.7.6. Pre-implantation genetic testing

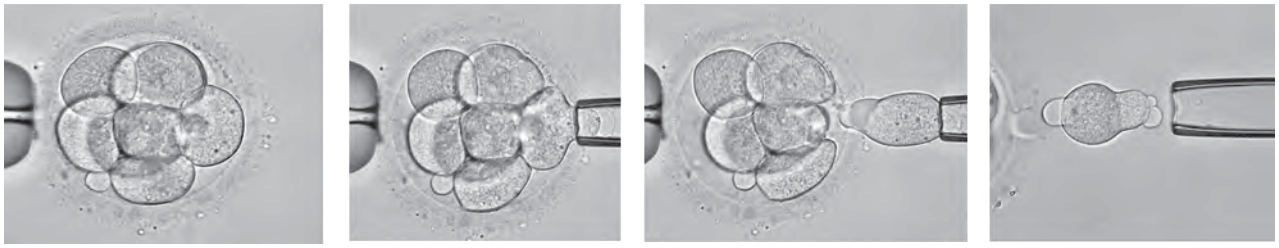
Oocytes and pre-implantation embryos can be biopsied and the obtained genetic material tested for certain monogenic disorders or chromosomal abnormalities. The biopsy procedure may be applied to [21]:

a. first and second polar body;
b. blastomeres of day 3 embryos (Figure 29.7);
c. trophectoderm at the blastocyst stage (Figure 29.8).

Cell(s) destined for genetic analysis are biopsied in the IVF laboratory using glass microtools on a micro-manipulation set. The embryology laboratory has the responsibility to provide unique identification between biopsied polar bodies, blastomeres or trophectoderm cells and the corresponding oocyte, embryo or blastocyst, respectively. All cells and embryos used for genetic investigation must be handled individually, avoiding DNA contamination from other cells, from the samples or from the operator. They must be identified and labelled carefully and be traceable during the entire procedure. During these steps, double identity checks must be adopted. The biopsy sample should be subject to analytical procedures in an accredited laboratory for medical genetics. Traceability for embryo identification must also be guaranteed during the analysis in the reference genetic laboratory.

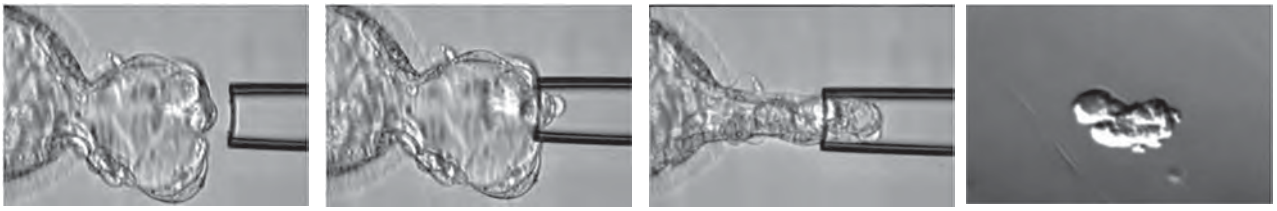
The purpose of PGT-M (for monogenic/single gene or mitochondrial disorders) and PGT-SR (for chromosomal structural rearrangements) is to identify embryos generated *in vitro* that carry certain hereditary genetic diseases or chromosomal abnor-

Figure 29.7. **Blastomere biopsy of an 8-cell stage embryo**



Source: Image provided by María José De los Santos Molina (Spain).

Figure 29.8. **Trophectoderm biopsy of blastocyst**



Source: Image provided by Cristina Magli (Italy).

malities and exclude those embryos from transfer [22-23].

Genetic counselling must be available to all couples known to carry a (severe) hereditary disease. The recipient must be informed that due to embryo mosaicism or limitations of the test, genetic testing on embryos does not substitute for prenatal analysis, such as amniocentesis.

PGT-A (pre-implantation aneuploidy testing) is used to assess whether a cell biopsy from an embryo, and by inference the whole embryo, has the correct number of chromosomes. Such screening is used particularly for women of advanced reproductive age and for women who have had recurrent miscarriages or implantation failures. It is considered as a complement to standard morphological selection of embryos for transfer. For women of advanced reproductive age, embryo selection using PGT-A may decrease the number of embryo transfers necessary to obtain a pregnancy, thus reducing the time to pregnancy. However, the cumulative results are similar as when no PGT-A is used; see e.g. [24]. Bearing in mind the scarcity of data from prospective clinical trials and meta-analyses, PGT-A should be offered with caution, and full information on its present value should be provided to the patients [25].

Another possible use for PGT could be to reduce the transgenerational risk of transmitting mitochondrial DNA disorder. Other minor applications, such as the generation of histocompatible siblings for cell/tissue donation can be considered, case by case.

In some countries, PGT may not be allowed or allowed only in specific circumstances according to national legislation.

29.7.7. *In vitro* maturation

IVM refers to the maturation in culture of immature oocytes in specialised media after being aspirated from follicles that may or may not have been exposed to exogenous gonadotropins before oocyte retrieval [26]. During IVM, such oocytes progress from prophase I (i.e. from germinal vesicle (GV) stage) to reach metaphase II (MII). However, reaching the morphological criterion for MII (release of the first polar body) does not necessarily imply that the oocyte is competent for normal development.

Bearing in mind the lack of sufficient data from prospective clinical trials and meta-analyses, IVM should be considered an innovative procedure and full information on its present value and the lack of long-term outcome data should be provided to patients.

29.7.8. Processing of samples from seropositive donors in partner donations

In couples with one or both partners being seropositive, MAR may still be applied for procreation, considering the risks of horizontal or vertical transmission of the infection, after appropriate counselling and with the informed consent of patients.

For couples with seropositive males, the process includes density-gradient separation of the semen sample and optional swim-up.

Processing of samples from seropositive partner donors should be handled according to specific SOPs to protect personnel and avoid cross-contamination.

Hepatitis B-seronegative individuals with seropositive partners should be offered vaccination before ART [27].

Good quality and safety laboratory practices in assisted reproductive technologies for serodiscordant couples must be in place and should include personal protection of patients and staff, protocols for risk reduction of cross-contamination and proper decontamination of the work area [2, 28].

29.8. Cryopreservation

29.8.1. Methods for cryopreservation of human gametes and embryos

Sperm, oocytes and embryos can be cryopreserved for future use in MAR treatments (supernumerary oocytes or embryos, fertility preservation, non-partner donor gametes for banking). At present, the two most used methods for cryopreserving gametes and embryos are slow freezing and vitrification (see Chapter 9).

Slow freezing is a method based on programmed step-wise decrease in the temperature of the solution in which the specimen is cryopreserved. This usually involves specific computerised equipment and programmes for cooling different types of tissues and cells in solutions with cryoprotectant substance(s).

Vitrification is an ultra-rapid cooling method that relies on very fast cooling rates (4 000-6 000 °C/s up to > 10 000 °C/s, depending on the volume and device used) of the solution in which the specimen is cryopreserved, in the absence of formation of ice crystals. This is a fast method that does not require special cooling equipment (although special consumables are required) and is performed with the addition of cryoprotectants at higher concentrations (compared with slow freezing) for shorter exposure times.

There are significant differences in the sensi-

tivity of different types of male and female gametes and embryos at different stages of development in response to low temperatures and cryoprotectant agents [29, 30, 31, 32].

Concerns about the safety and quality of cryopreserved human gametes and embryos are raised regarding cell damage (disassembly of meiotic spindles, membrane rupture), toxic effects of cryoprotectants, osmotic damage and incomplete dehydration, all having an effect on the cell viability [33]. Overall, however, laboratory and clinical data converge towards the notion that gamete and embryo cryopreservation is a safe procedure [34].

29.8.1.1. *Cryopreservation of sperm*

For sperm, slow freezing is still the method of choice, but rapid cooling is a possible alternative [29]. Freezing/thawing of human sperm is a well-proven technology. Sperm samples are usually cryopreserved in glycerol-based cryoprotectant solutions in cryovials or straws, frozen in a programmed cell-freezing device. Alternatively, it can be cooled at 4 °C, temporarily stored in liquid nitrogen vapour and finally plunged into liquid nitrogen.

Seminal plasma, immotile and damaged sperm may be removed (by sperm processing) before freezing to select a population of sperm with a better chance of survival. It is recommended to process testicular biopsy samples before freezing.

29.8.1.2. *Cryopreservation of oocytes*

In recent years, successful cryopreservation of MII human oocytes has been reported worldwide, with rapidly increasing success rates due to optimisation of protocols. These data strongly suggest that vitrification may be the method of choice in oocyte cryopreservation, since improved rates of survival, implantation and pregnancy have been obtained using this method compared to slow freezing [30, 31, 32].

29.8.1.3. *Cryopreservation of embryos*

Zygotes, embryos, morulae and blastocysts have been cryopreserved successfully and used later for 'frozen embryo transfer'. Slow freezing or vitrification can be used, with vitrification/warming in dimethyl sulphoxide-based cryoprotectants resulting in better survival rates; see e.g. [32]. Exposure time to the cryoprotectant before vitrification is crucial and manufacturers' instructions must be strictly respected.

29.9. Storage

Regarding cryostorage facilities, the main aspects to be considered are location, ventilation and construction materials.

From a practical point of view the storage room with liquid nitrogen tanks should be located close to the laboratory, so that cryopreserved gametes or embryos can be easily, rapidly and successfully transferred to the storage room and into the liquid nitrogen tanks. If cryopreserved gametes or embryos need to be transported prior to being stored, correct temperature should be adequately maintained.

It is recommended to have a system in place whereby a forced ventilation system can be automatically activated when low oxygen pressure is detected.

The type of construction materials should be similar to the ones used in the procurements and processing facilities, with smooth surfaces and easy to clean. A special consideration in the choice of construction materials is that the floor should be resistant to large changes in temperature caused by liquid nitrogen spills.

29.9.1. Storage limits

There is no scientific evidence that gametes, embryos and gonadal tissue, if kept under appropriate storage conditions, deteriorate after a certain time of storage; hence, they can be stored for long periods of time. Use of frozen sperm through assisted reproductive techniques has led to the birth of healthy offspring more than 20 years after initial storage [35], and successful storage over a long period for oocytes and embryos has also been published [36, 37]. However, at defined time points, contact with patients should be made to determine the destiny of their cryopreserved material. In some EU countries, national laws determine a maximum legal storage period. Patients must declare in writing the destiny of their reproductive material when this maximum storage period has ended (see also §29.3.2 on donor consent).

A periodic inventory of the cryobank is recommended, including cross-referencing contents with storage records.

29.9.2. Storage temperature

Optimal storage temperature is based on the type of tissue, cryoprotectant and freezing method used. However, a temperature < -140 °C for gonadal tissue, embryos and gametes is appropriate, and > -140 °C is detrimental to the survival and quality

of the material cryopreserved. Even though storage in liquid nitrogen phase or the vapour phase forming above it is common practice and ensures uniform temperatures, it is important to guarantee that the critical minimum temperature is also maintained when handling the stored samples (< −140 °C). Special attention should be given to temperature changes when handling vitrified material stored in liquid nitrogen vapour.

29.9.3. Storage devices

Several devices can be used to store reproductive material. Sperm can be stored in straws or vials, whereas gonadal tissue is stored mostly in vials. Oocytes and embryos are stored in straws, whereby one straw can hold one or more oocytes/embryos. It is, however, much better practice to store only one embryo per straw to encourage single frozen embryo transfer and to keep traceability between the quality of the oocyte or the development of the embryo frozen.

In the case of using straws for storage (and especially for storage of oocytes), open or closed systems can be used. Using open storage systems means that, at some point in the processing of reproductive tissues and cells, there is direct contact of the cells with liquid nitrogen. In a closed system, there is no direct contact between cells and liquid nitrogen.

29.9.4. Cross-contamination during storage

Introduction of contamination in the storage vessel is due to human manipulations during processing. Viral and microbial agents may survive during long periods of time in liquid nitrogen. However, no reports have shown cross-contamination between these environmentally-derived pathogens and the preserved reproductive material. Also, storage of reproductive material originating from patients carrying infectious diseases in liquid nitrogen has not led to cross-contamination of other frozen reproductive material residing in the same vessel [38, 39]. Even though evidence is lacking, it should be considered good laboratory practice to store reproductive material of patients with positive serology and negative serology separately. Vapour-phase storage containers have been proposed as an alternative to liquid nitrogen containers. Periodic thawing and cleaning of storage vessels is recommended for extending the lifetime of the vessel as well as periodic decontamination of viral and microbial agents.

29.9.5. Storage safety

Storage in liquid nitrogen or vapour nitrogen vessels is definitely the most common infrastructure used to store gametes, embryos and gonadal tissue. Cryopreservation and thawing of material is a daily process in a fertility clinic. Therefore, it is of the utmost importance that personnel working in the cryogenic room have received appropriate training on how to handle liquid nitrogen, and that they are aware of the potential hazards. Personnel must be equipped with specific protective garments (gloves, boots and goggles) and use special forceps for manipulation of straws. All rooms of the laboratory in which liquid nitrogen is used must be equipped with oxygen sensors to guarantee the safety of all laboratory personnel.

For electricity-dependent equipment (alarms etc.) the storage facility should be part of the clinic's general emergency plan whereby, in case of loss of electrical power, a generator or uninterrupted power supply (UPS) system should be in place; for more information, see Chapter 10 – Storage.

29.10. Distribution, import/export

Transport of tissues and cells within the EU is usually referred to as distribution (see Chapter 12). During transport of gametes and embryos, measures need to be taken to ensure the quality, safety and traceability of reproductive tissues and cells. Before transport, some specific actions need to be taken using the appropriate documents:

a. a signed transport agreement between distributing (or exporting when outside EU) and receiving (or importing when outside EU) TEs;
b. signed consent by the patients for sample transportation;
c. when a third party courier is used to perform the transportation of the material, then the distributing TE must have a contract or service-level agreement (SLA) with this qualified courier;
d. other relevant documents should include:
   i. medical history (including genetic disease, family history of disease and transfusion history);
   ii. a protocol for adequate sample handling during transport, for storage, thawing (slow freezing) and warming (vitrification);
   iii. a protocol of acceptance (checking for possible damage to container, for samples and patient identification and for presence of valid documentation);

iv. information on the labelling of the samples; and

e. in the case of distribution of donor gametes, the traceability chain needs to be kept complete at all times. This entails that the distributing and receiving TEs must inform all stakeholders of any severe adverse reactions and events (SARE) when donor gametes are used. Genetic or multifactorial diseases in children born from using the distributed donated gametes must be reported by the receiving TE and/or SARE in the donor file by the distributing TE.

It is also necessary to consider and strictly control the conditions during the actual transport because cryopreserved material is highly sensitive to any fluctuations in temperature. See also Chapter 15 and section 29.9.

For export to and import from countries outside the EU, different requirements need to be met; for details see Chapter 12.

29.11. Packaging and labelling in assisted reproductive technologies

As addressed in Chapter 15, the coding, packaging and labelling of tissues and cells have an important role during banking procedures. Packaging applies only to cryopreserved gametes and embryos in storage and transport. Frozen gametes and embryos are packaged and stored in straws/cryovials as described in section 29.9.3.

Labelling is intended to identify gametes and embryos unambiguously. Labelling and identification systems may vary between centres and countries. Procedures must be in place that ensure correct identification of patients at all stages of handling, using at least two points of identification (e.g. treatment number, SEC code, colour code and/or date of birth) and should include at least the names of partners (when relevant) and date of processing. For frozen samples, colour coding of cryovials and straws should also be used.

At the time of cryopreservation, documentation on biological material should include labelling of devices, cryopreservation method, date and time of cryopreservation, operator, embryo quality and stage of development, number of oocytes or embryos per device, number of devices stored per patient, location of stored samples (tank, canister). Cryodevices must be clearly and permanently labelled with a liquid nitrogen-proof method with reference to patient details, treatment number and/or a unique identification.

At thawing, documentation on biological material should include thawing method, date and time of thawing, identity of operator and post-thawing sample quality.

29.12. Traceability

Identification of patients and traceability of their reproductive cells are crucial aspects in MAR treatments. Each IVF laboratory must have an effective and accurate system to uniquely identify, trace and locate reproductive cells during each procedural step. A proper identification system should ensure that the main characteristics of patients (or donors) and their tissues and cells, together with relevant data regarding products and materials coming into contact with them, are available at all times.

Proper training in traceability procedures for all laboratory staff is highly recommended.

Before commencing any procedure, the laboratory must be provided with each patient's unique identification, with clear and easy reference to the patient's documentation. Each treatment cycle must be assigned a unique identification.

Relevant consent forms, clinical data and details of serological exams undertaken by patients/donors prior to admission to the treatment should be available to the laboratory staff.

Rules concerning correct identification and processing of reproductive cells must be established in the laboratory by a system of codes and checks that considers all the following:

a. Direct verification of patient identity and correspondence with their assigned unique identification is required at every critical step. Patients should be asked to give their own identifying information (at least full name and date of birth) before procurement or assisted insemination/embryo transfer.

b. Labelling of dishes/tubes containing gametes and embryos must be permanent and on the container itself, not only on the removable lid.

c. All devices containing biological material must be clearly and permanently labelled with the unique patient and cycle identification.

d. Biological material from different patients must not be processed in the same working area at the same time.

e. Incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein.

f. During critical steps, traceability must be veri-

fied through correct identification of the reproductive cells and tissues. This can, for example, be executed by use of the four-eye-principle of witnessing (i.e. a double check of the identification by a second person) and/or by use of an electronic identification system.

g. Products and materials used with biological materials must be traceable. The date and time of each manipulation and the identity of all operators and witnesses must be documented throughout the treatment. These records should be kept for a specified period of time according to European and/or national legislation.

h. Gametes and embryos from non-partner donation require specific coding for those countries that are regulated according to European Commission directives, specifically Directive 2015/565 amending Directive 2006/86/EC (see also Chapter 15 §15.2.3).

i. Transport of reproductive cells and tissue requires identification of distributing, importing and exporting institutions, as well as identification of the biological material and its conformity to clinical use. At both institutions, the accompanying documentation and sample identification on the storage device must be checked to ensure that they correspond with patient records.

j. TEs that store and distribute non-partner gametes must label containers with an appropriate unique donation identification. In the EU, the coding requirements for non-partner donation apply (see §15.2.3.1 and following).

29.13. Biovigilance in medically assisted reproduction procedures

Deviations from SOPs in TEs or other adverse events that may influence the quality and safety of tissues and cells should result in SARE reporting to the Health Authority. Adverse reactions can also be related to ovarian stimulation and surgical procurement of gametes, in partner and non-partner donors.

Examples of SARE reported for MAR are given below. In addition, the Notify Library (www.notifylibrary.org) includes many well-documented cases of adverse occurrences in MAR treatments. The database is publicly accessible and can be searched by substance, adverse occurrence and record number.

All patients involved in an SARE should be informed as soon as possible and should be offered counselling and support.

29.13.1. Serious adverse reactions and events

29.13.1.1. *Serious adverse events*

Serious adverse events (SAEs) may be, for example, mix-ups or loss of gametes, embryos or tissues, and may occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryopreservation). Reasons for mix-ups or loss of cells or tissues can be multiple processing steps, mislabelling, contamination, human factor involvement, misidentification, absence/failure of witnessing and/or poor-quality systems. The consequences may include reduced or no chance of pregnancy, (genetic) disease transmission, psychological impact and ethical/legal issues. Causal factors must always be investigated.

29.13.1.2. *Examples of SAEs reported in MAR*

29.13.1.2.1. Mix-ups/loss of traceability

a. mix-up of sperm samples during preparation/treatment;

b. sperm sample contaminated by another sample (e.g. with a used pipette);

c. oocytes fertilised with spermatozoa from the wrong person;

d. insemination of a woman with sperm cells from wrong donor;

e. wrong embryos thawed;

f. labelling error of tubes/dishes containing the oocytes/sperm/embryos.

29.13.1.2.2. Accidental loss of gametes and embryos

a. loss of gametes or embryos resulting in total loss of chance of pregnancy in one cycle (e.g. technical failure of incubator, cryo equipment or cryo tank, accident with culture dishes);

b. embryos destined for culture or freezing were instead destroyed (error in transmission of information);

c. gametes or embryos lost due to microbiological contamination.

29.13.1.2.3. Adverse events after treatment with donated gametes

a. genetic condition discovered in a sperm/oocyte donor years after the gamete donation (for further information see §29.13.2).

29.13.1.3. *Serious adverse reactions*

All serious adverse reactions (SARs) related to ovarian stimulation and procurement of the tissues and cells should be reported to the Health Authorities under the category of "SARs" in donors. Hospitalisation due to ovarian stimulation (OHSS) should be considered as an adverse reaction (non-serious adverse reaction if it is for observation only).

Although the birth of a child with a genetic disease inherited from the donor is an affected offspring, it should be reported under the category of SARs for recipients (see e.g. §29.13.1.3.2.d)

29.13.1.3.1. Examples of SARs reported in MAR for donors

a. severe OHSS leading to hospitalisation;
b. bleeding after oocyte retrieval;
c. ovarian torsion.

29.13.1.3.2. Examples of SARs reported in MAR for recipients

a. salpingitis after intra-uterine insemination;
b. bacterial infection of the recipient due to infected sperm;
c. ovarian torsion after ovarian stimulation;
d. mix-up of samples in the genetics or IVF laboratory (PGT-M treatment), causing the birth of a baby carrying a genetic disease.

29.13.2. Transmission of genetic diseases by MAR with non-partner donations

Donors may unknowingly carry genetic defects causing a (severe) disease. Thereby gamete banks, for example, when distributing sperm or oocytes from a non-partner donor to multiple recipients, could potentially be spreading a (severe) genetic disease. TEs should keep this in mind, especially when informing non-partner donors and recipients of non-partner donations. National registries to facilitate traceability of non-partner donors and offspring are strongly recommended.

Non-partner donors should be strongly advised to inform the procurement centre/TE if they are diagnosed with a genetic abnormality. It is recommended to contact the recipient in the case of a diagnosis that may seriously affect a child's health.

Recipients of non-partner donations should be advised to inform the clinic where they received fertility treatment and also any physician treating a child with a genetic disease that the child was conceived through a non-partner donation, so that appropriate investigations about the origin of the genetic defect can be put in place. Measures should be put in place to prevent the use of gametes from the same donor until an appropriate investigation and risk assessment has taken place. Subsequent measures may include launching international rapid alerts if gametes from the same donor have been distributed or exported to other countries.

These examples emphasise that forward and backward traceability is of the utmost importance in MAR treatments.

29.13.3. Cross-border management of serious adverse reactions and events

Individuals travel abroad to access fertility treatment for various reasons (legal restrictions, long waiting times, treatment costs, lack of expertise, quality of treatment). If patients travel home after treatment, there is a risk that an SAR might occur that might not get reported to the professionals who carried out the treatment or to the Health Authorities. As a consequence, no investigation of potential causes is done and no preventive measures are taken. It is strongly recommended that medical teams involved in both countries communicate with each other to ensure adequate treatment and follow-up. Healthcare professionals should report any SAR to their national Health Authorities, even for cross-border treatments.

29.14. Additional considerations

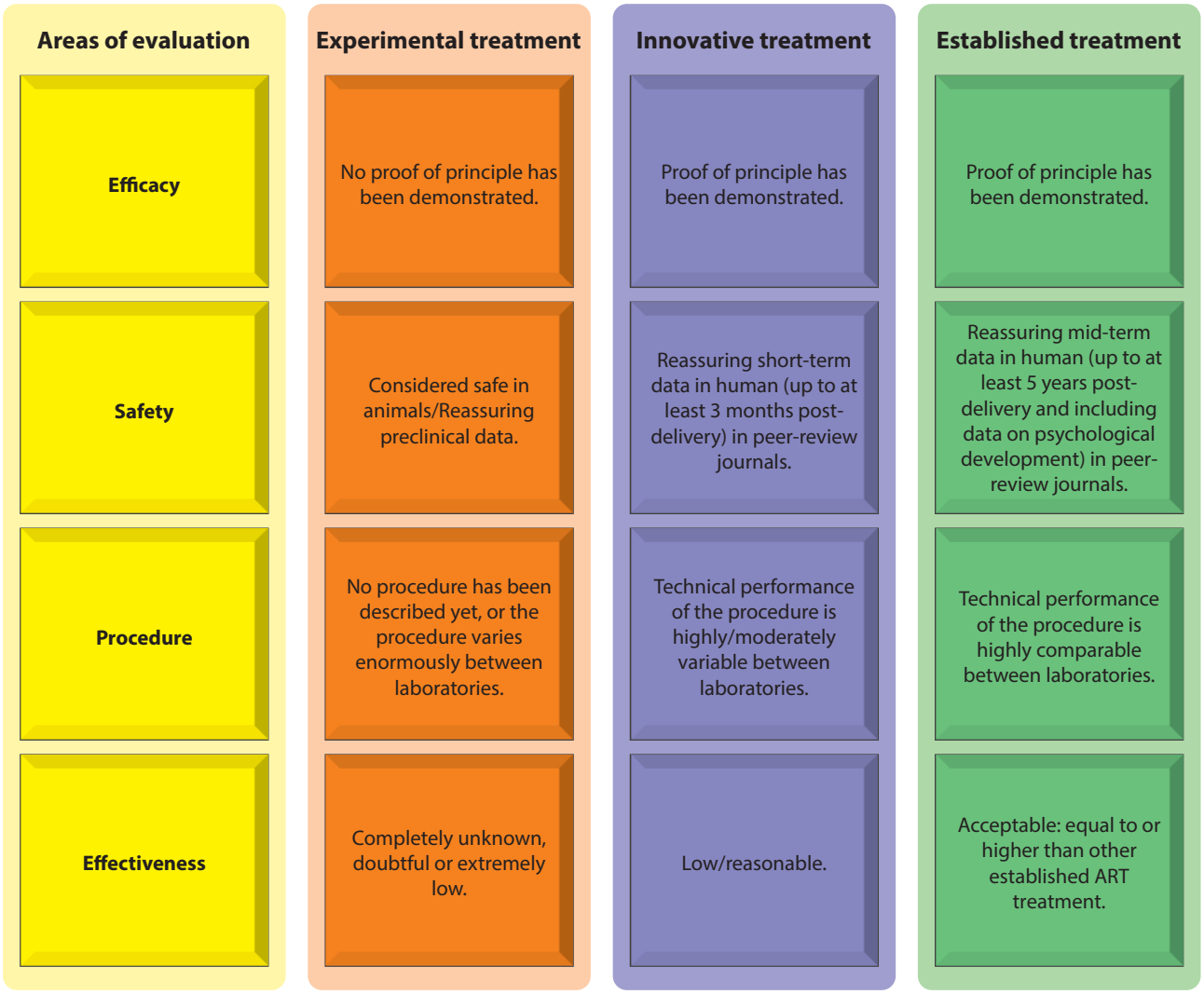
Fair, clear and appropriate information must be provided to donors and recipients at all stages of MAR treatments. The chances of success (including the live-birth rate) should be discussed appropriately. Clinicians, embryologists, technicians, nursing staff and all involved professionals need to communicate at all times to ensure optimal teamwork for the benefit of patients.

OHSS risk, appropriate selection of laboratory methods, the risk of multiple pregnancy and its complications, and the need for follow-up of children must all be addressed. In this sense the use of a unique European database for donor–recipient allocation would be critical in achieving prompt, rapid and reliable SAR report management.

All establishments are strongly encouraged to document internal data and results, and to benchmark with international standards [3, 5, 6]. It is also important to keep track of developments that may increase safety and quality.

In order to ensure global consistency and harmonisation when communicating regarding MAR, a consensus and evidence-driven set of terms and definitions has been generated [1].

Figure 29.9. **Experimental, innovative and established treatments**



Source: adapted from Provoost V *et al.* Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014 Mar;29(3):413-17 [5].

29.15. Developing clinical applications

MAR is a rapidly evolving field. Development and implementation of new technology may affect not only donors and recipients, but also future generations. It is therefore important that these procedures are proven to be safe and efficient.

A methodology for the introduction of new techniques and treatments into clinical practice has been proposed by Provoost *et al.* [8], involving three levels: from experimental, then innovative, to established. A scoring tool is used at each level to determine whether a threshold has been reached of sufficient efficacy, safety, procedure and effectiveness (see Figure 29.9). For more information on the introduction of new methodology see Chapter 18.

29.15.1. Time-lapse technology

Time-lapse technology (TLT) is a conceptually simple live cell microscopy technique, by which micrographs are acquired at short time intervals and viewed sequentially at accelerated pace in order to observe phenomena and processes that otherwise would go unnoticed. In association with phase contrast optics, TLT is particularly suited to observing dynamically and non-invasively the development of the preimplantation embryo *in vitro*, whose ability to establish a viable pregnancy is reflected in the achievement of specific developmental stages at precise time intervals. Events such as embryo cleavage, fragmentation, multinucleation, compaction, blastocoel formation and blastocyst development can be described temporally with unprecedented precision and therefore interpreted under a new light.

The debate on the ability of TLT to improve the efficiency of MAR treatments, by ranking embryos of

a cohort according to their developmental ability and prioritising their use for embryo transfer, remains currently unresolved [40, 41]. Regardless, TLT has revealed unsuspected developmental behaviours of the embryo, manifested in atypical modalities of polar body II (PBII) extrusion, deregulation of pronuclear formation and breakdown, abnormal cleavage and other uncommon phenomena. Access to such evidence has the potential to extend our appreciation of the biology of embryo development. In addition, TLT allows embryo assessment without removal of culture dishes from the incubator, thereby assuring ideal culture conditions, superior to standard embryo culture. Recent studies also indicate that the full potential of TLT to predict embryo developmental ability can be achieved by combination with advanced imaging and artificial intelligence approaches [42].

29.15.2. Non-invasive genetic testing of pre-implantation embryos

It is known that embryo morphology performs relatively poorly in determining embryo quality and predicting implantation. Many embryos that do not implant or end in miscarriage are indeed carriers of chromosomal abnormalities due to inherited meiotic and mitotic errors.

The discovery of cell-free nucleic acids in human embryo-culture media supported the research on new non-invasive biomarkers of embryo viability (niPGT) that could eventually replace the current invasive PGT screening methods [43]. This new technology could be able to determine the euploidy status or even single gene mutations of human embryos by measuring cell-free DNA in the spent culture media [44] or in blastocoel fluid [45]. Validation of the methods still needs to be undertaken. Recent studies have shown good prediction power with high sensitivity and specificity values in a specific situation of beta thalassaemia [46].

29.15.3. Whole genome screening

Recent research developments in the field of genomics have made possible the comprehensive testing of the human genome by combining the methods of next-generation sequencing with advanced bioinformatics. In this way, a complete picture of each individual genome, including single nucleotide- (SNV) and copy number- (CNV) variations, leads to expanded DNA screening. The application of this approach permits PGT and non-invasive prenatal testing, with expanded carrier screening, but also the disclosure of gamete donor anonymity. The comprehensive information derived from whole genome screening has benefits as well as limitations and risks, and its introduction into clinical practice requires prudence and genetic counselling [47].

29.15.4. Mitochondrial replacement therapy

Recently, novel reproductive technologies known as mitochondrial donation or mitochondrial replacement therapy (MRT) have been introduced into clinical practice. These enable women with mitochondrial DNA (mtDNA) disorders to have healthy, genetically related children [48]. Since mitochondrial transmission is uniparental and of maternal origin, the diseases caused by mitochondrial dysfunction are passed on from mother to offspring in a dominant way. Because there is no cure for mitochondrial diseases, prevention of germline transmission is a priority.

PGT appears a reliable procedure to reduce transmission of heteroplasmic mtDNA mutations although it can be ethically challenging to determine which embryos should be transferred [49].

MRTs, however, are able to avert transmission of mutations in the mtDNA. As the technique uses part of a donated egg to replace the maternal mutated mtDNA the child would inherit DNA from a "third person", the mitochondrial donor.

MRTs are a modification of conventional IVF and comprise two main strategies. In the pronuclear transfer procedure, the mother's oocyte with mutated mtDNA is fertilised with the father's sperm and simultaneously, a donated oocyte with wild-type mtDNA also is fertilised with the father's sperm. Then, the parental pronuclei are removed from the zygote with mutated mtDNA and transferred into the enucleated zygote with wild-type mtDNA. Finally, the embryo with wild-type mtDNA is transferred to the uterus.

The maternal spindle transfer technique starts at an earlier stage of oocyte development. The MII spindle and associated chromosomes are transferred from the mother's oocyte with mutated mtDNA into an enucleated donor oocyte with wild-type mtDNA. Then, the reconstructed oocyte is fertilised with the father's sperm and the embryo with wild-type mtDNA is transferred to the uterus. Maternal spindle transfer is technically more challenging than pronuclear transfer. A zygote, however, is discarded with the pronuclear transfer technique, which could raise ethical concerns, whereas no zygote is destroyed with the spindle transfer technique.

Mitochondrial replacement therapy is still con-

sidered on a case-by-case basis. Few successful case reports have been published and long-term results are lacking.

29.15.5. Gene editing

Gene editing includes a group of technologies that allow modifying the genome by inserting, deleting, replacing or modifying genetic material at specific locations in the DNA sequence. In cases of genes carrying a mutation, the enzyme cuts the mutation and replaces it with the correct DNA sequence, making it of great interest in the prevention and treatment of human diseases. This approach can be used for somatic gene editing for the treatment not only of a genetic disorder, but also of cancer and infectious diseases. Ethical concerns arise when gene editing is used to alter genes in gametes or in embryo, introducing changes that will be passed to future generations.

In 2015, a study from China was published, reporting genome editing by CRISPR-Cas9 on non-viable human embryos [50]. The following year, the United Kingdom issued the world's first endorsement of a national regulatory authority for research on human embryos using genome editing. Additional studies have demonstrated how human germline gene modification is rapidly progressing from the experimental field to clinical research applications [51].

The results obtained so far raise high expectations regarding possible therapeutic applications in humans, but much remains to be considered before clinical applications, including the reproducibility of the technique and possible long-term consequences [52].

29.16. References

1. Zegers-Hochschild F, Adamson GD, Dyer S *et al.* The international glossary on infertility and fertility care. *Hum Reprod* 2017;32(9):1786-1801, and *Fertil Steril* 2017;108(3):393-406.
2. ESHRE Guideline Group on Viral Infection/Disease, Mocanu E, Drakeley A *et al.* ESHRE guideline: medically assisted reproduction in patients with a viral infection/disease. *Hum Reprod Open* 2021;4:hoab037.
3. Wyns C, De Geyter Ch, Calhaz-Jorge C *et al.* ART in Europe, 2017: results generated from European registries by ESHRE. *Hum Reprod Open* (2021) 3:2021, hoab026. https://doi.org/10.1093/hropen/hoab026.
4. Rienzi L, Bariani F, Dalla Zorza M *et al.* Comprehensive protocol of traceability during IVF: the result of a multicentrefailure mode and effect analysis. Italian Society of Embryology, Reproduction and Research (SIERR), Italy. *Hum Reprod* 2017;32(8):1612-20.
5. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. *Hum Reprod Open* 2017:2 and *Reprod Biomed Online* 2017:35(5):494-510.
6. ESHRE Clinic PI Working Group, Vlaisavljevic V, Apter S *et al.* The Maribor concensus: report of an expert meeting on the development of performance indicators for clinical practice in ART. *Hum Reprod Open* 2021;3:hoab022.
7. Harper J, Magli MC, Lundin K *et al.* When and how should new technology be introduced into the IVF laboratory? *Hum Reprod* 2012;27(2):303-13.
8. Provoost V, Tilleman K, D'Angelo A *et al.* Beyond the dichotomy: a tool for distinguishing between experimental, innovative and established treatment. *Hum Reprod* 2014 Mar;29(3):413-17.
9. ESHRE Working Group on Reproductive Donation, Kirkman-Brown J, Calhaz-Jorge C, Dancet EAF *et al.* Good practice recommendations for information provision for those involved in reproductive donation. *Hum Reprod Open* 2022 Feb 16;2022(1):hoac001. https://doi.org/10.1093/hropen/hoac001. PMID: 35178481; PMCID: PMC8847071.
10. Gameiro S, Boivin J, Dancet E *et al.* ESHRE guideline: routine psychosocial care in infertility and medically assisted reproduction-a guide for fertility staff. *Hum Reprod* 2015;30(11):2476-85.
11. WHO Department of Reproductive Health and Research. *WHO laboratory manual for the examination and processing of human semen*. 6th edition. Geneva, Switzerland: World Health Organization; 2021.
12. Van Landeghem S, Tilleman K, Vanden Meerschaut F *et al.* Screening of chlamydia trachomatis in non-partner donors: situation of stored donations and proposal for periodic screening. *Reprod Biomed Online* 2021; https://doi.org/10.1016/j.rbmo.2021.11.025.
13. European Centre for Disease Prevention and Control. *Laboratory testing of non-partner sperm donors – an assessment of potential risks involved in changing the current testing protocols for HIV, hepatitis B and hepatitis C*. Stockholm: ECDC; 2018. ISBN 978-92-9498-246-9; https://doi.org/10.2900/952089; Catalogue number TQ-04-18-586-EN-N.
14. ESHRE Working Group on Ultrasound in ART, D'Angelo A, Panayotidis C, Amso N *et al.* Recommendations for good practice in ultrasound: oocyte pick up. *Hum Reprod Open* 2019; Dec 10;2019(4): hoz025. https://doi.org/10.1093/hropen/hoz025. eCollection2019.
15. ESHRE Guideline Group on Good Practice in IVF

Labs, De los Santos MJ, Apter S, Coticchio G *et al.* Revised guidelines for good practice in IVF laboratories: oocyte pick up. *Hum Reprod*. 2016 Apr;31(4): 685-6. https://doi.org/10.1093/humrep/dew016. Epub 2016 Feb.

16. Mortimer D, Cohen J, Mortimer ST *et al.* Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. *Reprod Biomed Online* 2018; 36(6):658-74.
17. Verheyen G, Popovic-Todorovic B, Herman Tournaye H. Processing and selection of surgically-retrieved sperm for ICSI: a review. *Basic Clin Androl* 2017;27:6.
18. Lundin K, Ahlström A. Quality control and standardization of embryo morphology scoring and viability markers. *Reprod Biomed Online* 2015;31(4):459-71.
19. ESHRE Working group on Time-lapse technology, Apter S, Ebner T *et al.* Good practice recommendations for the use of time-lapse technology. *Hum Reprod Open* 2020; Mar 19;2020(2):hoaa008. https://doi.org/10.1093/hropen/hoaa008. eCollection 2020.
20. Ciray HN, Campbell A, Agerholm IE *et al.* Proposed guidelines on the nomenclature and annotation of dynamic human embryo monitoring by a time-lapse user group. *Hum Reprod* 2014;29(12):2650-60.
21. ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, Kokkali G, Coticchio G, Bronet F *et al.* ESHRE PGT Consortium and SIG Embryology good practice recommendations for polar body and embryo biopsy for PGT. *Hum Reprod Open* 2020(3):2020, hoaa020. https://doi.org/10.1093/hropen/hoaa020.
22. ESHRE PGT-M Working Group, Carvalho F, Moutou C, Dimitriadou E *et al.* ESHRE PGT Consortium good practice recommendations for the detection of monogenic disorders. *Hum Reprod Open* 2020(3):2020, hoaa018. https://doi.org/10.1093/hropen/hoaa018.
23. ESHRE PGT-SR/PGT-A Working Group, Coonen E, Rubio C, Christopikou D *et al.* ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. *Hum Reprod Open* 2020(3):2020, hoaa017, https://doi.org/10.1093/hropen/hoaa017.
24. Rubio C, Bellver J, Rodrigo L *et al.* In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil Steril* 2017:107(5):1122-9.
25. Verpoest W, Staessen C, Bossuyt PM *et al.* Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. *Hum Reprod* 2018;33(9): 1767-76.
26. Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology. *In vitro* maturation: committee opinion. *Fertil Steril* 2013;99(3):663-6.
27. Savasi V, Mandia L, Laoreti A, Cetin I. Reproductive assistance in HIV serodiscordant couples. *Hum Reprod Update* 2013;19(2):136-50.
28. Jindal SK, Rawlins RG, Muller CH, Drobnis EZ. Guidelines for risk reduction when handling gametes from infectious patients seeking assisted reproductive technologies. *Reprod Biomed Online* 2016 Aug;33(2): 121-30.
29. Di Santo M, Tarozzi N, Nadalini M, Borini A. Human sperm cryopreservation: update on techniques, effect on DNA integrity, and implications for ART. *Adv Urol* 2012;2012:854837. https://doi.org/10.1155/2012/854837.
30. Potdar N, Gelbaya TA, Nardo LG. Oocyte vitrification in the 21st century and post-warming fertility outcomes: a systematic review and meta-analysis. *Reprod Biomed Online* 2014;29(2):159-76.
31. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online* 2005;11(3): 300-8.
32. Rienzi L, Gracia C, Maggiulli R *et al.* Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update* 2017; 23(2):139-55.
33. Kopeika J, Thornhill A, Khalaf Y. The effect of cryopreservation on the genome of gametes and embryos: principles of cryobiology and critical appraisal of the evidence. *Hum Reprod Update* 2015;21(2):209-27.
34. Cobo A, Serra V, Garrido N *et al.* Obstetric and perinatal outcome of babies born from vitrified oocytes. *Fertil Steril* 2014;102(4):1006-15.e4.
35. Feldschuh J, Brassel J, Durso N, Levine A. Successful sperm storage for 28 years. *Fertil Steril* 2005;84(4):1017.
36. Urquiza MF, Carretero I, Cano Carabajal PR *et al.* Successful live birth from oocytes after more than 14 years of cryopreservation. *J Assist Reprod Genet* 2014: 31:1553-5.
37. Revel A, Safran A, Laufer N *et al.* Twin delivery following 12 years of human embryo cryopreservation: case report. *Hum Reprod* 2004;19(2):328-9.
38. Pomeroy KO, Harris S, Conaghan J *et al.* Storage of cryopreserved reproductive tissues: evidence that cross-contamination of infectious agents is a negligible risk. *Fertil Steril* 2010;94(4):1181-8.
39. Cobo A, Bellver J, de los Santos MJ, Remohí J. Viral screening of spent culture media and liquid nitrogen samples of oocytes and embryos from hepatitis B, hepatitis C, and human immunodeficiency virus chronically infected women undergoing *in vitro* fertilization cycles. *Fertil Steril* 2012 Jan;97(1):74-8.

40. Armstrong S, Bhide P, Jordan V *et al.* Time-lapse systems for embryo incubation and assessment in assisted reproduction. *Cochrane Database Syst Rev* 2018 May 25;5:CD011320. https://doi.org/10.1002/14651858.CD011320.pub3. Review. [Update in *Cochrane Database Syst Rev* 2019 May 29;5:CD011320.]
41. ESHRE Working group on Time-lapse technology, Apter S, Ebner T, Freour T *et al.* Good practice recommendations for the use of time-lapse technology. *Hum Reprod Open* 2020 Mar 19;2020(2):hoaa008. https://doi.org/10.1093/hropen/hoaa008. eCollection 2020.
42. Berntsen J, Rimestad J, Lassen JT *et al.* Robust and generalizable embryo selection based on artificial intelligence and time-lapse image sequences. *PLoS One*. 2022 Feb 2;17(2):e0262661. https://doi.org/10.1371/journal.pone.0262661. eCollection 2022.
43. Leaver M, Wells D. Non-invasive preimplantation genetic testing (niPGT): the next revolution in reproductive genetics? *Hum Reprod Update* 2020 Jan 1;26(1):16-42.
44. Shamonki MI, Jin H, Haimowitz Z, Liu L. Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media. *Fertil Steril* 2016;106:1312-18.
45. Rubio C, Navarro-Sánchez L, García-Pascual CM *et al.* Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts. *Am J Obstet Gynecol* 223.5 (2020):751-e1. https://doi.org/10.1016/j.ajog.2020.04.035.
46. Liu WQ, Liu JQ, Du HZ *et al.* Non-invasive pre-implantation aneuploidy screening and diagnosis of beta thalassemia IVSII654 mutation using spent embryo culture medium. *Annals of Medicine* 2017;49(4):319-28.
47. Harper JC, Aittomäki K, Borry P *et al.* on behalf of the European Society of Human Reproduction and Embryology and European Society of Human Genetics. Recent developments in genetics and medically assisted reproduction: from research to clinical applications. *Eur J Hum Genet* 2018 Jan;26(1):12-33, and *Hum Reprod Open* 2017(3), 5 October 2017.
48. Herbert M, Turnbull D. Progress in mitochondrial replacement therapies. *Nat Rev Mol Cell Biol* 2018 Jan 23;19(2):71-2.
49. Sallevelt SCEH, Dreesen JCFM, Coonen E *et al.* Preimplantation genetic diagnosis for mitochondrial DNA mutations: analysis of one blastomere suffices. *J Med Genet*. 2017 Oct;54(10):693-7.
50. Liang P, Xu Y, Zhang X *et al.* CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein Cell* 2015 May;6(5):363-72. https://doi.org/10.1007/s13238-015-0153-5. Epub 2015 Apr 18.
51. Ma H, Marti-Gutierrez N, Park SW *et al.* Correction of a pathogenic gene mutation in human embryos. *Nature* 2017 Aug 24;548(7668):413-19. https://doi.org/10.1038/nature23305. Epub 2017 Aug 2.
52. Davies B. The technical risks of human gene editing. *Hum Reprod*. 2019;34(11):2104-11.

Related material

Appendix 11. Example of consent form: female (NHS, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (NHS, UK)

Chapter 30. **Fertility preservation**

30.1. Introduction

Despite some differences in technical approaches and expected results, today fertility preservation (FP) can be applied for both medical and non-medical reasons.

FP involves biomedical interventions undertaken in order to avoid, delay, diminish or circumvent the exhaustion of the germ-cell pool of the individual. In most current circumstances, either in anticipation of the effects of cytotoxic therapy applied to treat a severe disease or for a number of possible reasons causing postponement of parenthood, this involves cryopreservation of gonadal tissue, gametes or embryos.

FP techniques are usually offered to prepubertal girls and boys and to males and females of reproductive age at risk of losing their reproductive potential due to either malignant or non-malignant diseases. Gamete cryopreservation is also an option for non-medical conditions, such as postponement of parenthood, previous vasectomy, transgender treatments, or other reasons.

This chapter describes the indications for male and female FP and the techniques actually available for the cryopreservation of reproductive cells and germinal tissue. The collaboration between all medical specialities involved, like paediatricians, oncologists and reproductive specialists, is essential to ensure proper evaluation and counselling for each patient. Patient assessment and approach will depend on disease, age and treatment. Information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with patients, or parents (in the case of minors). It is important to realise that FP and subsequent restoration may include experimental techniques, whose availability may be restricted according to national legislation.

This chapter must be read in conjunction with Chapter 29 – Medically assisted reproduction and the following chapters (Part A) of this Guide:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

30.1.1. Female fertility preservation

Female FP should be considered whenever fertility loss is predicted as a consequence of a cytotoxic treatment for a specific disease (e.g. in cancer patients) [1, 2] or due to the disease itself (malignant or non-malignant, e.g. severe endometriosis) [3, 4]. This part includes indications for FP under medical conditions (oncological and non-oncological) as well as for non-medical reasons.

30.1.1.1. *Medical reasons*

All prepubertal girls and women of reproductive age newly diagnosed with specific medical conditions (e.g. certain cancers or rheumatoid arthritis) and whose treatment may cause premature ovarian insufficiency (POI) should be referred to a fertility expert, to be counselled about the risk of infertility and informed about fertility preservation. FP should ideally be offered before treatment is started, but should not delay treatment. The implications of undergoing FP, including possible delay of the cancer treatment, should be weighed against the benefits of having reproductive cells and/or tissues stored for future use. Factors such as age, ovarian reserve, gonadotoxicity of the treatment and time available before it starts will help in the decision [5].

30.1.1.1.1. Oncological reasons for fertility preservation

Chemotherapy and radiation therapy may cause depletion of the pool of primordial follicles in the ovaries of girls or premenopausal women and thus render them infertile. Once ovaries are exhausted of follicles, the patient will experience POI and infertility. In the case of pre-pubertal girls, loss of the entire stock of primordial follicles implies that the girl will not enter puberty spontaneously and that she will not be able to become pregnant with her own oocytes later on in life. This is of course a very serious side-effect of an otherwise efficient cancer treatment and is, by many, considered a significant reduction in the quality of life. As more and more girls and women at reproductive age survive a malignant disease today, these unwanted side-effects will affect an increasing number of adults in the population.

It is well known that chemotherapeutic drugs belonging to the group of alkylating agents cause the most severe damage to ovaries. Alkylating agents, such as cyclophosphamide or busulfan, are used to treat various forms of cancer, including breast cancer, lymphoma and sarcoma. They are also used in the preconditioning protocol before haematopoietic progenitor cells (HPC) transplantation. As alkylating agents cause damage to both dividing and resting cells, they are very toxic to the oocytes and granulosa cells of the primordial follicles, which are the most immature and 'dormant' type of follicle [6].

Radiation therapy, whether given to the abdomen or the spine, will also affect ovarian function. Radiation therapy is very toxic to oocytes, as doses as low as 2 Gy will destroy half of the pool of follicles. Whenever possible, the ovaries are shielded or moved away from the field of radiation, but scatter dose is inevitable [7].

Some genetic conditions may be also a reason for FP. Women carriers of mutations in the BRCA genes (BReast CAncer gene) may be counselled for the prophylactic removal of their ovaries due to an increased risk of developing ovarian cancer compared with the general population [8].

30.1.1.1.2. Non-oncological reasons for fertility preservation

Non-malignant diagnoses – such as kidney disease, autoimmune conditions or haematological diseases like aplastic anaemia or thalassaemia – can sometimes be life-threatening and require treatment with alkylating agents or even HPC. Women affected by any severe disease requiring these treatments may also need FP.

When ovarian surgery is required, as in the case of severe endometriosis or benign ovarian cysts, or borderline cysts, healthy ovarian tissue containing primordial follicles will inevitably be excised as an effect of the operative procedure. These procedures may pose some threat to the reproductive potential of the patient, and in these cases FP should also be offered [3, 4].

Certain genetic conditions – such as Turner mosaicism, galactosaemia, Fragile X mutation carrier status or blepharophimosis, ptosis or epicanthus inversus syndrome – are associated with premature exhaustion of the pool of primordial follicles in the ovaries, and girls and women at reproductive age suffering from any of these conditions can also be potential candidates for FP.

Several options exist to preserve fertility in post-pubertal girls and women. Oocytes, embryos and ovarian tissue can be cryopreserved depending on the characteristics of each individual case and considering the most efficient alternative for every patient [5]. Examples of consent forms for female FP are given in Appendix 11 and Appendix 12.

Also cross-hormone treatment for transgender persons is potentially harmful to their fertility. Therefore, transgender female-to-male patients may undergo oocyte collection and storage before cross-hormone treatment and sex-reassignment

surgery. In the case of female-to-male transgender treatment, patients should be informed of the possible use of their gametes in the future. This approach may be different in different countries according to national legislation.

30.1.1.2. *Non-medical reasons for fertility preservation*

Non-medical FP can be also considered in young women who want to postpone maternity (age-related fertility preservation) [4].

In all cases, women should be aware of relevant questions, such as the expected survival rate of oocytes after cryopreservation or the fact that the likelihood of successful live birth strictly depends on the number of stored oocytes; in this sense, the development of *ad hoc* prediction models is an interesting approach that may guide patients and clinicians [5].

Specifically, for age-related FP cases, women should be adequately informed about the biological limitation associated with pregnancy due to age-dependent oocyte aneuploidy.

30.1.2. Male fertility preservation

30.1.2.1. *Medical reasons*

FP is indicated in all boys and men facing gonadotoxic treatment or surgical procedures affecting semen production and collection. All patients at risk of fertility loss should be informed about FP options.

30.1.2.1.1. Oncological reasons for fertility preservation

Chemotherapeutic agents and radiation treatments can adversely affect the male gonadal epithelium. Thus, therapies used to cure cancer (but also used for several non-malignant conditions) may render the patient temporarily or permanently infertile. The amount of damage depends on the regimen, the cumulative dosage of treatments used and the individual capacity of recovery. Mitotically active spermatogonia are highly sensitive to cytotoxic treatments and radiation. Low doses of these treatments deplete the pool of differentiating spermatogonia, while spermatogonial stem cells (SSC) may initially survive, and spermatocytes and spermatids can continue their maturation into sperm. Testicular involution occurs when no new precursors are provided from the stem-cell pool and the differentiating germ cells mature into spermatids, which are released from the seminiferous epithelium [9, 10].

Significant damage is reported after treatment with alkylating agents, of which different threshold doses are reported in the literature (e.g. for cyclophosphamide and cisplatin-based drugs). Both alkylating and platinum-containing agents cause direct DNA and RNA damage, therefore also affecting non-dividing, reserve stem cells. The gonadal epithelium is highly susceptible to radiation-induced damage. Differentiating spermatogonia are sensitive to scattered doses of radiation as low as 0.1 Gy, leading to short-term cessation of spermatogenesis. Cumulative doses above 3 Gy affect SSC and cause long-term azoospermia, whereas doses in excess of 6 Gy deplete the SSC pool and can lead to permanent infertility. Fractionation of radiotherapy increases the germ-cell toxicity. Overall, post-treatment infertility problems are reported in up to 60 % of cancer patients [11].

30.1.2.1.2. Non-oncological reasons for fertility preservation

As in females, certain non-malignant pathologies such as haematological and autoimmune conditions requiring potentially gonadotoxic treatments could compromise male fertility. Transgender women may wish to store semen for FP. Transgender persons planning to start cross-hormone treatment and undergo sex-reassignment surgery can benefit from FP. Analogous considerations previously explained for trans men also apply for trans women [5].

30.1.2.2. *Non-medical reasons for fertility preservation*

These indications include groups such as men in military services, who are at risk of potential harm to their fertility.

30.2. Consent in fertility preservation

After referral of the patient, informed consent for FP should be obtained by a clinician. However, since pre-pubertal children can also benefit from FP techniques, informed consent should in this situation be discussed with and signed by the parents or legal guardians of the child. It is important that, in the case of FP for pre-pubertal children, care should be taken to explain the future use of banked gonadal tissue.

Individual countries may have their own legislation regarding FP, and therefore consent forms can differ. Examples of generic consent forms for cryopreservation of sperm appear in Appendix 13 and Appendix 40. The forms can be used as a template that can be adjusted according to national legislation or common practice of the MAR centre. Information with regard to the process, legal time of cryostorage

and potential risks can be reported in the consent form or in a related information document.

30.3. Patient evaluation

In addition to the clinical examination of the disease to be treated, patients who are candidates for fertility preservation undergo evaluation similar to that for patients undergoing MAR techniques (Chapter 29). The future use of the stored gonadal tissue or gametes will eventually involve the use of such material in MAR techniques with the aim of obtaining embryos in a (non) partner donation treatment.

Patient evaluation for pre-pubertal boys and girls needs special care in cases where gonadal tissue is removed and banked. In this regard, close collaboration between paediatric, surgical, oncologic and fertility specialists is essential. When patients are first seen in the oncology department, the impact of cancer treatment on the patient's future conditions should be discussed and the fertility and preservation options explained. The referral to a fertility clinic should be made possible in very timely fashion because of the short time interval available for certain patients to undergo FP before treatment for their specific disease. In the fertility clinic, detailed information on the possibility and the process of FP will be offered on an individual patient basis. Additionally, the future use of the preserved reproductive material must be addressed.

To improve patient compliance, certain aspects of the FP process should be carefully managed. For example, to minimise trauma to the patient, surgical recovery of gonadal tissue should be combined with other interventions requiring anaesthesia, such as bone marrow sampling or implantation of venous ports. To this end, close interdisciplinary co-operation between paediatric oncologists and gynaecologists, urologists, paediatric surgeons, psychologists or other medical specialists is required.

In the case of pre-pubertal boys, measurement of testicular volume is helpful to predict the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has initiated – are comparable to those of adult patients, irrespective of the underlying disease [12, 13]. In the case of pre-pubertal girls, the assessment of the ovarian reserve by measurement of blood levels of anti-Müllerian hormone (AMH) may be investigated [14]. No further gynaecological investigations should be performed, since they can be perceived as intrusive and an emotional and psychological burden for these patients.

30.4. Procurement

30.4.1. Female

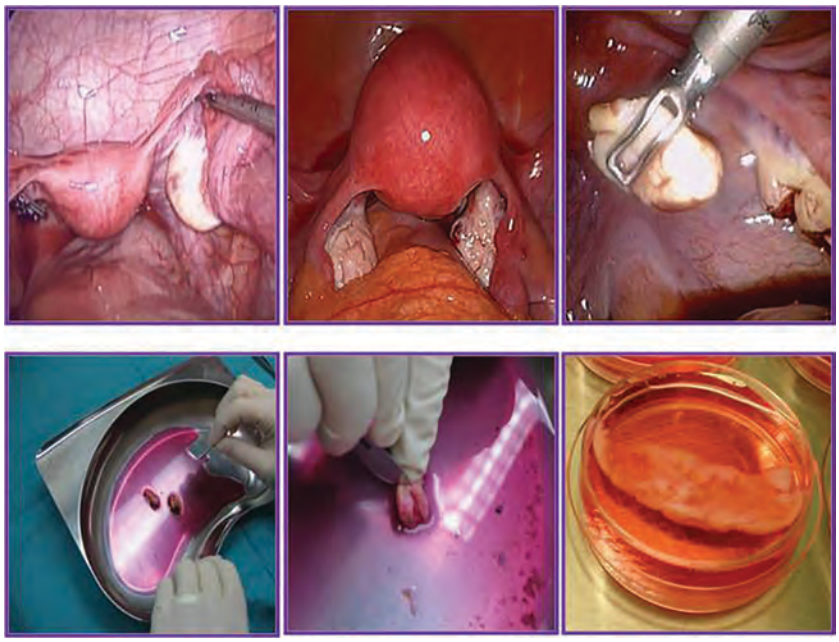
30.4.1.1. *Ovarian tissue*

Procurement of ovarian tissue can be performed at any time of the menstrual cycle and can be performed at short notice.

By the procurement of ovarian tissue, many thousands of primordial follicles can be preserved. Such follicles lie within the cortical tissue of an ovary, with the vast majority of follicles in the outermost 1-2 mm of the ovary. An entire ovary, a portion of it or ovarian cortical biopsies are removed by an operative procedure under general anaesthesia and pre-

Figure 30.1. **Ovarian tissue procurement by laparoscopy: stage 1**

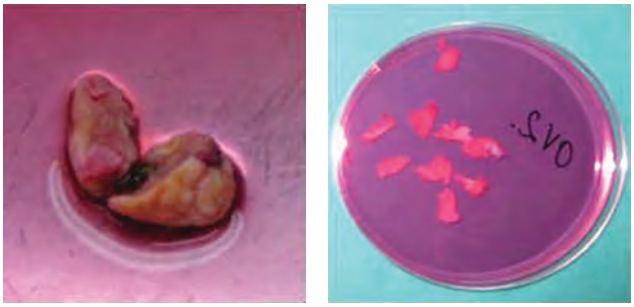
Images in the upper panel show the surgery steps taken to obtain the tissue. The lower panel shows processing steps: the medulla of the ovary is removed.



Source: Images provided by Sonia Herraiz, Valencia, Spain.

Figure 30.2. **Ovarian tissue procurement by laparoscopy: stage 2**

After the medulla removal the remaining ovarian cortex is then cut into small pieces of 10 × 5-10 mm



Source: Images provided by Sonia Herraiz, Valencia, Spain.

pared for cryopreservation [15]. See Figure 30.1. and Figure 30.2.

Ovarian tissue procurement is offered to pre-pubertal girls, and to post-pubertal girls not ready to undergo ovarian stimulation, trans-vaginal ultrasound monitoring and oocyte retrieval in order to procure and bank oocytes. Adult women who do not have the time to undergo stimulation for procurement of oocytes, either because cancer treatment is imminent or because the cancer is hormone-dependent, are also candidates for ovarian tissue cryopreservation.

Although, from a technical standpoint, it is possible to store ovarian tissue at the time of sex-reassignment surgery and thus during cross-hormone therapy, this approach is highly experimental [16]. For all patients, including transgender men, ovarian procurement should be performed before the start of gonadotoxic treatment.

30.4.1.2. *Oocytes*

In order to collect mature oocytes, controlled ovarian stimulation is needed. This stimulation is similar to the stimulation for *in vitro* fertilisation (IVF) (Chapter 29). The number of oocytes that can be collected depends on the age of the patient and her ovarian reserve. Special considerations are necessary to avoid high oestrogen production during ovarian stimulation in patients with oestrogen-dependent diseases. In cases of breast cancer, stimulation protocols have been developed to reduce the risk of an unwanted high level of oestradiol. Ideally, stimulation should start on the third day of the menstrual cycle, but can be started at any time in the menstrual cycle, including in the luteal phase, with apparently good results. In order to increase the yield of oocytes, two consecutive stimulations (collections) can be performed according to the approach referred to as dual stimulation [17]. Any pre-menopausal patient with a sufficient ovarian reserve is eligible for oocyte collection for FP. Some post-pubertal girls may be able to undergo ovarian stimulation and tolerate trans-vaginal ultrasound monitoring and oocyte retrieval.

Oocytes will be collected by aspiration via the transvaginal route following the same steps previously described in Chapter 29.

30.4.2. Male

30.4.2.1. *Testicular tissue*

Testicular tissue is mostly procured in pre-pubertal boys when there is no possibility to produce a sperm sample. Collection of testicular tissue can be performed at any time. In general, unilateral procurement takes place, with a maximum of half of the testis.

The procedure used for testicular biopsy in pre-pubertal boys is quite simple and similar to the technique described in adults. Basically, it should be performed at the cranial pole of the gonad, to avoid damage to the main testicular artery. After making a transverse or midline scrotal skin incision of 2-3 cm, the tunica vaginalis is opened and the lateral surface of the testis is exposed. The tunica albuginea is incised (0.5 cm in length) and the testes are compressed to make the testicular tissue protrude. A biopsy of 2-3 mm^3 is then cut with scissors. The tunica albuginea and the skin are closed over. Besides being useful in fertility preservation, testicular biopsy in pre-pubertal boys is a minor procedure that can provide valuable information for predicting the risk of malignancy and fertility, as described in Faure *et al.* 2016 [18] (see also Figure 30.3).

The amount of tissue procured for FP will have an effect on future testosterone production, and hormone replacement therapy could possibly be needed. However, it has been shown that the development of the testis in boys after biopsy of gonadal tissue for FP did not have an effect on the testicular growth [19].

A balance between the amount of tissue retrieved and the amount conserved is important to achieve adequate levels of testosterone. Since the testis volume in very young pre-pubertal children can be limited, one third of the testis is generally procured in this patient population. Immunohistochemical staining is necessary to assess the presence of SSC in the procured and stored tissue [20].

30.4.2.2. *Sperm*

Sperm samples are mostly obtained through masturbation. Sperm samples can be collected in adult men, postpubertal boys and in peri-pubertal

boys if the patient is capable of obtaining a sample through masturbation [13, 21-23]. In cases of failure to produce a semen sample by masturbation, assisted ejaculation techniques such as penile vibratory stimulation or electro-ejaculation under general anaesthesia could be considered as a second-line treatment option.

Special care should be taken to clearly explain to young post-pubertal boys how to produce a sample by ejaculation, since not all patients will be sexually active.

30.5. Processing

MAR TEs such as MAR centres and tissue banks can process and store gonadal tissue, gametes and embryos for FP. The techniques for processing and storage are described in Chapter 29. For further microbiological testing, refer to Chapter 11. Processing and storage of gonadal tissue require a tissue establishment with the facilities, licence and expertise to perform the procedure, and to process and store the tissue. These are described in more detail below.

Based on risk analysis, and offered testing for infectious disease (HIV, hepatitis), separate processing and storage of infectious material will be performed.

30.5.1. Female

30.5.1.1. *Ovarian tissue*

Ovarian tissue should be transported on ice in a transport medium supplemented with serum albumin [24]. Processing of the ovarian tissue starts with the ovarian biopsy or with bisecting the ovary, in the case of a whole ovariectomy. The medulla, the inner part of the ovarian tissue, is removed by careful scraping with a scalpel to prepare the cortical tissue with the required thickness of, on average, 1-2 mm. The cortex is subsequently cut into smaller fragments (5 × 5 mm) and 1 to 3 fragments of ovarian tissue are transferred to cryovials, immersed in cryoprotectant solution, and subsequently to controlled freezing for cryostorage. Each cryovial should be unequivocally identified [24]. Vitrification of the ovarian tissue is another optional methodology (see monograph 30.1) [5]. Microbiological quality control checks should be performed during processing to monitor any bacterial or fungal growth in the tissue previous to cryopreservation (aerobic and anaerobic bacteria, fungi assessment).

During ovarian tissue processing, the medulla should be further minced into small pieces in a petri dish with medium and examined under a stereomicroscope for the presence of cumulus oocyte complexes (COC). These COC can be collected and subjected to *in vitro* maturation (IVM] in order to obtain metaphase II oocytes that can be collected and stored. This collection, with IVM and storage of oocytes obtained during the processing of ovarian tissue, is considered a highly innovative and yet experimental FP technique, since so far only five live births have been described in Europe [25]. However, it opens up the possibility of cryopreservation of a few *in vitro* matured oocytes in the case of ovarian tissue procurement and storage.

Transport of the procured tissue from different centres to a centralised tissue establishment is a realistic and efficient logistic option [24].

30.5.1.2. *Oocytes*

Oocyte cryopreservation is the preferred option for FP in post-pubertal patients who can be submitted to controlled ovarian stimulation. Vitrification is the technique of choice, due to the excellent results obtained in IVF patients in terms of survival, embryo development and implantation [1, 26]. The methodology is described in Chapter 29.

30.5.1.3. *Embryos*

Although oocyte cryopreservation is generally practised today, embryo cryopreservation can also be considered for FP in the case of couples. Cryopreserved embryos will not be used for reproductive purposes if the couple separate; however, the situation can be different in various countries, depending on national regulations.

30.5.2. Male

30.5.2.1. *Ejaculate*

Sperm cryopreservation is performed for male FP in post-pubertal males. Semen characteristics may vary with both patient age and type of disease, with testicular cancer patients having the worst semen quality. For adolescents, in more than 80 % of cases semen can be cryopreserved. However, up to 20 % of adolescent or adult patients may either fail to produce a semen sample or may present with azoospermia. Measurement of testicular volume is helpful to predict the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been initiated – are comparable to those of adult patients, irrespective of the underlying disease.

The methodology for sperm cryopreservation is described in Chapter 29.

30.5.2.2. *Surgical sperm recovery from testicular tissue/epididymis*

For pre-pubertal boys, for azoospermic patients and for anejaculation, surgical sperm procured by sperm-extraction procedures should be offered. Sperm can be retrieved by microsurgical aspiration of the epididymal fluid (microsurgical epididymal sperm aspiration, MESA) or by testicular biopsy/testicular sperm aspiration (TESA) or testicular sperm extraction (TESE).

Testicular tissue should be transported on ice in a transport medium (e.g. Hepes-buffered DMEM/F12), supplemented with serum albumin (in general, 10 % HSA).

30.6. Conventional testicular biopsy and tissue processing for sperm recovery

Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging the pieces in medium supplemented with a cryoprotectant to protect the cells from cryodamage and then subjecting them to controlled slow freezing. However, no standardised protocol for cryopreservation of immature testicular tissue is available. Most groups are using DMSO-based cryoprotectants (0.7-1.4 M DMSO) with or without addition of sucrose. Slow-freezing protocols are mostly applied. Vitrification may also be effective when using higher doses of cryoprotectants. Vials/straws are thereafter submerged into liquid or vapour-phase nitrogen. Since the reproductive potential of cryopreserved immature testicular tissue has still to be demonstrated in humans, the technique remains experimental.

The legislation and recommendations for FP in males differ between countries. There are no strict limitations on semen quality or sperm numbers for FP strategies and there are no international guidelines for the duration of storage of spermatozoa, whether ejaculated or procured directly from the testis.

30.7. Storage

The permitted storage period of cryopreserved sperm, oocytes and embryos and reproductive tissues varies according to national legislation.

Long-term storage of ejaculated or testicular spermatozoa, ovarian cortex, oocytes or embryos does not negatively affect the quality of the frozen material, provided that it is stored at a temperature of ≤ −140 °C [27-31].

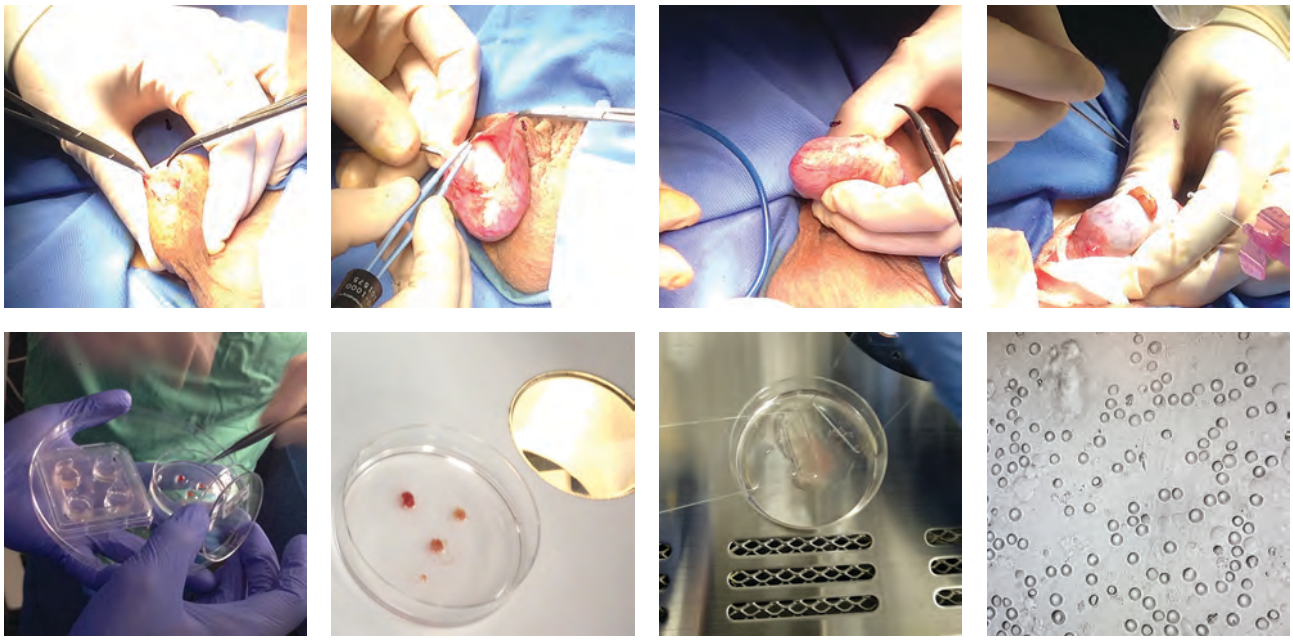
30.8. Clinical application

30.8.1. Female fertility restoration

When a patient wishes to use her preserved tissue or oocytes/embryos for MAR treatment, the physician who treated her with the gonadotoxic therapy should be consulted as to whether it is safe for the patient to attempt a pregnancy.

Figure 30.3. **Conventional testicular biopsy and tissue processing for sperm recovery**

Images in the upper panel show a surgical procedure for testicular tissue extraction. Lower panel shows processing steps: the small pieces of tissue are cut into smaller pieces. Isolated testicular spermatozoa and/or the tissue can be cryopreserved and thawed for future use.



Source: Images provided by Dina Pabón (Spain).

Recent reports comparing the efficacy of oocyte vitrification and ovarian tissue cryopreservation in women undergoing gonadotoxic treatments shows a trend towards higher live birth rates after oocyte vitrification [26].

In cases of transplantation of ovarian tissue, this can be done either orthotopically (at the remaining ovary or at the site of the removed ovary) or heterotopically to other sites such as the abdominal wall. These alternative options have implications for the modalities by which pregnancy can be achieved, spontaneously or by MAR. It takes approximately 20 weeks from the time of transplantation for the tissue to become active again as demonstrated by the return of menses and oestradiol production. Hence, restoration of fertility is combined with the restoration of the patient's endocrine environment. Although the primary reason for the use of stored reproductive material may be a future desire for a child, the restoration of endocrine function could also potentially be a reason for transplanting ovarian tissue. The latter has to be considered with caution as a recent review by Dolman *et al.* [32] showed that, of 258 patients undergoing ovarian tissue transplantation and for whom follow-up data were available, 204 received a diagnosis of premature ovarian insuffiency. Of those, 181 (88.7 %, ranging from 77.6 % to 97.2 % according to centre) experienced the recovery of ovarian endocrine function, as shown by the resumption of menstruation.

Spontaneous pregnancies can occur after the follicles start maturing and the patient regains her cycle, but sometimes IVF is needed. The duration of functionality varies from a few months to up to 10 years, with a mean of 3-4 years. If oocytes or embryos have been cryopreserved, an embryo replacement cycle must be planned. If the woman is menopausal, her endometrium will be prepared in a hormone replacement cycle.

It has to be emphasised that a significant percentage of women suffering from breast cancer will experience a spontaneous return of ovarian function months after chemotherapy. For these women spontaneous pregnancies may occur and they may not need their cryopreserved gametes or gonadal tissue [28]. Despite the number of cases still being scarce, some large series have shown 29 % conception rates following ovarian tissue transplantation. More recent publication have revealed similar live birth rates of 11 % after either spontaneous conception or IVF [26]. Premenarchal girls who lose all their ovarian tissue due to chemo- or radiation therapy will not enter puberty spontaneously. These girls will need to be induced with exogenous hormones in order to undergo normal pubertal development. After puberty, they will need to take hormonal replacement therapy to prevent osteoporosis and improve general well-being. Later on in life they can have their cryopreserved ovarian tissue transplanted in order to re-establish menstrual cycling and/or become pregnant [33].

30.8.2. Male fertility restoration

In most of the cases where chemotherapy and/or radiotherapy has been applied, spontaneous recovery of spermatogenesis is possible up to 10-15 years after the end of treatment; however, it cannot be accurately foreseen. Thus, regular semen analysis should be offered to patients after treatment. About 60 % of male cancer patients will face infertility problems after the end of the cancer therapy [23].

When cryopreserved samples are used, intracytoplasmic sperm injection (ICSI) is recommended to improve the chances of success. Before ICSI was implemented, the success rate of MAR procedures with cryopreserved semen samples (IUI or IVF) was low. When ICSI procedures are applied, the success rates using cryopreserved spermatozoa are comparable to standard IVF and ICSI procedures in infertile couples using fresh sperm.

30.9. Quality control and tissue evaluation

Quality control after tissue transplantation includes approaches aimed at decreasing the risk of cancer recurrence while maximising the tissue viability after thawing or warming.

As the autotransplantation of cryopreserved tissue of oncological patients may be associated with a risk of reintroduction of cancer cells, different approaches to detect such cells are under development [34-36]. Depending on the medical reason for tissue cryopreservation and the type of disease, the ovarian cortex and testicular tissue should be sampled and sent for histological examination to detect any malignant cells.

Post-cryopreservation viability of ovarian tissue depends on both possible damage caused by cryopreservation methodology and the original amount of primordial follicles. Follicle density in the tissue destined for cryopreservation may be assessed histologically to predict the chances of post-storage restoration of ovarian function. However, since primordial follicles are often distributed irregularly throughout the ovarian cortex, their absence or scarcity in small examined specimens does not nec-

essarily correspond to a contraindication for cryopreservation. After storage, the success of ovarian cortex transplantation, which depends on quality and quantity of the reintroduced tissue, can be evaluated by measuring the relevant endocrine function and fertility restoration [33, 37].

30.10. Biovigilance

Any adverse event or reaction should be notified, based on the general rules described in Chapter 17.

30.10.1. Female

A risk assessment for the re-introduction of malignant cells should be performed and transplanting of tissue must be with the agreement of the treating oncologist. This is to be considered especially in the case of leukaemia, since it is known that leukaemic cells can reside in the stroma of the cortical tissue. Women suffering from disseminated cancer with a risk of ovarian metastases should be advised against transplanting the tissue [36]. Since such transplantations are scarce, compared with other disciplines, limited cases of adverse events and reactions have been reported. Up to date the surgical related complications remain low (≈ 3 %), although one report of a major complication (intra-abdominal haemorrhage) was published in 2017 [37].

However, the reporting of serious adverse reactions and events affecting the offspring should follow the same rules as used for MAR (Chapter 29).

30.10.2. Male

When cryopreserved sperm samples are used, the ICSI technique increases the number of MAR treatments that can be performed. No adverse effect on the health of the offspring has been reported from the combination of cryopreservation of semen and subsequent MAR.

A number of studies have been performed regarding sperm quality in the man after spontaneous recovery of spermatogenesis. Both cancer and its treatment are associated with sperm DNA damage, although treatment-induced DNA damage seems to be modest and transient. In a large cohort study of offspring from male cancer survivors, a modest but statistically significant increase in the risk of major congenital abnormalities was observed. This was independent of whether the sperm were cryopreserved pre-treatment and used for MAR or if the children were conceived naturally [38, 39].

Any report of serious adverse reactions and events should also follow the same criteria used for assisted reproductive technologies (Chapter 29).

30.11. Developing applications

30.11.1. Female experimental approaches

30.11.1.1. *Oocyte regeneration*

In cases where no oocytes exist, current investigations are carried out on the generation of oocytes derived *in vitro* from pluripotent stem cells as a promising though still incipient therapy.

30.11.1.2. *In vitro maturation*

Female patients who seek FP but cannot undergo ovarian stimulation and oocyte/embryo preservation may consider using immature oocytes. IVM can be performed in antral follicles collected during the follicular or luteal phase [40]. Also small to mid-antral-sized follicles could be obtained as part of the ovarian cortex and medulla processing technique. The efficacy of IVM in the context of oestrogen-sensitive cancers, or in women with limited time for initiating fertility preservation before undergoing potentially gonadotoxic cancer treatment, is still not clear. Currently, however, success rates that may derive from IVM are expected to be low, since IVM has not been optimised as a laboratory process [39, 41].

30.11.1.3. *Organ culture*

Also, when re-implantation is contraindicated, other strategies are being explored. Some of them include the *in vitro* growth of structures containing theca cells and primordial follicles together This approach will require the use of IVM techniques as well as the means to determine to what extent cryopreservation will affect follicular viability [42].

Other lines of investigation could also involve *in vitro* perfusion and hormonal stimulation of the patient's removed whole ovary (or ovaries), where oocytes may be matured, aspirated and cryopreserved. As no ovarian stimulation is required, these techniques would shorten the time for starting oncologic treatment of the patient, as well as preventing the risk of reintroducing malignant cells via transplantation.

30.11.2. Male experimental approaches

30.11.2.1. *Sperm regeneration*

Development of the procedures used for the preservation of SSC and testicular tissues from boys and adolescents is far more advanced than research into the methods needed to realise the fertile poten-

tial of these cells. In principle, fertility restoration strategies in laboratory practice will include autotransplantation of a suspension of SSC by injection into the testis to restore spermatogenesis or autotransplantation of frozen-thawed testicular grafts back into the testis or an ectopic site. Should any risk of re-introduction of malignant cells exist via the transplant, then the only option is to grow and mature the SSC *in vitro*.

SSC transplantation was originally described in the mouse and is now an established research tool. SSC are infused through the efferent ducts into the *rete testis*, a technique which has been successfully applied in a number of species, including humans. The procedure is best performed under ultrasound guidance and presents a relatively non-invasive strategy for stem cell transfer. However, the colonisation efficiency after infusion of enzymatically digested testicular cells remains low. For future clinical applications, SSC need to be isolated, enriched and propagated *in vitro* before they can be autotransplanted in the numbers required to efficiently recolonise the testis and reinstate spermatogenesis.

Nonetheless, the principle of the procedure has been shown and offspring have been generated from transplanted spermatogonia in a number of species, including primates [10]. While the demonstration of functional donor spermatogenesis following SSC transplantation in primates is an important milestone on the way towards using SSC to restore human fertility, it remains vitally important to prove that the epigenetic programming and stability of SSC are not compromised following cryopreservation, culture and transplantation in humans.

Grafting of fragments of testicular tissue provides an alternative strategy to the use of cryobanked immature testis tissue. This approach maintains the SSC within their non-exposed natural niche, thus preserving the interactions between the germ cells and their supporting somatic cells. This procedure was successfully applied to retrieve sperm from ectopic and intra-testicular allografts, and insemination studies using ICSI have demonstrated that the spermatozoa were able to support full-term development of the progeny. This procedure is now tested in a number of species.

Similarly as in the case of oocytes, when no germ cells are available in the initial testis biopsy, an alternative option may be the *in vitro* derivation of sperm cells from the patient's somatic cells, such as skin fibroblasts, by induced pluripotency or transdifferentiation of these cells. This approach is, however, still in its infancy.

30.11.2.2. *Organ culture*

The major hurdle which must be overcome in patients with a haematological malignancy is the risk of re-introducing residual malignant cells via the testicular tissue. Sorting protocols using magnetic activated cell sorting (MACS), fluorescence activated cell sorting (FACS) or differential plating have been found to have variable efficiency when used to enrich human SSC. The risk of re-introduction of malignant cells via the graft may be circumvented by *in vitro* spermatogenesis. *In vitro*-derived spermatozoa that are free from residual disease can then be used to inseminate oocytes using ICSI. Various strategies – including standard 2D cultures, 3-dimensional culture of testicular cells or organ culture – have been tested and showed some promise [43]. Although encouraging results have recently been obtained regarding the genetic and epigenetic stability of human SSC during long-term culture, the fertility of *in vitro*-derived sperm has still to be established before the clinical value of this type of experimental approach can be fully assessed.

30.12. References

1. Cobo A, Garcia-Velasco J, Domingo J *et al.* Elective and onco-fertility preservation: factors related to IVF outcomes. *Hum Reprod* 2018;33(12):2222-31.
2. Jensen AK, Kristensen SG, Macklon KT *et al.* Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Hum Reprod* 2015;30(12):2838-45.
3. Cobo A, Giles J, Paolelli S *et al.* Oocyte vitrification for fertility preservation in women with endometriosis: an observational study. *Fertil Steril.* 2020;113(4):836-44.
4. Somigliana E, Vigano P, Filippi F *et al.* Fertility preservation in women with endometriosis: for all, for some, for none? *Hum Reprod* 2015;30(6):1280-6.
5. The ESHRE Guideline Group on Female Fertility Preservation, Anderson RA, Amant F, Braat D *et al.* ESHRE guideline: female fertility preservation. *Hum Reprod Open* 2020(4):2020, hoaa052. https://doi.org/10.1093/hropen/hoaa052.
6. Teinturier C, Hartmann O, Valteau-Couanet D *et al.* Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is a major cause of ovarian failure. *Bone Marrow Transplantation* 1998;22(10):989-94.
7. Wallace WH, Shalet SM, Crowne EC *et al.* Ovarian failure following abdominal irradiation in childhood: natural history and prognosis. *Clin Oncol* (R Coll Radiol) 1989 Nov;1(2):75-9.
8. Julian-Reynier C. [Genetic predisposition to breast and ovarian cancer: importance of test results]. *Med*

Sci (Paris) Jun/Jul 2011;27(6-7):657-61. https://doi.org/10.1051/medsci/2011276019 [in French].

9. Jahnukainen K, Ehmcke J, Hou M, Schlatt S. Testicular function and fertility preservation in male cancer patients. *Best pract Res Clin Endocrinol Metab* 2011;25(2):287-302.
10. Jahnukainen K, Ehmcke J, Nurmio M, Schlatt S. Autologous ectopic grafting of cryopreserved testicular tissue preserves the fertility of prepubescent monkeys that receive sterilizing cytotoxic therapy. *Cancer Res* 2012 Oct 15;72(20):5174-8.
11. Meirow D. Reproduction post-chemotherapy in young cancer patients. *Mol Cell Endocrinol* 2000;169(1-2):123-31.
12. Bahadur G, Ling KL, Hart R *et al.* Semen quality and cryopreservation in adolescent cancer patients. *Hum Reprod* 2002;17(12):3157-61.
13. Kliesch S, Behre HM, Jurgens H, Nieschlag E. Cryopreservation of semen from adolescent patients with malignancies. *Med Pediatr Oncol* 1996;26(1):20-7.
14. Fasano G, Dechene J, Antonacci R *et al.* Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients. *Reprod Biomed Online* 2017 Jun;34(6):575-82.
15. Newton H, Aubard Y, Rutherford A *et al.* Low temperature storage and grafting of human ovarian tissue. *Hum Reprod* 1996;11(7):1487-91.
16. De Roo C, Tilleman K, T'Sjoen G, De Sutter P. Fertility options in transgender people. *Int Rev Psychiatry* 2016;28(1):112-19.
17. Vaiarelli A, Cimadomo D, Trabucco E *et al.* Double stimulation in the same ovarian cycle (duostim) to maximize the number of oocytes retrieved from poor prognosis patients: a multicenter experience and SWOT analysis. *Front Endocrinol* (Lausanne) 2018 Jun 14;9:317. https://doi.org/10.3389/fendo.2018.00317.
18. Faure A, Bouty A, O'Brien M *et al.* Testicular biopsy in prepubertal boys: a worthwhile minor surgical procedure? *Nat Rev Urol* 2016 Mar;13(3):141-50.
19. Uijldert M, Meissner A, de Melker AA *et al.* Development of the testis in pre-pubertal boys with cancer after biopsy for fertility preservation. *Hum Reprod* 2017;32(12):2366-72.
20. Schlatt S, Ehmcke J, Jahnukainen K. Testicular stem cells for fertility preservation: preclinical studies on male germ cell transplantation and testicular grafting. *Pediatr Blood Cancer* 2009 Aug;53(2):274-80.
21. Kamischke A, Jurgens H, Hertle L *et al.* Cryopreservation of sperm from adolescents and adults with malignancies. *J Androl* 2004 Jul-Aug;25(4):586-92.
22. Nangia AK, Krieg SA, Kim SS. Clinical guidelines for sperm cryopreservation in cancer patients. *Fertil Steril* 2013;100(5):1203-9.
23. Picton HM, Wyns C, Anderson RA *et al.* A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. *Hum Reprod* 2015;30(11):2463-75.
24. Vilela JMV, Dolmans M-M, Amorim CA. Ovarian tissue transportation: a systematic review, *Reproductive BioMedicine Online* 2021;42:351-65.
25. Segers I, Bardhi E, Mateizel I *et al.* Live births following fertility preservation using in-vitro maturation of ovarian tissue oocytes. *Hum Reprod* 2020 Sep 1;35(9):2026-36. https://doi.org/10.1093/humrep/deaa175. PMID: 32829388.
26. Diaz-Garcia C, Domingo J, Garcia-Velasco JA *et al.* Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study. *Fertil Steril* 2018;109(3):478-85 e2.
27. Cobo A, Romero JL, Perez S *et al.* Storage of human oocytes in the vapor phase of nitrogen. *Fertil Steril* 2010;94(5):1903-7.
28. Dunlop CE, Brady BM, McLaughlin M *et al.* Re-implantation of cryopreserved ovarian cortex resulting in restoration of ovarian function, natural conception and successful pregnancy after haematopoietic stem cell transplantation for Wilms tumour. *J Assist Reprod Genet* 2016;33(12):1615-20.
29. Kelleher S, Wishart SM, Liu PY *et al.* Long-term outcomes of elective human sperm cryostorage. *Hum Reprod* 2001;16(12):2632-9.
30. Liu Q, Lian Y, Huang J *et al.* The safety of long-term cryopreservation on slow-frozen early cleavage human embryos. *J Assist Reprod Genet* 2014;31(4):471-5.
31. Urquiza MF, Carretero I, Cano Carabajal PR *et al.* Successful live birth from oocytes after more than 14 years of cryopreservation. *J Assist Reprod Genet* 2014;31(11):1553-5.
32. Dolmans MM, von Wolff M, Poirot C *et al.* Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. *Fertil Steril* 2021 May;115(5):1102-15. https://doi.org/10.1016/j.fertnstert.2021.03.008. PMID: 33933173.
33. Chow EJ, Stratton KL, Leisenring WM *et al.* Pregnancy after chemotherapy in male and female survivors of childhood cancer treated between 1970 and 1999: a report from the Childhood Cancer Survivor Study cohort. *Lancet Oncol* 2016 May;17(5):567-76.
34. Mouloungui E, Zver T, Roux C, Amiot C. A protocol to isolate and qualify purified human preantral follicles in cases of acute leukemia, for future clinical applications. *J Ovarian Res* 2018 Jan 5;11(1):4.
35. Rodriguez-Iglesias B, Novella-Maestre E, Herraiz S *et al.* New methods to improve the safety assessment of cryopreserved ovarian tissue for fertility preservation in breast cancer patients. *Fertil Steril* 2015;104(6):1493-502, e1-2.

36. Soares M, Saussoy P, Maskens M *et al.* Eliminating malignant cells from cryopreserved ovarian tissue is possible in leukaemia patients. *Br J Haematol* 2017 Jul;178(2):231-9.
37. Jadoul P, Guilmain A, Squifflet J *et al.* Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. *Hum Reprod* 2017;32(5):1046-54.
38. Romerius P, Stahl O, Moell C *et al.* Sperm DNA integrity in men treated for childhood cancer. *Clin Cancer Res* 2010 Aug 1;16(15):3843-50.
39. Wang X, Gook DA, Walters KA *et al.* Improving fertility preservation for girls and women by coupling oocyte *in vitro* maturation with existing strategies. *Women's Health* 2016;12(3):275-8.
40. Huang JY, Chian RC, Gilbert L *et al.* Retrieval of immature oocytes from unstimulated ovaries followed by *in vitro* maturation and vitrification: A novel strategy of fertility preservation for breast cancer patients. *Am J Surg* 2010 Jul;200(1):177-83.
41. ASRM opinion paper: Practice Committees of the American Society for Reproductive Medicine, the Society of Reproductive Biologists and Technologists, and the Society for Assisted Reproductive Technology. In vitro maturation: a committee opinion. *Fertil Steril*. 2021 Feb;115(2):298-304. https://doi.org/10.1016/j.fertnstert.2020.11.018. Epub 2020 Dec 24. PMID: 33358333.
42. Telfer EE. Fertility preservation: progress and prospects for developing human immature oocytes *in vitro*. *Reproduction* 2019 Nov;158(5):F45-F54. https://doi.org/10.1530/REP-19-0077.
43. Stukenborg JB, Schlatt S, Simoni M *et al.* New horizons for *in vitro* spermatogenesis? An update on novel three-dimensional culture systems as tools for meiotic and post-meiotic differentiation of testicular germ cells. *Mol Hum Reprod* 2009 Sep;15(9):521-9.

Related material

Appendix 11. Example of consent form: female (NHS, UK)
Appendix 12. Example of consent form: female (CNPMA, Portugal)
Appendix 13. Example of consent form: male (NHS, UK)
Appendix 40. Informed legal consent for cryopreserving and storing semen from a minor

Chapter 31. **Human milk**

31.1. Introduction

Human milk (HM) is produced by the human mammary glands of a postnatal mother to feed her baby. HM contains essential nutrients and bioactive components that promote the growth and development of the newborn. HM not only covers the nutritional needs of healthy term infants, but it also facilitates the process of maturation of various organs such as the gut and the brain [1]. It contains many unique nutritional components and a complex combination of immunological and anti-infective constituents that promote health, protect against infection and support a baby's immune system [2]. For these reasons, mother's own milk is universally accepted as the optimal feeding choice for neonates and infants, but it is considered of vital importance for preterm infants.

Unfortunately, not all preterm infants can receive milk from their mothers and some mothers are unable to provide enough milk for their infants. In these cases official bodies, such as the World Health Organization (WHO), the American Academy of Pediatrics and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, as well as many scientific associations, consider donated human milk, obtained and processed in human milk banks (HMBs), to be the clinically preferred option in the absence of sufficient maternal milk [3][4][5][6].

Donor HM (DHM) is the human milk expressed by a breastfeeding mother and donated voluntarily and freely to an HMB, and it should be considered the first option when milk from the infant's own mother is partially or totally unavailable. Given the significant impact that HMBs can have on infant health outcomes, the WHO has asked member countries "to promote the safe use of DHM through HMBs for vulnerable infants" [3].

In the European Union (EU), DHM may fall within different national legal frameworks (see §31.2 below) for which appropriate quality and safety requirements need to be applied, e.g. food, tissues and cells [6]. Regardless of the regulatory status of HM, ensuring quality and safety requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide to provide an appropriate framework for safe and effective services to patients. The ethical principles described in Chapter 1 of this Guide must be respected so that donors are not exploited and the human body itself is not the subject of trade.

There are two types of DHM:

a. Fresh (or raw) milk is HM which has not undergone any treatment and can be stored at +4 °C (refrigerated) or at −20 °C/−80 °C (frozen).
b. Pasteurised milk is HM that has undergone a pasteurisation process.

An HMB is a facility established with the purpose of selection, collection, processing, storage and distribution of human milk donated by lactating mothers who are not biologically related to the recipient infant, to meet the specific needs of individuals for whom DHM is prescribed by health care providers. The primary and by far the largest group of consumers of DHM are premature babies. Other

clinical situations in which the use of DHM has been described are: post-feeding intolerance, gastrointestinal surgery, congenital heart disease, metabolic disorders, chronic renal failure and allergies [7][8][4]. The use of DHM is also described in term and late preterm infants as the preferable option when supplementation due to insufficient maternal milk supply is needed to prevent dehydration and severe hyperbilirubinemia [9].

The first HMB was opened in Vienna in 1909. Since then, HMBs have been established in many countries: currently more than 280 HMBs exist in Europe [10], over 300 in South America – including 222 in Brazil [11] – and 29 in North America [12]. The numbers and activities of HMBs are growing, driven by studies indicating that premature infants show better development if they are fed HM rather than formula. With donated HM, there is a lower rate of necrotising enterocolitis with a better tolerance of enteral feeding [13], and protection against bronchopulmonary dysplasia. In addition, it has been shown that where new HMBs are established there is an increase in the rates of breastfeeding on discharge from hospital of these babies [14-15]. It is now widely accepted that HMBs and the availability of DHM encourage and support breastfeeding.

The theoretical microbiological risks associated with feeding with DHM are similar to those associated with transfusion and transplantation (i.e. transmission of viruses and other infective agents from the donor) as well as those associated with the food industry (i.e. contamination or cross-contamination with bacteria or fungi, with subsequent recipient infection). One of the most commonly used risk-assessment methods is Hazard Analysis Critical Control Points (HACCP), developed in the 1950s in the food industry but now widely used for many manufacturing processes, including biological control. The system consists of a detailed analysis of all steps of the production process to evaluate and control hazards and to avoid the possible errors (see Chapter 2). Using the HACCP system in the management of an HMB activity was first described in the Italian Guidelines for the Establishment and Operation of a Donor Human Milk Bank, in the 1st edition published in 2002 [16].

The following generic chapters (Part A) of this Guide all apply to milk banking and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 8. Premises
h. Chapter 9. Processing
i. Chapter 10. Storage
j. Chapter 11. Principles of microbiological testing
k. Chapter 12. Release, distribution and import/export
l. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
m. Chapter 14. Computerised systems
n. Chapter 15. Coding, packaging and labelling
o. Chapter 16. Traceability
p. Chapter 17. Biovigilance
q. Chapter 18. Introduction of novel processes and clinical applications

31.2. Legal status of human milk

Human milk does not have a uniform legal status in all countries; moreover, not all countries have a specific regulation for this activity. In some countries it is under the umbrella of food, whereas others assume the tissues and cell regulation as the best scenario. Like any other substances of human origin, there are some important principles to consider.

The World Health Organization classifies DHM used to feed premature infants as among a group of substances [17] derived wholly or in part from the human body and intended for clinical application, like organs for transplantation; blood; ocular, musculoskeletal or other types of tissue; haematopoietic or other types of cells; and ova or sperm used in assisted reproductive treatments. All these products share a basic characteristic that makes them different from other medical products or devices: all of them depend on the altruistic donation of biological materials from living or deceased persons. Thus, to preserve the dignity and human rights of donors, it is necessary to follow high ethical standards in obtaining these biological products, such as those standards established in the Oviedo Convention adopted by the Council of Europe in 1997 [18].

As commented by Cohen [19], despite the longstanding and seemingly natural classification of human milk as a food, it is unlike any other food product due to the ethical and safety issues it raises. For instance, it is necessary to obtain informed consent from an infant's parents before it can be administered, on the premise that it comes from another

person. However, no such consent is required to dispense formula based on cow's milk to a baby, even though it comes from the body of an animal.

Moreover, the human origin of these medical products also entails risks to public health, making it necessary to establish safety mechanisms such as donor selection, screening and testing, techniques that inactivate pathogens or render them less pathogenic, and adequate traceability of medical substances of human origin, so that sentinel events of disease transmission can be promptly investigated and linked to specific products, source individuals and recipients, enabling the development of new risk containment and mitigation strategies, including rapid product recalls. All of these apply to donor human milk.

However, following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed the questions related to the legal status of human milk in the EU and, despite confirming that Article 168 (4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances [20].

Currently, the collection of DHM is done following good quality rules in the majority of HMBs in EU member states, thanks to the existence of Guidelines in several countries. However, regulatory guidance at European level does not exist. Donor human milk is one of the few medical products of human origin that does not have legislation associated with it at a supranational level. Some countries, like France [21] and Italy [22], have laws and norms regulating the functioning of milk banks, whereas other countries have a variety of alternative sources of guidance, including locally, regionally and nationally developed documents. The question of how to regulate the allogeneic use of human milk remains open.

31.3. Minimal requirements of human milk banks

31.3.1. Facilities and equipment

HMBs should establish a service-level agreement at least with a Neonatal Unit that admits newborns of gestational age < 34 weeks.

The rooms required in an HMB should include adequate space to conduct donor interviews if necessary and to carry out administrative tasks, with a space for freezers and a room for handling and processing milk. The premises must be structured in such a way as to allow easy control, correct cleaning and effective disinfection. The dimensions will be in line with the HMB's workload.

An HMB should include the following basic equipment:

1. A commercially available pasteuriser or a water bath with adjustable temperature, preferably with agitation, able to work at a temperature of 62.5°± 1 °C for 30 minutes.
2. 1 or more freezers (< –15 °C) equipped with acoustic and/or visual alarms for the temperature and with a thermo-recorder.
3. 1 or more refrigerators with a working temperature between 2 °C and 8 °C, and with acoustic and/or visual alarms for the temperature and with a thermo-recorder.
4. 1 work counter.
5. Laminar flow hood to handle HM aseptically.
6. Electrical breast pumps, if milk is expressed in the HMB.
7. Dish-washing machine qualified for thermal disinfection and a system to seal the containers if they will be submerged at any point during the heating or cooling phases of the pasteurisation process.

31.3.2. Staff

The organisation and maintenance of a working system able to guarantee the quality of the milk from collection to processing and distribution is based on its staff, with a number of people adequate to the working load, well prepared, trained and motivated to perform all the activities related to the bank.

The HMB team must include a responsible person and sufficient staff to carry out the daily operations of the HMB. The tasks of a dedicated team (doctors, nurses, technicians, ancillary staff) are different according to the complexity of the service: responsibility, co-ordination, enrolment of donors, care of donors, home milk collection, quality control of the milk, thermal treatment and storage of the milk, checking and disinfection of the equipment and of the rooms, registration of the official documents, and distribution and traceability of the final product.

Staff must be trained on all the following: hygiene, quality control, safety and traceability, technical procedures (collection, storage, pasteurisation), legislation, ethical issues and infant nutrition. Staff

must receive continuing education to update their knowledge. Their work must be audited periodically.

31.3.3. Quality Management System

An HMB must implement a QMS that covers the scope of all its activities, especially (see Chapter 2):

1. collecting, testing, processing, storing and transporting milk;
2. recruitment and evaluation of donors, including home collection training;
3. external and internal auditing, non-conformance with processes and self-inspection;
4. coding of the milk collected and distributed, for identification of the HMB and of the donors (traceability);
5. records of the safety procedures utilised and of the final destination of the donated milk;
6. personnel, required documentation, premises and equipment;
7. presence of written standard operating procedures (SOPs) and an Internal Regulation specific to the HMB;
8. home milk collection service organised according to the established safety procedures, if applied.

31.4. Donor recruitment

The donation of HM must be voluntary and unpaid. In some countries, in accordance with national regulations, a sort of reimbursement is provided to meet the expenses of the donors.

Before accepting a donor's milk, written informed consent for its use in accordance with the HMB's protocols (including for approved research, if relevant) must be obtained.

Promoting HM donation is carried out through a variety of different channels: written material (e.g. in prenatal clinics, maternity hospitals, paediatric/primary care centres, pharmacies, shops for mother/infant items), media, social networks, associations for breastfeeding support or educational guidance, and direct contact with pregnant women by their physicians and midwives.

A woman should be of legal age and lawfully competent to take this decision in accordance with national regulations; she should be nursing her own baby, who must be adequately fed, before donating milk to a HMB. Bereaved mothers should be made aware of the possibility of donating their previously expressed and stored milk as well as continuing to lactate for the purposes of HM donation if desired. This has been shown to be of benefit to grieving mothers [23][24].

It is the responsibility of the HMB to provide all new donors with training, preferably face-to-face with additional information by telephone and in writing. This training should cover [25]: handwashing; good personal hygiene; collecting and expressing milk, including cleaning and using breast pumps and containers; storing donated milk (including cooling and freezing); labelling donated milk and documenting storage conditions; and transportation of donated milk (if needed).

Because HM donation is carried out frequently over a period of a few months, it must be stressed to the donor that certain health-related conditions – like infectious diseases or drugs (including nicotine and alcohol) – during the donation period would make her unsuitable as an HM donor. HMB staff have a duty of care to those who offer to donate HM, including a duty to those whose milk is not accepted because of, for example, medication or tobacco use. A mother who is unable to donate for whatever reason should be reassured that this should not affect her choice to feed her own baby – assuming that this is the case. HMB staff should take responsibility for ensuring that she understands the reasons for her deferral and how this affects or does not affect her own infant [26]. The value of breast milk and of breastfeeding her own infant in accordance with WHO guidance should be highlighted in all communications between HMB and prospective donors.

31.5. Donor evaluation

Screening of donors should include both oral interview (face to face or by telephone) and completion of a health questionnaire, using clear, non-technical language in printed material and digital media.

HMB processing cannot guarantee the complete elimination of toxic substances and potential infectious elements that may be contained in the milk. For this reason, HM, which has not undergone any treatment, should be as safe as possible from the point of its origin.

In addition to the general contraindications for donation specified in Chapter 5, the following conditions contraindicate the donation of milk [25][27][28][29].

a. *Donor's behavioural risks*
   i. smoking tobacco, use of snuff or use of nicotine-containing products to help stop smoking – wait for 7 days from the last exposure;
   ii. drug abuse or use of methadone;

iii. daily consumption of beer (≥ 200 mL), wine (≥ 100 mL) or spirits (≥ 30-40 mL) – occasional consumption may be accepted if milk collection is avoided for 12 hours;
iv. consumption of high quantities (> 300 mg) of substances containing caffeine (coffee, tea, cola or cacao) should be avoided – occasional consumption may be accepted;
v. if there is the suspicion of low vitamin B12 level (vegans or strict vegetarians, without vitamin B12 supplementation), the donor can be accepted if an adequate level of vitamin is verified in a blood test.

b. *Donor's treatments*
i. the use of drugs or other pharmacologically active substances (including herbal products) must be evaluated since most will be secreted into breast milk – the concentration and potential toxicity vary substantially depending on the substance and the dose (relevant information can be accessed at www.e-lactancia.org);
ii. women immunised with attenuated live virus should not donate milk for 4 weeks after the immunisation;
iii. the transfusion of blood and blood components should be evaluated by individual risk assessment;
iv. treatments with acupuncture needles that are not properly sterilised or disposable should be evaluated by an individual risk assessment;
v. transplantation of organs should exclude donation (organ transplantation is usually followed by long-term anti-rejection medication which excludes donation).

c. *Donor's medical history*
i. acute infections and diseases must be evaluated, depending on the type of infection for the appropriateness of temporary exclusion and the exclusion time itself;
ii. women who have recently been in contact with infectious patients (e.g. chickenpox, mumps, measles) unless they have been immunised; if they have not been immunised, they should be excluded for a period equivalent to the incubation period or, if not known, for 4 weeks;
iii. women with sexual contact with partners with HIV or viral hepatitis B or C should be excluded;
iv. women with mastitis or fungal infections of the nipple or areola should be excluded temporarily until all symptoms have disappeared and any course of medication completed;
v. women with reactivation of *Herpes simplex* (HSV) or varicella–zoster infections in the mammary or thoracic region should be excluded;
vi. women with a history of malignancy should be evaluated by individual risk assessment;
vii. during the interview, donors should be instructed to inform the HMB if there are any changes in their behaviour or health status, especially in relation to the presence of mastitis, temporary use of some pharmacologically active substances, the presence of acute infectious diseases and skin diseases such as *Herpes simplex* or varicella–zoster, or fungal infections of the nipple, areola, mammary or thoracic region.

31.6. Donor serological testing

In addition to the tests outlined in Chapter 5, testing for HTLV-I/II is recommended; at least it should certainly be performed for donors who live/d in or originate from high-prevalence areas [30] or who have known sexual partners originated from those areas, or if the donor's parents originated from those areas.

The risk of transmission of *Trypanosoma cruzi* through breastfeeding has not clearly been established. Pasteurisation destroys *T. cruzi* [27]. If there is a suspicion of this disease during the health interview, serological screening for *T. cruzi* should be done.

While a donor continues to donate, ask regularly about her general health and the exclusion criteria detailed in section 31.5 to consider whether repetition of serological testing is indicated. If there is no change in the donor's lifestyle, it should not be necessary to repeat donor testing during the period of milk donation. However, if it is not feasible to evaluate carefully a change in donor's lifestyle, donors should be tested every 3 months.

Seropositivity for *Cytomegalovirus* (CMV) is not considered a contraindication as long as the milk is pasteurised.

31.7. Human milk expression and collection

Donors should be trained in handwashing and hygiene procedures for expressing, handling, storing and transporting the DHM. The milk can be expressed by hand or with a breast pump. Good breast hygiene should be encouraged, and at least once daily washing of the breasts is recommended. Additional washing prior to expressing has also been

shown to reduce bacterial contamination of the milk, together with cleaning and disinfecting all the components of the breast pumps [29].

Most recommended containers are in rigid plastic, made from a variety of food-contact materials such as polyethylene or polypropylene, and should be bisphenol A free. It is recommended that containers should be sterile and single-use. Re-using containers requires cleaning and sterilisation. The use of containers sterilised with ethylene oxide is regulated in the EU at the European level, and the HMB should ascertain that manufacturers respect this EU regulation (or users should avoid devices sterilised with ethylene oxide, as shown on the label). Glass containers can be used; however, they should be purpose-designed and sold as intended for breast milk storage, including freezing and high temperatures, and made from glass that is chosen to resist breakage [31].

Some milk banks use plastic bags of polyethylene as an alternative to rigid containers, but these bags can easily break with the risk of loss of milk and contamination. Use of a double bag is therefore recommended if this type of container is used.

If the milk is to be frozen, the container should not be filled completely. The containers must be labelled with the donor code provided by the HMB (or, if that is not possible, with a donor's given name and family name and date of birth), and the date of collection.

31.8. Milk storage at home and transportation to the bank

Collected milk has to remain at room temperature for the shortest possible time. After collection, the container should be sealed, labelled and frozen as soon as possible in order to avoid bacterial growth and degradation of the milk. If this is not possible, donors may refrigerate (2 °C to 8 °C) samples collected over 24 hours, and then freeze (< 15 °C) the batch [25][32].

If a woman has accumulated milk before being accepted as a donor, her medical and behavioural history must be evaluated retrospectively for their suitability, and the milk can be accepted only after a risk assessment considering how the milk was collected, stored and identified.

Frozen expressed milk should be transported to the milk bank as soon as possible. However, if necessary DHM can be stored before transport for less than 1 month in a domestic freezer (< 18 °C). In this case, the HMB should ensure that donors can check and document any temperature change on the freezer and communicate any problem with the home fridge or freezer to the HMB. After a risk assessment, the HMB will decide about the acceptance of the milk.

The milk must be transported while maintaining the cold chain. The HMB is responsible for transportation of the milk. If third parties are used, a formal agreement should be in place, with the milk bank establishing and checking the transport conditions to ensure the safety and quality of the milk.

When the milk arrives, the HMB should check the label of each bottle as well as the accompanying documentation. In addition, it should check the integrity of the bottles and the container. A record of this should be maintained as part of the HMB records.

31.9. Milk storage in the human milk bank prior to processing

The lipase activity present in DHM is active while stored at < – 18 °C and, therefore, there is active lipolysis, which degrades triglycerides, reducing their content and increasing the content of diglycerides, monoglycerides and free fatty acids [33]. In contrast, when milk is stored at < –70 °C, no measurable lipolysis is observed [34]. In addition, fat and energy content was significantly higher in the samples stored at < –70 °C compared to the paired samples at < – 18 °C, while there was no significant difference when protein and carbohydrate concentration were compared [35]. Despite these data, most HMBs store DHM prior to pasteurisation at < – 18 °C, mainly because the equipment is cheaper and more affordable than those at < –70 °C. Therefore, if a temperature of < – 18 °C is used, the DHM must be processed within 3 months from the date of collection. In cases where the DHM is stored at < –70 °C, this period could last up to 1 year.

It has been shown that, due to heat inactivation of the breast milk lipases, the increase of free fatty acid levels is not observed after pasteurisation [36]. Therefore, it is recommended that storage at < – 18 °C prior to pasteurisation should be minimised to prevent free fatty increases. Alternatively, if it is possible, it is recommended to freeze and store at the lowest possible temperature (ideally < –70 °C) prior to pasteurisation.

31.10. Processing

The first control for donated milk, before processing, is the evaluation of its organoleptic characteristics: in the case of inappropriate aspect or smell, the milk should be discarded. Human milk varies widely in colour and may be clear (early colostrum), yellow (colostrum and early transitional milk),

creamy white or white tinged with blue or green, depending on the period of lactation, the extent to which the breast has been emptied and the mother's diet. If the milk exhibits unusual colouring or if it contains any foreign bodies or visible impurities, these should be noted, and the milk should be discarded.

Milk must be processed under aseptic conditions. Individuals handling milk in an HMB must wear a hair covering, gloves and a clean gown to prevent contamination of the milk. HM should be handled under a Class A laminar-flow cabinet or a bio-safety cabinet, which must be qualified and periodically certified (including regular analyses of the particulate and microbiological contamination of the cabinet).

Thawing the milk before processing can be obtained by defrosting the milk rapidly in a water bath at a temperature not exceeding 37 °C [25][28], or in the refrigerator (2 °C-8 °C) for a maximum period of 24 hours. Human milk should not be thawed in a microwave oven [28].

After thawing, some HMBs combine or pool milk from multiple donors to provide more consistent nutrient content and to increase uniformity in the product after pooling [37]. The HMBs that combine or pool milk should support this practice with a comprehensive risk–benefit assessment. Moreover, traceability must be fully ensured because, if there is any contamination of pooled milk, it may be difficult to trace the source of the contamination. In addition, they must state in their protocol the maximum number of donors recommended for pooling. The Human Milk Bank Association of North America recommends using the milk from three to five donor mothers [33].

It is common practice in HMBs to increase safety and reduce the risk of contamination by pasteurising the milk. However, in very specific situations raw milk – from no-high-risk donors with all serological determinations, including CMV negative – can be used [38]. In those situations, each HMB must have performed a risk analysis, including the initial level of bacterial count, before they decide to accept the distribution of raw milk, and the decision taken must be documented.

Currently, a pasteurisation process performed at a temperature of 62.5 ± 1 °C for 30 minutes, which is known as Holder pasteurisation (HoP) method, is recommended in all international guidelines for the establishment and management of HMBs [29][34]. This process can be carried out in a shaker water bath, or with equipment specifically designed for pasteurising milk. The equipment used for pasteurisation must be calibrated at least every 12 months. Regular qualification of pasteurisers is needed to optimise pasteurisation and to improve milk quality. A few quality criteria for the HoP process have been proposed: temperature plateau in the range of 62.5-64.5 °C, duration of the plateau between 30 and 35 minutes, exposure time over 58 °C < 50 min, and rapid cooling from 62.5 °C to 10 °C (≤ 1 h) [35][39].

Before pasteurisation, a milk sample should be taken from each batch for microbiological testing (bioburden). The European Milk Bank Association recommends accepting the milk if it contains 10^5 CFU/mL or less of non-pathogenic organisms and if no pathogens are detected [34]. Each HMB should define the contamination level in accordance with their national guidelines if they apply. In addition, this sample may also be used for a macronutrient analysis of the milk to evaluate the necessity of fortification.

During pasteurisation, bottle caps must remain above water level to prevent contamination, unless caps and equipment designed for submersion are used, in which case additional checks should be put in place to ensure seals are effective and end users should be cautioned to discard containers with an incomplete seal and should notify the milk bank immediately. A control bottle containing the same amount of milk or water as all the other bottles in the batch must be fitted with a calibrated thermometer to record milk temperature during pasteurisation. The control bottle should follow the same process as the rest of the batch at all times. In addition to the milk temperature, the water bath temperature should be monitored and recorded.

Pasteurised human milk is known to retain many beneficial and protective components of fresh HM [38]. However, pasteurisation also affects some of the nutritional and biological properties of HM and eliminates the beneficial microbiota of fresh HM, thus resulting in the reduction of some bacteriostatic mechanisms that render milk more susceptible to post-heating bacterial contamination and decrease its nutritional and biological value [39] [40]. In order to provide the same level of safety with improved preservation of the nutritional and bioactive components of human milk, new techniques of processing are under development as presented in the last section of this chapter.

At the end of the process, a sample of the pasteurised milk should be taken for microbiological testing. It is advisable to keep a sample of each batch of pasteurised milk for further tests should the need arise.

31.11. Microbiological evaluation

The microbiological evaluation of DHM includes the testing of each batch before and after pasteurisation, to identify unusual or heavy contamination and pathogens. The European Milk Bank Association recommends discarding the milk if, before pasteurisation, total bacterial growth is more than 105 CFU/mL or if pathogenic micro-organisms are detected [34]. However, at the moment there is no consensus in the international guidelines on the microbiological criteria (bioburden) to be used to define the acceptability of the milk before and after pasteurisation [21][25][28][29][34][42][43][44]. Most of these international guidelines agree that the criterion for discarding pasteurised human milk should be the presence of any microbial growth.

31.12. Labelling and packaging

In addition to the information about labelling set out in Chapter 15, labels for packaging pasteurised milk may contain information about the nutritional value, such as the concentration of protein, fats and carbohydrates, and the energy content. The volumes of the final storage containers may vary depending on the needs of the recipient. The most common volumes are 50, 100 and 200 mL.

31.13. Milk storage in human milk bank after processing preservation

The most common method of milk preservation in an HMB is storage in a freezer at a temperature of < −18 °C. Devices used for freezing should be qualified and the temperature recorded and controlled using calibrated probes. Milk that has not been frozen is acceptable for use (or for freezing) only if kept in the range of 2 °C to 8 °C for less than 24 h from collection.

After pasteurisation, DHM is refrozen at < −18 °C for up to six months or at < −70 °C for up to twelve months [25] [45]. Before feeding, thawed pasteurised DHM can be stored at 4 °C for up to 24 h. These criteria may vary depending on national guidelines, although similar recommendations can be found in the guidelines of the Human Milk Bank Association of North America [44] and similar bodies in Australia [46], Italy [28] and Spain [32], among others.

Despite its advantages, very few milk banks use lyophilisation after pasteurisation as a method of preservation. Donor milk that has been lyophilised after pasteurisation can be stored at ambient temperature as a powder, for up to 18 months, versus 6 months after HoP. In France, this technology is used to supply donor human milk to French overseas territories. The HMB of Bordeaux-Marmande in France uses Holder pasteurisation followed by lyophilisation and processes more than 12 000 litres of milk per year [47].

31.14. Distribution and traceability

Distribution of milk must be conducted so as to ensure product traceability between donor and recipient, as described in Chapter 16 of this Guide. When the donation occurs, the HMB gives an exclusive ID number to the donor mother and to the donated milk, so that it is possible to guarantee an appropriate identification of the donor mother in order to assure the safety of the administration of the donated milk and the traceability of the donation itself. The ID number of the donor mother and the data related to the donation are recorded in a specific register.

During distribution (transportation), milk must remain frozen (except lyophilised milk), and dry ice may be used for this purpose. The freezing point of milk is at a lower temperature than that of water and, consequently, use of water ice as a coolant during transportation can result in partial melting of the milk. The use of validated, easily cleaned insulated transport containers is recommended. The transport procedure must be validated, and the temperature of the transport container should be monitored at all times during transportation. In the case of transport by third parties, a formal agreement is required with the milk bank to ensure appropriate transport conditions are maintained.

Pasteurised milk when distributed, after being thawed, should be used for feeding as soon as possible; it can be kept in the refrigerator for a maximum period of 24 h [28] and cannot be refrozen again.

The HMB must keep records of the documentation of each donor, the processing pool, qualification, storage and final destination (distribution, disposal, expiry date), and the hospital must document how the milk has been used. The registry data of the donor mother are kept separately from their health data. The data recorded in the register are strictly confidential and treated according to the rules of privacy in order to protect information about the health conditions of the donor mother.

Access to these data is made possible only for specific and motivated reasons, and it is limited to people responsible for collection and storage of the

milk, and to the authorities in charge of inspection and control. In current practice, the identity of the receiver must not be revealed to the donor and vice versa. All personal data collected during the process of human milk donation are kept securely, guaranteeing that the donor and the recipient are identifiable only for health reasons.

31.15. Biovigilance

As described in Chapter 17, deviations from the standard operating procedure (SOP), from donation to the administration of human milk, should be recorded and documented, as well as adverse reactions after application [48]. In addition to milk banks, hospitals should also have appropriate SOPs for the storage, thawing and handling of milk containers to avoid degradation of the quality of the milk and the possibility of adverse reactions in recipients.

31.16. New technologies for processing donor human milk

Donor human milk delivered to HMBs should be pasteurised to inactivate viral and bacteriological agents. The Holder method (HoP), a pasteurisation process performed at a temperature of 62.5 °C for 30 min, is currently recommended in all international guidelines. However, this methodology affects some of the nutritional and biological properties of human milk. The European Milk Bank Association (EMBA) recognises that HoP is at present the safest compromise for the treatment of donor human milk; however, further studies are needed to improve this technology in order to minimise its effects on the biological components of human milk [49][50].

Due to the limitations of HoP, several new techniques are under investigation with the purpose of eliminating pathogens in milk and better preserving the biological properties of human milk [51].

The most studied technologies include:

- high temperature short time (HTST) or ultra-high short time (UHST) [52][53],
- conventional pasteurisation below 60 °C [50].

There are also methods for reducing microorganisms in food that do not use heat. They achieve the same effect as HoP and are known as cold pasteurisation. These include:

- high-pressure processing (HPP) or pascalisation [54],
- ultraviolet (UV) irradiation [55] [56],
- ultrasonication [57],
- high intensity pulsed electric field (PEF) [58].

Even if such techniques are shown to be effective and maintain important bioactive components of HM better than HoP, they may be difficult to translate into practice, given the lack of appropriately scaled equipment for use in HMBs. Furthermore, these developing devices need to be validated in real conditions, with milk volumes that are currently treated in milk banks, and to be compared with Holder pasteurisation performed with qualified pasteurisers. When testing new technologies, a precise description of the process and recording of the process parameters are necessary [49].

31.17. References

1. Boquien C-Y. Human milk: An ideal food for nutrition of preterm newborn. *Front Pediatr* 2018;6(295):1-9. https://doi.org/10.3389/fped.2018.00295.
2. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am* 2013 Feb;60(1):49-74.
3. WHO (World Health Organization)/UNICEF. *Global strategy for infant and young child feeding.* Report. Geneva: World Health Organization; 2003.
4. Abrams SA, Landers S, Noble LM, Poindexter BB. Donor human milk for the high-risk infant: preparation, safety, and usage options in the United States. *Pediatrics* 2017 Jan;139(1):e20163440. https://doi.org/10.1542/peds.2016-3440.
5. Arslanoglu S, Corpeleijn W, Moro GE *et al.* Donor human milk for preterm infants: current evidence and research directions. *J Pediatr Gastroenterol Nutr* 2013 Oct;57(4):535-42.
6. World Health Organization. Feeding of low-birthweight infants in low- and middle-income countries: recommendations. Geneva: WHO; 2017.
7. Anderson A, Arnold LDW. Use of donor breastmilk in the nutrition management of chronic renal failure: three case histories. *J Hum Lact.* 1993 Dec;9(4):263-4.
8. Tully MR, Lockhart-Borman L, Updegrove K. Stories of success: the use of donor milk is increasing in North America. *J Hum Lact.* 2004 Feb;20(1):75-7.
9. Kair LR, Nidey NL, Marks JE *et al.* Disparities in donor human milk supplementation among well newborns. *J Hum Lact.* 2020 Feb;36(1):74-80.
10. European Milk Bank Association (2022), website at https://europeanmilkbanking.com, accessed 19 April 2022.
11. Rede Brasileira de Bancos de Leite Humano, website at https://rblh.fiocruz.br/pagina-inicial-rede-blh, accessed 19 April 2022.
12. Human Milk Bank Association of North America (HMBANA), website at www.hmbana.org, accessed 19 April 2022.

13. Quigley M, Embleton ND, Mcguire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev* 2018 Jun 20;6(6):CD002971.
14. Arslanoglu S, Moro GE, Bellù R *et al.* Presence of human milk bank is associated with elevated rate of exclusive breastfeeding in VLBW infants. *J Perinat Med*. 2013 Mar;41(2):129-31.
15. Marinelli KA, Lussier MM, Brownell E *et al.* The effect of a donor milk policy on the diet of very low birth weight infants. *J Hum Lact*. 2014 Aug;30(3):310-16.
16. De Nisi G, Ambruzzi AM, Bertino E *et al. Linee Guida per la costituzione e l'organizzazione di una Banca del Latte Umano Donato*. Trento: New Magazine edizioni, Via dei Mille 69; 2002, publication available at www.sin-neonatologia.it/wp-content/uploads/2020/04/Linee-Guida-per-la-costituzione-e-lorganizazione-di-una-banca-del-latte-umano-donato.pdf, accessed 19 April 2022.
17. World Health Assembly 70. Principles on the donation and management of blood, blood components and other medical products of human origin: report by the Secretariat. Geneva: WHO, 2017.
18. Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: convention on human rights and biomedicine, Council of Europe Treaty Series (CETS) No. 164, Oviedo, 4.IV.1997. Article 21—Prohibition of financial gain. Text available at https://rm.coe.int/168007cf98, accessed 19 April 2022.
19. Cohen M. Should human milk be regulated? *UC Irvine Law Rev.* 2019;9:557-634.
20. Competent Authorities on Substances of Human Origin Expert Group (CASoHO E01718). Summary report of meeting on 3-4 December 2014, available at https://ec.europa.eu/health/system/files/2016-11/ev_20141203_sr_en_0.pdf. accessed 19 April 2022.
21. Ministére de la Santé de la J et des S. Décision du 3 décembre 2007 définissant les règles de bonnes pratiques prévues à l'alinéa 3 l'article L.2323-1 du code de la santé publique. *J Off la République Fr.* 2008;texte 22 sur 165.
22. Ministero della Salute – Comitato Nazionale Multisettoriale per l'allattamento materno. Linee di Indirizzo Nazionale per l'organizzazione e la gestione delle banche del latte umano donato nell'ambito della protezione, promozione e sostegno dell'allattamento al seno. *Gazz Uff.* 2014;(32).
23. Welborn JM. The experience of expressing and donating breast milk following a perinatal loss. *J Hum Lact*. 2012 Nov;28(4):506-10.
24. Carroll KE, Lenne BS, McEgan K *et al.* Breast milk donation after neonatal death in Australia: a report. *Int Breastfeed J*. 2014 Dec;9(1):1-9.
25. NICE (National Institute for Health and Clinical Excellence). *Donor milk banks: service operation*. Centre for Clinical Practice, editor. London: NICE 2010 (revised 2018), available at www.nice.org.uk/guidance/CG93, accessed 19 April 2022.
26. Hartmann BT. Ensuring safety in donor human milk banking in neonatal intensive care. *Clin Perinatol*. 2017 Mar;44(1):131-49.
27. Norman FF, López-Vélez R. Chagas disease and breast-feeding. *Emerg Infect Dis*. 2013 Oct;19(10):1561-6.
28. Arslanoglu S, Bertino E, Tonetto P *et al.* Guidelines for the establishment and operation of a donor human milk bank. *J Matern Fetal Neonatal Med*. 2010 Sep; 23(Suppl 2):1-20.
29. HMBANA (Human Milk Bank Association of North America). *Guidelines for the establishment and operation of donor human milk banking*. 9th edn. Raleigh NC: HMBANA; 2018.
30. Carneiro-Proietti ABF, Amaranto-Damasio MS, Leal-Horiguchi CF *et al.* Mother-to-child transmission of human T-cell lymphotropic viruses-1/2: What we know, and what are the gaps in understanding and preventing this route of infection. *J Pediatric Infect Dis Soc*. 2014 Sep;3(Suppl1):S24-9.
31. PATH. *Strengthening human milk banking: a global implementation framework*. Version 1. Seattle WA, USA: Bill & Melinda Gates Foundation Grand Challenges Initiative, PATH; 2013.
32. Calvo J, García Lara NR, Gormaz M *et al.* Recommendations for the creation and operation of maternal milk banks in Spain. *An Pediatría* (English edn) 2018 May;(xx):1-6.
33. HMBANA (Human Milk Banking Association of North America). Our work, 2022, available at www.hmbana.org/about-us/our-work/overview.html, accessed 2 August 2022.
34. Weaver G, Bertino E, Gebauer C *et al.* Recommendations for the establishment and operation of human milk banks in Europe: a consensus statement from the European Milk Bank Association (EMBA). *Front Pediatr.* 2019 Mar;7:53.
35. Buffin R, Pradat P, Trompette J *et al.* Air and water processes do not produce the same high-quality pasteurization of donor human milk. *J Hum Lact*. 2017 Nov;33(4):717-24.
36. Nessel I, Khashu M, Dyall SC. The effects of storage conditions on long-chain polyunsaturated fatty acids, lipid mediators, and antioxidants in donor human milk – a review. *Prostaglandins Leukot Essent Fatty Acids*. 2019 Oct;149:8-17.
37. Young BE, Murphy K, Borman LL *et al.* Milk bank pooling practices impact concentrations and variability of bioactive components of donor human milk. *Front Nutr.* 2020 Oct;7.

38. Peila C, Moro GE, Bertino E *et al.* The effect of Holder pasteurization on nutrients and biologically-active components in donor human milk: a review. *Nutrients*. 2016;8(Dm):477.
39. Picaud J-CC, Buffin R. Human milk. Treatment and quality of banked human milk. *Clin Perinatol.* 2017 Mar;44(1):95-119.
40. Czank C, Prime DK, Hartmann B *et al.* Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. *Pediatr Res.* 2009 Oct;66(4):374-9.
41. Picaud JC, Buffin R. Human milk – treatment and quality of banked human milk. Vol. 44, *Clinics in Perinatology*. Philadelphia PA, USA: W.B. Saunders; 2017, pp. 95-119.
42. Omarsdottir S, Casper C, Åkerman A *et al.* Breastmilk handling routines for preterm infants in Sweden: a national cross-sectional study. *Breastfeed Med*. 2008 Sep;3(3):165-70.
43. Bharadva K, Tiwari S, Mishra S *et al.* Human milk banking guidelines. Vol. 51(6), *Indian Pediatrics*. Springer; 2014, pp. 469-74.
44. Human Milk Bank Association of North America. Best practice for expressing, storing and handling human milk in hospitals, homes and child care settings. 2019.
45. Berkow SE, Freed LM, Hamosh M *et al.* Lipases and lipids in human milk: Effect of freeze–thawing and storage. *Pediatr Res.* 1984;18(12):1257-62.
46. Hartmann BT, Pang WW, Keil AD *et al.* Best practice guidelines for the operation of a donor human milk bank in an Australian NICU. *Early Hum Dev.* 2007 Oct;83(10):667-73.
47. Lactarium du CHU de Bordeaux [Internet]. Available at www.chu-bordeaux.fr/Patient-proches/Maternité/Lactarium/, accessed 2 August 2022.
48. Project Notify. Notify Library [Internet]. Available at www.notifylibrary.org/, accessed 2 August 2022.
49. Moro GE, Billeaud C, Rachel B *et al.* Processing of donor human milk: update and recommendations from the European Milk Bank Association (EMBA). *Front Pediatr.* 2019;7(February):1-10.
50. Gaya A, Calvo J. Improving pasteurization to preserve the biological components of donated human milk. *Front Pediatr.* 2018;6(October):1-6.
51. Peila C, Emmerik NE, Giribaldi M *et al.* Human milk processing: A systematic review of innovative techniques to ensure the safety and quality of donor milk. *J Pediatr Gastroenterol Nutr.* 2017;64(3):353-61.
52. Giribaldi M, Coscia A, Peila C *et al.* Pasteurization of human milk by a benchtop high-temperature short-time device. *Innov Food Sci Emerg Technol.* 2016 Aug; 36:228-33.
53. Escuder-Vieco D, Espinosa-Martos I, Rodríguez JM *et al.* Effect of HTST and Holder pasteurization on the concentration of immunoglobulins, growth factors, and hormones in donor human milk. *Front Immunol*. 2018;9(September):1-12.
54. Wesolowska A, Sinkiewicz-Darol E, Barbarska O *et al.* New achievements in high-pressure processing to preserve human milk bioactivity. *Front Pediatr.* 2018 Nov;6:323.
55. Barbarska O, Strom K, Oledzka G *et al.* Effect of non-thermal processing on human milk bactericidal activity against *Escherichia coli*. *J Pediatr Gastroenterol Nutr.* 2020 Feb;1.
56. Christen L, Lai CT, Hartmann B *et al.* Ultraviolet-C irradiation: a novel pasteurization method for donor human milk. *PLoS One*. 2013 Jan;8(6):e68120.
57. Czank C, Simmer K, Hartmann PE. Simultaneous pasteurization and homogenization of human milk by combining heat and ultrasound: effect on milk quality. *J Dairy Res.* 2010 May;77(2):183-9.
58. Xu S, Walkling-Ribeiro M, Griffiths MW, Corredig M. Pulsed electric field processing preserves the antiproliferative activity of the milk fat globule membrane on colon carcinoma cells. *J Dairy Sci.* 2015 May;98(5): 2867-74.

Chapter 32. **Intestinal microbiota**

32.1. Introduction

The intestinal microbiota comprises a resilient, stable and integral part of the human organism [1, 2]. Transfer of minimally manipulated human faeces from a healthy donor to a recipient with the intent to re-establish normal composition and function of the intestinal microbiota is termed faecal microbiota transplantation (FMT) [3]. The use of FMT was first reported in 1958 by Eiseman and co-workers who treated four patients who suffered from pseudomembranous colitis [4]. Since then, observational studies, randomised clinical trials and systematic reviews and meta-analyses have provided high-level evidence that FMT is effective for recurrent *Clostridioides* (formerly *Clostridium*) *difficile* infection (rCDI) unresponsive to antibiotic treatments [5-13]. Due to the rising prevalence, severity and mortality of this infection, embedding the use of FMT in modern healthcare is pivotal to save human lives and to decrease the economic burden on healthcare systems [14-19]. Based on the current evidence, both the European Society for Microbiology and Infectious Disease and the American College of Gastroenterology recommend FMT as a treatment for recurrent *C. difficile* infection [20, 21]. Accordingly, stool banks and FMT services are being implemented.

The following generic chapters (Part A) of this Guide all apply to FMT and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

32.2. Legal status

The regulatory classification of intestinal microbiota is challenging [22]. Most experts agree that faecal preparations that are minimally processed solely with the aim of preserving the intestinal microbiota must be classified as a transplantation preparation [23]. While minimally processed donor faeces may thereby be comparable to a tissue and regulated according to the tissue and cells legislation

[24], a standardised microbiota-based product may in addition be regarded as a drug due to its content or commercialisation [25]. This dual regulation opens the way for both safe procedures and innovation.

Following a Competent Authorities expert group meeting on substances of human origin in June 2014, the European Commission addressed questions related to the legal status of faecal microbiota in the EU. Despite confirming that Article 168 (4) of the Treaty on the Functioning of the European Union provides a legal basis for future regulation of these substances of human origin in terms of their quality and safety, it was clarified that member states are free to decide on the most suitable framework (e.g. tissues and cells; medicinal products) either by creating a specific regulatory framework at national level or by applying an existing national legislative framework, including the tissues and cells quality and safety requirements, to these substances. Several countries have introduced national rules and others require compliance with the EU tissue and cells directive [24] (see also §32.6 below). Regardless of the regulatory status of faecal microbiota, ensuring the safety and quality of its use requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide.

Given that commercial interests may drive the development of microbiota-based drugs on a for-profit basis, it is essential that the ethical principles described in Chapter 1 of this Guide are respected so that donors are not exploited and the human body itself is not the subject of trade. Promotion of altruistic unpaid donation of faecal microbiota by means of advertisement or public appeal may be undertaken in accordance with domestic regulations.

32.3. Donor recruitment

Donation of stool is a voluntary act and should not become a subject of trade. The use of unpaid anonymous donors reduces the risk that applicants withhold information during the screening process. Reimbursement of expenses for travel to the donation centre may be justified. Anonymous donors must be used, to ensure anonymity and independence between donor and recipient. The timeline and tasks to be fulfilled for donor recruitment, donor screening, procurement, processing and release of faecal microbiota are illustrated in Figure 32.1.

The selection of faeces donors for FMT has two main aims. The first aim is to prevent adverse events associated with the transfer of pathogenic bacteria or viruses from faecal material. The second aim is to avoid the transmission of impaired microbiota that could be ineffective or even harmful to the recipient.

The structure of donor recruitment influences eligibility and safety [26-30]. Eligibility rates vary from 3 % following public advertising to 30 % when recruiting donors from healthy subgroups of the general population, such as blood donors [27-33]. Current evidence does not support the superiority of related donors over unrelated ones, at least when FMT is administered to cure *C. difficile* infection [10]. Anonymous donors are preferred to maintain the ethical separation between the donor and recipient and maintain the donation as an impartial act of altruism.

Recruitment and selection of donors for FMT mainly aims to avoid transferring potential pathogens. While donor microbiota richness may impact efficacy in chronic inflammatory bowel disease [34, 35], this seems to play a minor role when administering FMT for rCDI.

Healthcare workers may be approved as faeces donors if they fulfil all necessary requirements. Donors younger than 60 years may be preferred because older individuals are more likely to suffer from chronic diseases.

32.3.1. Donor pre-screening

Initial pre-screening may be performed, using pre-defined criteria for donor age, body mass index, absence of chronic disease, etc. Donors who fulfil

Figure 32.1. **Timeline and tasks in donor recruitment, donor screening, procurement, processing and release of faecal microbiota**

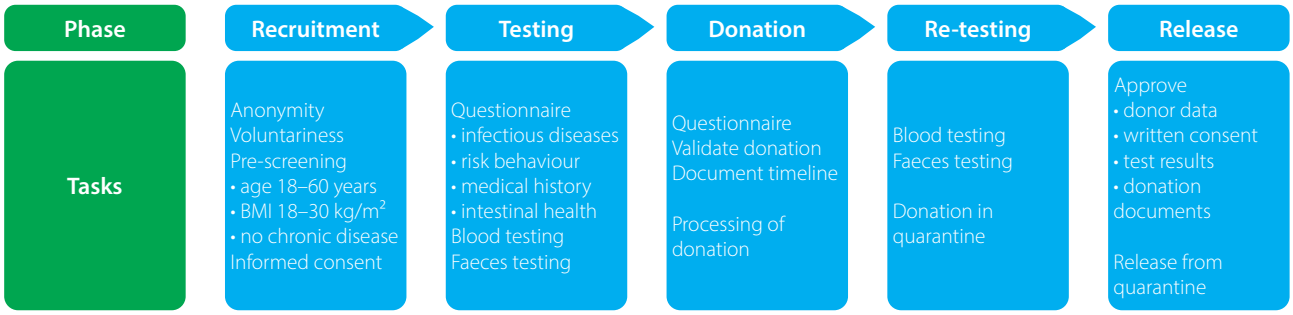


Table 32.1. **Items to address in screening of potential faeces donors before approval for FMT, in addition to those mentioned in Chapter 4**

Domain	Exclusion criteria
Infectious diseases	• History of or exposure to infectious diseases with chronic activity: particularly human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV), non-successfully eradicated *Helicobacter pylori*, syphilis, malaria, trypanosomiasis, tuberculosis, Chagas disease, strongyloidiasis • Any currently active infection or those of relevance within the last 6 months • Live attenuated vaccine within the last 4 weeks • Country of birth (if high risk)
At-risk behaviour	• Current or previous intravenous drug use • Ongoing high-risk sexual behaviour within the last 6 months • Travel to high-risk foreign countries within the last 6 months • Current occupation in a setting facilitating acquisition of potential pathogens (e.g. veterinarian, animal attendant, gamekeeper, prison worker) • Tattoo, piercing or acupuncture within the last 6 months • Major surgery within the last 6 months • Contact with human blood (e.g. accident, needle stick injury) within the last 6 months • Previous prison term • Previous tissue/organ transplantation • Transfusion of blood products (e.g. packed red cells, plasma, platelets, immunoglobulins) within the last 6 months
Medical history	• Chronic diseases • (Risk of) Creutzfeld–Jakob disease • Allergies or atopy (e.g. food or drug allergies, asthma) • Hospitalisation within the last 4 months • Ongoing pregnancy • Antibiotic treatments or chemotherapy ongoing, scheduled or received within the last 3 months • Regular medication or nutritional supplements • Body mass index (BMI) (accepted if ≥ 18 and ≤ 30 kg/m^2) • Age (accepted if ≥ 18 and ≤ 60 years)
Intestinal health	• Previous or scheduled gastrointestinal surgery • Gastrointestinal symptoms within the last 3 months (e.g. diarrhoea, constipation, haematochesia, vomiting, abdominal pain) • Any other relevant clinical sign or symptom within the last 3 months (e.g. fever or rash) • First-degree history of large bowel cancer

Source: adapted from European FMT stool bank consensus [36]. Some national, regional or institutional amendments may be possible.

these pre-screening criteria may be approached for information and a request for informed consent to undergo detailed donor screening, as outlined below.

32.3.2. Donor consent

All donors who undergo screening must be informed about the associated risks and benefits and provide written informed consent that covers the provision of personal information, the screening processes, the revealing of screening results, the provision of multiple donations, the storage of donor data in a donor registry, and future unscheduled contacts from the tissue establishment in case of adverse events or for research purposes.

Particular attention should be paid to identifying traits or risk factors during donor screening, e.g. intestinal carriage of multidrug resistant organisms.

32.3.3. Initial questionnaire for donor selection

Potential donors should undergo two steps to be selected as faeces donors: 1. medical history and risk assessment; 2. blood and stool testing.

The medical history and risk behaviour of potential donors must be assessed using a dedicated questionnaire or interview. The results must be evaluated by a qualified health professional. To objectify evaluation, there should be a document clearly identifying the consequence of a specific response for the screening process.

The questionnaire should be designed both to exclude the risk factors for infectious diseases, to select allogeneic living donors of human tissue transplants [24]), and to identify subjects suffering from recent or chronic diseases or who take drugs that may alter the donor microbiota (Table 32.1). A positive response to any of these questions would usually lead to exclusion from further consideration as a donor, although this would depend upon the particular circumstances or answers given.

Table 32.2. **Blood and faeces screening for faecal microbiota transplantation**

Sample type	Analyses
General blood testing	• General laboratory: CRP, creatinine, ALT, bilirubin, blood cell count • Viruses: Hepatitis A (IgM), Hepatitis B (HBs Ag), Hepatitis C (anti-HCV), Hepatitis E, HIV 1 and 2 • Bacteria: *Treponema pallidum* (TPHA)
Blood testing for use in immunocompromised patients	• CMV, EBV, HTLV 1 and 2, toxoplasmosis
General stool testing	• Bacterial enteral pathogens: Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2, *Shigella* spp., *Campylobacter jejuni* and *coli*, *Salmonella* spp., *Yersinia enterocolitica* and *Clostridioides difficile*, *Helicobacter pylori*, *Vibrio* species (if visited or residing in tropical country within the last 6 months) • Antibiotic-resistant bacteria: Extended spectrum beta-lactamase-producing bacteria (ESBL), Multidrug-resistant Gram-negative bacteria (MRGN) including carbapenemase-producing *Enterobacterales*, Vancomycin-resistant *Enterococci* (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA) • Viruses: Norovirus, Rotavirus, SARS-CoV-2 • Parasites: *Cryptosporidium* spp., *Giardia lamblia*, helminths, *Entamoeba histolytica*, *Di-entamoeba fragilis*, *Strongyloides stercoralis*, *Cyclospora* and *Isopsora* (if visited or residing in tropical country within the last 6 months), *Blastocystis hominis*** • **colonisation with *Blastocystis hominis* is not considered an exclusion criterion. However, it is advised to monitor for the effects of transmission.
Stool testing for use in immuno-compromised patients	• *Plesiomonas shigelloides, adenovirus, parechovirus, astrovirus, enterovirus, sapoviruscy-clospora, isospora, microsporidia*

Source: European FMT stool bank consensus [36].

32.4. Donor testing

The blood and faeces test panels are dynamic and should be updated on a regular basis according to known risk factors for disease transmission. Taking into account emerging diseases, additional testing or methods may need to be applied. Local adjustments may be applied, and the screening panel should be evaluated regularly. A suggested test panel is listed in Table 32.2. Because donor faeces are not pathogen-free, Table 11.1 (listing micro-organisms that should result in discard of tissues or cells) does not apply in the evaluation of faeces donors.

32.4.1. Donor screening for treatment of patients with severe immunosuppression

A distinction should be made between severely immunocompromised and moderately immunocompromised patients. FMT preparations for severely immunocompromised patients should be made with additional safety precautions. Matching the *Cytomegalovirus* (CMV) and Epstein–Barr virus (EBV) status of donor and recipient or using CMV-negative donors may be considered. In particular, seropositive donors (IgM and/or IgG) should not be matched with seronegative patients.

32.4.2. Donation-specific questionnaires and donor re-testing

Once a donor has been approved, he or she should complete a second short questionnaire before each donation to assess any event that may have occurred between donor approval and donation (see Table 32.3).

Complete donor screening based on blood and faecal analyses should be repeated at minimum every 3 months. A donation period should have a defined maximum duration, not exceeding 3 months, and should start and end with a complete donor screening. Complete post-donation period re-screening before any use of the preparations allows for an exemption from blood screening at each donation. Blood tests pertaining to the close-out screening should be performed a minimum of 4 weeks after the last donation thus taking incubation times into account.

If donor screening is performed as recommended in this document, direct testing of each preparation is not mandatory. It may, however, be necessary according to local regulation, or in severely immunocompromised patients.

32.4.3. Quarantine of faeces donations

FMT preparations that have been processed during a donation period should be put under quarantine until the repeat donor screening results are approved.

Table 32.3. **Issues to be addressed by questionnaire on the day of the donation**

Issue
• any new gastrointestinal signs and symptoms (e.g. diarrhoea, nausea, vomiting, abdominal pain)
• new-onset or systemic signs (e.g. fever, throat pain, swollen lymph nodes)
• direct contact with individuals with Covid-19 (during pandemic)
• use of antibiotics or other drugs that may alter gut microbiota
• new sexual partners or travel abroad since the last screening
• recent ingestion of a substance that may result in harm for the recipients
• travel in tropical areas
• contact with human blood (sting, wound, showing, piercings, tattoos)
• sexual high-risk behaviour

Source: European FMT stool bank consensus [36].

32.5. Procurement and processing

32.5.1. Procurement

According to the European directives, donation is voluntary and unpaid, a factor which may contribute to high safety standards and therefore to the protection of human health [22]. The procurement must be authorised only after an informed consent procedure.

Preferably, defecation would take place at the FMT facility, but in most cases, faeces are collected by the donor at his or her home. The travel distance to the processing facility is important because donors should be able to deliver their donation to the FMT facility within 2 hours following defecation [37]. If the duration from defecation to delivering the faeces to the tissue establishment is longer than 30 minutes, temporary storage at 2-8 °C is preferred because faecal storage without stabilisation buffer significantly changes taxa abundances from 30 minutes onwards [38-40]. The processing facility should provide sterile faecal containers validated for transportation of human material.

The data related to the donation must be handled with respect for confidentiality of any health-related information provided to the authorised personnel, the results of tests on their donations, and details of traceability from donor to recipient and vice versa.

Faeces have traditionally been processed for immediate 'fresh' use, but the use of processed and cryopreserved donor faeces is equally effective for rCDI [8, 41-43]. Because waiting time for post-donation screening results abrogates the use of fresh donor faeces, FMT that complies with the standards of the present guide can only be performed using frozen donor faeces. In addition to allowing post-donation screening, the use of frozen FMT has advantages, mainly from a logistical point of view, e.g. selection and screening of donors, quality of stool etc. [44].

Faeces donors may provide donations within a specified time period with screening of the first and last donation [36, 44, 45]. This documents a safe period without subjecting the donor to excessive interventions.

32.5.2. Laboratory processing

Visual inspection of the donor faeces must be undertaken to document stool consistency and to confirm the absence of blood and urine. To preserve the donated intestinal microbiota, stool should be processed within 6 hours of defecation [46, 47]. A biosafety cabinet should be used to protect personnel from unpleasant odours and contamination during manufacture, although it may not be mandatory from a regulatory perspective, depending on the local authority. Prior to the processing procedures, the designated spaces and non-disposable equipment should be sterilised, if possible using measures effective against sporulating bacteria [36]. Protective gloves and facial masks should be used during preparation. If a faecal donation cannot be processed immediately, it should be stored at 2-8 °C until initiating the procedures [37].

Preparation of faecal preparations under aerobic conditions is considered suitable when preparing FMT for use in patients with rCDI, because anaerobic processing does not increase clinical cure rate [41, 48, 49]. This may be explained by findings that a considerable part of the bacterial genera tolerate oxygen for a limited amount of time or produce resilient spores that allow transfer of at least a proportion of anaerobic intestinal bacteria [50].

It is preferable to use ≥ 50 grams of crude faeces per FMT preparation, although lower amounts may also be effective [51, 52].

Donor faeces for liquid FMT preparation should be homogenised with cooled, sterile 0.9 % saline [53], using a blender, smasher or stomacher bag [54]. The amount of added saline depends on the viscosity required for clinical application. Donor faeces intended for infusion may be sieved or centrifugated to remove debris and thereby prevent clogging during administration. Donations for encapsulated

FMT may undergo further centrifugation steps to concentrate the donated sample and reduce the required number of capsules [55].

Before freezing, glycerol should be added as a cryoprotectant up to a final concentration of 10 % for liquid-based preparations [41, 56]. The amount of glycerol used for microbiota cryopreservation has no independent effect on the recipient [57]. For lyophilisation of FMT preparations, a lyoprotectant, e.g. trehalose, sucrose or skimmed milk, should be added in order to protect the intestinal microbiota during the freeze drying process [51, 58].

The final preparations must be clearly labelled and traceable. The material used for storage must be validated for freezing at low temperatures and for storage of human material. Preparations should be stored at – 60 °C to – 80 °C. Soon-to-be-used preparations may be stored at – 20 °C in relation to clinical application [59, 60]. Freeze dried preparations may be stored at room temperature, but storage at – 60 °C to – 80 °C prevents moisture permeation. The preparation labels must include a unique donor code and number, processing and expiration date, volume and storage instructions.

32.5.3. Quality control

Quality control needs to be differentiated from quality management, as it specifically serves to ensure that materials, processes and the final preparation meet the pre-determined specifications. For FMT preparations, such specifications must include time from donation to completion of processing, completeness of documentation used for donor assessment and release, intact appearance of the FMT preparation, including primary and secondary FMT preparation packaging, weight of donation and final FMT preparation and adequate storage conditions. Further specifications may be subject to local regulatory requirements including exclusion of potential pathogens from the final preparation, diversity in the final preparation, and quantity and viability of bacteria in the final preparation. There are currently no easily accessible and rigorously validated tests to assess this latter specification. To ensure a high level of quality control, FMT preparations should be stored until review of all specifications and release of the preparations by the responsible person.

32.6. Quality management

Stool banks and FMT services for the treatment of *C. difficile* infection should be implemented in healthcare settings with appropriate expertise and facilities [61]. Access to all relevant facilities, including a ward or clinic that is able to provide the treatment itself as well as the appropriate care in case of treatment-related complications, is essential to the implementation of an FMT service. FMT administration may be performed in either an inpatient or an outpatient setting. A stool bank may be established within or separate from the infrastructure of an existing tissue bank and with similar principles of function [31, 37, 44, 62].

32.6.1. Organisation, personnel and premises

Management of a stool bank and a clinical FMT service should be regulated by the responsible local or national health authority to ensure that the fundamental European effective rights concerning protection of personal data (see EU regulation 2016/679) and any other relevant aspects, including applicable national legislation, are fully guaranteed. Currently, no general European legal framework covers the entirety of faecal microbiota by itself.

Concerning laboratory processing standards, a formalised quality control system must be implemented. The Good Practice Guidelines for tissue establishments included in the present Guide provide a suitable and sufficient framework.

Laboratory space for FMT preparation should meet the required standards to minimise the risk of contamination of the preparation with potential pathogens. In general, the dedicated use of a laboratory for FMT processing purposes is required. Other measures may need to be established according to local regulation. In addition to a manufacturing area, the stool bank should be able to provide a dedicated space for donor communications and storage of retained samples and FMT preparations. The set-up of the stool bank should enable discreet delivery of donations and communication between donors and personnel.

32.6.2. Computerised systems (software, coding, packaging, labelling, traceability)

While paper-based documentation of all aspects of FMT processing and application is feasible, computerised documentation should be preferred to minimise risks. All necessary information concerning the implementation and maintenance of computerised systems for the running of tissue establishments, as described in Chapter 14 of this Guide, fully apply to stool banks and FMT services. Regular software updates should be possible without system shutdown. Because FMT centres in Europe are often

initially established on a small scale, the computerised system should allow for appropriate growth over time. Computerised systems may further be of help in maintaining local, national and international registries to follow up adverse reactions and clinical effectiveness of FMT preparations.

All data on donors and recipients should be pseudonymised to maintain a principle of voluntary altruism between donor and recipients without any revealing elements. This also applies to third-party data access. All utensils and packaging used for the donation, manufacture, transport and storage of FMT preparations and their associated retained samples should therefore be uniquely coded and linked using specific donor identifiers, donation identifiers and donation preparation identifiers. Labels should include a unique donor code and number, with processing and expiration date, volume and storage instructions.

Storage of FMT preparations and retained samples requires appropriate freezing-resistant (down to −60 °C to −80 °C) containers and labels. Primary packaging includes plastic syringes or other receptacles suitable for FMT preparations used for endoscopic or nasogastric/nasojejunal administration or administration by enema, or acid-resistant capsules for orally administered FMT. Secondary packaging refers to the direct outer packaging of the FMT preparations and the accompanying labels.

32.7. Labelling and packaging

When labelling and distributing FMT preparations, the stool bank (if in the EU) must comply with the standards defined in the EU Tissues and Cells Directive [24]. All FMT preparations must be marked at the time of release. The primary container must be marked with donation identification data or code, preparation type and expiry date. The container with the FMT preparation must be marked with:

a. preparation type, preparation identification number or code, and lot or batch number, tissue centre identification and expiry date;
b. who the donation is intended for, if the donation is for a specific recipient;
c. a unique code, if the preparation is distributed for human use by another legal entity (hospital unit) other than the stool bank.

If there is no space for the information on the label of the container, it may be entered on a separate delivery note accompanying the preparation. The delivery note must be packed together with the preparation in a way that ensures that they are together at all times. In addition to the above, the label or delivery note must contain the following information:

d. description (definition) and, if applicable, the volume of the final FMT preparation,
e. date of distribution of the FMT preparation,
f. selected biological determinations performed on the donor and the results, if relevant,
g. recommendations regarding storage,
h. instructions for opening the container, packing and any handling or reconstitution required,
i. shelf life after opening/handling.

When distributing FMT preparations, the procedures must meet the following criteria:

i. Critical transport conditions, e.g. temperature and time limit, are determined so that the required tissue and cell properties are maintained.
ii. The container/package is preserved under the specified conditions. All containers and packages must be validated as being fit for the purpose.
iii. If the distribution is carried out by a third party under contract, there must be a documented agreement which ensures that the required conditions are present.
iv. Tissue establishments involved in FMT must have approved staff who can assess the need for recall and also implement and co-ordinate the necessary measures.
v. There must be an effective recall procedure that includes a description of the division of responsibilities and the measures to be taken.
vi. There must be a documented system for handling returned preparations, including any criteria for including them in the stock statement.

32.8. Storage and release

The frozen preparations should be stored in sealed, clean plastic containers, with a unique code ensuring traceability of the sample. Preparations should be stored in dedicated −60 °C to −80 °C freezers with logging and alert systems, and separate sections for quarantined and released preparations. Recently processed preparations should be placed in the quarantined sections, and moved to the released section when all release criteria are met. Documenting this release should conform to standard operating procedures (SOPs).

All processed FMT preparations must be placed into quarantine and stored in clearly distinguishable sections until all donor screening results are available. Another section must be reserved to

retain samples of FMT preparations. The release of preparations must be documented and, following these quarantine measures, the FMT preparations must be cleared and transferred to another distinct storage section. Only cleared FMT preparations must be used for patient treatment.

Storage at −60 °C to −80 °C for up to 10 months does not affect the clinical utility [42, 43, 48], and storage up to 24 months is safe and associated with equal effect [63].

32.9. Distribution

The distribution of faecal preparations from a tissue establishment to an FMT service requires a written agreement between the tissue establishment and the FMT service in which each party's responsibilities are described. The faecal preparation must be ordered by a clinician or other authorised person. Prior to distribution, the third party must confirm that it is in possession of sufficient facilities to safely conduct an FMT. Clinical administration and patient follow-up is the responsibility of the treating physician.

Distribution of FMT preparations should be performed using dry-ice shipment through a certified courier service. If preparations are to be used immediately following transport, this may be done at 2-8 °C.

32.10. Clinical application

The primary aim of a stool bank is to centralise the process of donor screening and the processing and storage of FMT preparations. Given the relative novelty of this treatment modality, guidance for treating physicians may be required to optimise the safety and efficacy of large-scale FMT implementation. Stool banks can offer expert consultation at the request of the treating physician, or they can incorporate consultation as part of a standard procedure to verify the correct indication for FMT before delivering FMT preparations.

To offer appropriate consultation, an expert panel is required. As indications for FMT are likely to become more numerous in the future, medical specialists in the field pertaining to the added indication should be included in the FMT expert panel [61]. In the context of CDI, a standardised patient questionnaire is required with key information about the current episode of CDI, previous episodes of CDI, treatment effects and microbiological testing. The expert panel may assess whether the indication is appropriate and advise about additional diagnostics or an alternative therapy. Patient data must be shared and stored appropriately, in accordance with European General Data Protection Regulation (GDPR) guidance and other local regulations.

A standardised treatment protocol for rCDI will enable the comparison of outcomes among FMT centres, and subsequently increase safety and facilitate quality assurance. However, variation in almost all steps of the FMT treatment protocol is conceivable.

Currently, FMT is indicated only for CDI, excepting clinical trials. In a research or compassionate use setting, other indications may be considered. FMT preparations must not be sold or delivered to patients at their own request. Lastly, the need for documentation and quality improvement requires a strong and well-defined infrastructure.

32.10.1. Clinical activity documentation and responsibility

A tissue establishment must maintain a registry of its activities and must be able to collect and report data on the patients treated with the faeces preparations distributed by the tissue establishment. Reporting should include the number of patients, the treatment modality used (e.g. colonoscopy, enema, nasogastric or nasoenteric tube, gastroscopy, capsules), as well as follow-up data including safety and clinical outcomes. From these data, stool banks should be able to produce annual reports of procedure-related adverse events. In the near future, such data should also be entered into a national or international registry.

Modulation of the human intestinal microbiota is regarded as a medical treatment carried out by registered physicians who have the associated medical qualifications and obligations. Because FMT, as well as other predefined microbial consortia, acts to modulate the indigenous host microbiota by concurrently engrafting and the long-term safety profile for this engraftment is unknown, traceable long-term follow-up is pivotal. This is irrespective of whether a "whole" FMT or predefined microbial consortia are given.

According to the EUTCD, the treating physician carries the responsibility for the patient and the long-term follow-up. The treating physician should also have access to treatment facilities capable of handling any effect or complication following the modulation. The EUTCD-specified provision for a 30-year long-term traceability may be ensured by the affiliated stool bank to facilitate safety in practice.

32.10.2. Preparation of the recipient

Patients with CDI should be pre-treated with antibiotics against CDI for at least 3 days and until 12 to 48 hours before FMT, if the patients do not display signs of life-threatening disease. This pre-treatment aims both to reduce bowel movements, allowing longer persistence of the donor microbiota in the bowel of the recipient, and to provide a bridging therapy to recipients while they are waiting for the procedure.

Before FMT treatment, risk factors for treatment failure should be reduced, including the use of proton pump inhibitor and concomitant antibiotic use [64]. Donor faeces should not be administered to patients with a history of anaphylactic reaction secondary to food allergy.

Bowel cleansing is required in patients who receive FMT at colonoscopy, and inadequate bowel preparation has been identified as a risk factor for failure of colonoscopically administered FMT [65]. For upper gastrointestinal delivery, bowel lavage is generally also performed [5], although evidence for its use is lacking. Application via enema does not require pre-treatment for clinical efficacy [8].

32.10.3. Thawing of FMT preparations

Thaw times depend on the volume and type of FMT preparations. A 200 mL preparation may be thawed overnight in a 2 °C to 8 °C refrigerator, or during 5 hours at room temperature. Warm water baths or incubators (30 °C to 37 °C) can be used to speed up thawing of FMT preparations, but are not applicable to the thawing of capsules [61]. Only clean water baths with fresh water should be used to avoid cross-contamination, particularly with water-dwelling bacteria such as *Pseudomonas*. Thawed faecal preparations should be infused the same day, and should NOT be refrozen, because freeze–thaw cycles adversely affect the viability of the microbial communities in the faecal preparation [66]. The faecal preparation should be at room temperature when infusing into the recipient. FMT capsules do not require thawing, but may be used after removal from the freezer.

32.10.4. Application methods

FMT can be performed through different routes of delivery including colonoscopy, upper gastrointestinal endoscopy, nasogastric, nasoduodenal/nasojejunal tube, enema or capsule. For each route of delivery, application may be repeated if a single treatment fails to prevent *C. difficile* recurrence.

During colonoscopy, the faecal preparation should be administered into the right colon of the recipient, when possible [9, 67]. In patients with severe *C. difficile* infection and high risk of colonic perforation, donor faeces may be infused into the left hemicolon for safety reasons.

Enema application is inferior to other application methods in clinical efficacy following one application and on average requires a higher treatment frequency for a sustained response [8].

Upper gastrointestinal administration by gastroscopy, by nasoduodenal or nasojejunal tube, or through a gastrostomy, requires the recipient to be positioned at least 45° upright, and to keep this position for at least two hours after the procedure, to reduce the risk of aspiration [45, 68].

The ideal volume for instillation of liquid faeces preparation has not been established. Small volumes of 25-50 mL could be used for upper gastrointestinal delivery; larger volumes (e.g. 250-500 mL) could be used for instillation via colonoscopy.

Capsule ingestion confers minimal procedural cost and risk and may be equally effective as other application methods [51, 55, 56, 69, 70].

When repeated treatments are necessary, further administrations may be indicated from the same donor only [45].

32.10.5. Use of FMT in severely immunocompromised patients

FMT appears safe in immunocompromised patients [71-73]. A report of a severely immunocompromised patient's death following transfer of multi-drug-resistant organisms calls for particular caution in this patient group [74]. The risk of developing complications after transfer of potential pathogens appears to be increased in severely immunocompromised patients, defined as:

i. current or foreseeable neutropenia (< 500 neutrophils/µl) within the next 14 days;
ii. scheduled or recent (< 100 days) allogeneic stem cell transplantation (SCT); or
iii. active graft-*versus*-host disease (GVHD) requiring immunosuppressive treatment.

For severely immunocompromised patients, additional screening tests of the donor(s) and testing of the FMT preparation or donated stool used for treatment of the individual patient should be carried out.

For less severely immunocompromised patients (e.g. > 200 CD4 T-cells/µl; or prolonged use of corticosteroids at a mean dose of ≥ 0.3 mg/kg/d

of prednisone equivalent for > 3 weeks; or treatment with other recognised T-cell immunosuppressants, such as cyclosporine, TNF-alpha blockers, specific monoclonal antibodies (e.g. alemtuzumab), methotrexate or nucleoside analogues during the last 90 days); or inherited severe immunodeficiency (e.g. chronic granulomatous disease or severe combined immunodeficiency), normal donor screening appears sufficient. More stringent follow-up is justified in all immunocompromised patients, and careful reporting of outcomes and side effects is needed to confirm the safety of FMT in those patients.

32.11. Traceability

The tissue establishment must keep donor and recipient records from FMT procedures. The FMT procedure and donors' and recipients' records must be stored for at least 10 years. According to EUTCD, data required for full traceability must be kept for a minimum of 30 years after clinical use [24]. This may differ from local requirements, and a longer storage time could be needed. In case of discrepancies, sufficient protocols for procedural handling and reporting should be in place.

32.12. Biovigilance

Recipients should be monitored for the occurrence of acute complications related to the FMT procedure. An overall treatment plan for patients with rCDI should be adjusted for disease severity and comorbidities. The need for hospitalisation of patients with other underlying diseases depends on the diagnosis and clinical condition. The duration of the observation period has not been defined yet, as it depends on the route of delivery, the underlying diseases and the general condition of the patient.

Adverse events and reactions are not rare and should be carefully monitored throughout FMT [75]. The vast majority are mild, self-limiting and gastrointestinal in nature [76]. Severe adverse reactions such as death, viral and bacterial infections, or transient relapse of inflammatory bowel disease, have been reported in several studies and may even be under-reported [75]. This mandates reporting to biovigilance registries at the local level and to the Health Authority, with the recording of well-defined and standardised data. In order to trace a potential causal link between FMT and newly developed diseases, keeping appropriate registries would be a wise process to trace and learn about potential long-term side effects.

32.12.1. Adverse reaction documenting and reporting

Adverse reactions (AR) and serious adverse reactions (SAR), as defined by the ICH Harmonised tripartite guidelines [77], must be documented. The FMT centre should be able to communicate data related to performance, outcome and safety in an annual report for the national health authorities or other auditing authorities. Quality auditing should be organised in collaboration with local or national authorities. Complications of FMT are generally under-reported [75, 76]. Side effects are expected events that may occur following FMT. They include short-term (including abdominal pain, dyspepsia, diarrhoea, fever, constipation) and long-term (for example, weight gain, small intestinal bacterial overgrowth) outcomes [76].

SARs may be procedure-related (aspiration, ileus, perforation, sepsis, death) or not clearly related to the procedure. They may be expected or unexpected, and the reporting of suspected unexpected serious adverse reactions (SUSARs) is encouraged. Rare SUSARs include hyperammoniaemic hepatic encephalopathy as recently described [78]. A distinction between procedure-related and unrelated complications should be made according to established guidelines [79]. Assessment of severity and causality may follow the "common terminology criteria for adverse events" (CTCAE version 5.0, 2017) and be graded accordingly into mild, moderate, severe and life-threatening/disabling [80].

Each tissue establishment and FMT service at an organisation responsible for human application must have an SOP to describe how to handle SARs. In the case of any SAR where a connection to the adverse reaction and application of FMT is made, the unit is obligated to notify the appropriate authority immediately. Also, an SAE occurring during the laboratory processing of donated faeces or distribution should prompt an immediate notification to the health authorities. The stool bank is responsible for ensuring notification to the health authorities and confirming that sufficient actions and adaptations are being made. All relevant personnel should be notified in case of an SAR to facilitate traceability and control of quality and safety. Effective and rapid procedures for recall and disposal must be in place in the case of an SAR.

32.13. Developing applications

While the use of FMT is established for *C. difficile*-associated disease, potential future indications are being investigated in clinical trials. These

include, but are not restricted to, ulcerative colitis [81-84], irritable bowel syndrome [85-90], relapsing pouchitis [91], carrier status with multidrug-resistant organisms [92-94], hepatic encephalopathy [95] and graft-*versus*-host disease [96]. The use of FMT for these and other developing indications outside a clinical trial setting is discouraged.

The question of an optimal donor–recipient match, in particular the hypothesis of the existence of so-called 'super donors', remains elusive [97]. While faeces donor selection for FMT in patients with *C. difficile*-associated disease may mainly be based on the absence of risk factors, optimised donor selection may determine the therapeutic efficacy in conditions such as irritable bowel syndrome [88]. Microbiota signature profiles may predict therapeutic outcomes in patients with ulcerative colitis [34, 35], and the presence of particular micro-organisms may confer beneficial therapeutic outcomes even in *C. difficile* infection [98]. The use of multidonor preparations [83] may ensure a high microbial diversity in the treatment preparation, but it challenges basal principles of traceability and the concept of transplanting a complete and balanced viable microbial ecosystem.

32.14. References

1. Sommer F, Anderson JM, Bharti R *et al.* The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol* 2017;15(10):630-8.
2. Dominguez-Bello MG, Godoy-Vitorino F, Knight R *et al.* Role of the microbiome in human development. *Gut* 2019;68(6):1108-14.
3. Allegretti JR, Mullish BH, Kelly C *et al.* The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet* 2019; 394(10196):420-31.
4. Eiseman B, Silen W, Bascom GS *et al.* Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958;44(5):854-9.
5. van Nood E, Vrieze A, Nieuwdorp M *et al.* Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368(5):407-15.
6. Cammarota G, Masucci L, Ianiro G *et al.* Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. *Aliment Pharmacol Ther* 2015;41(9):835-43.
7. Kelly CR, Khoruts A, Staley C *et al.* Effect of fecal microbiota transplantation on recurrence in multiply recurrent Clostridium difficile infection: a randomized trial. *Ann Intern Med* 2016;165(9):609-16.
8. Lee CH, Steiner T, Petrof EO *et al.* Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent Clostridium difficile infection: a randomized clinical trial. *JAMA* 2016; 315(2):142-9.
9. Hvas CL, Jørgensen SMD, Jørgensen SP *et al.* Fecal microbiota transplantation is superior to fidaxomicin for treatment of recurrent Clostridium difficile infection. *Gastroenterology* 2019;156:1324-32.
10. Drekonja D, Reich J, Gezahegn S *et al.* Fecal microbiota transplantation for Clostridium difficile infection: A systematic review. *Ann Intern Med* 2015;162(9):630-8.
11. Quraishi MN, Widlak M, Bhala N *et al.* Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Aliment Pharmacol Ther* 2017;46(5):479-93.
12. Ianiro G, Eusebi LH, Black CJ *et al.* Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther* 2019;50(3):240-8.
13. Baunwall SMD, Lee MM, Eriksen MK *et al.* Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis. *EClinicalMedicine* 2020 Nov 23;29-30: 100642. https://doi.org/10.1016/j.eclinm.2020.100642.
14. McGlone SM, Bailey RR, Zimmer SM *et al.* The economic burden of Clostridium difficile. *Clin Microbiol Infect* 2012;18(3):282-9.
15. Varier RU, Biltaji E, Smith KJ *et al.* Cost-effectiveness analysis of treatment strategies for initial Clostridium difficile infection. *Clin Microbiol Infect* 2014;20(12): 1343-51.
16. Heimann SM, Vehreschild JJ, Cornely OA *et al.* Economic burden of Clostridium difficile associated diarrhoea: a cost-of-illness study from a German tertiary care hospital. *Infection* 2015;43(6):707-14.
17. Le Monnier A, Duburcq A, Zahar JR *et al.* Hospital cost of Clostridium difficile infection including the contribution of recurrences in French acute-care hospitals. *J Hosp Infect* 2015;91(2):117-22.
18. Shah DN, Aitken SL, Barragan LF *et al.* Economic burden of primary compared with recurrent Clostridium difficile infection in hospitalized patients: a prospective cohort study. *J Hosp Infect* 2016; 93(3):286-9.
19. van Beurden YH, Nieuwdorp M, van de Berg P *et al.* Current challenges in the treatment of severe Clostridium difficile infection: early treatment potential of fecal microbiota transplantation. *Therap Adv Gastroenterol* 2017;10(4):373-81.
20. Debast SB, Bauer MP, Kuijper EJ: European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. *Clin Microbiol Infect* 2014;20 Suppl 2:1-26.

21. McDonald LC, Gerding DN, Johnson S *et al.* Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). *Clin Infect Dis* 2018;66(7):987-94.
22. Khoruts A, Hoffmann DE, Palumbo FB. The impact of regulatory policies on the future of fecal microbiota transplantation. *J Law Med Ethics* 2019;47(4):482-504.
23. Keller JJ, Vehreschild MJ, Hvas CL *et al.* Stool for fecal microbiota transplantation should be classified as a transplant product and not as a drug. *United European Gastroenterol J* 2019;7(10):1408-10.
24. European Commission. Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32004L0023, accessed 19 April 2022.
25. EU directive relating to medicinal products for human use (2001/83/EC): European Union; 2001.
26. Paramsothy S, Borody TJ, Lin E *et al.* Donor recruitment for fecal microbiota transplantation. *Inflamm Bowel Dis* 2015;21(7):1600-6.
27. Kazerouni A, Burgess J, Burns LJ *et al.* Optimal screening and donor management in a public stool bank. *Microbiome* 2015;3:75.
28. Tariq R, Weatherly R, Kammer P *et al.* Donor screening experience for fecal microbiota transplantation in patients with recurrent C. difficile infection. *J Clin Gastroenterol* 2018;52(2):146-50.
29. Jørgensen SMD, Erikstrup C, Dinh KM *et al.* Recruitment of feces donors among blood donors: Results from an observational cohort study. *Gut Microbes* 2018;9:540-50.
30. Lai CY, Sung J, Cheng F *et al.* Systematic review with meta-analysis: review of donor features, procedures and outcomes in 168 clinical studies of faecal microbiota transplantation. *Aliment Pharmacol Ther* 2019; 49(4):354-63.
31. Terveer EM, van Beurden YH, Goorhuis A *et al.* How to: Establish and run a stool bank. *Clin Microbiol Infect* 2017;23(12):924-30.
32. Woodworth MH, Carpentieri C, Sitchenko KL *et al.* Challenges in fecal donor selection and screening for fecal microbiota transplantation: A review. *Gut Microbes* 2017;8(3):225-37.
33. Kassam Z, Dubois N, Ramakrishna B *et al.* Donor screening for fecal microbiota transplantation. *N Engl J Med* 2019;381(21):2070-2.
34. Kump P, Wurm P, Grochenig HP *et al.* The taxonomic composition of the donor intestinal microbiota is a major factor influencing the efficacy of faecal microbiota transplantation in therapy refractory ulcerative colitis. *Aliment Pharmacol Ther* 2018;47(1):67-77.
35. Vermeire S, Joossens M, Verbeke K *et al.* Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis* 2016;10(4):387-94.
36. Keller J, Ooijevaar R, Hvas CL *et al.* A standardised model for stool banking for faecal microbiota transplantation: a consensus report from a multidisciplinary UEG working group. *United European Gastroenterol J* 2020 Mar;9(2):229-47. https://doi.org/10.1177/2050640620967898.
37. Jørgensen SMD, Hansen MM, Erikstrup C *et al.* Faecal microbiota transplantation: establishment of a clinical application framework. *Eur J Gastroenterol Hepatol* 2017;29:36-45.
38. Gorzelak MA, Gill SK, Tasnim N *et al.* Methods for improving human gut microbiome data by reducing variability through sample processing and storage of stool. *PLoS One* 2015;10(8):e0134802.
39. Guo Y, Li SH, Kuang YS *et al.* Effect of short-term room temperature storage on the microbial community in infant fecal samples. *Sci Rep* 2016 May 26;6: 26648. https://doi.org/10.1038/srep26648.
40. Loftfield E, Vogtmann E, Sampson JN *et al.* Comparison of collection methods for fecal samples for discovery metabolomics in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2016;25(11):1483-90.
41. Hamilton MJ, Weingarden AR, Sadowsky MJ *et al.* Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. *Am J Gastroenterol* 2012;107(5):761-7.
42. Youngster I, Sauk J, Pindar C *et al.* Fecal microbiota transplant for relapsing Clostridium difficile infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. *Clin Infect Dis* 2014;58(11):1515-22.
43. Satokari R, Mattila E, Kainulainen V *et al.* Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difficile infection – an observational cohort study. *Aliment Pharmacol Ther* 2015;41(1):46-53.
44. Costello SP, Tucker EC, La Brooy J *et al.* Establishing a fecal microbiota transplant service for the treatment of Clostridium difficile infection. *Clin Infect Dis* 2016; 62(7):908-14.
45. Cammarota G, Ianiro G, Tilg H *et al.* European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 2017;66(4):569-80.
46. Ott SJ, Musfeldt M, Timmis KN *et al.* In vitro alterations of intestinal bacterial microbiota in fecal

samples during storage. *Diagn Microbiol Infect Dis* 2004;50(4):237-45.

47. Peri R, Aguilar RC, Tuffers K *et al.* The impact of technical and clinical factors on fecal microbiota transfer outcomes for the treatment of recurrent Clostridioides difficile infections in Germany. *United European Gastroenterol J* 2019;7(5):716-22.
48. Costello SP, Conlon MA, Vuaran MS *et al.* Faecal microbiota transplant for recurrent Clostridium difficile infection using long-term frozen stool is effective: clinical efficacy and bacterial viability data. *Aliment Pharmacol Ther* 2015;42(8):1011-18.
49. Emanuelsson F, Claesson BE, Ljungstrom L *et al.* Faecal microbiota transplantation and bacteriotherapy for recurrent Clostridium difficile infection: a retrospective evaluation of 31 patients. *Scand J Infect Dis* 2014;46(2):89-97.
50. Browne HP, Forster SC, Anonye BO *et al.* Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature* 2016;533(7604): 543-6.
51. Staley C, Hamilton MJ, Vaughn BP *et al.* Successful resolution of recurrent Clostridium difficile infection using freeze-dried, encapsulated fecal microbiota; pragmatic cohort study. *Am J Gastroenterol* 2017; 112(6):940-7.
52. Allegretti JR, Fischer M, Sagi SV *et al.* Fecal microbiota transplantation capsules with targeted colonic versus gastric delivery in recurrent Clostridium difficile infection: A comparative cohort analysis of high and low dose. *Dig Dis Sci* 2019;64(6):1672-8.
53. Liao CH, Shollenberger LM. Survivability and long-term preservation of bacteria in water and in phosphate-buffered saline. *Lett Appl Microbiol* 2003; 37(1):45-50.
54. McCune VL, Quraishi MN, Manzoor S *et al.* Results from the first English stool bank using faecal microbiota transplant as a medicinal product for the treatment of Clostridioides difficile infection. *EClinicalMedicine* 2020 Mar 16:100301. https://doi.org/10.1016/j.eclinm.2020.100301.
55. Kao D, Roach B, Silva M *et al.* Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent Clostridium difficile infection: a randomized clinical trial. *JAMA* 2017;318(20):1985-93.
56. Youngster I, Mahabamunuge J, Systrom HK *et al.* Oral, frozen fecal microbiota transplant (FMT) capsules for recurrent Clostridium difficile infection. *BMC Med* 2016 Sep 9;14(1):134.
57. Thornit DN, Sander B, la Cour M *et al.* The effects of peroral glycerol on plasma osmolarity in diabetic patients and healthy individuals. *Basic Clin Pharmacol Toxicol* 2009;105(5):289-93.
58. Jiang ZD, Ajami NJ, Petrosino JF *et al.* Randomised clinical trial: faecal microbiota transplantation for recurrent Clostridum difficile infection – fresh, or frozen, or lyophilised microbiota from a small pool of healthy donors delivered by colonoscopy. *Aliment Pharmacol Ther* 2017;45(7):899-908.
59. Takahashi M, Ishikawa D, Sasaki T *et al.* Faecal freezing preservation period influences colonization ability for faecal microbiota transplantation. *J Appl Microbiol* 2019;126(3):973-84.
60. Satokari R, Pietilä L, Mattila E *et al.* Faecal banking at −20 °C facilitates faecal microbiota transplantation for recurrent Clostridioides difficile infection in clinical practice. *Infect Dis (Lond)* 2020;52(9):662-5.
61. Cammarota G, Ianiro G, Kelly CR *et al.* International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. *Gut* 2019; 68(12):2111-21.
62. Jørgensen SMD, Hvas CL, Dahlerup JF *et al.* Banking feces: a new frontier for public blood banks? *Transfusion* 2019;59:2776-82.
63. Terveer EM, Vendrik KE, Ooijevaar RE *et al.* Faecal microbiota transplantation for Clostridioides difficile infection: Four years' experience of the Netherlands Donor Feces Bank. *United European Gastroenterol J* 2020:2050640620957765.
64. Garey KW, Sethi S, Yadav Y *et al.* Meta-analysis to assess risk factors for recurrent Clostridium difficile infection. *J Hosp Infect* 2008;70(4):298-304.
65. Ianiro G, Valerio L, Masucci L *et al.* Predictors of failure after single faecal microbiota transplantation in patients with recurrent Clostridium difficile infection: results from a 3-year, single-centre cohort study. *Clin Microbiol Infect* 2017;23(5):337.e1-.e3.
66. Sleight SC, Wigginton NS, Lenski RE: Increased susceptibility to repeated freeze-thaw cycles in Escherichia coli following long-term evolution in a benign environment. *BMC Evol Biol* 2006;6:104.
67. Mattila E, Uusitalo-Seppala R, Wuorela M *et al.* Fecal transplantation, through colonoscopy, is effective therapy for recurrent Clostridium difficile infection. *Gastroenterology* 2012;142(3):490-6.
68. van Beurden YH, de Groot PF, van Nood E *et al.* Complications, effectiveness, and long term follow-up of fecal microbiota transfer by nasoduodenal tube for treatment of recurrent Clostridium difficile infection. *United European Gastroenterol J* 2017;5(6):868-79.
69. Hirsch BE, Saraiya N, Poeth K *et al.* Effectiveness of fecal-derived microbiota transfer using orally administered capsules for recurrent Clostridium difficile infection. *BMC Infect Dis* 2015;15:191.
70. Iqbal U, Anwar H, Karim MA: Safety and efficacy of encapsulated fecal microbiota transplantation for recurrent Clostridium difficile infection: a systematic review. *Eur J Gastroenterol Hepatol* 2018;30(7):730-4.

71. Kelly CR, Ihunnah C, Fischer M *et al.* Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. *Am J Gastroenterol* 2014;109(7):1065-71.
72. Shogbesan O, Poudel DR, Victor S *et al.* A systematic review of the efficacy and safety of fecal microbiota transplant for Clostridium difficile infection in immunocompromised patients. *Can J Gastroenterol Hepatol* 2018:1394379.
73. Abu-Sbeih H, Ali FS, Wang Y. Clinical review on the utility of fecal microbiota transplantation in immunocompromised patients. *Curr Gastroenterol Rep* 2019;21(4):8.
74. DeFilipp Z, Bloom PP, Torres Soto M *et al.* Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. *N Engl J Med* 2019;381(21): 2043-50.
75. Wang S, Xu M, Wang W *et al.* Systematic review: adverse events of fecal microbiota transplantation. *PLoS One* 2016;11(8):e0161174.
76. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. *J Hosp Infect* 2016;92(2):117-27.
77. International Council for Harmonisation. Clinical safety data management: definitions and standards for expedited reporting, E2A, available at www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html, accessed 19 April 2022.
78. Eriksen LL, Baunwall SMD, Eriksen PL *et al.* Deep hyperammonemic hepatic encephalopathy precipitated by fecal microbiota transplantation for fulminant Clostridioides difficile infection. *Gastroenterol Rep (Oxford)* 2022;10:goab043, available at https://doi.org/10.1093/gastro/goab043.
79. Edwards IR, Biriell C. Harmonisation in pharmacovigilance. *Drug Saf* 1994;10(2):93-102.
80. Kelly CR, Kunde SS, Khoruts A. Guidance on preparing an investigational new drug application for fecal microbiota transplantation studies. *Clin Gastroenterol Hepatol* 2014;12(2):283-8.
81. Moayyedi P, Surette MG, Kim PT *et al.* Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 2015;149(1):102-9.
82. Rossen NG, Fuentes S, van der Spek MJ *et al.* Findings rom a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology* 2015;149(1):110-8.
83. Paramsothy S, Kamm MA, Kaakoush NO *et al.* Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet* 2017;389(10075):1218-28.
84. Costello SP, Hughes PA, Waters O *et al.* Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: A randomized clinical trial. *JAMA* 2019;321(2):156-64.
85. Halkjaer SI, Christensen AH, Lo BZS *et al.* Faecal microbiota transplantation alters gut microbiota in patients with irritable bowel syndrome: results from a randomised, double-blind placebo-controlled study. *Gut* 2018;67(12):2107-15.
86. Johnsen PH, Hilpusch F, Cavanagh JP *et al.* Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. *Lancet Gastroenterol Hepatol* 2018;3(1): 17-24.
87. Aroniadis OC, Brandt LJ, Oneto C *et al.* Faecal microbiota transplantation for diarrhoea-predominant irritable bowel syndrome: a double-blind, randomised, placebo-controlled trial. *Lancet Gastroenterol Hepatol* 2019;4(9):675-85.
88. El-Salhy M, Hatlebakk JG, Gilja OH *et al.* Efficacy of faecal microbiota transplantation for patients with irritable bowel syndrome in a randomised, double-blind, placebo-controlled study. *Gut* 2019;69(5):859-867.
89. Holster S, Lindqvist CM, Repsilber D *et al.* The effect of allogenic versus autologous fecal microbiota transfer on symptoms, visceral perception and fecal and mucosal microbiota in irritable bowel syndrome: A randomized controlled study. *Clin Transl Gastroenterol* 2019;10(4):e00034.
90. Lahtinen P, Jalanka J, Hartikainen A *et al.* Randomised clinical trial: faecal microbiota transplantation versus autologous placebo administered via colonoscopy in irritable bowel syndrome. *Aliment Pharmacol Ther* 2020;51(12):1321-31.
91. Selvig D, Piceno Y, Terdiman J *et al.* Fecal microbiota transplantation in pouchitis: Clinical, endoscopic, histologic, and microbiota results from a pilot study. *Dig Dis Sci* 2019.
92. Singh R, de Groot PF, Geerlings SE *et al.* Fecal microbiota transplantation against intestinal colonization by extended spectrum beta-lactamase producing Enterobacteriaceae: a proof of principle study. *BMC Res Notes* 2018;11(1):190.
93. Grosen AK, Povlsen JV, Lemming L *et al.* Faecal microbiota transplantation eradicated extended-spectrum beta-lactamase-producing Klebsiella pneumoniae from a renal transplant recipient with recurrent urinary tract infections. *Case Rep Nephrol Dial* 2019;9(2): 102-7.
94. Huttner BD, de Lastours V, Wassenberg M *et al.* A five-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrug-resistant Enterobacteriaceae: a randomized clinical trial. *Clin Microbiol Infect* 2019;25:830-38.
95. Bajaj JS, Kassam Z, Fagan A *et al.* Fecal microbiota

transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology* 2017;66(6):1727-38.

96. Kakihana K, Fujioka Y, Suda W *et al.* Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood* 2016; 128(16):2083-8.

97. Wilson BC, Vatanen T, Cutfield WS *et al.* The super-donor phenomenon in fecal microbiota transplantation. *Front Cell Infect Microbiol* 2019;9:2.

98. Terveer EM, van Gool T, Ooijevaar RE *et al.* Human transmission of Blastocystis by fecal microbiota transplantation without development of gastrointestinal symptoms in recipients. *Clin Infect Dis* 2019: https://doi.org/10.1093/cid/ciz122.

Chapter 33. **Blood components for topical use or injection**

33.1. Introduction

Serum eye drops and platelet preparations are examples of substances of human origin where there is wide variation in approach to regulation in Europe [1]. Platelet preparations are defined as autologous and allogeneic platelets with a concentration higher than the baseline used for transfusion. They include platelet-rich plasma (PRP), platelet gel, platelet-rich fibrin (PRF) and platelet lysate eye drops.

Within the European Union (EU), blood used for the manufacture of serum eye drops must meet the standards of quality and safety specified in Commission Directive 2004/33/EC of 22 March 2004, which implements Directive 2002/98/EC of the European Parliament and of the Council regarding certain technical requirements for blood and blood components, including donor selection. For umbilical cord blood serum, the selection criteria for living donors of tissues and cells specified in Annex I/III of Directive 2006/17/EC are applicable for EU member states. Serum eye drops *per se* and platelet preparations may fall within different national legal frameworks in the EU for which the appropriate quality, safety and vigilance requirements need to be applied (these may include blood, tissues and cells, medicinal products).

The following generic chapters (Part A) of this Guide all apply to serum eye drops and platelet preparations and must be read in conjunction with this chapter where applicable:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage
k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

33.2. Serum eye drops

33.2.1. Introduction

Serum eye drops are prepared from the serum component of whole blood or umbilical cord blood for use by patients affected by severe ocular surface diseases, specifically for patients who have either not responded to, or who in their clinician's opinion are unlikely to benefit from, conventional treatments. Serum eye drops can be prepared for autologous use

from the patient's own serum or they can be allogeneic [2], prepared from allogeneic blood donors or from umbilical cord blood. The serum, either undiluted or diluted in physiological saline, is dispensed in small aliquots into dropper bottles or suitable dispensers as eye drops, for application either by the healthcare professional or for home treatment by the patient.

Serum eye drops have a potential advantage over traditional therapies for dry eye syndrome and persistent epithelial defects because human serum not only replicates the mechanical functions of tears (lubricating the eyelid, and rinsing particles from the ocular surface), but also serves as a lacrimal substitute, containing many of the same growth factors and other biochemical components that are present in natural tears. This is the reason why serum eye drops have become a popular second-line therapy in dry eye treatment [3-5]. The Cochrane review of eye drops made from autologous serum concluded that autologous serum *versus* artificial tears might provide benefit for treatment of dry eye in the short term. The overall benefit seems unclear at this time although some countries have reported good results [6]; however, much more research is needed in this area.

33.2.2. Donor evaluation

33.2.2.1. *Autologous setting*

Suitability criteria for autologous blood donation are less strict than for allogeneic blood donors and the risks posed by blood donation must be carefully considered on an individual basis against the potential benefits from the treatment. Special attention should be paid to avoiding the development of anaemia, especially where there is repeated collection of blood to prepare serum eye drops. Active viral or fungal infection and certain medications that may injure the cornea are contraindications to donation for serum eye drops.

Infectious disease markers in autologous donors must be tested as required by applicable laws and regulations (see Chapter 6). In cases where the results of the required tests are reactive or positive, the autologous serum eye drops should only be processed and released after careful assessment of the risk to the tissue establishment's staff and their safety from processing reactive or positive donations, the cross-contamination during storage and the risk of infecting the patient's household members with that specific serum eye drop preparation.

To overcome problems of co-existing medical conditions related to autologous donors and delays in treatment because of autologous serum eye drops preparation, the use of allogeneic eye drops can be considered.

33.2.2.2. *Allogeneic setting*

Allogeneic serum eye drops can be prepared in advance and be ready for use in emergency cases, or if patients are not eligible to donate for themselves. Allogeneic serum eye drop donors must meet the same eligibility criteria as voluntary blood donors [7]. Preferably, male donors with AB blood type should be selected to ensure ABO compatibility and minimise anti-HLA antibody titres. Additional selection criteria over and above these can be applied according to national requirements. Since topical application of serum eye drops is quite different from transfusion, and since eye tissues are susceptible to additional infectious agents, donor screening for additional infectious agents beyond government rules should be considered carefully.

In addition to general donor selection applicable for allogeneic blood donors, specific factors such as medications that may change the physiological or immunological state of the eye or that might injure the cornea should be considered in determining donor suitability for serum eye drops. As allogeneic serum eye drops are not lifesaving products, quarantining of the products for 4 months, followed by a negative nucleic acid test (NAT) and/or antibody screen on the donors' subsequent donation, should be recommended to enhance product safety. At the end of the quarantine period, the donor should be re-tested for relevant infectious disease markers, and if the outcome of this further screening is negative, the serum can be released for clinical application. If the initial screening includes NAT, and if appropriate donor-referral criteria and donor-compliance monitoring are in place to cover the risk of window-period infections, this quarantine period may not be necessary. Pooling of donations increases donor exposure; therefore the rationale for pooling must be justified with a risk assessment that must include consideration of further measures to mitigate the risk of infection transmission from donor to recipients (see §9.4.2.1).

33.2.2.3. *Umbilical cord blood serum*

Umbilical cord blood can be obtained during delivery, and laboratory testing of maternal blood for infectious diseases is required. Umbilical cord blood serum contains a higher concentration of growth factors and neurotropic factors compared with the levels in adult peripheral blood [8]. There is no definitive evidence demonstrating which components

of serum are essential for serum eyedrop efficacy. In comparison to standard serum, there is limited published evidence available on the use of cord blood serum in the treatment of various ocular surface diseases that demonstrates efficacy [9, 10].

33.2.3. Processing and storage

There must be written protocols for all procedures related to blood processing and eye drops production. All measures should be taken to minimise the risk of microbiological contamination, including disinfection of the phlebotomy site using methods accepted for collection of blood for transfusion. Blood must be collected in a sterile container/blood bag, without anticoagulant. The collected blood volume depends on local procedure but cannot be more than for regular blood donors.

The collected blood must be allowed to clot, and the serum must be separated, following validated protocols used for preparing serum eye drops [11]. The serum can be used undiluted, or diluted with physiological saline solution or balanced salt solution to different concentrations, based on the requirements of the clinician responsible for treating the patient. No standard production protocol or optimal serum concentration has been established to deliver maximal clinical benefit.

Eye-drop preparation must be carried out using aseptic technique. If the process involves open dispensing, it is required that clean rooms be used for manufacture of eye drops. It is strongly recommended to use a closed system for aliquoting. Microbiological control for each batch is mandatory (see Chapter 11). The volume of one aliquot should be adjusted to be no more than one daily dose to minimise microbiological growth in the thawed serum during the application period. All bags that are used in the collection, processing and aliquots of final packaging must be properly labelled.

Eye drops should be stored frozen at $< -15\,°C$ and transported in an appropriate container, to maintain the required temperature. Establishments must specify the shelf life of serum eye drop products at a defined temperature(s) based on internal validation data and/or literature data. This shelf life could be based on studies of the presumptive active components of serum eye drops at the designated storage temperature [12, 13].

The same principles apply to preparing umbilical cord serum eye drops. There must be a written protocol for the preparation of cord blood serum eye drops as described in this section for serum eye drops prepared from whole blood.

A large number of components present in the serum, as well as in tears, have been specified to explain the beneficial effects of serum eye drops. The risk of bacterial contamination occurring during donation and the manufacturing process, as well as during the application period of the thawed product by the patient, should be considered. To improve the risk–benefit ratio, precautions should be taken to avoid bacterial contamination and growth of harmful bacteria by sterile filtration of the final product before freezing the aliquots. However, filtration could also remove some of the presumptive active components of serum eye drops, and this risk should be considered. Other risk-reduction methods, such as a short shelf life, training of the patient and secure product packaging, can be implemented to reduce the bacterial contamination risk during the application period. The TE should fully risk assess its production protocol for serum eye drops to ensure that risks of contamination, or reducing the clinical efficacy of the eye drops, are adequately mitigated.

33.2.4. Application

The patient must be given appropriate information about the blood collection and testing, and about eye-drop preparation. The patient must be provided with written instructions for storage and handling of the eye drops at home, as well as information about the risk of, and signs of, potential bacterial contamination of the product and whom to contact, if they have any concerns.

33.2.5. Vigilance

Guidelines published by the Royal College of Ophthalmologists strongly recommend that the ophthalmologist monitors the patient's progress in a systematic way to enable collection of data regarding the benefits of using serum eye drops [14].

Any serious adverse reaction that occurs during usage of eye drops must be notified to the Health Authority following national regulations (See Chapter 17).

33.3. Platelet preparations

33.3.1. Introduction

Platelet preparations are used in regenerative medicine as source of growth factors and cytokines for the treatment of soft and hard tissue lesions. Each growth factor is involved in a phase of the healing process, such as inflammation, collagen synthesis,

tissue granulation and angiogenesis, collectively promoting tissue restitution.

The use of platelet preparations is an emerging field and its efficacy remains controversial. Several techniques for the preparation of platelet preparations are available; however, the results of applications have been confusing because each preparation method yields a different product with different composition of biologically active substances and potential uses. Platelet preparations have been prepared as platelet-rich plasma (PRP), platelet gel, platelet-rich fibrin (PRF) and platelet lysate eye drops, and they vary in consistency and in composition, for example in the concentration of growth factors and cytokines. Depending on the leukocyte and fibrin content, platelet preparations could be classified into four categories: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF) [15].

They are usually used in an autologous setting and can be prepared at the time of application. When they are prepared in advance and stored for future application, this should be done by a blood or tissue establishment. Allogeneic platelet preparations can be collected from healthy donors or produced from umbilical cord blood.

PRP is a concentrated source of autologous platelets, and it contains several different growth factors and other cytokines, in concentrations 5 to 10 times higher than in standard plasma; PRP is used to stimulate healing of soft tissue by injecting this concentrated plasma in the tissue where the healing effect is desired. There are primarily 3 isomers of platelet-derived growth factor (PDGF), namely αα, ββ and αβ, 2 transforming growth factors, TGF-β1 and TGF-β2, endothelial growth factor (EGF) and vascular epidermal growth factor (VEGF). PRP also contains proteins responsible for cell adhesion: fibrin, fibronectin and vitronectin [16]. The content of bioactive molecules depends on the production protocol [17]. All the products of this family can be used as liquid solutions or in an activated gel form. It can therefore be injected, for example in sports medicine, or placed during gelling on a skin wound or suture.

PRP is used to promote healing of injured tendons, ligaments, muscles and joints, and can be applied to various musculoskeletal problems. In addition to orthopaedics, other uses include dermatology, ophthalmology, plastic surgery and dentistry, including oral and maxillofacial surgery. As of 2020, no large-scale randomised controlled trials have confirmed the efficacy of PRP as a treatment for musculoskeletal or nerve injuries, the accelerated healing of bone grafts or the reduction of androgenic hair loss.

The main advantages so far identified in platelet gel derived from umbilical cord blood (CBPG), as compared with platelet gel from adult platelets, relate to a different profile of growth factor concentrations, such as a higher content of VEGF and lower content of TGF-β in CBPG. Recent developments have led to a procedure in which cord blood platelet gel can be prepared, stored in a cryopreservation bag and applied to the skin ulcer without breaking the sterility chain [18].

Platelet-rich fibrin (PRF) is a second-generation PRP where autologous platelets and leukocytes form a strong natural fibrin matrix or three-dimensional scaffold. This 'scaffolding' helps localise the growth factors, essentially increasing their concentration at the desired location to guide tissue regeneration [19]. PRF has a dense fibrin network with leukocytes, cytokines and structural glycoproteins, as well as growth factors (e.g. TGF β1, PDGF, VEGF) and glycoproteins, such as thrombospondin-1. Leukocytes that are concentrated in PRF scaffold play an important role in growth factor release, immune regulation, anti-infectious activities and matrix remodelling during wound healing. In addition, due to their elasticity and viscosity, these membranes adhere to the bone surface, acting as mechanical barriers against the penetration of the epithelium, which has faster regeneration potency than connective tissues [20].

Topical application of a platelet lysate, administered as eye drops, is an alternative therapeutic option for treatment of ocular surface disorders that do not respond to standard treatment [21]. The plasma component contains proteins essential for surface lubrication, whereas platelets provide growth factors (PDGF, EGF and TGF-β) and fibronectin that can promote ocular re-epithelialisation [22]. Eye drops comprising PRP have been used to treat dry eye syndrome for patients with Sjögren disease, and ocular chronic graft-*versus*-host disease (cGvHD) [23], and are used during macular hole surgery. So far, only studies of small case series have been published exploring the use of platelet preparations in ophthalmology, and further large-scale studies are necessary to demonstrate efficacy.

33.3.2. Donor evaluation

In the case of autologous donation, special attention should be paid to the status of donor's coagulation systems. The use of autologous platelet preparations avoids the ethical and legal implications of exposing the patient to the risks (albeit low)

of transmission of blood-borne pathogens, although the risk of infection related to contamination during collection and handling still remains. Disadvantages of autologous products include a larger individual variability in the quality of platelet preparations compared with allogeneic products that are prepared from healthy donor blood through standardised working procedures.

33.3.3. Procurement and processing

Depending on the type of platelet preparations, they can be prepared from whole blood or from apheresis product, or by using other methods of collection, such as small volume bags, tubes or various types of medical devices. Different blood volumes can be used, but the volume of anticoagulant should be proportional to the amount of blood collected. All manipulations during processing carried out in open system must be performed under aseptic processing.

33.3.3.1. *Procurement and processing of platelet-rich plasma*

For the preparation of PRP, the blood is drawn with the addition of an anticoagulant, such as citrate dextrose A (ACD-A), to prevent platelet activation prior to its use. The platelets are separated from other blood cells using the two-step centrifugation method. A 30 mL venous blood draw will yield 3-5 mL of PRP, depending on the patient's baseline platelet count, the device used and the technique employed. An initial centrifugation separates red blood cells from PRP, and is followed by a second centrifugation that concentrates platelets in 3-5 mL of the final plasma volume.

After the first centrifugation step, the whole blood is separated into three layers: an upper layer that contains mostly platelets and white blood cells, an intermediate thin layer that is known as the buffy coat and is rich in white blood cells, and a bottom layer that consists mostly of erythrocytes. To produce pure PRP, the upper layer and superficial buffy coat are transferred to an empty sterile tube. The second centrifugation process should be adequate to generate the formation of soft platelet pellets at the bottom of the tube. The upper portion of the volume, composed mostly of platelet-poor plasma, is removed. Platelet pellets are re-suspended in the lower third part of plasma to create the PRP.

Many automated systems for the preparation of PRP facilitate the preparation of ready-to-apply platelet-rich suspensions in a reproducible manner and are commercially available. These systems widely differ in their ability to collect and concentrate platelets, depending on the method and time of its centrifugation. As a result, suspensions of different concentration of platelets and leukocytes are obtained. Differences in the concentrations in platelets and white blood cells influence the diversity of growth factors concentration.

33.3.3.2. *Procurement and processing of platelet-rich fibrin*

For the preparation of PRF, a sample of blood is collected from the patient in tubes without anticoagulant and the blood is immediately centrifuged. During centrifugation, the platelets are activated when the blood contacts the tube wall.

The duration of time between blood collection and centrifugation is an important factor affecting the success and clinical outcome of this procedure. The majority of PRF preparation protocols recommend immediate (within 2 minutes of collection) centrifugation after blood collection. Delay in centrifugation will result in diffuse polymerisation of fibrin, leading to the formation of a small blood clot with irregular consistency. Therefore, a reproducible protocol for PRF production should be followed to obtain a clinically usable fibrin clot with substantial enmeshment of platelets.

After centrifugation, the uppermost of the three layers consists of acellular platelet-poor plasma, the PRF clot is in the middle layer and red blood cells are at the bottom of the tube. After centrifugation, the fibrin clot is removed from the tube and any attached red blood cells are scraped off and discarded.

PRF can also be applied as a membrane; the membrane can be formed in different shapes by squeezing out the fluids present in the fibrin clot using, for example, the stainless steel PRF compression device composed of two spoon-shaped parts [24].

33.3.3.3. *Procurement and processing of platelet lysate eye drops*

Platelet lysate eye drops are prepared using PRP after freezing–thawing at a final dilution of 30 %. A volume of 40 to 60 mL of peripheral blood anticoagulated with anticoagulant citrate dextrose solution A (ACD-A) is collected and centrifuged to obtain an autologous PRP. The platelet preparation is afterwards exposed to thermal shock by freezing at – 60 ° to – 80 °C for at least 60 min and then thawing to induce platelet lysis. The lysate can be diluted with sterile saline solution, and aliquoted into defined doses. A sample for microbiological control must be taken at the end of the processing (see Chapter 11). The final product is then frozen again at – 15 °C and stored in a freezer. Patients are usually provided with

a monthly supply of doses and trained how to thaw the dose, store it for the day from to 2 to 8 °C and safely instil eye drops.

33.3.4. Quality control

The quality of platelet preparations could be evaluated according to platelet recovery and growth factor contents. Further investigations are required to define standardised protocols for the preparation of high-quality platelet preparations suitable for different clinical applications, thus making it possible to compare results [25].

It is recommended that, if the platelet preparations are going to be stored, tissue establishment should have a microbiological testing protocol and acceptance/rejection criteria, similar to other cell and tissue products.

33.3.5. Biovigilance

Studies that have evaluated the topical use of platelet preparations have shown that the application is safe, and no serious adverse events were observed [26, 27]. According to a current literature search on platelet preparations use, there is no evidence of systemic effects that might limit the use of platelet preparations, provided that the possible risk of infections is excluded [15]. Few randomised controlled trials have reported adverse events after injection of platelet product; where these occur, they are mostly local side-effects related to venipuncture required for blood collection or (rarely) bad scarring or calcification at the application sites after injection of platelet product.

33.4. References

1. Competent Authorities on Substances of Human Origin Expert Group (CASoHO E01718), Summary report of meeting on 3-4 December 2014; available at https://ec.europa.eu/health/system/files/2016-11/ev_20141203_sr_en_0.pdf, accessed 5 August 2022.
2. Hennerbichler-Lugscheider. S. Setting up a serum eye drops program. *Int J Eye Banking* 2020 Dec;8(3), available at https://eyebankingjournal.org/article/setting-up-a-serum-eye-drops-program/, accessed 20 April 2022.
3. Soni NG, Jeng BH. Blood-derived topical therapy for ocular surface diseases. *Br J Ophthalmol* 2016;100(1): 22-7.
4. Pan Q, Angelina A, Zambrano A *et al.* Autologous serum eye drops for dry eye. *Cochrane Database Syst Rev* 2013;Aug 27;8:CD009327.
5. Anitua E, Muruzabal F, Tayebba A *et al.* Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies. *Acta Ophthalmol* 2015;93(8):605-14.
6. Lomas RJ, Chandrsekar A, Macdonald-Walis C *et al.* Patient reported outcome measures for a large cohort of serum eye drops recipients in the UK. Royal College of Ophthalmologists. *Eye* 2021 September 16;35: 3425-32, available at www.nature.com/articles/s41433-021-01560-8, accessed 20 April 2022.
7. European Directorate for the Quality of Medicine & HealthCare (EDQM). *Guide to the preparation, use and quality assurance of blood components*, 20th edition. Strasbourg: Council of Europe, 2020, available at www.edqm.eu/en/blood-guide, accessed 21 January 2022.
8. Buzzi M, Versura P, Grigolo B *et al.* Comparison of growth factor and interleukin content of adult peripheral blood and cord blood serum eye drops for cornea and ocular surface diseases. *Transfus Apher Sci.* 2018 Aug;57(4):549-55. https://doi.org/10.1016/j.transci.2018.06.001.
9. Yoon KC. Use of umbilical cord serum in ophthalmology. *Chonnam Med J* 2014;50:82-5.
10. Versura P, Profazio V, Buzzi M *et al.* Efficacy of standardized and quality-controlled cord blood serum eye drop therapy in the healing of severe corneal epithelial damage in dry eye. *Cornea* 2013 Apr;32(4):412-18.
11. Marks DC, van der Meer PF. Serum eye drops: a survey of international production methods. *Vox Ang* 2017 May;112(4):310-17.
12. Bradley JC, Simoni J, Bradley RH *et al.* Time- and temperature-dependent stability of growth factor peptides in human autologous serum eye drops. *Cornea* 2009 Feb;28(2):200-5.
13. Fischer KR, Opitz A, Boeck M, Geerling G. Stability of serum eye drops after storage of 6 months. *Cornea* 2012 Nov;31(11):1313-18.
14. Rauz S, Koay SY, Foot B *et al.* The Royal College of Ophthalmologists guidelines on serum eye drops for the treatment of severe ocular surface disease: executive summary. *Eye* 2018; 32(1):44-8.
15. Dohan Ehrenfest DM, Andia I, Zumstein MA *et al.* Classification of platelet concentrates (platelet-rich plasma—PRP, plateletrich fibrin—PRF) for topical and infiltrative use in orthopedic and sportsmedicine: current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J* 2014;4:3-9.
16. Lacci KM, Dardik A. Platelet-rich plasma: support for its use in wound healing. *Yale J Biol Med* 2010;83(1):1-9.
17. De Pascale MR, Sommese L, Casamassimi A *et al.* Platelet-derivatives in regenerative medicine: an update. *Transfus Med Rev* 2015;29(1):52-61.
18. Rebulla P, Pupella S, Santodirocco M *et al.* Italian

Cord Blood Platelet Gel Study Group. Multicentre standardisation of a clinical grade procedure for the preparation of allogeneic platelet concentrates from umbilical cord blood. *Blood Transfus* 2016;14:73-9.

19. Naik B, Karunakar P, Jayadev M *et al.* Role of platelet rich fibrin in wound healing: a critical review. *J Conserv Dent* 2013;16(4):284-93.
20. Simon BI, Zatcoff AL, Kong JJW *et al.* Clinical and histological comparison of extraction socket healing following the use of autologous platelet-rich fibrin matrix (PRFM) to ridge preservation procedures employing demineralized freeze dried bone allograft material and membrane. *Open Dent J* 2009;3:92-9.
21. Anitua E, Muruzabal F, Tayebba A *et al.* Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies. *Acta Ophthalmol* 2015;93(8):605-14.
22. Hartwig D, Harloff S, Liu L *et al.* Ephiteliotrophic capacity of a growth factor preparation produced from platelet concentrate on corneal epithelial cells: a potential agent for the treatment of ocular surface defects? *Transfusion* 2004;44:1724-31.
23. Zallio F, Mazzucco L, Monaco F *et al.* A single-center pilot prospective study of topical application of platelet-derived eye drops for patients with ocular chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2016;22:1664-70.
24. Kobayashi M, Kawase T, Horimizu M *et al.* A proposed protocol for the standardized preparation of PRF membranes for clinical use. *Biologicals* 2012;40(5): 323-9. https://doi.org/10.1016/j.biologicals.2012.07.004.
25. Aprili G, Gandini G, Guaschino R *et al.* SIMTI Working Group. SIMTI recommendations on blood components for non-transfusional use. *Blood Transfus* 2013t;11:611-22.
26. Picardi A, Lanti A, Cudillo L *et al.* Platelet gel for treatment of mucocutaneous lesions related to graft-versus-host disease after allogeneic hematopoietic stem cell transplant. *Transfusion* 2010 Feb;50(2):501-6.
27. Pezzotta S, Del Fante C, Scudeller L *et al.* Long-term safety and efficacy of autologous platelet lysate drops for treatment of ocular GvHD. *Bone Marrow Transplant* 2017 Jan;52(1):101-6.

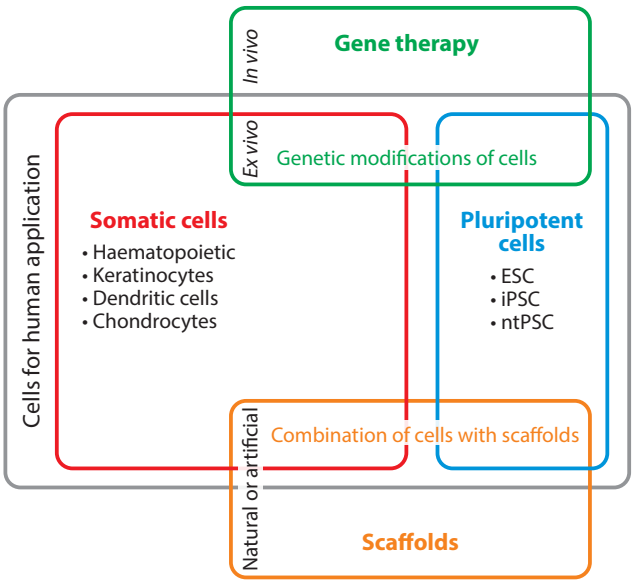
Chapter 34. **Tissues and cells as starting materials**

34.1. Introduction

In recent years, increasing numbers of tissue establishments (TEs) have expanded their activities, engaging in the preparation of more complex products based on human tissues and cells and/or providing starting materials for the manufacture of therapies classified under different regulatory frameworks (Figure 34.1).

These therapies rely on a supply of cells and tissues of appropriate safety and quality. This chapter provides guidance for authorised TEs on quality and safety aspects, mainly in donation, procurement, testing and distribution of starting material for further processing for the production of novel therapies involving human tissues and cells. A starting material is considered to be any procured human cell or tissue that will constitute an integral part of an active substance in an advanced therapy medicinal product (ATMP).

Figure 34.1. **Some novel therapies involving human tissues and cells**



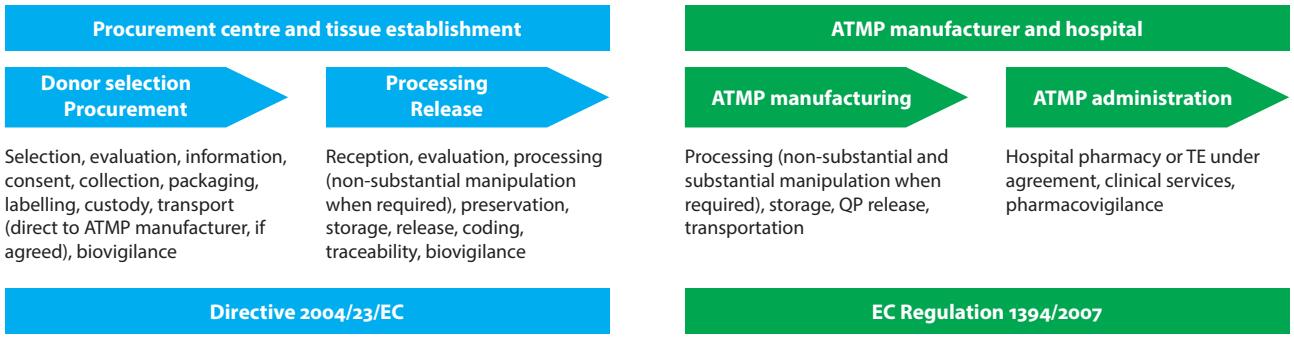
Note: ESC = embryonic stem cells; iPSC = induced pluripotent stem cells; ntPSC = nuclear transfer pluripotent stem cells.

The general quality and safety demands in Chapters 1 to 18 (Part A) apply, but some specific considerations for these cells are also relevant. The special considerations for donor selection, procurement, processing, testing and release, distribution and biovigilance are described in these sections, and an overview of the different steps is provided in Figure 34.2.

The following chapters of this Guide all apply to these cells and must be read in conjunction with this chapter:

a. Chapter 1. Introduction
b. Chapter 2. Quality management and validation
c. Chapter 3. Risk management
d. Chapter 4. Recruitment of potential donors, identification and consent
e. Chapter 5. Donor evaluation
f. Chapter 6. Donor testing – markers for infectious diseases
g. Chapter 7. Procurement
h. Chapter 8. Premises
i. Chapter 9. Processing
j. Chapter 10. Storage

Figure 34.2. **Tissue and cell collection and processing *versus* ATMP manufacturing and application.**



k. Chapter 11. Principles of microbiological testing
l. Chapter 12. Release, distribution and import/export
m. Chapter 13. Interaction between tissue establishments and organisations responsible for human application
n. Chapter 14. Computerised systems
o. Chapter 15. Coding, packaging and labelling
p. Chapter 16. Traceability
q. Chapter 17. Biovigilance
r. Chapter 18. Introduction of novel processes and clinical applications

It is important to note that, in different countries, these tissue/cell-based therapies may fall under different regulatory frameworks, including those for transplantation, medicinal products or medical devices. Irrespective of the content of this document, any operator active in the field should carefully consider the legal requirements that apply to the activities they are undertaking and it is advisable that, before starting any activities, they consult with the relevant authorities to understand the regulatory environment and seek any licence/authorisation that may be required. An overview of the legal framework for the development of ATMPs in the European Union (EU) is provided in Appendix 41.

34.2. Role of tissue establishments in providing starting materials

When ATMP preparation takes place in the EU, or where products meeting the ATMP classification are intended to be used in the EU, there are specific requirements, as described in Appendix 41 [1, 2]. Donation, procurement and testing of such cells as starting material must comply with the requirements of Directive 2004/23/EC. Acceptance criteria for cells and tissues used as starting materials for the manufacture of ATMPs, such as minimal cell number, viability or cell composition or tissues characteristics – besides viral (as per Commission Directive 2006/17/EC) and microbiological controls – must be established by ATMP manufacturers, who may also need additional controls.

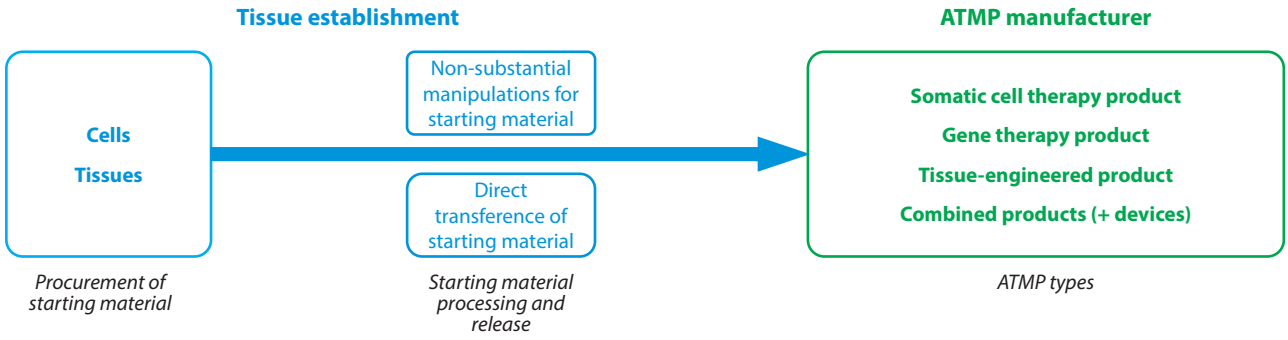
In order to link both responsibilities, appropriate third-party agreements, including quality agreements, must be in place. When requested, tissue- or cell-derived starting materials are transferred to a licensed ATMP manufacturer. Appropriate TE release and well-defined transportation arrangements and import/export authorisation are required. TEs must comply with specific guidance for good distribution practice of active substances for medicinal products for human use [3]. Thereafter, ATMP processing, quality control, storage, packaging, release, distribution, traceability, use and pharmacovigilance must be done in accordance with ATMP legislation, specifically EC Regulation 1394/2007. For all other requirements in the EU, there is specific guidance on GMP for ATMPs [1].

Figure 34.2 summarises which aspects are regulated by tissues and cells legislation and which aspects are controlled under ATMP regulation in the EU.

TEs participate in procurement, processing (non-substantial manipulation, if required), storage, testing and release of starting material after minimal manipulation. Particular attention should be paid to avoid contamination and to minimise variability of these materials. Traceability is required for all substances used in non-substantial manipulation/processing of donated cells and tissues as starting material for further ATMP manufacturing. Specifications requested by ATMP manufacturers related to these products will dictate whether and to what stage substances and materials can have a defined level of bioburden or need to be sterile. Prior to introduction in the manufacturing process, the conformity to the relevant requirements should be checked.

Figure 34.3 shows an example of how TEs and

Figure 34.3. **Example of possible interactions between tissue establishment and ATMP manufacturer**



ATMP manufacturers can be linked. This procedure can be valid for any type of starting material to be used in the manufacture of ATMP for clinical application.

Importantly, although blood banks and TEs are authorised in accordance with Directive 2002/98/EC and Directive 2004/23/EC respectively, ATMP manufacturers can be requested to perform quality audits to ensure that all steps performed in blood banks or TEs follow the specifications of the applicable directive.

Lastly, good communication between biovigilance and pharmacovigilance systems is essential to facilitate effective investigation and corrective/preventive actions if ATMPs are associated with adverse outcomes, including the donor and patient follow-up (see §17.6)

34.3. Cell and tissue types used in the development of novel therapies

Many cell and tissue types are currently being used or researched for the development of new therapeutic solutions (see Table 34.1). Due to their undifferentiated nature, stem cells can be expanded *in vitro* and differentiated into various tissue-specific cells. This unique characteristic can be exploited to support the development of new therapies for the treatment of a number of conditions. However, before considering a new cell-based therapy, it is necessary to understand the physiological properties of each stem cell or progenitor cell type. In addition, it is important to take into consideration that in some countries the use of some of these stem cells may not be permitted by law.

In addition to cell source, tissues can also serve as scaffolds to mimic extracellular matrix (ECM) in the context of tissue engineering. The ECM has a heterogeneous composition of proteoglycans, proteins and signalling molecules, and can thus influence cell differentiation, proliferation, survival and migration. These structures, alone or in combination with cells and bioactive agents, bring the mechanical support during *in vitro* cell growth and *in vivo* implantation. A diversity of biomaterials and manufacturing methods, including 3D bioprinting, have been used to create novel alternatives to traditional tissue engineering strategies (see §34.6.2.6). Natural and synthetic polymers, bioceramics or combinations of these as composites have been used.

34.4. Donor selection

Recent and rapid advances in biological and medical research are allowing human tissues and cells to be used increasingly in new ways. However, their expanding potential therapeutic benefits and their unique source bring their own challenges, requiring a delicate balance between three factors: the duty of care owed to donors; the duty of care owed to recipients; and the development and regulation of the technologies themselves.

Human tissues and cells can only be obtained from the body of a person, hence the ethical principles that should remain the cornerstone of their use. The person providing the material may be living or deceased, and the material may be used almost immediately or stored for long periods. It may also be used following minimal manipulations or heavily processed. Due to this diversity of circumstances, it is essential to clearly define the limits of consent. Donors must be given appropriate information beforehand as to the intended use and nature of the intervention, as well as its consequences and risks, in a complete and understandable fashion. Donors must be explicitly informed if their donations may be used to develop therapeutic products by commercial manufacturers or for research purposes, and in accordance with ethical and legal rules they cannot themselves expect to benefit financially if this occurs (see Chapter 4). The principle of prohibiting financial gain from the human body and its parts, as such, whether the donor

Table 34.1. **Some cell types being used to develop novel cell-based therapies**

	Cell type	Source	Processing
Pluripotent	Embryonic stem cells (ESC)	Obtained from the inner cell mass of blastocyst which have been cryopreserved and are no longer to be used for fertility treatment.	Cultured embryos (maximum 14 days) are dissociated and the inner cell mass is removed and cultured for a few more days. Then inner cell mass outgrowths consisting of potential ESC are isolated and expanded to create stem cell lines.
	Reprogrammed stem cells	Somatic cells which are reprogrammed to an embryonic stem cell-like state.	Reprogramming is achieved by introducing into adult cells (e.g. epithelial cells) a defined and limited set of transcription factors (e.g. stemness transcription factors) giving rise to induced pluripotent stem cells (iPSC). Cell reprogramming can also be achieved by nuclear transfer, giving rise to nuclear-transfer pluripotent stem cells (ntPSC).
Multipotent (lineage-restricted)	Somatic stem cells	Found in various tissues and may be isolated from extra-embryonic tissues, fetal specimens and adult tissues. Generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, neural stem cells, etc.)	Specific protocols have been developed, depending on the cell type.
Lineage-committed	Progenitor cells (e.g. haematopoietic progenitor cells), immune cells	Somatic cells that are committed to a specific mature cell fate and can divide only a limited number of times.	Can be transplanted without *in vitro* expansion (e.g. bone marrow, peripheral blood, cord blood), or cultured *in vitro* and differentiated into more restricted cell types for clinical application. Tissues and cells used for advanced therapies could be applied to two main areas: (1) to restore or produce immunological functions in patients (immunotherapy) and (2) to restore organ- or tissue-specific functions (regenerative medicine).

is living or deceased, and by extension the need to ensure voluntary and unpaid donation, must remain central in any consideration of the donation or use of tissues and cells of human origin. Cell therapy files documenting the development of a cellular product must ensure that consent remains valid at all stages of the process.

Recruitment, evaluation, testing and procurement of cell and tissue donors should follow at minimum existing EDQM guidance (see Chapter 4, 5, 6 and 7) and, depending of the nature of the tissues and cells collected, some additional tests may be performed either for quality or safety of the future ATMPs. The possibility of infection from administered cell therapies remains one of the greatest risks to potential recipients. Therefore, it implies a particular focus on the selection and assessment of donors, and on risk assessment for communicable diseases.

An additional issue to consider is the need for genetic screening. Currently, the recommendation is that no routine genetic screening be carried out on donors, providing that relevant genetic tests will be done on the stem cell lines or derived product. The reason for this is that there is a significant genetic distance between the donor and the final cellular products.

34.5. Procurement and processing

The process flow for the collection of starting material for the manufacture of novel therapies involving human tissues and cells usually starts with the referral of a donor (living or deceased) by the clinical site or donor team. An assessment of the donor's suitability, including actions to ensure their health and wellbeing are not compromised, must be undertaken and the findings communicated to the ATMP manufacturer to ensure their requirements are met.

Procurement must be carried out according to the general requirements detailed in Chapter 6, taking into account any additional instructions that might have been detailed in the manufacturer's protocol. All deviations must be reported, documented and investigated (see Chapter 2).

Collection products may be distributed to the TE for further preparation processes or directly to the ATMP manufacturer. Aspects of labelling, storage (if necessary) and transport must apply as stated in Chapter 15, Chapter 10 and Chapter 12 respectively. In the case of exceptional release, approval by a qualified person and acceptance by the ATMP manufacturer should be disclosed.

The collection facility or TE and the ATMP manufacturer must agree in advance on the extent of additional documentation that must accompany

the collection at distribution. Adverse events associated with the collection episode must be recorded, reviewed and reported through the biovigilance process. And finally, living donors should undergo post-collection follow-up.

For some ATMPs, the final steps before administration (e.g. thawing, washing and checks/controls) may be performed by TEs following validated procedures provided by the ATMP manufacturer. The roles and responsibilities of each party in these final stages of preparation and final checks before administration must be explicitly set out in the contractual agreement between the TE and the hospital pharmacy and/or the ATMP manufacturer. The responsibility for auditing and ensuring that the preparation and checks/controls meet the predefined standards lies with the ATMP manufacturer. These steps are important to ensure viability, to avoid microbiological contamination and to ensure the efficacy of the product.

34.6. Considerations for some common starting materials

34.6.1. Blood-derived mononuclear cells for immunotherapy

Several immunotherapy applications – like CAR-T-cell therapy [4, 5], virus-specific T-cells [6, 7], 'natural killer' (NK) cell therapy [8] or dendritic cell production [9] among others – are increasingly used for therapy. Normally, they use the patient's own blood-derived cells; however, registered stem-cell donors, cord blood banks or other third-party donor registries can also be employed to create individualised therapies by related donors for recipients of allogeneic haematopoietic progenitor cell (HPC) transplantation, or to develop universal, off-the-shelf therapies.

Donors must be evaluated for their eligibility for an apheresis procedure, bone marrow harvest or blood donations. Currently, apheresis is the most common source of starting material, and collection sites specialising in HPC collection are used for these procedures. In the case of unrelated donors, a licensed donor organisation should be responsible of their care and wellbeing. For eligibility, the medical evaluation of the donor must take into account the burden of collecting a large amount of leukocytes using apheresis. Special procedures must be in place for paediatric donors. Depending on the method used to obtain blood cells, a predetermined level of target cells may be relevant before procurement.

Other assessments may be relevant for specific applications. For example, for virus-specific T-cells, tests should determine the presence of circulating antibodies (immunological memory) against the specific target antigen or, in the case of NK cells, donor selection for protocols with adoptive transfer of allogeneic NK cells could include specific major histocompatibility complex (MHC) typing like HLA-C, HLA-E, and possibly also HLA-F and HLA-G, as well as killer Ig-like receptors or KIRs. As mentioned, regarding transmissible disease testing, consideration should be given both to the possible presence of a wild-type virus of the same type as the basis of the vector employed and to the likelihood of the formation of a replication-competent virus. In cases of autologous use, a donor viral positive test result may not be an exclusion criterion for the manufacture of a product if an appropriate risk-based analysis is performed (see §5.5.4).

The mononuclear cell fraction isolated through an apheresis procedure is normally used as starting material to ensure a higher dose of target-cells for cell culture. The blood volume processed, in order to obtain a sufficient number of cells, depends on the patient's peripheral blood counts and should be calculated to avoid unnecessary apheresis time with the increasing risks of serious adverse reactions (see also Chapter 24 on HPC).

34.6.2. Cells for regenerative medicine applications

34.6.2.1. *Mesenchymal stromal cells*

Mesenchymal stromal cells (MSC) are multipotent stem cells with immuno-regulatory and regenerative properties [10, 11]. They can normally be isolated from bone marrow and, recently, from other non-bone-marrow sources, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth. After growing in adherence cultures, they consist of a defined population with the ability to differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes. Due to their unique characteristics, they can be used either in immunotherapy through their modulating properties, or in regenerative medicine through their paracrine effects on tissue repair.

Under resting conditions, MSCs express HLA class I but not class II alloantigens. When cultured *in vitro* with allogeneic lymphocytes, MSC do not generally stimulate immune responses. Based on these findings, it has been assumed that MSC can be transfused across HLA barriers; and therefore cells from HLA-identical siblings, HLA-haplo-identical relatives or third-party HLA-mismatched healthy volunteer donors have been used in clinical protocols.

Taking into account their regenerative capacity function, MSCs are to be used preferably from an autologous source, although they can also be used in allogeneic settings.

The three main sources of MSC presently used for human application are bone marrow, adipose tissue and umbilical cord, and can be obtained in brief as follows. Bone marrow is normally obtained after aspiration from the anterior iliac crest with the patient in a supine position. The aspiration syringe is loaded with heparin to prevent clotting of the marrow sample. The technique is similar to that used in bone marrow procurement for HPC transplantation. In the case of adipose tissue, MSCs are found in subdermal adipose tissue and are normally obtained through abdominal liposuction using the techniques of aesthetic surgery. Finally MSCs can be isolated from all perinatal tissues, such as placenta, amniotic membrane, umbilical cord and Wharton's jelly. These tissues, which are the less invasive source for MSCs, are aseptically collected during caesarean section and can be obtained from different areas of cord and placental tissues, which are dissected before further processing.

34.6.2.2. *Chondrocytes*

In cartilage repair, autologous chondrocyte implantation (ACI) therapy is widely used for the treatment of isolated cartilage defects [12]. This technique is based on an implantation of a suspension of *in vitro* expanded chondrocytes. At the present time ACI is not indicated for patients with severe osteoarthritis, active rheumatoid arthritis or active autoimmune connective-tissue diseases, or patients with concomitant malignancies.

For donor evaluation, the patient is examined by an arthroscopic procedure where the location, depth and size of the defect and the quality of the surrounding cartilage are evaluated. A typical patient is a young patient with large ($> 2\ cm^2$) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. The ACI technique includes a two-stage procedure, with an initial procurement of a cartilage biopsy, which is sent for chondrocyte culture, followed by a second-stage operation that includes the cell application. A full-thickness cartilage biopsy (about 200-400 mg) is procured from a low-weight-bearing area of the knee during arthroscopy. The biopsy is transferred to a sterile transport tube with biopsy medium. The biopsy tube and blood tubes should then be placed in an outer secondary packaging that ensures the sterility and maintenance of the temperature, and is approved for transport of biological substances (see also Chapter 15). The biopsy should be kept cold – at about 5-15 °C – during transport to ensure the quality of the biopsy specimen. It should be sent directly to the TE for further processing, which should start within 48 hours. For culture conditions with autologous serum instead of fetal bovine serum, up to 50 mL of autologous blood should accompany the biopsy.

34.6.2.3. *Keratinocytes*

Keratinocytes are used in skin repair [13]. In the autologous setting, donor-site selection and timing are important. To get access to as many adult progenitor cells as possible, the donor site should preferably be in a hair-bearing area of healthy skin. The sooner (after trauma) the skin biopsy is taken, the better because the patient (and tissue) might be contaminated by microbes, which will affect the subsequent cell culture.

However, the skin areas available for donor-site selection are principally determined by the extent and location of the lesional skin. For allogeneic application, donor selection must include – apart from general donor evaluation criteria (see Chapter 4 and Chapter 5) – the tissue-specific criteria defined in Chapter 21. The transplanted allogeneic keratinocytes will be a temporary wound coverage, stimulating wound healing; thus human leukocyte antigen (HLA) typing or ABO blood grouping are not necessary. When procuring the skin for culture of keratinocytes, it is essential that the site for the biopsy is located in an area with healthy skin as remote from the lesional skin as possible. The biopsy can be either full-thickness or split-thickness. A full-thickness skin biopsy is preferred because of the amount of progenitor-cell-like keratinocytes in the appendages (hair follicles, sweat glands, etc.). The procurement should yield as many non-differentiated keratinocytes as possible.

The biopsy site should first be cleaned properly with disinfectant ethanol (70 %) with no additives and, after the site has dried, given a second wash with sterile saline solution (9 mg/mL) before the biopsy is procured (with e.g. a scalpel). Local anaesthetics can be used *ad lib*.

Immediately after procurement, the biopsy is placed in a medium suitable for transportation to the TE (basal culture medium with or without serum – or similar – and antibiotics in normal cell-culture concentrations). The primary container should be sterile, closed and appropriately labelled. Transport the biopsy to the culture facility and initiate the cell-isolation process as soon as possible, < 24-48 hours after surgical removal of the biopsy. Minimising the time will increase the likelihood of the successful culture

of cells. It is crucial to keep in mind that handling and culturing the skin biopsy/keratinocytes *in vitro* opens up a risk of contributing (microbiological) contaminants to the cells, both from the skin itself and from culture conditions. Those risks can only be avoided by adequate facilities with controlled environment (see Chapter 8), and skilful, excellent handling techniques by the staff (see Chapter 18).

34.6.2.4. *Limbal stem cells*

Limbal stem cell (LSC) deficiency refers to acquired pathological deficiencies such as chemical burns and inherited ones such as aniridia, may lead to ocular surface disease, including persistent epithelial defects with chronic inflammatory conditions, vascularisation and scarring of the cornea and conjunctiva, and corneal conjunctivalisation [14]. LSCs are being used to treat these conditions. Donors must be tested for infectious diseases as described in Chapter 6, although in the case of autologous use a positive test result may not be an exclusion criterion.

The treatment of unilateral LSC deficiency may involve *ex vivo* expansion of a tissue explant or isolated LSCs from the unaffected limbal region of a patient's healthy eye. In this case the autologous cell population is isolated and the final aim is to expand the limbal epithelial cells for application into the affected eye. Human amniotic membrane or human fibrin gel are typically used as a scaffold for supporting the *ex vivo* expansion of LSC and used as a carrier for the cells. Bilateral LSC deficiency, on the other hand, is a devastating pathological condition affecting both eyes; in this case, autologous limbal tissue or cells cannot be sourced from the same patient since both eyes are affected. Alternative sources of tissue include the culture of epithelial cells lining the autologous oral mucosa. Allogeneic sources of tissue, from deceased donors, may be an option for restoring the function of the ocular surface, although procedures with autologous healthy tissue are always preferable to those using heterologous sources [15, 16].

34.6.2.5. *Stromal vascular fraction*

Stromal vascular fraction (SVF) is isolated from adipose tissue and consists of a heterogeneous cell population that includes endothelial cells (10-20 %), haematopoietic lineage cells (25-45 %), stromal cells (15-30 %) and pericytes (3-5 %), as well as adipose stromal/stem cells (1-10 %). SVF may be used either directly or as a source material to isolate regenerative cells for treating various clinical conditions including musculoskeletal, neurological, immunological, cardio-pulmonary and immunological disorders, as well as soft tissue defects [17, 18]. Commonly, SVF cells are of autologous origin. Donor testing includes assays for transmissible diseases as described in Chapter 6; although their presence is not an exclusion criterion, it must be documented and special actions should be taken to avoid cross-contamination to other cells and to ensure the safety of personnel. The SVF can be isolated from either resected adipose tissue or aspirated adipose tissue using tumescent liposuction. Further details can be found in Chapter 28 on adipose tissue. There is no general consensus on what is the best area to obtain these cells. Although a common procedure is still lacking, in general, minced adipose tissue is digested by enzymes including collagenase, dispase, trypsin or the like [33]. However, mechanical procedures have also been reported [19]. After neutralising the enzymes, the released elements defined as SVF are separated from mature adipocytes by differential centrifugation.

34.6.2.6. *Bioprinting*

Bioprinting is an emerging technology in the regenerative medicine field and it is being explored as a way to tackle the need for tissues and organs suitable for the treatment of patients. It allows the generation of 3D structures by controlled disposition of biological materials and supporting components, and functional elements as biochemicals or living cells. The technique itself arranges these components layer-by-layer with a particular spatial placement of functional components [20]. The final aim of the technique is to mimic, to the maximum extent, the natural environment of living tissues at both structural and cellular levels to generate and use complex 3D functional living tissues. For example, multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures have been implanted [20]. Importantly, the combination of natural biomaterials (that possess the appropriate biochemical and biomechanical signals) with bioprinting (which can design complex structures with specific shape and size at macroscopic and microscopic levels) opens the door to obtaining a new set of tissues with defined and specific characteristics.

Natural tissue matrices, also known as decellularised extracellular matrices (ECM), have been used for bioprinting [20-23] and are very useful as source material for the 'building blocks' required for bioprinting; many of the attributes of decellularised tissues – such as their biocompatibility, their ability to support the attachment and differentiation of cells, and their capacity to be remodelled by the recipient's innate biology – make them ideal for this purpose. It is therefore important that TEs develop an awareness and appreciation of this novel use of donated tissues.

The ability to procure an effective custom-made graft, with specific shape, size, porosity and mechanical properties, is of great interest for personalised medicine in treating specific patient pathologies. Ideally, the composition of this *de novo* built graft should accomplish certain specific needs, such as the induction of new tissue formation and regeneration, without activating the immunological response [24]. Several studies show that decellularised ECM from different tissues can promote regeneration in damaged areas, such as demineralised bone [25], amniotic membrane [26], nerve [27] and skin [28, 29]. In recent years, due to the biological composition of ECM, the use of decellularised tissues as starting material has been considered for the generation of an ECM-based biomaterials pool to develop bio-inks in 3D bioprinting. The main hypothesis is that, if decellularised ECM promotes tissue regeneration, so the ECM pool from digested tissue could be used as the starting materials in a 3D bioprinting technique to develop *de novo* personalised grafts.

Moreover, the bioprinting technique offers the opportunity to combine different natural materials, such as collagens [30], fibrin [31], gelatine [32] or fibrinogen [33], to obtain hybrid scaffolds, in order to enhance bioactivity in the implantation site. The advantage of using natural polymers is that they fully satisfy the biochemical requirements of the tissue in terms of composition and biochemical signalling. Nowadays, the natural polymers used are mainly collagen and gelatin obtained from tendons and ligaments from tissue banks. This approach allows clinicians and patients to benefit from the biological properties of human tissues that have been increasingly used in recent decades, and gives the opportunity to tissue establishments to integrate the new 3D bioprinting technologies for more personalised grafts.

3D bioprinting involves different levels of complexity, such as the choice of biological or synthetic materials, cell types, or growth and differentiation factors, and technical challenges related to the sensitivities of living cells and the rheology of the substances of human origin used for the construction of the tissues. Dealing with these complexities requires a combination of different fields of expertise, from engineering and biomaterials science to cell biology, physics and medicine [20]. The process of bioprinting is composed typically of various steps: imaging of the native tissue or organ, design approach, material and cell selection, bioprinting itself and application [20]. Clinical trials have been made with bioprinting technology for the regeneration of tracheal [34] and craniofacial defects [35].

But it must be repeated that these approaches have not yet been deeply studied for human application. Therefore, a lot of effort is still needed in this field.

34.7. References

1. European Union. EudraLex [legislation]. Rules governing medicinal products in the European Union, Volume 4: Good Manufacturing Practice, Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products, available at https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf, accessed 20 April 2022.
2. European Medicines Agency. Guideline on human cell-based medicinal products, 2008, available at www.ema.europa.eu/en/documents/scientific-guideline/guideline-human-cell-based-medicinal-products_en.pdf, accessed 29 November 2022.
3. European Commission, Guidelines of 19 March 2015 on principles of Good Distribution Practice of active substances for medicinal products for human use (Text with EEA relevance), available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A52015XC0321(01), accessed 29 November 2022.
4. Davila ML, Sadelain M. Biology and clinical application of CAR T-cells for B cell malignancies. *Int J Hematol* 2016;104(1):6-17.
5. Dai H, Wang Y, Lu X, Han W. Chimeric antigen receptors modified T-cells for cancer therapy. *J Natl Cancer Inst* 2016;108(7):1-14.
6. O'Reilly RJ, Hasan A, Doubrovina E *et al.* Novel strategies for adoptive therapy following HLA disparate transplants. *Best Pract Res Clin Haematol.* 2011 Sep; 24(3):381-91.
7. Bollard CM, Heslop HE. T cells for viral infections after allogeneic hematopoietic stem cell transplant. *Blood* 2016; 127(26):3331-40.
8. Cheng M, Chen Y, Xiao W *et al.* NK cell-based immunotherapy for malignant diseases. *Cell Mol Immunol* 2013;10(3):230-52.
9. Bregy A, Wong TM, Shah AH *et al.* Active immunotherapy using dendritic cells in the treatment of glioblastoma multiforme. *Cancer Treat Rev* 2013;39(8): 891-907.
10. Dominici M, Le Blanc K, Mueller I *et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8(4):315-17.
11. Brown C, McKee C, Bakshi S *et al.* Mesenchymal stem cells: Cell therapy and regeneration potential. *J Tissue Eng Regen Med.* 2019 Sep;13(9):1738-55.
12. Brittberg M, Lindahl A, Nilsson A *et al.* Treatment of deep cartilage defects in the knee with autologous

chondrocyte transplantation. *N Engl J Med* 1994; 331(14):889-95.

13. Fredriksson C, Kratz G, Huss F. Transplantation of cultured human keratinocytes in single cell suspension: a comparative *in vitro* study of different application techniques. *Burns* 2008;34(2):212-19.
14. Jeschke MG, Finnerty CC, Shahrokhi S *et al.* Wound coverage technologies in burn care: established techniques. *J Burn Care Res* 2013;34(6):612-20; and more recently Jeschke MG, Shahrokhi S, Finnerty CC *et al.* Wound coverage technologies in burn care: established techniques. *J Burn Care Res* 2018 Apr 20;39(3): 313-18. https://doi.org/10.1097/BCR.0b013e3182920d29.
15. Baylis O, Figueiredo F, Henein C *et al.* 13 years of cultured limbal epithelial cell therapy: a review of the outcomes. *J Cell Biochem* 2011;112(4):993-1002.
16. Menzel-Severing J, Kruse FE, Schlötzer-Schrehardt U. Stem cell-based therapy for corneal epithelial reconstruction: present and future. *Can J Ophthalmol* 2013; 48(1):13-21.
17. Gimble JM, Bunnell BA, Guilak F. Human adipose-derived cells: an update on the transition to clinical translation. *Regen Med* 2012;7(2):225-35.
18. Bourin P, Bunnell BA, Casteilla L *et al.* Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013;15(6):641-8.
19. Oberbauer E, Steffenhagen C, Wurzer C *et al.* Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. *Cell Regen (Lond)* 2015 Sep 30;4:7. https://doi.org/10.1186/s13619-015-0020-0.
20. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nature Biotechnology* 2014;32(8):773-85.
21. Hoch E, Tovar GEM, Borchers K. Bioprinting of artificial blood vessels: Current approaches towards a demanding goal. *Eur J Cardiothoracic Surg* 2014;46: 767-78.
22. Hinderer S, Layland SL, Schenke-Layland K. ECM and ECM-like materials – Biomaterials for applications in regenerative medicine and cancer therapy. *Adv Drug Deliv Rev* 2016;97:260-9.
23. Pati F, Jang J, Ha DH *et al.* Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat Commun* 2014;5:3935. https://doi.org/10.1038/ncomms4935.
24. Swinehart IT, Badylak SF. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. *Dev Dyn* 2016;245:351-60.
25. Gruskin E, Doll BA, Futrell FW *et al.* Demineralized bone matrix in bone repair: History and use. *Adv Drug Deliv Rev* 2012;64:1063-77.
26. Zelen CM, Serena TE, Denoziere G, Fetterolf DE. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. *Int Wound J* 2013;10:502-7.
27. Whitlock EL, Tuffaha SH, Luciano JP *et al.* Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve* 2009;39(6):787-99.
28. Aldekhayel SA, Sinno H, Gilardino MS. Acellular dermal matrix in cleft palate repair: An evidence-based review. *Plast Reconstr Surg* 2012;130:177-82.
29. Chang M, Ahn SE, Baek S. The effect and applications of acellular dermal allograft (AlloDerm®) in ophthalmic plastic surgery. *J Cranio-Maxillofacial Surg* 2014;42:695-9.
30. Chang CC, Krishnan L, Nunes SS *et al.* Determinants of microvascular network topologies in implanted neovasculatures. *Arterioscler Thromb Vasc Biol* 2012; 32(1):5-14.
31. Cui X, Boland T. Human microvasculature fabrication using thermal inkjet printing technology. *Biomaterials* 2009;30:6221-7.
32. Hoch E, Hirth T, Tovar GEM, Borchers K. Chemical tailoring of gelatin to adjust its chemical and physical properties for functional bioprinting. *J Mater Chem B* 2013;1:5675.
33. Melo BAG de, Jodat YA, Cruz EM *et al.* Strategies to use fibrinogen as bioink for 3D bioprinting fibrin-based soft and hard tissues. *Acta Biomaterialia* 117;2020:60-76.
34. Zopf DA, Hollister SJ, Nelson ME *et al.* Bioresorbable airway splint created with a three-dimensional printer. *N Engl J Med* 2013;368:2043-5.
35. O'Brien CM, Holmes B, Faucett S, Zhang LG. Three-dimensional printing of nanomaterial scaffolds for complex tissue regeneration. *Tissue Eng Part B Rev* 2015;21:103-14.

Related material

Appendix 41. Legal framework for the development of advanced therapy medicinal products

Part C. Good Practice Guidelines for tissue establishments

Good Practice Guidelines for tissue establishments

Introduction

High-quality, safe and efficacious procedures for the donation, procurement, importation, testing, processing, preservation, storage and distribution of human tissues and cells for human application are essential for donors and recipients alike. Tissues and cells are health products of an exceptional nature, and all member states should endeavour to safeguard public health by promoting a high level of safety and quality in these substances when used for human application. This objective should be attained, maintained and continually optimised through the identification and implementation of key quality and safety criteria in relation to all the above-mentioned procedures in tissue establishments.

In the field of blood and blood components, Good Practice Guidelines (GPG) have been included in the *Guide for the preparation, use and quality assurance of blood components* since the 18th edition, published in 2015. In 2016, Directive 2005/62/EC was amended by Directive 2016/1214 to require EU member states to take into account the GPG jointly developed by the Commission and the European Directorate for the Quality of Medicines & Healthcare of the Council of Europe and published by the Council of Europe.

Following this approach, and aiming to promote and assure a high level of quality in the field of human tissues and cells, the European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM) took the decision to incorporate equivalent GPG in the 4th edition of the *Guide to the quality and safety of tissues and cells for human application*.

These guidelines do not introduce new requirements, but rather consolidate the guidance that is already defined in existing legislation and scientific guidelines. The GPG are therefore intended to elaborate on the basic requirements set out in the European Union Tissues and Cells directives and to detail the key elements that should be defined and controlled within the quality system of tissue establishments that are required to comply with those directives. The GPG incorporate and amplify not only the associated recommendations from the main chapters of the *Guide to the quality and safety of tissues and cells for human application*, but also relevant elements from the detailed principles of Good Manufacturing Practice (GMP) as referred to in Article 47 of EU Directive 2001/83/EC, the results of relevant EU-funded projects and expert opinion consistent with current scientific knowledge.

The GPG should be seen as a complementary document for tissue establishments (and their inspectors or auditors) that describes in detail, and from a practical point of view, the key elements for achieving comprehensive quality management in a tissue establishment.

1. General principles

1.1. Tissue establishments

1.1.1. The term 'tissue establishment' (TE) became widely used in Europe following publication of EU Tissues and Cells Directive 2004/23/EC, which defines it as: "a tissue bank or a unit of a hospital or another body where activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement or testing of tissues and cells".

1.1.2. In the field of medically assisted reproduction (MAR), the term 'TE' refers to the laboratories in MAR centres or clinics, but also to banks of gametes and embryos. These centres or clinics often include clinical units in which the patients are treated. In the context of these guidelines, the term 'TE' will be used to refer to all the banks, units, centres and clinics mentioned above.

1.2. EU tissues and cell legislation

1.2.1. The EU Tissues and Cells directives created a benchmark for the standards that must be met if carrying out any activity involving tissues and cells for human application, including gametes, embryos and germinal tissue. The directives also require that systems be put in place to ensure that all the tissues and cells used in human applications are traceable from donors to recipients and vice versa.

1.2.2. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 applies to the donation, procurement, testing, preservation, storage and distribution of human tissues and cells intended for human application (including reproductive cells used in MAR procedures). It introduced obligations on EU member states and their authorities to supervise human tissue and cell procurement, to authorise and inspect TEs, to ensure traceability and vigilance and to maintain a publicly accessible register of national TEs. This directive also laid down the rules on donor selection and evaluation and on the quality and safety of tissues and cells (e.g. quality management, tissue and cells reception, processing and storage conditions).

1.2.3. Commission Directive 2006/17/EC established specific technical requirements for each step in the human tissue and cell preparation process, in particular the requirements for the procurement of human tissues and cells, selection criteria for donors of tissues and cells, laboratory tests required for donors, tissue and/or cell donation, procurement and reception procedures at the TE and requirements for the direct distribution to the recipient of specific tissues and cells. Directive 2006/17/EC was amended in 2012 by Commission Directive 2012/39/EC about certain technical requirements for the testing of human tissues and cells.

1.2.4. Commission Directive 2006/86/EC includes traceability requirements and notification of serious adverse reactions and events (SAREs), as well as certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

1.2.5. In 2015, two new Commission Directives were adopted. Directive 2015/565 amended Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells. Directive 2015/566 was an implementing directive on the procedures for verifying equivalent standards of quality and safety of imported tissues and cells.

1.3. Using this Guide

1.3.1. These guidelines are based on a quality management system (QMS) approach. They form the basis of good practice in all TEs and should be used in preparation for both inspection and continuous improvement.

1.3.2. For each topic, the guidelines aim to provide sufficient detail to make TEs aware of the essential matters which should be considered as a minimum in the context of a risk-based analysis which takes full account of the specific protocols and risk-mitigation strategies of each TE that are relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

2. Quality management system

2.1. General requirements

2.1.1. Quality management is a wide-ranging concept covering all matters that individually or collectively influence the quality of tissues and cells. It is the sum total of the organised arrangements made with the objective of ensuring that tissues are of the quality required for their intended use. Quality management therefore incorporates good practice.

2.1.2. Each TE must develop and maintain a quality system which facilitates meeting all the relevant minimum requirements identified in the EU tissues and cells directives and which is based on the principles of good practice, incorporating quality risk management and taking into account the relevant elements of EU GMP as in Directive 2001/83/EC.

2.1.3. Quality must be recognised as being the responsibility of all persons involved in the processes of the TE, with management ensuring a systematic approach towards quality and the implementation and maintenance of a quality system.

2.1.4. Attainment of this quality objective is the responsibility of senior management. It requires the participation and commitment both of staff, in many different departments and at all levels in the organisation, and of the organisation's suppliers and distributors. To achieve this quality objective reliably there must be a comprehensive, well-designed and correctly implemented quality system incorporating good practice and quality risk management.

2.1.5. Each actor in the supply chain should establish, document and fully implement a comprehensive and well-designed quality system to deliver quality assurance based on the principles of quality risk management by incorporating good practice and quality control.

2.1.6. The basic concepts of quality management, good practice and quality risk management are interrelated. They are described here in order to emphasise their relationships and their fundamental importance to all stages, from identification of a potential donor through processing and storage of the tissues or cells to the final preparation for application to patient.

2.2. **Quality system**

2.2.1. The quality system encompasses quality management, quality assurance, continuous quality improvement, personnel, premises and equipment, documentation, donation, procurement, testing and processing, storage, release for circulation including distribution, quality control, change control, traceability, tissues and cells recall, external and internal auditing, contract management, validation, verification and qualification, and self-inspection. The design of the system should incorporate appropriate risk-management principles, including the use of appropriate tools.

2.2.2. The quality system must ensure that all critical processes are specified in appropriate procedures and/or instructions and are carried out in accordance with the standards and specifications of good practice and that they comply with appropriate regulations as set out in the chapters in this Guide.

2.2.3. The quality system must be designed to assure the quality and safety of processed tissues and cells, as well as to ensure donor and staff safety and end-user service. This strategy requires the development of clear policies, objectives and responsibilities. It also requires implementation, by means of quality planning, quality control, quality assurance and quality improvement, to ensure the quality and safety of tissues and cells, and to provide end-user satisfaction.

2.2.4. Senior management has the ultimate responsibility for ensuring that an effective quality system is in place and resourced adequately, and that roles and responsibilities are defined, communicated and implemented throughout the organisation. Senior management's leadership and active participation in the quality system is essential. This leadership should ensure the support and commitment to the quality system of staff at all levels and sites within the organisation.

2.2.5. Senior managers should establish a quality policy that describes the overall intentions and direction of the TE in relation to quality. They should ensure quality system management and governance of good practice through reviews of the performance of the QMS at regular intervals so as to verify its effectiveness, and they should ensure continuous and systematic improvement of all processes affecting the quality and safety of tissues and cells and the quality system itself and introduce corrective measures if

deemed necessary. Senior management is also responsible for regulatory supervision to ensure compliance with all legal and regulatory frameworks.

2.2.6. The quality system must be defined and documented. A quality manual or equivalent document should be established and contain a description of the quality system (including management responsibilities).

2.2.7. All TEs should be supported by an independent quality-assurance programme for fulfilling quality assurance. That programme must be involved in all quality-related matters, and must review and approve all appropriate quality-related documents.

2.2.8. All procedures, premises and equipment that have an influence on the quality and safety of tissues and cells (and tissues and cells components) must be validated, verified or qualified before introduction and must be periodically re-validated or re-qualified, as determined on the basis of quality risk management and change management.

2.2.9. A general policy regarding qualification of facilities and equipment, as well as validation or verification of processes, automated systems and laboratory tests, must be in place. The formal objective of qualification, validation or verification is to ensure compliance with the intended use and regulatory requirements.

2.3. **Change control**

2.3.1. A formal change-control system must be in place to describe the actions to be taken to plan, evaluate and document any planned change which is proposed for the range and/or specifications of procured or processed tissues and cells, the processes, equipment, environment (or site), method of processing or testing or any other change that may affect the reproducibility of a process, the quality and safety of tissues and cells, or the health of donors or recipients/patients.

2.3.2. Change-control procedures should ensure that sufficient supporting data are generated to demonstrate that the revised process results in a tissues or cells product of the desired quality, consistent with the approved specifications. Supporting data, e.g. copies of documents, should be reviewed to confirm that the impact of the change has been demonstrated prior to final approval.

2.3.3. The potential impact of a proposed change should be evaluated, and the degree of revalidation or additional testing, qualification, verification and validation needed should be determined, based on the principles of quality risk management.

2.3.4. Changes should be authorised and approved by the Responsible Person(s) or relevant personnel in accordance with the TE's quality system.

2.3.5. After implementation of any change, an evaluation should be undertaken to confirm that the quality objectives were achieved and that there was no unintended deleterious impact.

2.3.6. Where temporary and time-limited changes are implemented, provisions should be in place to ensure and verify that the changes are reversed as appropriate.

2.3.7. All changes should be evaluated for any requirement of notification to, or approval from, a national Health Authority.

2.4. **Deviations**

2.4.1. Procedures must be in place for notifying the Responsible Person in a timely manner of any significant deviations, non-compliance with regulatory commitments (e.g. in submissions or responses to regulatory inspections), tissues and cells defects, or testing errors and related actions (e.g. recalls, regulatory actions). Adequate resources should be made available for the timely resolution of deviations.

2.4.2. The investigation of deviations must include an assessment of component impact, including a review and evaluation of relevant operational documentation and an assessment of deviations from specified procedures. Deviations should be categorised depending on how critical they are to the quality and safety of tissues and cells, and how frequently they occur.

2.4.3. If it is determined that deviations could recur and/or there is doubt about compliance with quality and safety requirements, based on the estimated frequency of occurrence and severity (e.g. risk matrix) of the deviations, a root cause analysis should be performed to identify the underlying cause or causes of the deviation. This can be determined using quality risk-management principles. In cases where the true root cause(s) of the issue cannot be determined, consideration should be given to identifying and addressing the most likely root cause(s).

2.4.4. An associated system for the implementation of corrective and preventive actions must be in place. Appropriate corrective and/or preventive actions (CAPAs) should be identified and taken in response to investigations, with a view to ensuring that existing quality problems are identified and corrected, and that recurrence of the problem is prevented. The need to consider a recall of tissues and cells or the need to quarantine materials should also be considered. CAPAs must be completed in a timely and effective manner.

2.4.5. The effectiveness of CAPAs should be monitored and assessed, in line with quality risk-management principles.

2.4.6. The systems in place for the management of deviations should be linked as appropriate to the systems in place for the management of SAREs.

2.4.7. Where it is considered that a deviation or associated SARE may have the potential to impact another procurement organisation or TE, or an organisation responsible for human application (ORHA), the details of the deviation should be formally communicated to them so that they may undertake such investigations and actions as they may consider necessary.

2.4.8. Data relating to deviations should be routinely analysed to identify quality problems that may require corrective action or to identify trends that may require preventive action.

2.5. **Process quality review**

2.5.1. Regular process quality reviews should be conducted with the objective of verifying the consistency of the existing preparation process and the appropriateness of current specifications for all starting materials (including tissues and cells) and for processed tissues and cells, as well as highlighting trends and identifying improvements which may be required. Such a review should normally be conducted annually, taking into account the conclusions of previous reviews; it should be documented and include all tissues and cells which are imported, exported or intended for use in the manufacture of other products. Quality reviews may be grouped by tissue/cell type, where scientifically justified.

2.5.2. A quality review of tissues and cells that are ready for circulation may also be considered as an instrument for surveying the overall quality status of tissues and cells processing, including procurement. It may include:

2.5.2.1. review of starting materials, including tissues and cells;

2.5.2.2. review of critical in-process controls;

2.5.2.3. review of results of quality control and quality monitoring;

2.5.2.4. review of all changes;

2.5.2.5. review of the qualification status of equipment;

2.5.2.6. review of technical agreements and contracts;

2.5.2.7. review of all significant deviations and the CAPAs implemented;

2.5.2.8. review of the findings of internal and external audits and inspections;

2.5.2.9. review of complaints and recalls;

2.5.2.10. review of donor-acceptance criteria;

2.5.2.11. review of donor deferrals;

2.5.2.12. review of look-back cases.

2.5.3. The results of process quality reviews should be evaluated, and an assessment should be made whether any CAPA or any revalidation or re-verification should be undertaken. Reasons for any such CAPA should be documented. There should be management procedures for the ongoing management and review of these actions, and the effectiveness of these procedures should be verified during self-inspection.

2.6. **Good practice**

2.6.1. Good practice is the part of quality management that ensures that tissues and cells are consistently processed and controlled according to quality standards appropriate to their intended use. Good practice is concerned with donation, procurement, processing, preservation, storage (all hereinafter included in the generic term 'preparation'), import, release for circulation (including distribution) and quality control. The basic requirements are as follows:

2.6.1.1. All processes are defined clearly and reviewed systematically in the light of experience and shown to be capable of consistently delivering tissues and cells of the required quality and complying with their specifications. This strategy includes ensuring that:

2.6.1.1.1. critical steps and significant changes to the process are verified or validated;

2.6.1.1.2. all requirements are provided including:

2.6.1.1.2.1. appropriately qualified and trained personnel;

2.6.1.1.2.2. adequate premises and space;

2.6.1.1.2.3. suitable equipment and services;

2.6.1.1.2.4. correct materials, containers and labels;

2.6.1.1.2.5. approved procedures and instructions;

2.6.1.1.2.6. suitable storage and transport;

2.6.1.1.3. instructions and procedures are written in an instructional form in clear and unambiguous language, and are applicable specifically to the facilities provided;

2.6.1.1.4. operators are trained to carry out procedures correctly;

2.6.1.1.5. records are made, manually and/or by recording instruments, during preparation which demonstrate that all the steps required by the defined procedures and instructions were in fact taken and that the quantity and quality of the tissues and cells was as expected;

2.6.1.1.6. any significant deviations are fully recorded and investigated;

2.6.1.1.7. records of preparation processes, storage and release for circulation (including distribution) enable the complete history of the tissues and cells to be traced and these records are retained in a comprehensible and accessible form;

2.6.1.1.8. the release for circulation (including distribution) of the tissues and cells minimises any risk to their quality;

2.6.1.1.9. a system is available to recall any tissues and cells (including those processed using a batch of critical materials that have been distributed or issued);

2.6.1.1.10. complaints about tissues and cells are examined, the causes of quality defects are investigated and appropriate measures are taken in respect of the defective tissues and cells components to prevent recurrence.

2.6.1.2. Quality control is the part of good practice that is concerned with sampling, specifications and testing, as well as with the organisation, documentation and release procedures which ensure that materials are not released for use in processing, and tissues and cells are not released for circulation, including distribution, until their quality has been judged to be satisfactory and also that the necessary and relevant tests have been carried out. The basic requirements are:

2.6.1.2.1. adequate facilities, trained and qualified personnel and approved procedures are available for sampling, inspecting and/or testing starting materials (including tissues and cells), packaging materials, intermediate components and finished/ready-for-circulation tissues and cells, and also, if appropriate, for monitoring environmental conditions;

2.6.1.2.2. samples of starting materials (including tissues and cells), packaging materials, intermediate and processed tissues and cells are taken by approved personnel and methods;

2.6.1.2.3. test methods are verified or validated;

2.6.1.2.4. records are made, manually and/or by recording instruments, which demonstrate that all the required sampling, inspecting and testing procedures were actually carried out;

2.6.1.2.5. the processed tissues and cells comply with the specifications and are correctly labelled;

2.6.1.2.6. records are made of the results of inspection and of the testing of materials and intermediate and processed tissues and cells. to show they have been formally assessed against specifications;

2.6.1.2.7. no tissues and cells are released for circulation, including distribution, that do not comply with the requirements of the relevant authorisations, except in the case of an exceptional release (see 2.8).

2.7. **Quality risk management**

2.7.1. A quality risk-management approach, consisting of a systematic process for the assessment, control, communication and review of risks to quality across the life-cycle of tissues and cells, should be applied. Where appropriate, statistical tools should be used in the assessment of ongoing process capability.

2.7.2. It must be ensured that the risks inherent in the use and handling of biological material are identified and minimised, consistent with maintaining adequate quality and safety for the intended purpose of the tissues and cells (Directive 2006/86/EC Annex I A.5). Donor selection, and tissues and cells procurement, processing, storage and distribution activities, should therefore be subjected to a comprehensive risk assessment encompassing all the process steps with respect to the procedures, materials, tests, personnel, premises and equipment involved.

2.7.3. All components of the risk-management process should be linked to the authorised activities of the TE and all elements of the QMS should incorporate the principles of quality risk management.

2.7.4. Any evaluation of the risk to quality should be based on scientific knowledge, experience with the process and, ultimately, protection of the donor and recipient/patient.

2.7.5. The level of effort, formality and documentation of the quality risk-management process should be commensurate with the level of risk.

2.7.6. Risk assessments should be based on an analysis of the risks related to the application of the specific type(s) of tissues and cells and should be undertaken with the primary objective of identifying, where relevant, all those factors which could result in cross-contamination, contamination with adventitious agents, the transmission of disease or infectious agents, the transmission of inherited conditions or mix-ups, or which could render the tissues or cells clinically ineffective or harmful to the recipient/patient.

2.7.7. Such risks could, for example, derive from but are not limited to:

2.7.7.1. the sensitivity of donor screening protocols and tests;

2.7.7.2. procurement procedures;

2.7.7.3. biological properties of the procured tissues and cells;

2.7.7.4. the absence of standardised quality-control tests;

2.7.7.5. the use of potentially infective or known infective materials;

2.7.7.6. processing, storage and transport procedures and environment.

2.7.8. Risk assessments and associated management plans should identify and describe the principal activities of the TE and the circumstances to which the different phases of the plan apply.

2.7.9. Risk-mitigation strategies should be developed on the basis of prospective risk analysis in order to maximise the quality and safety of tissues and cells and to protect recipients/patients, personnel and the process itself, as well as other linked or proximal processes.

2.7.10. Risk management should serve as documentation of the rationale for key safety- or quality-related decisions, such as in the case of actions to be taken in relation to deviations and to determine the eligibility of impacted tissues and cells for clinical use.

2.7.11. All risk-assessment and risk-management plans should include documentation on:

2.7.11.1. the scope of, and circumstances for conducting, the assessment;

2.7.11.2. the individuals assigned to the work programme;

2.7.11.3. identification of the hazards associated with the scope/circumstances;

2.7.11.4. an estimate of their severity and probability of occurrence;

2.7.11.5. the risk analysis, evaluation and control measures for these hazards;

2.7.11.6. the scientific grounds for acceptance / rejection of the decision;

2.7.11.7. a rationale for the acceptability of the residual risk;

2.7.11.8. a statement of acceptance or otherwise of the residual risk.

2.7.12. Risk management should be used to support decision making regarding the specific qualification/validation activities that need to be performed. The associated risk assessment should highlight the critical points in the processes, thus allowing the development of an appropriate validation plan.

2.7.13. Risk-management principles and methodologies should be incorporated into staff training programmes.

2.8. Exceptional release

2.8.1. In exceptional circumstances, an ORHA may agree with a TE, or a procurement organisation in the case of direct distribution, that tissues or cells which do not meet defined release criteria can be released for use in a specific recipient. Although directives 2004/23/EC, 2006/17/EC and 2006/86/EC lay down a number of specific requirements for performing a risk assessment when managing specified aspects of the quality and safety of tissues or cells for human use, nonetheless, in exceptional circumstances where any other defined requirements of the directives have not been complied with or cannot be complied with, and where clinical use of the impacted tissues and cells is required due to urgent medical need, the limited availability of alternative therapeutic options and the expected clinical benefit, a comprehensive documented risk assessment must be used to inform the decision of the Responsible Person as to whether the tissues or cells may be released for use.

2.8.2. In such circumstances, the physician treating the intended recipient should work with the nominated registered medical practitioner who advises on and oversees the medical activities of the TE, in conducting the risk assessment and a risk–benefit analysis for the intended recipient. All associated discussions and conclusions must be documented and the treating physician must sign their agreement with the exceptional release and their acceptance of any implied risk for the intended recipient.

2.8.3. In the case of microbiological contamination of autologous tissues and cells, or tissues and cells received from a specific allogenic donor whereby a repeated procurement cannot be conducted or involves a high degree of risk, the risk assessment and risk–benefit analysis should be based on the nature and extent of the contamination and should specifically consider the risk based on identification of the contaminating micro-organisms and the potential for adequate prophylaxis of the intended recipient.

2.9. Self-inspection, audits and improvements

2.9.1. Self-inspection or audit systems must be in place for all elements of operations to verify compliance with the standards. They must be carried out regularly by trained and competent persons, in an independent way, and according to approved procedures.

2.9.2. All results must be documented, and appropriate CAPAs must be implemented in a timely and effective manner.

3. Management of outsourced activities (contractual agreements)

3.1. General principles of outsourcing

3.1.1. Outsourced activities that may impact on the quality and safety or efficacy of the tissues and cells must be correctly defined, agreed and controlled in order to avoid misunderstandings which could result in tissues and cells or work of unsatisfactory quality. There must be a written contract covering these activities, tissues and cells or processes to which they are related, and any technical arrangements made in connection with it.

3.1.2. All outsourced arrangements for tissues or cells that influence their quality or safety, including any proposed changes, must be done in accordance with a written contract, with reference to the specification for the tissues or cells concerned.

3.1.3. The responsibilities of each party must be clearly documented to ensure that the principles of good practice are maintained.

3.1.4. The contract giver is the establishment or institution that subcontracts particular work or services to a different institution and is responsible for setting up a contract defining the duties and responsibilities of each party.

3.1.5. The contract acceptor is the establishment or institution that performs particular work or services under a contract for a different institution.

3.2. The contract giver

3.2.1. The contract giver is responsible for assessing the competence of the contract acceptor to successfully carry out the work being outsourced and for ensuring, by means of the contract, that the principles and guidelines of good practice are followed.

3.2.2. The contract giver must provide the contract acceptor with all the information necessary to carry out the contracted operations correctly and in accordance with the specification and any other legal requirements. The contract giver must ensure that the contract acceptor is fully aware of any problems associated with the materials, samples or the contracted processes that might pose a hazard to the premises, equipment, personnel, other materials or other tissues or cells of the contract acceptor.

3.2.3. The contract giver must ensure that all tissues or cells, analytical results and materials delivered by the contract acceptor comply with their specifications and that they have been released under a quality system approved by the Responsible Person or other authorised person.

3.3. **The contract acceptor**

3.3.1. The contract acceptor must have adequate premises, equipment, knowledge, experience and competent personnel to satisfactorily carry out the work requested by the contract giver.

3.3.2. The contract acceptor must ensure that all products, materials or test results delivered by the contract giver are suitable for their intended purpose.

3.3.3. The contract acceptor must not pass to a third party any of the work entrusted under the contract without the contract giver's prior evaluation and approval of the arrangements. Arrangements made between the contract acceptor and any third party must ensure that the relevant information is made available in the same way as between the original contract giver and contract acceptor.

3.3.4. The contract acceptor must refrain from any activity that may adversely affect the quality of the tissues or cells processed and/or analysed for the contract giver.

3.4. **The contract**

3.4.1. A contract must be drawn up between the contract giver and the contract acceptor that specifies their respective responsibilities relating to the contracted operations. All arrangements for tissues or cells procurement, processing and testing must be in compliance with the requirements of good practice and with regulatory requirements, and must be agreed by both parties.

3.4.2. The contract must specify the procedure, including the necessary evidence to be provided by the contract acceptor, by which the Responsible Person or other authorised person releasing the tissues or cells can ensure that each component has been processed and/or distributed in compliance with the requirements of good practice and regulatory requirements.

3.4.3. The contract must clearly describe who is responsible for purchasing materials, for testing and releasing materials, for undertaking tissues or cells procurement and for processing and testing (including in-process controls). In the case of subcontracted analyses, the contract must state the arrangements for the collection/procurement of samples, and the contract acceptor must agree that they can be subject to inspections by the Health Authorities.

3.4.4. Processing and distribution records, including reference samples if relevant, must be kept by, or be available to, the contract giver. Any records relevant to assessment of the quality of the tissues or cells in the event of complaints or a suspected defect must be accessible and specified in the defect/recall procedures of the contract giver.

The contract must permit the contract giver to audit the facilities of the contract acceptor.

4. Personnel and organisation

4.1. Personnel must be available in sufficient numbers and with the necessary qualifications and experience to carry out the activities related to the procurement, testing, processing, storage and release for circulation (including distribution) of tissues and cells for human application and they must be trained and assessed as competent to perform their tasks.

4.2. Management has the ultimate responsibility to determine and provide adequate and appropriate resources (human, financial, materials, facilities and equipment) to implement and maintain the QMS and continually improve its suitability and effectiveness through participation in management reviews. The responsibilities placed on any one individual should not be so extensive as to present any risk to quality.

4.3. There should be an organisation chart in which the relationships between key personnel are clearly shown in the managerial hierarchy.

4.4. All personnel should have up-to-date job descriptions, which clearly set out their tasks and responsibilities. The correct understanding of responsibilities by individuals should be assessed and recorded.

4.5. Personnel in responsible positions should have adequate authority to carry out their responsibilities. Their duties may be delegated to designated deputies of a satisfactory qualification level. There should be no gaps or unexplained overlaps in the responsibilities of those personnel concerned with the application of good practice.

4.6. Traceability must be ensured to personnel performing critical processing tasks or approving critical results or documents. If such personnel sign paper documents, whether regularly or occasionally, personnel signature lists should be available.

4.7. All personnel must receive initial and continued training appropriate to their specific tasks. Training records must be maintained. Training programmes must be in place and must include the principles of good practice.

4.8. Training should be provided for all personnel whose duties take them into processing areas or into laboratories (including the technical, maintenance and cleaning personnel). Dedicated training on nitrogen risks is required when storage in nitrogen for cells and tissues is in place.

4.9. There should be written policies and procedures to describe the approach to training, including a record of training that has taken place, its contents and an assessment of its effectiveness.

4.10. Personnel must be provided with initial/basic training, updated training as required when procedures change or when scientific knowledge develops, and adequate opportunities for relevant professional development. The training programme must ensure and document that each individual:

4.10.1. has demonstrated competence in the performance of their designated tasks;

4.10.2. has an adequate knowledge and understanding of the scientific/technical processes and principles relevant to their designated tasks;

4.10.3. understands the organisational framework, quality system and health and safety rules of the establishment in which they work; and

4.10.4. is adequately informed of the broader ethical, legal and regulatory context of their work.

4.11. The competence of personnel must be evaluated regularly and the contents of training programmes should be periodically assessed.

4.12. Only personnel who are authorised by defined procedures and documented as such may be involved in the procurement, processing, testing and distribution processes, including quality control and quality assurance.

4.13. There must be written safety and hygiene instructions in place, adapted to the activities to be carried out.

4.14. It is the organisation's responsibility to provide instructions on hygiene and health conditions that may be relevant to the quality of tissues and cells (e.g. during procurement) and to ensure that staff report any relevant health problems.

4.15. Steps should be taken to ensure as far as is practicable that no person affected by an infectious disease or having open lesions on the exposed surface of their body is engaged in the processing of tissues and cells. Medical examinations should be carried out when necessary to assure fitness for work and personal health. There should be instructions ensuring that health conditions that can be of relevance to the quality of tissues and cells are reported by personnel.

4.16. Visitors or untrained personnel should, preferably, not be taken into the procurement, processing and laboratory areas. If this is unavoidable, they should be given information in advance, particularly about personal hygiene and the prescribed protective clothing. They should be closely supervised.

4.17. Eating, drinking, chewing or smoking, or the storage of food, drink, smoking materials or personal medication in the processing, testing and storage areas should be prohib-

ited. In general, any unhygienic practice within the processing areas or in any other area where the tissues or cells might be adversely affected should be forbidden.

4.18. There should be a written policy outlining the requirements for wearing protective garments in the different areas. The requirements should be appropriate to the activities to be carried out.

4.19. Personnel must be trained in the gowning requirements appropriate to various area classifications. The competence of personnel working in Grade A/B areas with regards to the gowning requirements should be reassessed at least annually.

4.20. Every person entering the processing areas should wear clean clothing suitable for the processing activity with which they are involved and this clothing should be changed when appropriate. Additional protective garments appropriate to the operations to be carried out (e.g. head, face, hand and/or arm coverings) should be worn when necessary. Jewellery and make-up must be removed before entering a cleanroom.

4.21. The clothing and its quality should be appropriate for the process and the grade of the working area. It should be worn in such a way as to protect the operator and tissues and cells from the risk of contamination.

4.22. The description of clothing required for clean areas is as follows:

- **Grade D**: Hair and, where relevant, beard and moustache should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.
- **Grade C**: Hair and where relevant beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.
- **Grade A/B**: Sterile headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a sterile face mask should be worn to prevent the shedding of droplets and particles. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-legs should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

4.23. Outdoor clothing should not be brought into changing rooms leading to Grade B and C rooms. For every worker in a Grade A/B area, clean (sterilised) protective garments (including face masks) should be provided every time there is an entry into the clean area; the need to exit and re-enter the clean area for a different processing step/different batch should be determined by the risk of the activity. Upon exit from a clean area there should be a visual check of the integrity of the garment.

4.24. Clean area clothing should be cleaned and handled in such a way that it does not gather additional contaminants which can later be shed. When working in a contained area, protective clothing should be discarded before leaving the contained area.

4.25. Personnel working in clean areas must be given specific training on aseptic processing, including the basic aspects of microbiology.

4.26. Particular attention should be given to the qualification of the aseptic technique of personnel working in Grade A environments with Grade B backgrounds. Prior to participating in routine aseptic processing operations, personnel should be qualified through participation in successful process-simulation tests. The usual approach is to conduct simulated processes using culture medium in place of, or added to, tissues or cells.

4.27. Microbial monitoring of personnel working in A/B areas should be performed after critical operations and when leaving the A/B area. A system of disqualification of personnel should be established based on the results of the monitoring programme, as well as other parameters that may be relevant. Once disqualified, retraining/requalification is required before the operator can be involved in aseptic operations. It is advised that the retraining/requalification includes participation in a successful process-simulation test.

5. Premises

5.1. General requirements

5.1.1. Premises must be suitable for carrying out the intended procedures in order to prevent errors (e.g. mix-ups, contamination, cross-contamination or improper labelling of tissues and cells).

5.1.2. When required, environmental conditions such as lighting, temperature, humidity and ventilation should be appropriate and controlled to assure safety and comfort to patients, donors and personnel, and to assure the accurate functioning of equipment during processing and storage.

5.1.3. There must be adequate equipment and materials for the activities performed.

5.1.4. Premises must be secure to prevent the entrance of unauthorised people and should not be used as if by right as a routeway by personnel who do not work there.

5.1.5. Facilities should have an appropriate design to permit ease of maintenance and cleaning. Cleaning and sanitation must be performed on a regular basis and documented. The efficacy of the methods used must be validated and monitored.

5.1.6 A written sanitation programme should be available, describing the frequency and methods of cleaning. Additionally, a pest control system should be in place, where necessary.

5.1.7. A written safety manual and personal protective equipment must be available to minimise the risks to the health of personnel and visitors.

5.1.8. All waste generated by the facilities must be disposed of in accordance with applicable laws and regulations.

5.2. **Donor area**

5.2.1. There must be a suitable space for the confidential interviews of living donors or the relatives of deceased donors, and for physical examination of the donor.

5.3. **Procurement area**

5.3.1. The facility must be of adequate size to allow proper operations and ensure donor privacy and anonymity.

5.3.2. Facilities in which tissues or cells are procured must meet appropriate grades of air quality and cleanliness where applicable. The appropriate standard of cleanliness will depend on the type of tissues or cells being procured, the degree of exposure of the tissues or cells during the procurement process, and the decontamination or sterilisation processes that will subsequently be applied to the tissues or cells during processing.

5.3.3. The procurement facility should be divided into different areas of adequate size to prevent improper labelling and packaging, mix-ups or cross-contamination of tissues or cells.

5.3.4. In cases of tissue/cell donation from living donors (except tissues which are surgical by-products), tissues and cells procurement must be carried out in an area appropriately equipped for the initial treatment of donors experiencing adverse reactions associated with the donation. Access to an intensive care unit and or emergency service must be available, where applicable.

5.4. **Processing area**

5.4.1. The adequacy of the processing and in-process storage areas should permit the orderly and logical positioning of equipment and materials so as to minimise the risk of cross-contamination and to minimise the risk of errors or omission or wrong application of any of the processing or control steps.

5.4.2. Processing of tissues and cells that are exposed to the environment, but are not subject to a subsequent microbial inactivation process, must take place in an environment with specified air quality with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current EU GMP Annex I and Directive 2003/94/EC and with a background environment appropriate for the processing of the tissues and cells concerned, but at least equivalent to GMP Grade D in terms of particles and microbial counts.

5.4.2.1. While Grade D is specified as the minimum background environment, the actual background environment which is utilised should be selected and justified on the basis of an

evaluation of the risks associated with the processing, testing and implantation of the types of tissues and cells concerned. Some national requirements may specify Grade C or B backgrounds for certain processes or types of tissue or cell.

5.4.2.2. A less stringent processing environment than Grade A with background D may be acceptable where:

5.4.2.2.1. a validated microbial inactivation or validated terminal sterilisation process is applied, or

5.4.2.2.2. where it is demonstrated that exposure in a Grade A environment has a detrimental effect on the required properties of the tissue or cell, or

5.4.2.2.3. where it is demonstrated that the mode and route of application of the tissue or cell to the recipient implies a significantly lower risk of transmitting bacterial or fungal infection to the recipient than with cell and tissue transplantation, or

5.4.2.2.4. where it is not technically possible to carry out the required process in a Grade A environment.

5.4.2.3. The risk assessment for determination of the processing environment should consider potentially significant factors, including:

5.4.2.3.1. tissue or cell contamination during open *versus* closed processing;

5.4.2.3.2. effectiveness of the processing method to remove contaminants;

5.4.2.3.3. suboptimal detection of contaminants due to the sampling method;

5.4.2.3.4. transfer of contaminants at transplantation.

5.4.2.4. The associated guidelines on environmental monitoring, relevant to the determined classification for the processing environment, should be considered at least in the context of a risk-based analysis which takes full account of the specific protocols and risk-mitigation strategies relevant to the risks associated with the processing, testing and implantation of the types of tissues and cells concerned.

5.4.3. Processing environments should be qualified and monitored in accordance with EN ISO 14644, EN 17141 and EU GMP Annex 1.

5.4.4. Critical facility parameters identified to be a risk to the tissues and cells, such as temperature, humidity, air-supply conditions, pressure differentials, particle numbers and microbial contamination, must be checked, monitored and recorded.

5.4.5. Environmental monitoring programmes are an important tool by which the effectiveness of contamination-control measures can be assessed. The environmental monitoring programme should include an assessment of non-viable and viable contamination and air-pressure differentials.

5.4.6. The monitoring locations should be determined having regard to the risks (e.g. at locations posing the highest risk of contamination) and the results obtained during the qualification of the premises.

5.4.7. The number of samples, volume, frequency of monitoring, alert and action limits should be appropriate, taking into account the risks and the overall control strategy for the establishment. Sampling methods should not pose a risk of contamination to the processing activities.

5.4.8. **Non-viable particulate monitoring**

5.4.8.1. Airborne particle monitoring systems should be established to obtain data for assessing potential contamination risks and to ensure maintenance of the designated environment in the cleanroom. Environmental monitoring is also expected for isolators and biosafety cabinets.

5.4.8.2. The degree of environmental control of non-viable particulates and the selection of the monitoring system should be adapted to the specific risks of tissues and cells and of the preparation process/processing (e.g. live organisms). The frequency, sampling volume or duration, alert limits and corrective actions should be established case by case having regard to the risks. It is not necessary for the sample volume to be the same as that used for qualification of the cleanroom.

5.4.8.3. Appropriate alert and actions limits should be defined. With a view to identifying potential changes that may be detrimental to the process, the alert limits for grades B to D should be lower than those specified as action limits and should be based on the area performance.

5.4.8.4. The monitoring system should ensure that, when alert limits are exceeded, the event is rapidly identified (e.g. alarm settings). If action limits are exceeded, appropriate corrective actions should be taken. These should be documented.

5.4.8.5. The maximum permitted particle concentrations in accordance with Annex 1 of EU GMP and ISO 14644 are shown in the table here:

Classification		Maximal number of particles/m^3					
ISO 14644-1	EU GMP	ISO 14644-1	EU GMP				
				at rest		in operation	
		≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
ISO 5	A	3 520	not applicable	3 520	20	3 520	20
	B			3 520	29	352 000	2 900
ISO 6		35 200	293				
ISO 7	C	352 000	2 930	352 000	2 900	3 520 000	29 000
ISO 8	D	3 520 000	29 300	3 520 000	29 000	not defined*	not defined*

* Limits have to be determined by the Tissue Establishment as defined in 5.4.8.3

5.4.8.6. When the risk assessment (see 5.4.2.3) concludes that the most stringent air quality standard is required to achieve an acceptable level of risk, then normally Grade A in full compliance with GMP for aseptic processing (i.e. with a Grade B background, accessed via grades C and D) should be applied. This implies that particle monitoring should be undertaken for the full duration of critical processing, including equipment assembly, except where duly justified (e.g. contaminants in the process that would damage the particle counter, production of particles by a process itself, e.g. bone cutting or grinding, or when this would present a hazard to the tissues or cells). In such cases, monitoring during equipment set-up operations should take place (i.e. prior to exposure of the tissues and cells to the hazard). For this most stringent standard, monitoring should also be performed during simulated operations.

5.4.8.7. For Grade B areas, there should be particle monitoring during critical operations, within the limitation referred to in 5.4.8.6, albeit the monitoring does not need to cover the entire duration of the critical processing. The Grade B area should be monitored at an appropriate frequency and with suitable sample size to permit the detection of changes in levels of contamination.

5.4.8.8. The monitoring strategy for grades C and D should be set having regard to the risks and in particular the nature of the operations conducted.

5.4.8.9. When there are no critical operations ongoing (i.e. at rest), sampling at appropriate intervals should be conducted. While at rest, the heating, ventilating and air-conditioning (HVAC) system should not be interrupted, as this may trigger the need for requalification. In the event of an interruption, a risk assessment should be conducted to determine any actions that may be required, taking account of the activities performed in the affected areas (e.g. additional monitoring).

5.4.8.10. While not required for qualification purposes, the monitoring of the ≥ 5.0 µm particle concentration in Grade A and B areas is required for routine monitoring purposes as it is an important diagnostic tool for early detection of failures. While the occasional indication of ≥ 5.0 µm particle counts may be false counts, consecutive or regular counting of low levels is an indicator of a possible contamination and it should be investigated. Such events may, for example, be indicative of early failure of the HVAC system or filling equipment failure, or it may be diagnostic of poor practices during machine set-up and routine operation.

5.4.9. **Microbiological monitoring**

5.4.9.1. Checks to detect the presence of specific micro-organisms in the cleanroom (e.g. bacteria and fungi) should be performed as appropriate. Viable particle monitoring is also expected for isolators and biosafety cabinets.

5.4.9.2. Where aseptic operations are performed, monitoring should be frequent, using methods such as settle plates, volumetric air and surface sampling (e.g. swabs and contact plates).

Rapid microbial monitoring methods should be considered and may be adopted after qualification of the premises.

5.4.9.3. The frequency of sampling should take into account the processes and activities of the staff. Aseptic process operations performed in a Grade A or B environment must be monitored routinely, during every process. Background and surrounding areas should be monitored periodically, at a pre-defined frequency.

5.4.9.4. The following recommended maximum limits apply for microbiological monitoring of clean areas during activity in accordance with Annex 1 of EU GMP:

	Recommended limits for microbial contamination			
Grade	***Air sample (CFU/m³)***	***Settle plates, diam. 90 mm (CFU/4 hours)****	***Contact plates, diam. 55 mm (CFU/plate)***	***Glove print, 5 fingers (CFU/glove)***
A†	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	not applicable
D	200	100	50	not applicable

* Individual settle plates may be exposed for less than 4 h. Where settle plates are exposed for less than 4 h the limits in the table should still be used. Settle plates should be exposed for the duration of critical operations and changed as required after 4 h.

† For grade A the expected result should be 0 CFU recovered; and recovery of 1 CFU or greater should result in an investigation.

5.4.9.5. Appropriate alert and actions limits should be defined. With a view to identifying potential changes that may be detrimental to the process, the alert limits for grades B to D should be lower than those specified as action limits and should be based on the characteristics of the work area.

5.4.9.6. If micro-organisms are detected in a Grade A and B areas, they should be identified to genus and species level and the impact thereof on tissues and cells quality and on the suitability of the premises for the intended operations should be assessed.

5.4.9.6.1. Whatever the grade, fungi detection leads to a deviation and requires an investigation.

5.4.10. **Air pressure**

5.4.10.1. An essential part of contamination prevention is the adequate separation of areas of operation. To maintain air quality, it is important to achieve a proper airflow from areas of higher cleanliness to adjacent less clean areas. It is fundamental for rooms of higher air cleanliness to have a substantial positive pressure differential relative to adjacent rooms of lower air cleanliness. For cleanrooms these pressure cascades should be clearly defined and continuously monitored with appropriate methods (e.g. alarm settings). Adjacent rooms of different grades should have a minimum pressure differential of at least 10 Pa.

5.4.10.2. Negative pressure may be required in specific areas for containment reasons (e.g. handling of viral-positive material). In such cases, the negative pressure areas should be surrounded by a positive pressure clean area of appropriate grade.

5.5. Storage areas

5.5.1. Storage rooms should be located in a secure area and access must be limited to authorised personnel.

5.5.2. Storage areas should be of appropriate size to allow orderly storage of materials and reagents, and of tissues and cells.

5.5.3. Dedicated areas must be available for storing tissues and cells in quarantine, and/or for storing unqualified materials.

5.5.4. Storage areas for tissues and cells should be maintained within defined temperature limits. Where special storage conditions are required (e.g. temperature, humidity) these must be checked, monitored and recorded.

5.5.5. An alarm system must be in place to alert users in a timely manner to any deviation from the pre-defined storage conditions. Alarm systems placed in storage devices must be continuously active and able to alert personnel on a 24-hour basis. The functionality of the alarm systems must be checked regularly.

5.5.6. Provision must be in place in the event of equipment or power failure.

5.5.7. Oxygen sensors must be appropriately placed and personal protection equipment must be available in areas where liquid nitrogen is present.

5.6. Ancillary areas

5.6.1. Suitable facilities for changing clothes and for washing hands should be readily accessible.

5.6.2. Staff rest and refreshment areas should be separate from other rooms.

5.6.3. Archive store and administrative areas should be protected against unauthorised access to ensure that records and documents are maintained in a confidential manner as required by applicable laws and regulations.

6. Equipment and materials

6.1. General requirements

6.1.1. TEs must have equipment and materials appropriate to the activities for which they are authorised.

6.1.2. All critical equipment must be designed, located, qualified and maintained to suit its intended purpose and must comply with the general safety requirements of this Guide and the specific requirements relevant to the type(s) of tissues and cells.

6.1.3. Equipment with an appropriate range and precision for measuring, weighing, recording and control should be available, and must be calibrated and checked at defined intervals using appropriate methods.

6.1.4. All critical equipment must be identified and qualified, regularly inspected and preventively maintained in accordance with the manufacturers' instructions. The qualification and maintenance status of each item of equipment must be available.

6.1.5. Where equipment or materials affect critical processing or storage parameters (e.g. temperature, pressure, particle counts, microbial contamination levels), they must be identified and must be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times.

6.1.6. A temperature monitoring system must be utilised to document temperatures and to alert staff when temperatures have deviated from acceptable limits. Procedures should be in place for reviewing temperatures. If storage utilises liquid nitrogen, either liquid nitrogen levels or temperature must be monitored and documented at an interval specified in the standard operating procedures (SOPs) and determined by validation.

6.1.7. Procedures for the operation of each piece of critical equipment, detailing the action to be taken in the event of malfunctions or failure, must be available and appropriate records must be kept.

6.1.8. The services that could impact on the tissues/cells quality (i.e. compressed air, heating, ventilating and air conditioning) should be qualified and scheduled in a maintenance programme.

6.1.9. Equipment must be selected to minimise any hazard to donors, personnel or tissues and cells.

6.1.10. All validated processes must use qualified equipment. For any equipment used in validated processes, qualification results must be documented, regular maintenance and calibration must be carried out and documented according to established procedures, and the qualification and maintenance status of each item of equipment must be available.

6.1.11. Records of maintenance activities should be clear and comprehensible and should detail the specific activities performed as part of maintenance.

6.1.12. All modifications, enhancements or additions to qualified systems and equipment should be managed through the change-control procedure of the TE. The effect of each change to the system or equipment, as well as its impact on quality and safety, should be determined to identify the extent of revalidation required.

6.1.13. Instructions for use, maintenance, servicing, cleaning, disinfection and sanitation must be available. These activities must be performed regularly and recorded accordingly.

6.1.14. Repair and maintenance operations should not present any hazard to the donor or staff, or to the quality of the tissues and cells or tissues and cells components.

6.1.15. Equipment should be designed or selected so that it can be thoroughly cleaned and, where necessary, decontaminated. This should be performed according to detailed and written procedures.

6.1.16. Washing/cleaning solutions and equipment should be chosen and used so that they are not sources of contamination or toxicity.

6.1.17. Equipment should be installed in such a way as to prevent any risk of error or of contamination.

6.1.18. Fixed pipework should be clearly labelled to indicate the contents and, where applicable, the direction of flow.

6.1.19. Distilled, deionised and, where appropriate, other water pipes should be sanitised according to written procedures that detail the action limits for microbiological contamination and the measures to be taken.

6.2. **Calibration and monitoring of equipment**

6.2.1. There should be a calibration plan that defines the principles that decide which equipment needs to be calibrated and the frequency of recalibration.

6.2.2. All equipment with a critical measuring function must be calibrated against a traceable standard if available. Adequate records of such tests should be maintained, including the values obtained prior to any adjustment. The measurement result of each piece of critical measuring equipment or system should be traceable through a documented and unbroken chain of calibrations back to the International System of Units or to a recognised standard. Calibration reports should include confidence limits of the calibration method. The report and/or calibration certificate must be reviewed and signed to show acceptance of the document. Any failed calibrations require investigation of the potential impact.

6.2.3. Observation of trends and analyses of calibration and monitoring results should be a continuous process. Intervals of calibration and monitoring should be determined for each item of equipment to achieve and maintain a desired level of accuracy and quality. The calibration and monitoring procedure should be based on a recognised international standard. The calibration status of all equipment that requires calibration should be readily available.

6.2.4. To ensure appropriate performance of a system or equipment, a monitoring plan should be developed and implemented. The plan should take into account the criticality of the system or equipment, and should specify monitoring, user-notification and problem-resolution mechanisms. When appropriate, equipment should be subject to continuous monitoring linked to an alarm system. If an unusual event is observed,

personnel should follow the standard response described in the monitoring plan. The standard response should involve the notification of affected personnel and, where appropriate, initiation of a resolution response to the problem and risk assessment of the affected tissues and cells. Depending on the severity of the problem and the criticality of the system or equipment, a back-up plan should be in place and be implemented to keep the process or system operating.

6.2.5. Assessment of the performance of equipment should occur in the following situations:

6.2.5.1. upon commissioning of new equipment, which must include the design, installation, and operational and performance qualifications;

6.2.5.2. after any relocation, repairs or adjustments that might potentially alter the functioning of equipment;

6.2.5.3. after any major repair or modification, when the equipment should be checked and qualified before its release;

6.2.5.4. if ever a doubt arises that the equipment is not functioning appropriately.

6.2.6. The ability of a supplier to maintain its activities relating to a system or equipment must be requalified on a regular basis; notably to anticipate weaknesses in services or to manage changes in the system, equipment or supplier. The periodicity and detail of the requalification process should be linked to the level of risk of using the system or equipment and should be planned for each supplier.

6.2.7. Defective equipment should be labelled clearly as such and, if possible, removed from processing areas.

6.3. **Data-processing systems**

6.3.1. If computerised systems are used, software, hardware and back-up procedures should be validated/qualified before use, be checked regularly to ensure reliability and be maintained in a validated/qualified state. Hardware and software must be protected against unauthorised use or unauthorised changes. The back-up procedure must prevent loss of or damage to data at expected and unexpected downtimes or function failures.

6.3.2. Systems must be properly maintained at all times. Documented maintenance plans must be developed and implemented.

6.3.3. Changes in computerised systems must be validated; applicable documentation must be revised, and relevant personnel trained appropriately, before any critical change is introduced into routine use. Computerised systems must be maintained in a validated /qualified state. This must include user testing to demonstrate that the system is correctly performing all specified functions at initial installation. If re-verification analysis is needed, it should be based on risk assessment and conducted not only for verification of the individual change, but also to determine the extent and impact of that change on the entire computerised system.

6.3.4. There must be a hierarchy of permitted user access to enter, amend, read or print data. Methods of preventing unauthorised entry must be in place, such as personal identity codes or passwords that are changed regularly.

6.3.5. All necessary measures must be taken to ensure protection of data. These measures must ensure that safeguards against unauthorised additions, deletions or modifications of data and transfer of information are in place, to resolve data discrepancies and to prevent unauthorised disclosure of such information.

6.3.6. Computer systems designed to control decisions related to inventories and release of tissues and cells should prevent the release of all tissues and cells considered not acceptable for release. Preventing release of any tissues and cells from a future donation from a deferred donor should be possible.

6.4. **Equipment and materials for procurement and processing and storage**

6.4.1. Critical equipment and materials must meet documented requirements and specifications and, when applicable, Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC and Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. In this context, 'critical' means those items of equipment and materials that come in contact with the tissues or cells or influence the critical quality/safety attributes of the tissues and cells directly (e.g. an additive) or indirectly (e.g. donor testing kits).

6.4.2. Procurement, processing and storage equipment must be managed in accordance with the standards and specifications laid down in the directives and with due regard to relevant national and international regulation, standards and guidelines covering the sterilisation of medicines and medical devices.

6.4.3. Sterile instruments and devices must be used for tissue and cell procurement. Instruments or devices must be of good quality, qualified or specifically certified and regularly maintained for the procurement of tissues and cells.

6.4.4. Wherever possible, only Conformité Européenne (CE) marked medical devices should be used, and all relevant staff must have received appropriate training on the use of such devices.

6.4.4.1. Medical devices manufactured within a health institution, and lacking CE marking, may be used within the institution only if they comply with the requirements in Regulation (EU) No. 2017/746, Article 5, 5.

6.4.4. When reusable instruments must be used, a validated cleaning and sterilisation procedure for removal of infectious agents should be in place.

6.4.6. Materials and parts of equipment that come into contact with tissues and cells should not be reactive, additive or absorptive to such an extent that they affect the quality of the tissues and cells and thus present any hazard.

6.4.7. A written SOP must be in place to regulate the specific materials that come into contact with tissues and cells during processing, the addition of therapeutic products to tissues and cells, the choice of those media and products, their characteristics, their source and control and the associated requirements for asepsis and labelling. A procedure to select the materials must be in place.

6.4.8. A controlled list should be constructed of all materials that come into contact with the tissues or cells or that influence the quality or safety of the tissues or cells. Detailed specifications for such critical reagents and consumables must be documented.

6.4.9. Only materials from qualified suppliers that meet the documented specifications should be used.

6.4.10 All changes to equipment and materials should be managed in accordance with the principles of change control.

6.4.11. Documented systems for purchasing equipment and materials should be available. These should identify the specific requirements for establishing and reviewing contracts for the supply of both equipment and materials.

6.4.12. The contracting process should include:

6.4.12.1. checks prior to awarding the contract to help ensure that suppliers meet the organisation's needs;

6.4.12.2. appropriate checks on received goods to confirm that they meet specifications;

6.4.12.3. the requirement for manufacturers to provide a certificate of analysis for critical material;

6.4.12.4. checks to ensure that goods in use continue to meet specifications;

6.4.12.5. regular contact with suppliers to help understand and resolve problems;

6.4.12.6. performance of periodic risk-based audits.

6.4.13. Specifications for the material in contact with tissues and cell should include, if applicable:

6.4.13.1. a description of the materials, including the designated name and the internal code reference; the reference, if any, to a pharmacopoeia monograph; the approved suppliers and, if possible, the original producer of the products; a specimen of printed materials;

6.4.13.2. directions for sampling and testing or reference to procedures;

6.4.13.3. qualitative and quantitative requirements with acceptance limits;

6.4.13.4. storage conditions and precautions;

6.4.13.5. the maximum period of storage before re-examination.

6.4.14. When using processing media and/or added therapeutic products, their source, lot number and expiry date must be recorded in the relevant processing documentation.

6.4.15. Materials used during the processing of tissues and cells should be qualified as being:

6.4.15.1. free of viral contamination (certificate should be available);

6.4.15.2. free of transmissible spongiform encephalopathy (TSE) contamination (certificate should be available);

6.4.15.3. produced under Medical Device Regulation conditions when available;

6.4.15.4. suitable for human use when available;

6.4.15.5. of defined identity, purity, sterility and quantification of endotoxins;

6.4.15.6. free of human/animal origin reagents if possible;

6.4.15.7. free of antibiotics whenever possible;

6.4.15.8. with quantified final residues of reagents whenever possible;

6.4.15.9. with risk assessment of potential residues in the final tissues and cells.

6.4.16. The specifications of the materials used to perform any evaluation of the donor should be described and these materials should not have any negative impact on the maintenance of the donors and reliability of the evaluation results.

6.4.17. Only materials from approved suppliers that meet their documented requirements and specifications must be used. Batch acceptance testing or checking of each delivery of materials should be carried out, and documented, before release for use in tissue or cell procurement or processing. Critical materials should be released by a person trained and authorised to perform this task.

6.4.18. Manufacturers of sterile materials should provide a certificate of release for each batch. The TE should define acceptance criteria for such certificates in writing, and should include at least the name of the material, manufacturer, compliance with relevant requirements (e.g. pharmacopoeias or regulations for medical devices) and confirmation that the materials are sterile and pyrogen-free as appropriate.

6.4.19. All incoming materials should be checked to ensure that the consignment corresponds to the order.

6.4.20. The status of materials (quarantined, released, rejected) should be indicated clearly.

6.4.21. Materials should be stored under the conditions established by the manufacturer and in an orderly manner that permits segregation by status, batch and lot as well as stock rotation.

6.4.22. Storage and use of materials should follow the 'first-in first-out' principle (i.e. the material that entered storage first should be used first) taking into account the expiry date of materials.

7. Qualification, verification and validation

7.1. General principles

7.1.1. TEs should establish a system to document evidence that provides a high degree of assurance that a specific process, piece of equipment or environment will consistently produce processed tissues and cells meeting pre-determined specifications and quality attributes.

7.1.2. All critical equipment and technical devices must be identified and qualified.

7.1.3. All critical processing procedures must be verified or validated and must not render the tissues and cells clinically ineffective or be harmful to the recipient.

7.1.4. TEs should identify what verification or validation work is needed to prove control of the critical aspects of their particular processes.

7.1.5. Any significant changes to the facilities, equipment or processes that might affect the quality of the tissues and cells should be qualified/verified/validated, and authorised by a Health Authority if applicable.

7.1.6. A risk-assessment approach should be used to determine the scope and extent of validation/verification/qualification. Such risk assessment should take into account all the equipment (e.g. autoclave, incubator, freeze dryer), facilities (e.g. cleanrooms, laminar flow module), electronic systems (e.g. cleanrooms environmental monitoring system, tissues processing system) and processes (e.g. musculoskeletal processing, skin processing, cleanrooms disinfection, tissue transport, analytical methods) which may affect the quality of processed tissues and cells.

7.1.7. The results from the risk-assessment study of the scope of validation/verification/qualification activities within a TE should be covered in a Validation Master Plan.

7.2. Documentation

7.2.1. As a minimum, the Validation Master Plan should consist of:

7.2.1.1. description of the TE;

7.2.1.2. list of equipment, facilities, electronic systems and processes that need to be qualified , verified or validated;

7.2.1.3. current state of validation/verification/qualification of each element within the scope of the Plan;

7.2.1.4. validation/verification/qualification programme;

7.2.1.5. validation/verification/qualification activities and who is responsible;

7.2.1.6. procedures related to validation/verification/qualification activities;

7.2.1.7. criteria for requalification, reverification or revalidation.

7.2.2. The activities of qualification verification or validation should be described in a protocol containing at least:

7.2.2.1. the objective(s);

7.2.2.2. scope of the activities;

7.2.2.3. who is responsible;

7.2.2.4. any related documents;

7.2.2.5. the stages of qualification, verification or validation;

7.2.2.6. the acceptance criteria.

7.2.3. A validation/verification/qualification report should be issued reflecting the results of the activities containing at least:

7.2.3.1. the objective(s);

7.2.3.2. scope of the activity;

7.2.3.3. who was responsible;

7.2.3.4. any related documents;

7.2.3.5. any deviations from the protocol;

7.2.3.6. detailed results;

7.2.3.7. conclusions.

7.3. **Facility, system and equipment qualification**

7.3.1. **Qualification for new facilities, systems and equipment**

7.3.1.1. The qualification of new facilities, systems or equipment begins with Design Qualification (DQ) and progresses successively through Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ).

7.3.1.1.1. DQ is the documented verification that the proposed design of the facilities, equipment or system is suitable for the intended purpose. During DQ the compliance of the design with good practice should be demonstrated and documented.

7.3.1.1.2. IQ is the documented verification that the equipment or system, as installed or modified, complies with the approved design, the manufacturer's recommendations and/or

user requirements. IQ should be performed on all critical facilities, systems and equipment. The IQ protocol should include, but need not be limited to, the following:

7.3.1.1.2.1. verification that all facilities and equipment comply with the requirements of the purchase order;

7.3.1.1.2.2. verification of CE-approval where required;

7.3.1.1.2.3. verification that the location and environmental conditions of the equipment/installation are correct according to the manufacturer's recommendations and internal specifications;

7.3.1.1.2.4. verification that items are installed in accordance with internal specifications and identified correctly with the manufacturer;

7.3.1.1.2.5. verification of serial numbers of all items/parts;

7.3.1.1.2.6. verification that all parts of the equipment are free from defects;

7.3.1.1.2.7. verification that the connections of electricity, water, steam, pressure, vacuum, etc. are functional and that their operating ranges are appropriate to the proper functioning of the installation;

7.3.1.1.2.8. identification of the items that require calibration, with checks of appropriate calibration certificates and documentation of the programme and procedure for periodic calibration;

7.3.1.1.2.9. verification of the existence of instructions for performing preventive maintenance.

7.3.1.1.3. OQ is the documented verification that the equipment or system, as installed or modified, performs as intended throughout the anticipated operating ranges. OQ should follow successful completion of IQ. The OQ protocol should include, but need not be limited to, the following:

7.3.1.1.3.1. tests that have been developed from knowledge of processes, systems and equipment;

7.3.1.1.3.2. tests to include a condition or a set of conditions encompassing upper and lower operating limits, sometimes referred to as 'worst case' conditions;

7.3.1.1.3.3. identification of critical operating variables, tests performed, alarms, security devices and acceptance criteria;

7.3.1.1.3.4. verification that the operation of various items of equipment/installation that are connected to the mains and put into operation is correct.

7.3.1.1.4. The completion of a successful OQ should allow the finalisation of calibration, operating and cleaning procedures, operator training and preventive maintenance requirements. It should permit a formal 'release' of the facilities, systems and equipment.

7.3.1.1.5. PQ is the documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly, based on the approved

process method and specifications. PQ should follow successful completion of IQ and OQ. Although PQ is described as a separate activity, it may in some cases be appropriate to perform it in conjunction with OQ, or concurrently with processing activities. The PQ protocol should include, but need not be limited to, the following:

7.3.1.1.5.1. tests, using materials, qualified substitutes or simulated tissues and cells, that have been developed from knowledge of the process and the facilities, systems or equipment;

7.3.1.1.5.2. tests that include a condition or set of conditions encompassing upper and lower operating limits;

7.3.1.1.5.3. a process description or reference to protocol development;

7.3.1.1.5.4. a list of equipment involved;

7.3.1.1.5.5. the critical parameters and operating ranges;

7.3.1.1.5.6. a description of the tests to be performed, or control variables, sample taking, time and reference method sampling and analytical methods;

7.3.1.1.5.7. the acceptance criteria.

7.3.2. **Qualification of established (in-use) facilities, systems and equipment**

7.3.2.1. Evidence should be available to support and verify the operating parameters and limits for the critical variables of the operating equipment.

7.3.2.2. The procedures and records for calibration, cleaning, preventive maintenance, operating and operator training of the facilities, systems and/or equipment in use should be documented.

7.3.3. **Qualification of cleanrooms**

7.3.3.1. Processing environments should be qualified and monitored in accordance with the requirements of EU GMP Annex 1 and ISO EN 14644 and EN 17141. Depending on the risk assessment and the structure of the environment, optional test methods to consider are:

7.3.3.1.1. air change (renewal) rate per hour within one room checked against specification;

7.3.3.1.2. airflow-visualisation test;

7.3.3.1.3. absolute filters integrity – the grade of sealing of the filters and the absence of leaks in the filter material checked;

7.3.3.1.4. total count of airborne particles (viable or not) be checked according to specifications;

7.3.3.1.5. temperature and relative humidity recorded during the test and checked according to specifications;

7.3.3.1.6. pressure differential between areas checked according to specifications;

7.3.3.1.7. recovery test (normally tested for Grade A and B cleanrooms), i.e. check the time required for a cleanroom to recover the specified classification after an out-of-specification period;

7.3.3.1.8. laminar flow velocities in laminar flow areas;

7.3.3.1.9. HVAC system operations and alarms;

7.3.3.1.10. electricity back-up systems.

7.3.3.2. All these tests should be performed at least in an 'at rest' situation. The particle counting test should also be performed in an 'in operation' situation.

7.3.4. **Qualification of laminar flow hoods**

7.3.4.1. The tests to be carried out for laminar flow hoods should include:

7.3.4.1.1. speed and uniformity of the airflow; check that the airflow speed, when measured at a set distance from the HEPA filter, is within a defined range. This measurement should be repeated at a specified number of locations within the laminar flow hood, which each measurement being within the defined range;

7.3.4.1.2. absolute filters integrity – check the grade of sealing of the filters and the absence of leaks in the filter material;

7.3.4.1.3. particle counting – check the total count of airborne particles (viable or not) according to specifications;

7.3.4.1.4. electronic test – check all the operating controls (light, ultra-violet light, fan) and alarms;

7.3.4.1.5. airflow visualisation (for biological safety cabinets), where the test objective is to study the behaviour of air inside and outside the cabin with the help of a smoke generator.

7.3.4.2. All these tests should be performed at least in an 'at rest' situation; the particle-counting test should also be performed in an 'in operation' situation.

7.4. **Process verification or validation**

7.4.1. Facilities, systems and equipment to be used should be qualified before use, and analytical testing methods should be verified or validated.

7.4.2. Processes that have been in use for some time should also be validated.

7.4.3. Staff taking part in the validation work should have been appropriately trained.

7.4.4. All facilities, systems, equipment and processes should be periodically evaluated to verify that they are still operating in a valid manner.

7.4.5. **Prospective verification or validation**

7.4.5.1. Process verification or validation should normally be completed prior to the distribution of any tissue or cell; this is called prospective verification or validation.

7.4.5.2. Prospective verification or validation should include, but need not be limited to, the following:

7.4.5.2.1. short description of the process;

7.4.5.2.2. summary of the critical processing steps to be investigated;

7.4.5.2.3. list of the equipment/facilities to be used (including measuring, monitoring and/or recording equipment) together with its calibration status;

7.4.5.2.4. specifications for release of the processed tissues and cells;

7.4.5.2.5. list of analytical methods, as appropriate;

7.4.5.2.6. proposed in-process controls with acceptance criteria;

7.4.5.2.7. additional testing to be carried out, with acceptance criteria and analytical verification/validation, as appropriate;

7.4.5.2.8. sampling plan;

7.4.5.2.9. methods for recording and evaluating results;

7.4.5.2.10. teams/departments and persons responsible;

7.4.5.2.11. proposed timetable.

7.4.5.3. Using this defined process (including specified components) of prospective verification/validation, a series of batches of the final tissues or cells may be produced under routine conditions.

7.4.5.4. The number of process runs carried out and observations made should be sufficient to allow the normal extent of variation and trends to be established and to provide sufficient data for evaluation.

7.4.5.5. Batches, where applicable, made for process verification or validation should be the same size as the routine scale batches.

7.4.6. **Concurrent validation**

7.4.6.1. In exceptional circumstances it may be acceptable not to complete a validation programme before routine processing starts and instead to validate a process during routine processing (concurrent validation). The decision to carry out concurrent validation must be justified, documented and approved by authorised personnel.

7.4.6.2. Documentation requirements for concurrent validation are the same as specified for prospective validation.

7.4.7. **Retrospective verification**

7.4.7.1. Retrospective verification is only acceptable for well-established processes. Verification of such processes should be based on historical data. The steps involved require prepa-

ration of a specific protocol and reporting of the results of the data review, leading to a conclusion and a recommendation.

7.4.7.2. Retrospective verification is not appropriate where there have been recent changes in relation to starting materials, the tissues or cells, operating procedures or equipment.

7.4.7.3. The source of data for this verification should include, but need not be limited to:

7.4.7.3.1. batch processing and packaging records;

7.4.7.3.2. process-control charts;

7.4.7.3.3. maintenance records;

7.4.7.3.4. records of personnel changes;

7.4.7.3.5. process-capability studies;

7.4.7.3.6. processed tissues and cells data;

7.4.7.3.7. storage-stability results.

7.4.7.4. Batches selected for retrospective verification should be representative of all batches made during the review period, including any batches that failed to meet specifications, and should be sufficient in number to demonstrate process consistency.

7.4.7.5. Additional testing of retained samples may be needed to obtain the necessary amount or type of data to retrospectively verify the process.

7.4.7.6. Retrospective verification generally requires that data from ten to thirty consecutive batches be examined to assess process consistency, but fewer batches may be examined if this can be justified; the justification must be documented.

7.5. Cleaning and disinfection verification or validation

7.5.1. Cleaning and disinfection verification or validation should be performed in order to confirm the effectiveness of a cleaning or disinfection procedure.

7.5.2. Cleaning or disinfection intervals and methods should be determined.

7.5.3. The rationale for selecting the limits for carry-over of tissues and cells residues, cleaning agents and microbial contamination should be logically based on the materials involved. The limits should be achievable and verifiable.

7.5.4. The potential risk of residues used during disinfection remaining on the tissues or cells after processing has been completed, should be considered subject to a risk assessment.

7.5.5. For cleaning and disinfection procedures for tissues and cells and processes which are similar, it is considered acceptable to select a representative range of similar tissues and cells and processes. A single validation or verification study utilising a 'worst case' approach can be carried out which takes account of the critical issues.

7.5.6. The number of consecutive replicate applications necessary to validate the cleaning and disinfection procedure should be determined, based on a risk assessment.

7.6. **Revalidation or requalification**

7.6.1. Revalidation or requalification should be performed when there is a change, in any equipment, facilities or process, that is considered significant because it affects the quality of the tissues and cells. These changes should be approved through a change-control procedure.

7.6.2. When the tissues and cells quality review confirms that the system or process is consistently producing material meeting its specifications, there is no need for revalidation.

8. Donation

8.1. **General requirements**

8.1.1. All necessary measures should be taken to ensure that any promotion and publicity activities in support of the donation of human tissues and cells comply with guidelines or legislative provisions laid down by the member states and the associated restrictions or prohibitions on advertising the need for, or availability of, human tissues and cells with a view to offering or seeking financial gain or comparable advantage.

8.1.2. The donation of tissues and cells must be voluntary and unpaid. No financial gain, or inducement, or any other compensation should be given to the living donor or the deceased donor's family. In the case of unrelated living donors, an allowance to cover any costs incurred should be acceptable if justifiable and transparent. Member states must define the conditions under which compensation may be granted.

8.1.3. Any extra medical costs related to the donation process of tissues or cells (e.g. serological or bacteriological testing) must not be charged to the donor or to a deceased donor's family. These costs must be met by the TE.

8.1.4. The activities related to tissue and cell procurement must be carried out in such a way as to ensure that donor evaluation and selection is carried out in accordance with the requirements referred to in Article 28(d) and (e) of Directive 2004/23/EC.

8.1.5. In the case of an autologous donation, the suitability criteria must be established in accordance with the requirements referred to in Article 28(d) of Directive 2004/23/EC.

8.1.6. The results of the donor evaluation and testing procedures must be documented, and any major anomalies must be reported in accordance with the requirements referred to in the Annex of Directive 2004/23/EC.

8.1.7. TEs must take all necessary measures to ensure that tissue and cell procurement, packaging and transportation comply with the requirements referred to in Article 28(b), (e)

and (f) of Directive 2004/23/EC. The tests required for donors must be carried out by a laboratory accredited, designated, authorised or licensed by the Health Authority or authorities.

8.1.8. TEs must implement a system for the identification of human tissues and cells, in order to ensure the traceability of all human tissues and cells.

8.1.9. The procurement of human tissues or cells must be authorised only after all mandatory consent or authorisation requirements in force in the member state concerned have been met.

8.1.10. TEs must, in keeping with the national legislation, take all necessary measures to ensure that donors, their relatives or any persons granting authorisation on behalf of the donors are provided with all appropriate information as referred to in the Annex of Directive 2004/23/EC.

8.1.11. Before the procurement of tissues and cells proceeds, an authorised person must confirm and record:

8.1.11.1. that consent for the procurement has been obtained in accordance with Article 13 of Directive 2004/23/EC;

8.1.11.2. how and by whom the donor has been reliably identified.

8.2. **Donor recruitment/referral**

8.2.1. Procedures for recruiting living donors in an ethical manner, for ensuring their safety and well-being and for the identification and referral of all potential deceased donors should be implemented and maintained in accordance with the applicable legislation.

8.2.2. Professionals involved in the recruitment of living donors, and in the identification and referral of deceased donors, must be trained and appropriately qualified.

8.2.3. In the case of both living and deceased donors, screening must be performed to exclude any contraindications to donation and, in the case of a living donor, to exclude any medical situation that could potentially harm the donors themselves.

8.2.4. A coding system must be in place, with unique codes physically attached and recorded in documents and/or electronic data, to guarantee traceability and biovigilance at all stages from donor screening until tissue and cell application (e.g. the donor is identified with a wrist band and/or different labels attached to the body). A donation code must be applied to all tissues procured. The donation code must be traceable to the donor code.

8.2.5. The coding system must be designed to relate all transplants of a certain donor to a unique donor number in order to guarantee traceability and biovigilance from donor screening until tissue transplantation.

8.2.6. A potential donor should receive a donor identification number/donor code before any further procedures are started. All documentation and/or electronic data that are collected from the donor should be traceable to this number. All body materials (e.g. blood, tissue, fluid) that are procured from this donor must be given a donation number, traceable to the donor identity number/donor code.

8.2.7. The method of verifying the donor's identity should be described in an identification procedure. This procedure should be followed before starting the procurement and should enable the identity of the donor to be established beyond any doubt. The verification should be performed based on at least two independent factors like date of birth and name, or name and hospital patient number.

8.2.8. The source of the donor's identity should be documented. For living donors this should include officially recognised means of identification such as identity cards, passports, etc. For deceased donors, the presence of toe tags, wrist bands or other confirmation of the deceased's identity should be noted. At least two independent forms of identification, such as name, date of birth, address or hospital number, must be used to verify a deceased donor's identity.

8.2.9. **Living donors**

8.2.9.1. Recruitment of donors must be voluntary and unpaid, and informed consent must be obtained in advance.

8.2.9.2. Recruitment of non-partner donors in MAR, whether performed by public health system or by private clinics (where allowed by national legislation), must be authorised by the Health Authority, and the donation of reproductive material should strictly follow the same principles of voluntary and unpaid donation.

8.2.9.3. Recruitment of persons not able to consent should never be done through public registries. In addition, in some countries, specific regulations restrict donation in these circumstances (e.g. some countries do not allow procurement of peripheral blood progenitor cells from minors and/or administration of growth factors).

8.2.10. **Deceased donors**

8.2.10.1. Identification and referral of deceased tissue donors must be in compliance with the national deceased donation programme.

8.2.10.2. A system should be established between the procurement organisation and the corresponding TE to ensure that any deceased individual who is a potential donor can be detected within an adequate period of time to allow an effective donation.

8.3. **Donor consent**

8.3.1. Procedures or protocols for expressing consent to donation, depending on the type of donor, the specific circumstances and the different legal systems for consent, must be implemented and maintained.

There should be an authorised person who confirms and records that consent for the procurement has been obtained in accordance with the legislation in place in the member state and (where applicable) Article 13 of Directive 2004/23/EC.

8.3.3. The request for donation must be explained in understandable terms by a healthcare professional familiar with the donation process.

8.3.3.1. The discussion about consent should be conducted in a suitable environment. The person who requests the consent of the donor should have received specific training for this purpose.

8.3.4. Consent should be recorded and/or documented in the donor/patient's record.

8.3.4.1. Informed consent must be obtained for living donors. The informed consent must include an explanation, in understandable terms, of all the reasonable risk and potential harm, both for the donor and recipient, as well as all the tests to be performed.

8.3.4.2. Informed consent must be obtained from all donors for the use of their tissue and/or cell for specific purposes and for serological testing.

8.3.4.3. In the case of a living donor the person in charge of the donation process must ensure that the donor has been properly informed of at least those aspects relating to the donation and procurement process outlined in point 8.3.4.5. This information must be given prior to the procurement.

8.3.4.4. The information must be given by a trained and appropriately qualified person able to transmit it in an appropriate and clear manner, using terms that are easily understood by the donor.

8.3.4.5. The information must cover: the purpose and nature of the procurement, its consequences and risks; analytical tests, if they are performed; the recording and protection of donor data and medical confidentiality; the therapeutic purpose and potential benefits of the donation; and information on the applicable safeguards intended to protect the donor.

8.3.4.6. The living donor or their legal representative must be informed that he/she has the right to receive the confirmed results of the analytical tests, clearly explained.

8.3.4.7. Information must be given to the donor about the necessity for obtaining the applicable mandatory consent, certification and authorisation in order that the tissue and/or cell procurement can be carried out.

8.3.5. Tissue or cell procurement must not be carried out on a person who does not have the capacity to consent, other than as specified in point 8.3.5.1.

8.3.5.1. In the case of a donor who is a minor or a donor with no legal capacity, the consent must be obtained from parents or the legal representative, provided the following conditions are met:

8.3.5.1.1. there is no compatible donor available who has the capacity to consent;

8.3.5.1.2. the recipient is a brother or sister of the donor;

8.3.5.1.3. the donation has the potential to be life-saving for the recipient;

8.3.5.1.4. the authorisation of the donor's representative, or of an authority or a person or body provided for by law, has been given specifically and in writing and with the approval of the competent body;

8.3.5.1.5. the potential donor concerned does not object.

8.3.6. In the case of discarded tissue or surgical by-product, the donor must be made aware that he or she can express any intention as to how he or she desires such surgical residues to be dealt with, or else the surgical residues must be handled by the healthcare institution as it deems fit.

8.3.7. All information must be given and all necessary consents and authorisations must be obtained in accordance with the legislation in force in member states.

8.3.8. In cases where a legal process applies, judicial consent must be obtained according to local regulations before starting any procurement activities. When judicial authorisation is needed due to an unknown cause of death, the transplant co-ordinator should be the person responsible to ask consent from the judge on call and in charge of the investigation. Procurement should only be carried out if it does not affect the judicial autopsy.

8.3.9. The use of tissue from a deceased donor must be in accordance with relevant national and international legislation.

8.4. **Donor selection and evaluation**

8.4.1. Procedures for donor selection and evaluation should be implemented and maintained. They should take place before each procurement and comply with the requirements referred to above.

8.4.2. Selection criteria for donors should be based on an analysis of the risks related to the application of the specific cells/tissues. Indicators of these risks must be identified by review of the medical and behavioural history, biological testing, physical examination (for living donors) or *post mortem* examination (for deceased donors) and any other appropriate investigation.

8.4.3. The TE or procurement organisation must ensure that donors comply with the selection criteria set out in these two annexes to Directive 2006/17/EC:

8.4.3.1. Annex I for donors of tissues and cells, except donors of reproductive cells;

8.4.3.2. Annex III for donors of reproductive cells.

8.4.4. The TE or procurement organisation must ensure that:

8.4.4.1. donors of tissues and cells, except donors of reproductive cells, undergo the biological tests set out in point 1 of Annex II of Directive 2006/17/EC;

8.4.4.2. the tests referred to in point 8.4.4.1. are carried out in compliance with the general requirements set out in point 2 of Annex II of Directive 2006/17/EC;

8.4.4.3. donors of reproductive cells undergo the biological tests set out in points 1, 2 and 3 of Annex III of Directive 2006/17/EC;

8.4.4.4. the tests referred to in point 8.4.4.3 above are carried out in compliance with the general requirements set out in point 4 of Annex III of Directive 2006/17/EC.

8.4.5. In the case of living donors, the health professional responsible for obtaining the health and social history must ensure that the donor has:

8.4.5.1. understood the information provided;

8.4.5.2. had an opportunity to ask questions and been provided with satisfactory responses;

8.4.5.3. confirmed that all the information provided is true to the best of his/her knowledge.

8.4.6. An authorised person must collect and record the donor's relevant medical and behavioural information according to the requirements described in section 1.4 of Annex IV of Directive 2006/17/EC.

8.4.7. In order to acquire the appropriate information, different relevant sources must be used, including at least an interview with the donor, for living donors, and the following when appropriate:

8.4.7.1. the medical records of the donor;

8.4.7.2. an interview with a person who knew the donor well, for deceased donors;

8.4.7.3. an interview with the treating physician;

8.4.7.4. an interview with the general practitioner;

8.4.7.5. the autopsy report, for deceased donors.

8.4.8. In the case of a deceased donor, and in the case of a living donor when justified, a physical examination of the donor must be performed to detect any sign that may be sufficient in itself to exclude the donor or which must be assessed in the light of the donor's medical and personal history.

8.4.9. The complete donor records must be reviewed and assessed for the donor's suitability and signed by a qualified health professional.

8.4.10. All donor data must be recorded and kept for 30 years after the use of the donated tissue and cells or after their utilisation. Data must be protected from unauthorised viewing.

8.4.11. The donor selection and evaluation process must be performed by trained and appropriately qualified personnel in accordance with SOPs, and described in detail in records. A set of authorised SOPs should define responsibilities and describe how procedures should be carried out and by whom.

8.4.12. The following list of actions for donor selection and evaluation must be conducted and verified in accordance with point 8.4.11:

8.4.12.1. donor identification;

8.4.12.2. donor/donor family consent details;

8.4.12.3. donor's medical history (including genetic disease, a family history of disease, exclusion criteria, additional exclusion criteria for deceased child donors);

8.4.12.4. donor's social history (including personal, travel, behavioural, risk assessment);

8.4.12.5. donor's physical examination (including exclusion signs);

8.4.12.6. donor's psychological examination for living haematopoietic progenitor cell (HPC) donors and medically assisted reproduction donors;

8.4.12.7. blood sample procurement;

8.4.12.8. evaluation of test results for markers of transmissible disease;

8.4.12.9. final decision making about eligibility.

8.4.13. In the case of living donors, a face-to-face interview must be conducted, during which a standardised questionnaire should be completed. The interviews should be done, documented and signed by a trained and appropriately qualified person. For deceased donors, alternative sources of information should be used.

8.4.14. **Living donors**

8.4.14.1. *Autologous living donor*

8.4.14.1.1. If the removed tissues and cells are to be stored or cultured, the same minimum set of biological testing requirements must apply as for an allogeneic living donor.

8.4.14.1.2. Positive test results will not necessarily prevent the tissues or cells or any product derived from them being stored, processed and reimplanted, if appropriate isolated storage facilities are available to ensure no risk of cross-contamination with other grafts and/or no risk of contamination with adventitious agents and/or mix-ups.

8.4.14.2. *Allogeneic living donor (except donors of reproductive cells)*

8.4.14.2.1. Allogeneic living donors must be selected on the basis of their health and medical history, an interview by a qualified and trained healthcare professional with the donor, and clinical investigations and tests in compliance with point 8.4.14.2.3. This assessment must include relevant factors that may assist in identifying and screening out persons

whose donation could present a health risk to others, such as the possibility of transmitting diseases or health risks to themselves.

8.4.14.2.2. For any donation, the procurement process must not interfere with or compromise the health or care of the donor. In the case of cord blood or amniotic membrane donation, this applies to both mother and baby.

8.4.14.2.3. Selection criteria for allogeneic living donors must be established and documented by the TE (and the transplanting clinician in the case of direct distribution to the recipient), based on the specific tissue or cells to be donated, together with the donor's physical status and medical and behavioural history and the results of clinical investigations and laboratory tests establishing the donor's state of health.

8.4.14.2.4. The same exclusion criteria must be applied as for deceased donors with the exception of factors related to an unknown cause of death. Depending on the tissue or cell to be donated, other specific exclusion criteria may need to be added, such as:

8.4.14.2.4.1. in the case of HPC, the potential for transmission of genetic diseases.

8.4.14.3. *Donor of reproductive cells*

8.4.14.3.1. In case of direct use of reproductive cells for partner donation, applicability of laboratory testing is assessed on a case-by-case basis.

8.4.14.3.2. Reproductive cells that are processed and/or stored and reproductive cells that will result in the cryopreservation of embryos must meet the following criteria:

8.4.14.3.2.1. the clinician responsible for the donor must determine and document, based on the patient's medical history and therapeutic indications, the justification for the donation and its safety for the recipient and any child(ren) that might result;

8.4.14.3.2.2. biological tests must be carried out to assess the risk of cross-contamination in compliance with the general requirements set out in point 2 of Annex III of Directive 2006/17/EC;

8.4.14.3.2.3. positive results will not necessarily prevent partner donation in accordance with national rules.

8.4.14.3.3. In the case of donations of reproductive cells other than by partners, donor-selection criteria and laboratory testing must be applied in accordance with Annex III, point 3 of Directive 2006/17/EC.

8.4.14.3.3.1. Donors must be selected on the basis of their age, health and medical history, provided on a questionnaire, and through a personal interview performed by a qualified and trained healthcare professional.

8.4.14.3.3.2. This assessment must include relevant factors that may assist in identifying and screening out persons whose donation could present a health risk to others, such as the possibility of transmitting diseases (such as sexually transmitted infections), or health

risks to themselves (e.g. superovulation, sedation or the risks associated with the oocyte procurement procedure or the psychological consequences of being a donor).

8.4.14.3.3.3. Complete information on the associated risk and on the measures undertaken for its mitigation must be communicated and clearly explained to the recipient.

8.4.15. **Deceased donor**

8.4.15.1. National and local requirements for confirmation of death must be complied with before tissue procurement begins.

8.4.15.2. In the case of deceased donors, the cause, time and circumstances of death must be verified and recorded. The sources of information used for deceased donors must be reviewed. Transferring information from donation records to a new document should be carried out by trained and appropriately qualified staff from the TE.

8.4.15.3. Donor age criteria should be established, documented and recorded.

8.4.15.4. Donor's identity must be confirmed with at least two forms of identifiable information, such as given name and family name, date of birth or medical history number, and documented.

8.4.15.5. *General criteria for exclusion*

8.4.15.5.1. Cause of death unknown, unless autopsy provides information on the cause of death after procurement and none of the general criteria for exclusion set out in the present section applies.

8.4.15.5.2. History of a disease of unknown aetiology

8.4.15.5.3. Malignancies:

8.4.15.5.3.1. Haematological malignancies should be considered as absolute contraindications;

8.4.15.5.3.2. Non-haematological malignancies. Donors with malignancy must be excluded from donation unless justified on the basis of a documented risk assessment approved by the responsible person. A history of malignancy should be evaluated carefully to determine its effects on the quality and safety of tissue, due to the risk of either the presence of a tumour, or the treatment given to the donor for the malignancy.

8.4.15.5.3.3. Donors with malignant diseases can be evaluated and considered for cornea donation, except for those with retinoblastoma, haematological neoplasm and malignant tumours of the anterior segment of the eye. Malignant melanoma with known metastatic disease also excludes use of ocular tissue, including avascular cornea. Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should be evaluated as discussed above.

8.4.15.5.4. Risk of transmission of diseases caused by prions. This risk applies, for example, to:

8.4.15.5.4.1. history of transmissible spongiform encephalopathies (TSE), which include Creutzfeldt–Jakob disease (CJD), Gerstmann–Stäussler–Scheinker (GSS), Kuru and fatal familial insomnia (FFI);

8.4.15.5.4.2. people with a history of rapid progressive dementia or diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination) or degenerative or demyelinising disease or a disorder of unknown aetiology involving the central nervous system;

8.4.15.5.4.3. recipients of hormones derived from the human pituitary gland (such as growth hormones) and recipients of grafts of cornea, sclera and *dura mater*, and persons that have undergone undocumented neurosurgery (where *dura mater* may have been used).

8.4.15.5.5. Systemic infection which is not controlled at the time of donation, including bacterial diseases, systemic viral, fungal or parasitic infections, or significant local infection in the tissues and cells to be donated. Donors with bacterial septicaemia may be evaluated and considered for eye donation but only where the corneas are to be stored by organ culture to allow detection of any bacterial contamination of the tissue.

8.4.15.5.6. History, clinical evidence or laboratory evidence of active HIV, hepatitis B (HBV), hepatitis C (HCV) and human T-lymphotrophic virus (HTLV) I/II, including evidence of transmission risk or evidence of risk factors for these infections.

8.4.15.5.7. History of chronic, systemic autoimmune disease that could have a detrimental effect on the quality of the tissue to be retrieved.

8.4.15.5.8. Indications that test results of donor blood samples will be invalid due to:

8.4.15.5.8.1. the occurrence of haemodilution, according to the specifications in Annex II, section 2 of Directive 2006/17/EC, where a pre-transfusion sample is not available;

8.4.15.5.8.2. treatment with immunosuppressive agents that can influence the reliability of serological tests. NAT testing may be helpful in such circumstances.

8.4.15.5.9. Evidence of any other risk factors for transmissible diseases on the basis of a risk assessment, taking into consideration donor travel and exposure history and local infectious disease prevalence.

8.4.15.5.10. Presence on the donor's body of physical signs implying a risk of transmissible disease(s) as described in Annex IV, point 1.2.3 of Directive 2006/17/EC.

8.4.15.5.11. Ingestion of, or exposure to, a substance (such as cyanide, lead, mercury, gold) that may be transmitted to recipients in a dose that could endanger their health.

8.4.15.5.12. Recent history of vaccination with a live attenuated virus where a risk of transmission is considered to exist.

8.4.15.5.13. History of xenotransplantation that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues, or organs from a non-human

animal source, or (b) human body fluids, cells, tissues or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs (unless justified on the basis of a documented risk assessment).

8.4.15.6. *Additional exclusion criteria for deceased child donors*

8.4.15.6.1. Any children born from mothers with HIV infection or that meet any of the general exclusion criteria must be excluded as donors until the risk of transmission of infection can be definitely ruled out.

8.4.15.6.1.1. Children aged less than 18 months born from mothers with HIV, HBV, HCV or HTLV infection, or at risk of such infection, and who have been breastfed by their mothers during the previous 12 months, cannot be considered as donors regardless of the results of the analytical tests.

8.4.15.6.1.2. Children of mothers with HIV, HBV, HCV or HTLV infection, or at risk of such infection, and who have not been breastfed by their mothers during the previous 12 months and for whom analytical tests, physical examinations and reviews of medical records do not provide evidence of HIV, HBV, HCV or HTLV infection, can be accepted as donors.

8.4.16. Procedures should be in place to ensure that abnormal findings arising from the donor-selection and evaluation process are properly reviewed by a qualified health professional and that appropriate action is taken. The reason for rejection of a donor should be recorded.

9. Donor testing

9.1. Testing of donations for infectious markers and agents is a key factor in ensuring that the risk of disease transmission is minimised and that tissues and cells are suitable for their intended purpose.

9.2. Each donation must be tested in conformity with the requirements laid down in EU directives, especially in Annex II and III Directive 2006/17 EC.

9.3. The tests must be carried out by a qualified laboratory, authorised as a testing centre by the Health Authority, using CE-marked testing kits where appropriate. The type of test used must be validated or verified for the purpose in accordance with current scientific knowledge.

9.4. Additional donor testing for other markers or agents may be required, taking into account the epidemiological profile in any given region or country.

9.5. The tests must be carried out on the donor's serum or plasma; they must not be performed on other fluids or secretions unless specifically justified clinically using a validated or verified test for such a fluid.

9.6. TEs may accept tissues and cells from donors with haemodilution of more than 50 % only if the testing procedures used are validated or verified for such diluted plasma or if a pre-transfusion sample is available.

9.7. **Donation samples**

9.7.1. **Deceased donors**

9.7.1.1. In the case of a deceased donor, blood samples must have been obtained just prior to death or, if not possible, as soon as possible after death (within 24 hours after death). If the period is extended a validation of each infectious disease test kit using such specimens is necessary under the acceptance by national regulations.

9.7.2. **Living donors**

9.7.2.1. In the case of HPC donors, blood samples must be taken for testing within 30 days prior to donation.

9.7.2.2. In the case of non-HPC and non-reproductive cell living donors:

9.7.2.2.1. where tissues and cells are going to be stored for less than 180 days, blood samples must be obtained at the time of donation or, if not possible, within 7 days after donation;

9.7.2.2.2. where tissues and cells are going to be stored for 180 days or longer, blood samples can be taken up to 30 days prior to and 7 days after donation, and repeat sampling and testing is required after an interval of 180 days.

9.7.2.2.3. Repeat blood sampling and testing are not required after an interval of 180 days if:

9.7.2.2.3.1. the blood sample, taken at the time of procurement or within 7 days after procurement, is additionally tested by the nucleic acid amplification technique (NAT) for HIV, HBV and HCV;

9.7.2.2.3.2. the processing includes an inactivation step that has been validated for HIV, HBV and HCV.

9.7.2.3. In the case of neonatal donors, tests can be carried out on the donor's mother to avoid medically unnecessary procedures upon the infant.

9.7.2.4. In the case of reproductive non-partner donors:

9.7.2.4.1. all serum samples must be obtained at the time of donation;

9.7.2.4.2. sperm donations must be quarantined for ≥ 180 days after the last procurement, after which repeat testing is required, but quarantine is not necessary if at each donation serology testing is combined with NAT for HIV, HBV and HCV;

9.7.2.4.3. the same testing approach must be used for oocyte donors, allowing for the safe use of cryopreserved oocytes (after quarantine and re-testing after 180 days) or fresh oocytes (if NAT is done at the time of donation); oocyte donation may be considered as starting at the first day of stimulation, and the sample for testing may be taken at that time.

9.7.2.5 In the case of reproductive partner donors:

9.7.2.5.1 blood samples must be taken before the first donation and this must be done ≤ 3 months before the first donation. For further partner donations, additional blood samples must be obtained according to national legislation, but ≤ 24 months from the previous sampling.

9.8. The procedure used for the labelling of laboratory samples with donation numbers must be designed to avoid any risk of identification error and mix-up.

9.9. Upon receipt of samples at the laboratory, positive identification of the samples received against those expected should be carried out.

9.10. Laboratory personnel must be thoroughly instructed, trained and competent to operate the test system.

9.11. Each step of the handling and processing of samples should be described, as should the conditions of pre-analytical treatment of specimens (e.g. centrifugation), storage and transportation (duration, temperature, type of container, storage after testing).

9.12. There must be data confirming the suitability of any laboratory reagents used in testing of donor samples.

9.13. All laboratory testing procedures must be validated or verified before use.

9.14. Screening algorithms should be defined precisely in writing (by SOPs) for dealing with initially reactive specimens and for resolving discrepancies in results after re-testing.

9.15. If additional NAT testing is performed, a thoroughly qualified system of labelling/identification of samples should be in place.

9.16. There must be a reliable process in place for transcribing, collating and interpreting results.

9.17. There must be clearly defined procedures to resolve discrepant results. Appropriate confirmatory testing must take place. In the case of confirmed positive results, appropriate donor management must take place, including the provision of information to the donor and follow-up procedures.

9.18. The quality of the laboratory testing must be assessed regularly by participation in a formal system of proficiency testing, such as an external quality-assurance programme.

10. Procurement

10.1. General requirements

10.1.1. Procurement activities must be authorised by the appropriate Health Authority.

10.1.2. The procurement of human tissues or cells must be authorised only after all mandatory consent or authorisation requirements in force in the member state concerned have been met.

10.1.3. Procurement of human tissues or cells must take place only after donor consent/authorisation requirements have been satisfied.

10.2. **Procurement procedures**

10.2.1. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured and must be standardised. There must be procedures in place to protect the safety of the living donor.

10.2.2. The procurement procedures must protect those properties of the tissue/cells that are required for their ultimate clinical use, and at the same time minimise the risk of microbiological contamination during the process, particularly when tissues and cells cannot subsequently be sterilised.

10.2.3. Policies and procedures must be in place to minimise the risk of tissue or cell contamination by staff who might be infected with transmissible diseases.

10.2.4. The sequence in which the various tissues are procured must be well defined to assure the quality of each type of tissue.

10.2.5. If deceased donation occurs after organ donation, aseptic conditions should be ensured throughout the whole procedure, including during organ procurement.

10.2.6. Sampling for microbiological testing must be performed prior to the commencement of processing, at any point between the end of the procurement process and the commencement of any processing protocols. A validated or verified test method must be used.

10.2.7. It is recommended that tissue should be recovered within 24 h after death if the body has been cooled or refrigerated (with the aim of reducing tissue degeneration and microbiological growth) in the first 6 h after death, or within 12 h of death if the body has not been refrigerated.

10.2.8 Extension of these time limits should be validated or verified by quality assessments and by bioburden testing to guarantee quality and microbiological safety. See section 11.3.5.

10.2.9. Once the tissue is procured and until it arrives at the TE, critical variables related to maintaining the quality of the tissues or cells (e.g. temperature, sterile packaging) must be controlled and recorded.

10.2.10. Once the tissues and cells have been retrieved from a deceased donor body, the donor body must be reconstructed so that it is as similar as possible to its original anatomical appearance.

10.2.11. Where appropriate, the staff and equipment necessary for body reconstruction of deceased donors must be provided. Such reconstruction must be completed effectively.

10.3. **Procurement report**

10.3.1. The organisation performing the procurement must produce a procurement report, which is passed on to the TE. This report must contain at least:

10.3.1.1. the identification, name and address of the TE to receive the cells/tissues;

10.3.1.2. donor identification data (including how and by whom the donor was identified);

10.3.1.3. description and identification of procured tissues and cells (including samples for testing);

10.3.1.4. identification of the person who is responsible for the procurement session, including signature;

10.3.1.5. date, time (where relevant, start and end) and location of procurement, the procedure (SOP) used and any incidents that occurred; also, where relevant, environmental conditions at the procurement facility (description of the physical area where procurement took place);

10.3.1.6. for deceased donors, conditions under which the body is kept, i.e. refrigerated (or not), time of start and end of refrigeration;

10.3.1.7. ID/batch numbers of reagents and transport solutions used.

10.3.2. The report must also contain the date and time of death where possible.

10.3.3. Where sperm is procured at home, the procurement report must state this and must contain only the following:

10.3.3.1. the name and address of the TE to receive the cells/tissues;

10.3.3.2. the donor identification.

10.3.4. The date and time of procurement may be included, where possible.

10.4. Following procurement, all recovered tissues and cells must be packaged and labelled as described in section 12: Packaging, coding and labelling.

11. Processing

11.1. **General requirements**

11.1.1. Each tissue and cell preparation process must be authorised by the Health Authority after evaluation of the donor-selection criteria and procurement procedures, the protocols for each step of the process, the quality-management criteria and the final quan-

titative and qualitative criteria for the cells and tissues. This evaluation must comply at least with the requirements set out in Directive 2006/86 Annex II.

11.1.2. TEs are responsible for determining the suitability of the received tissues/cells for processing and for assessing the quality and safety of the processed tissue/cell products before distribution. Decisions on suitability should be made by a person who is appropriately qualified.

11.2. **Reception of the tissues/cells at the TE**

11.2.1. Each TE must ensure that the tissue and cells received are quarantined until they, along with the associated documentation, have been inspected or otherwise verified as conforming to requirements. The review of relevant donor/procurement information and thus acceptance of the donation needs to be carried out by specified/authorised persons.

11.2.2. Tissue and cells must be held in quarantine until such time as the requirements relating to donor testing and information have been met in accordance with Directive 2004/23/EC Article 15. Received tissues and cells awaiting final test results, or subject to additional testing or confirmatory medical assessment, must be held in quarantine until such test results or medical data become available.

11.2.3. When the procured tissues/cells arrive at the TE, there must be documented verification that the consignment, including the transport conditions, packaging, labelling and associated documentation and samples, meets the requirements of Annex IV of Directive 2006/17/EC and the specifications of the receiving establishment.

11.2.4. Each TE must have a documented policy and specifications against which each consignment of tissues and cells, including samples, can be verified. These must include the technical requirements and other criteria considered by the TE to be essential for the maintenance of acceptable quality. The TE must have documented procedures for the management and segregation of non-conforming consignments, or those with incomplete test results, to ensure that there is no risk of contamination of other tissues and cells being processed, preserved or stored.

11.2.5. TEs must ensure that all donations of human tissues and cells are subjected to tests in accordance with the requirements referred to Directive 2004/23/EC Article 28(e) and that the selection and acceptance of tissues and cells comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

11.2.6. TEs must ensure that human tissue and cells and associated documentation comply with the requirements referred to in Directive 2004/23/EC Article 28(f).

11.2.7. TEs must verify and record the fact that the packaging of human tissue and cells received complies with the requirements referred to in Article 28(f). All tissues and cells that do not comply with those provisions must be discarded.

11.2.8. The procurement report and shipping record (if the donation was transported by a third party) must be cross-checked with the contents of the package.

11.2.9. The acceptance or rejection of received tissues or cells must be documented.

11.2.10. The data that must be registered at the TE (except for donors of reproductive cells intended for partner donation) include:

11.2.10.1. confirmation that the consent/authorisation has been obtained, including the purpose(s) for which the tissues and cells may be used (i.e. therapeutic or research, or both therapeutic use and research) and any specific instructions for disposal if the tissues or cells are not used for the purpose for which consent was obtained;

11.2.10.2. all required records relating to the procurement and the taking of the donor history, as described in the donor documentation section;

11.2.10.3. results of physical examination, of laboratory tests and of other tests (such as the autopsy report, if used in accordance with Directive 2006/17/EC Annex IV point 1.2.2);

11.2.10.4. for allogeneic donors, a properly documented review of the complete donor evaluation against the selection criteria by an authorised, trained and qualified person;

11.2.10.5. in the case of cell cultures intended for autologous use, documentation of the possibility of medicinal allergies (such as to antibiotics) of the recipient.

11.2.11. In the case of reproductive cells intended for partner donation, the data to be registered at the TE include:

11.2.11.1. consent, including the purpose(s) for which the tissues and cells may be used (such as reproductive only and/or for research) and any specific instructions for disposal if the tissue or cells are not used for the purpose for which consent was obtained;

11.2.11.2. partner identification;

11.2.11.3. place of procurement;

11.2.11.4. tissues and cells obtained and relevant characteristics.

11.3. **Processing**

11.3.1. TEs must include in their SOPs all processes that affect quality and safety and must ensure that they are carried out under controlled conditions. TEs must ensure that the equipment used, the working environment, the process design, validation and control conditions are all in compliance with the requirements referred to in Directive 2004/23/EC Article 28 (h).

11.3.2. Any modifications to the processes used in the preparation of tissues and cells must also meet the criteria laid down in paragraph 11.3.1 above. Any substantial modification to the processes and parameters in reference to tissue and cell processing should be

reported to the national Health Authority and, if required, authorised by that authority before processing begins.

11.3.3. The procedures must be documented in SOPs, which must conform to the validated or verified method and to the standards laid down in Directive 2006/86/ Annex I(E), points 1 to 4.

11.3.4. TEs must ensure that all processes are conducted in accordance with the approved SOPs.

11.3.5. The critical processing procedures must be validated or verified and must not render the tissues or cells clinically ineffective or harmful to the recipient. This validation or verification may be based on studies performed by the establishment itself, or on data from published studies or, for well-established processing procedures, by retrospective verification of the clinical results for tissues supplied by the establishment. Processing methods should be designed to ensure the safety and biological functionality of processed tissues and cells.

11.3.6. If verification is based on retrospective evaluation of the clinical results for tissues or cells supplied by the establishment, data should be collected and analysed that include the number of tissues or cells implanted following processing by the method under consideration, and the time period (start and end dates/times) during which these implantations occurred.

11.3.7. It should be demonstrated that the validated or verified process can be carried out consistently and effectively in the TE environment by the staff available.

11.3.8. If physicochemical methods are to be applied, these procedures must be adapted to the type of tissue or cell and should be validated or verified.

11.3.9. The processing procedures must undergo regular critical evaluation to ensure that they continue to achieve the intended results.

11.3.10. Before implementing any significant change in processing, the modified process must be validated/verified and documented.

11.3.11. Pooling of different tissues and cells from two or more donors during processing must only be done where it has been demonstrated to be the only way of providing sufficient clinically effective tissues or cells, and is subject to a comprehensive risk–benefit assessment.

11.3.12. In the case of MAR, pooling of tissues and cells is prohibited.

11.3.13. Pooled tissues or cells should be treated as a single batch while also ensuring that the original donations are fully traceable.

11.3.14. In the case of pooling during the process, a risk evaluation should be in place to preclude cross-contamination.

11.3.15. If the process includes a sterilisation or viral-inactivation step, process-specific validation/verification studies should be completed to demonstrate the log reduction achieved by the process.

11.3.16. Where a microbial inactivation procedure is applied to the tissue or cells, it must be specified, documented and validated.

11.3.17. In order to avoid cross-contamination, the tissues or cells from one donor should not come into contact, at any time during processing or storage, with tissues or cells from another donor, unless they are intentionally pooled.

11.3.18. A separate set of clean, sterile instruments should be used for each batch.

11.3.19. The procedures used to prevent or reduce contamination during processing should be validated/verified depending on the type of tissue and how it is processed.

11.3.20. The use of antibiotics during procurement, processing and preservation must be recorded, and the end must should be aware of the use of antibiotics.

11.3.21. Maximum times from procurement until processing and storage must be defined. For deceased donors, maximum times from circulatory arrest (cardiac arrest or, for organ donors, cross-clamp time) to procurement must also be defined.

11.3.22. Procurement, processing and storage times must be documented in the records for tissues and cells.

11.3.23. TEs must ensure that human tissues and cells are correctly identified at all times. Each delivery of tissues or cells must be assigned a unique identifying code, in accordance with Directive 2006/86/EC Art. 10, 10a, 10b.

11.3.24. Each processed tissue or cell product should have a unique identifying code, i.e. the Single European Code (SEC), that is also recorded in the processing records.

11.3.25. The acceptance, rejection or disposal of tissues and cells, including those to be discarded, must be properly documented.

11.4. **Storage**

11.4.1. Maximum storage time and storage conditions must be defined and should be validated/verified for each type of tissue and cell.

11.4.2. There must be a system in place to separate and distinguish tissues and cells prior to release/in quarantine from those that are released and from those that are rejected, in order to prevent mix-ups and cross-contamination.

11.4.3. A documented risk assessment approved by the Responsible Person must be undertaken to determine the fate of all stored tissues and cells following the introduction of any new donor selection or testing criterion or any significantly modified processing step that enhances safety or quality.

11.4.4. A written agreement must be in place each time that a tissue establishment entrusts storage of tissues and cells to a third party.

11.5. **Disposal**

11.5.1. TEs must include in their SOPs special provisions for the handling of tissues and cells to be discarded, in order to prevent the contamination of other tissues or cells, the processing environment or personnel. These procedures must comply with national regulations.

11.5.2. Disposal of human tissues should be carried out in a manner that shows respect for fundamental rights and for the human body.

11.5.3. There should be documentation to show that the conditions for disposal of HPC, as defined prior to procurement, have been met, including (where applicable) the option to transfer the cells to another facility if the designated recipient is still alive after the agreed storage period.

12. Packaging, coding and labelling

12.1. **General principles**

12.1.1. Labelling and packaging operations must be designed to prevent any cross-contamination or mix-ups. Simultaneous operations should be avoided or adequate measures should be taken to ensure that no cross-contamination or mix-ups occur.

12.1.2. Facilities where packaging or labelling operations have taken place should be inspected and documented before starting any other operation so as to guarantee that all the previous materials have been removed.

12.1.3. There must be written procedures describing the receipt, identification, quarantine, sampling, examination and/or testing, release and handling of packaging and labelling materials.

12.1.4. Records should be maintained for each shipment of labels and packaging materials showing receipt, examination or testing, and whether accepted or rejected.

12.1.5. All packaging and labelling materials should be stored and managed in a safe manner in order to avoid any cross-contamination or mix-up, which could result in incorrectly identified/packaged tissues/cells.

12.2. **Packaging and labelling**

12.2.1. Storage containers must be appropriate for the type of tissue or cells, the temperature and method of storage, and the intended application. They must withstand sterilisation

(where this is to be applied), not produce toxic residues during storage and be adequately robust to remain intact when handled during transport.

12.2.2. Labels must be designed to adhere firmly to the container under all storage and transport conditions and the processing techniques used.

12.2.3. Type of label to be used, as well as the labelling methodology, must be defined and established in written procedures.

12.2.4. Labels applied to containers, equipment or premises should be clear, unambiguous and in the agreed format of the TE.

12.2.5. All excess labels must be destroyed or maintained in a secure manner, when necessary, to prevent cross-contamination or mix-ups.

12.2.6. Obsolete labels must be destroyed according to written procedures.

12.2.7. Printed labels must be carefully examined to ensure that the information on the label conforms to the corresponding tissue/cells. The results of this examination should be documented.

12.2.8. A printed label, representative of those used, should be included in the processing records.

12.2.9. A unique donation code must be allocated to the donated tissues and cells, during procurement or at the end of the recovery process, to ensure proper identification of all donated material. The registration system must ensure traceability to the donor identity/donor code and must comply with the coding requirements of Directive 2006/86/EC.

12.2.10. Following procurement, all recovered tissues and cells must be packaged in a manner which minimises the risk of contamination and must be stored at temperatures that preserve the required characteristics and biological function of the cells/tissues. The packaging must also prevent contamination of those persons responsible for packaging and transportation of the tissues and cells.

12.2.11. The packaged cells/tissues must be shipped in a container which is suitable for the transport of biological materials and which maintains the safety and quality of the contained tissues or cells.

12.2.12. Any accompanying tissue or blood samples for testing must be accurately labelled to ensure identification with the donor, and must include a record of the date and time when the sample was drawn and should include the place where the specimen was taken.

12.2.13. **Primary packaging and labelling operation after procurement**

12.2.13.1. Every package containing tissues and cells must be labelled at the time of procurement. The primary tissue/cell container must indicate the donation identification or code and the type(s) of tissue and cell.

12.2.13.2. Where the size of the package permits, the following information must also be provided:

12.2.13.2.1. date (and time where possible) of donation;

12.2.13.2.2. details of any blood transfusion before the procurement, and the haemodilution risk;

12.2.13.2.3. hazard warnings;

12.2.13.2.4. nature of any additives/transport medium (if used);

12.2.13.2.5. in the case of autologous donations, the label must state 'for autologous use only';

12.2.13.2.6. in the case of directed donations, the label must identify the intended recipient;

12.2.13.2.7. If any of the information above cannot be included on the primary package label, it must be provided on a separate sheet accompanying the primary package.

12.2.14. **Secondary packaging and labelling operation after procurement**

12.2.14.1. When tissues/cells are shipped by an intermediary, every shipping container must be labelled at least with the following:

12.2.14.1.1. **TISSUES AND CELLS** and **HANDLE WITH CARE;**

12.2.14.1.2. the identification of the establishment from which the package is being transported (address and phone number) and a contact person in the event of problems;

12.2.14.1.3. the identification of the TE of destination (address and phone number) and the person to be contacted to take delivery of the container;

12.2.14.1.4. the date and time of the start of transportation;

12.2.14.1.5. specifications concerning conditions of transport relevant to the quality and safety of the tissues and cells;

12.2.14.1.6. in the case of living tissues and cells, the indication **DO NOT IRRADIATE;**

12.2.14.1.7. when tissues and cells are known to be positive for a relevant infectious disease marker, the indication **BIOLOGICAL HAZARD;**

12.2.14.1.8. in the case of autologous donors, the indication **FOR AUTOLOGOUS USE ONLY;**

12.2.14.1.9. specifications concerning storage conditions (such as **DO NOT FREEZE**).

12.2.15. **Final labelling for distribution**

12.2.15.1. The primary tissue/cell container must provide the following:

12.2.15.1.1. type of tissues and cells, identification number or (SEC) code of the tissue/cells, and lot or batch number where applicable;

12.2.15.1.2. identification of the TE from which the tissues/cells are sent;

12.2.15.1.3. expiry date of the tissues/cells;

12.2.15.1.4. in the case of an autologous donation, this information has to be specified (**FOR AUTOLOGOUS USE ONLY**) and the donor/recipient has to be identified;

12.2.15.1.5. in the case of directed donations, the label must identify the intended recipient;

12.2.15.1.6. when tissues and cells are known to be positive for a relevant infectious disease marker, they must be marked as **BIOLOGICAL HAZARD.**

12.2.15.2. If any of the information under points 12.2.15.1.4 and 12.2.15.1.5 above cannot be included on the primary container label, it must be provided on a separate sheet accompanying the primary container. This sheet must be packaged with the primary container in a manner that ensures that they remain together.

12.2.15.3. The following information must be provided either on the label or in accompanying documentation:

12.2.15.3.1. description (definition) and, if relevant, dimensions of the tissues;

12.2.15.3.2. morphology and functional data where relevant;

12.2.15.3.3. date of distribution of the tissue/cells;

12.2.15.3.4. biological investigations carried out on the donor and results;

12.2.15.3.5. storage recommendations;

12.2.15.3.6. instructions for opening the container or package, and any required manipulation/reconstitution;

12.2.15.3.7. expiry dates after opening/manipulation;

12.2.15.3.8. instructions for reporting serious adverse reactions and/or events;

12.2.15.3.9. presence of potential harmful residues (e.g. antibiotics, ethylene oxide).

12.2.16. **External labelling of the shipping container**

12.2.16.1. For transport, the primary container must be placed in a shipping container that must be labelled with at least the following information:

12.2.16.1.1. identification of the originating TE, including an address and phone number;

12.2.16.1.2. identification of the destination organisation responsible for human application (ORHA), including address and phone number;

12.2.16.1.3. a statement that the package contains human tissue/cells and the warning **HANDLE WITH CARE;**

12.2.16.1.4. where living cells are required for the function of the graft, such as stem cells, gametes and embryos, the warning **DO NOT IRRADIATE** must be added;

12.2.16.1.5. recommended transport conditions (e.g. keep cool, in upright position);

12.2.16.1.6. safety instructions/method of cooling (when applicable).

12.3. **Coding**

12.3.1. The TE must have effective and accurate systems to uniquely identify and label cells/tissues received and distributed. An identifying code (SEC) must be allocated by the TE to all procured tissues and cells, including tissues and cells imported from countries outside the EU, to ensure proper identification of the donor and the traceability of all donated material and to provide information on the main characteristics and properties of the tissues and cells.

12.3.2. There are some general exemptions:

12.3.2.1. partner donation of reproductive cells;

12.3.2.2. tissues and cells distributed directly for immediate transplantation to the recipient (e.g. HPC);

12.3.2.3. tissues and cells imported from non-EU countries into the EU in cases of emergency that are authorised directly by the Health Authorities;

12.3.2.4. when these tissues and cells remain in the same centre;

12.3.2.5. when tissues and cells that are imported from non-EU countries into the EU remain within the same healthcare facility from importation to application (provided that the healthcare facility is a tissue establishment authorised to import tissues and cells).

12.3.3. The code must incorporate at least:

12.3.3.1. donation identification;

12.3.3.2. unique ID number;

12.3.3.3. identification of the TE;

12.3.3.4. tissues and cells identification;

12.3.3.5. tissues and cells code (basic nomenclature);

12.3.3.6. split number (if applicable);

12.3.3.7. expiry date.

13. Quality control (including microbiological control)

13.1. General principles

13.1.1. A quality-control system must be in place to ensure that tissues and cells are not released for use until their quality has been assessed as satisfactory. Activities such as verification steps, sampling and testing should be carried out to assess that the tissues and cells, and also materials, equipment and processes, comply with established acceptance criteria.

13.1.1.1. All records which are critical to the safety and quality of the tissues and cells must be protected from unauthorised amendment and kept so as to ensure readability and access throughout their specified retention period, after expiry date, clinical use or disposal.

13.1.1.2. Samples for quality control should be representative of the tissues and cells from which they are taken and should be procured and recorded in accordance with written procedures that describe the method of sampling, including the amount of sample to be taken, precautions to be observed and storage conditions.

13.1.1.3. At all stages of quality-control testing, sampling containers must be labelled with relevant information on their identity and date of sampling.

13.1.2. There must be a person responsible for quality control who supervises all quality-control procedures, who ensures that the premises and equipment where quality-control operations are carried out are appropriate and maintained under suitable conditions and who also ensures that all personnel working under their responsibility are adequately trained.

13.1.3. Written procedures (SOPs) must be in place that govern quality control at key stages during processing. The SOPs should include at least:

13.1.3.1. the test method;

13.1.3.2. the sample size and sampling plan;

13.1.3.3. the accepted criteria.

13.1.4. Sampling and testing methods must be validated/verified to show the representativeness of the sample and the suitability of the selected methods.

13.1.4.1. The performance of the testing procedures should be regularly assessed.

13.1.5. Records related to quality-control testing should be part of the documentation of tissues and cells. Data to be recorded and maintained (by either the TE or the testing laboratories) should include:

13.1.5.1. name, manufacturer and batch number of the material or products used;

13.1.5.2. reference to the relevant specifications and testing procedures, and to the equipment used;

13.1.5.3. test results, including observations and calculations, and reference to any certificates of analysis;

13.1.5.4. date of testing, and date and time when the sample was obtained;

13.1.5.5. identification of the persons who performed the testing;

13.1.5.6. identification of the person who verified the testing;

13.1.5.7. statement of approval or rejection of the test results;

13.1.6. Minimum acceptance criteria should be based on a defined specification for each type of tissue and cell.

13.1.7. Non-conforming tissues and cells must be identified and separated from conforming tissues and cells. The fate of non-conforming tissues and cells must be decided by the Responsible Person in charge of the TE, according to written procedures.

13.2. **Microbiological control**

13.2.1. The microbiological safety of tissues and cells is based on donor selection and minimisation of initial contamination, with control and monitoring of contamination during the entire procurement process. All facilities that procure, process or store tissues and cells should have access to the services of a microbiology laboratory with a fully implemented QMS and to the advice of a suitably qualified expert microbiologist.

13.2.2. Microbiological testing for bacteria and fungi should be carried out at least on representative samples collected prior to the commencement of processing, at any point from the end of the procurement process and the commencement of any processing protocols, and at the end of the processing protocols, immediately before final packaging of the tissue or cell graft.

13.2.2.1. In exceptional cases, if sampling of the finished tissues and cells is not feasible, the storage medium or rinsing or washing solutions can be tested as surrogates.

13.2.2.2. In cases where the nature of the procured tissues and cells does not allow sampling of the starting material, an alternative sampling approach, such as liquids in contact with the starting material, may be followed.

13.2.2.3. Where applicable, a sample of the tissues/cells storage, transport or rinsing solution should be tested.

13.2.2.4. In-process testing should be performed at relevant steps of the preparation process, according to a risk assessment that has to take into consideration the nature of the tissues and cells, their origin and procurement, the critical steps during processing and their intended application.

13.2.2.5. For cells and tissues in which a closed system is used, pre- and post-process testing should be carried out. In this context, in-process testing does not yield more information on the microbiological status of the cell or tissue graft.

13.2.2.6. For processed tissues and cells sterilised in their final container by a validated sterilisation process, if the release is intended to rely on process data only, and not on final product testing for sterility, then validated procedures for all critical processing steps and a fully validated sterilisation method must be applied. It should be shown that adequate precautions have been implemented to minimise the microbial contamination before sterilisation and that tissues and cells with an acceptably low degree of microbial contamination have been used as determined by regular bioburden testing.

13.2.2.7. For tissue and cells obtained from processing that includes decontamination, such as treatment with antibiotics and anti-fungal agents, methods for finished tissue and cell testing must be evaluated carefully to assess possible inhibition of microbial growth due to decontaminating agents or their residues.

13.2.2.8. Microbiological testing methods for the detection of bacteria and fungi should follow the procedures outlined in the *European Pharmacopoeia* (*Ph. Eur.*), according to the method employed and the type of tissue or cell analysed. Chapter 2.6.27 of *Ph. Eur.* deals specifically with cell-based preparations or products where the examination for sterility cannot be applied according to Chapter 2.6.1 of *Ph. Eur.*, while General Monograph 5.1.6 gives indications on implementation and the use of alternative microbiological methods. Bioburden testing of non-sterile tissues and cells should be performed according to indications given in Chapters 2.6.12 and 2.6.13 of *Ph. Eur.*

13.2.2.9. Whatever the applied method, its suitability must be shown with respect to specificity, sensitivity and robustness.

13.2.2.10. If release of the tissues and cells is necessary before the end of the officially verified/required incubation period, a 'negative-to-date' reading of the results may be carried out. In this case, intermediate results of the final testing in combination with final results of in-process controls should be used for tissues and cells release.

13.2.2.11. If micro-organisms are detected after tissues and cells release, pre-defined measures such as identification and antibiotic sensitivity of the species should be carried out, and information must be provided to clinicians caring for the patient.

13.2.3. Depending on the type of preparation process, it may be necessary to complement the microbial test concept by additional tests for specific infectious agents such as mycoplasma (*Ph. Eur.* 2.6.7) and mycobacteria (*Ph. Eur.* 2.6.2).

13.2.3.1. Testing should be conducted at processing steps at which mycoplasma (*Ph. Eur.* 2.6.7) or mycobacteria (*Ph. Eur.* 2.6.2) contaminations would most likely be detected, such as after collection, but before washing steps.

13.2.3.2. As mycoplasmas are cell-associated micro-organisms that may locate within the cell, testing should always include the tissues or cells.

13.2.4. Depending on the intended application of the tissues and cells, and the estimated impact of pyrogens on the recipient, routine testing for pyrogens may be required. If risk assessment reveals the necessity of bacterial endotoxin control, methods *Ph. Eur.* 2.6.14 (Bacterial endotoxins) or 2.6.32 (Test for bacterial endotoxins using recombinant factor C) should be used. Depending on risk assessment, testing of non-endotoxin pyrogens in addition to endotoxin or mixtures of both can be performed with the monocyte activation test (MAT; *Ph. Eur.* 2.6.30).

13.2.4.1. Because raw materials are potential sources of pyrogens, raw materials declared by their manufacturers to be free of pyrogens should preferably be used for the culture of tissues and cells if available.

13.2.4.2. For certain cells that must be administered immediately and that cannot be cryopreserved without damaging the viability and quality of the cells, a rapid method for pyrogen testing may be employed.

13.2.5. Each batch of the microbiological culture medium and plates to be used should be tested for its growth-promoting capacities by using a 'growth-promotion test' in accordance with *Ph. Eur.*

13.2.6. The microbiological test methods employed for sterility testing should be verified in the presence of the intended sample material (method suitability test). The method suitability test should be carried out using the bacterial and fungal species indicated in the chapters applicable from *Ph. Eur.*

13.2.7. Contamination with specific pathogenic micro-organisms should result in the discard of the tissues or cells unless treated with a sterilisation process validated to eliminate the infectivity of such organisms. If applicable, a non-exhaustive list of pathogens whose presence necessarily excludes the procured tissue or cells from further processing should be established for each tissue.

14. Distribution, import/export and recall

14.1. Release

14.1.1. The distribution process, meaning transportation and delivery of cells or tissues intended for human application or for use in further manufacture, must be validated or verified.

14.1.2. Prior to distribution a comprehensive record review must ensure that all elements of procurement, processing and storage have met the established quality criteria, including the identity of the tissues and cells.

14.1.3. Packaged tissues or cells should be examined visually for appropriate labelling and container integrity.

14.1.4. Tissues and cells for clinical application must not be distributed without an order from a clinician or other authorised person at the OHRA.

14.1.5. In cases of incomplete eligibility of the donor, the tissues and cells must be released only for documented urgent medical need and after a risk assessment has been performed.

14.1.6. Distribution for clinical application must be restricted to authorised ORHAs or to authorised health professionals and must comply with all applicable national laws and regulations. In cases where tissues or cells require additional procedures such as thawing to be undertaken by the ORHA, the associated specific instructions must be provided to the ORHA by the TE.

14.2. **Transport**

14.2.1. Equipment used to ensure the maintenance of critical transport or shipment must be qualified.

14.2.2. The capacity of the transport container to maintain the required environmental conditions must be qualified, and the length of time that these conditions can be maintained, must be determined by validation or verification and documented.

14.2.3. The container/package must be secure, and shipment conditions such as temperature and time limit must be defined to ensure maintenance of the required properties of the tissues and/or cells.

14.2.4. Data loggers or temperature indicators should be used when it is mandatory to monitor temperature during transport or shipment of tissues or cells requiring a continuous temperature-controlled environment.

14.2.5. The mode of transport or shipment must comply with the applicable laws and regulations on transportation of biological substances.

14.2.6. An alternative plan of transport or shipping should be available in case of emergency situations, to prevent possible clinical complications for the recipient.

14.2.7. Couriers should be appropriately trained and should be able to contact the receiving facility on a 24-hour basis in cases of delay during transit.

14.2.8. Viable tissues and cells, including stem cells, gametes and embryos, must not be exposed to irradiation. Appropriate arrangements in accordance with relevant national legislation should be in place to ensure that such tissues and cells are not exposed to irradiation during transport, including at security screenings and border crossings.

14.3. **Agreements**

14.3.1. Written agreements for the shipment of tissues and cells must be in place between the shipping company and the TE.

14.3.2. A service-level agreement between the exporting TE and importing TE must clearly define the relevant roles and responsibilities, including procedures for transport, packaging and required environmental conditions.

14.3.3. The agreement should specify how tissues and cells will be identified, and a unique identifying code must allow unambiguous identification and traceability.

14.4. **Export**

14.4.1. Exported tissues and cells must be procured, handled, stored, transported, used and disposed of in accordance with the consent that has been given by the donor.

14.4.2. Tissues and cells should be exported only to countries that have proper controls on the use of donated material and only for the purposes for which they can lawfully be used in the country of destination.

14.5. **Import**

14.5.1. TEs must be authorised for the import of tissues and cells from non-EU countries by their respective Health Authorities.

14.5.2. The importing TE should assess and document that the exporting TE has applied the fundamental ethical principles of consent, non-remunerated donation, anonymity and respect for public health.

14.5.3. The importing TE must evaluate the general quality and safety systems at the exporting establishment, along with its licences and accreditations and the donor blood testing.

14.5.4. The importing TE must require that any changes to authorisation status be immediately communicated by the exporting facility.

14.5.5. The agreements between an importing TE and suppliers in other non-EU countries should include provisions for the performance of audits at the exporting facility.

14.5.6. Acceptance at the TE must include a documented procedure to verify compliance with the written agreement in place with the exporter.

14.5.7. Containers should be examined for any evidence of tampering or damage during transport.

14.5.8. Tissues and cells must stored in quarantine in an appropriate secure location under defined conditions until they, along with the accompanying documentation, have been verified as conforming to requirements.

14.5.9. The importing TE must identify and code the imported tissues and cells with the appropriate SEC.

14.6. **Records**

14.6.1. The courier must provide records of pick-up and delivery to the TE to ensure complete traceability of the tissues and cells.

14.6.2. Documentation obtained from the exporting TE must be archived for the time period required by national regulations (e.g. 30 years in EU member states).

14.7. **Recall**

14.7.1. The TE must have personnel authorised to assess the need for recall and to initiate and co-ordinate the necessary actions.

14.7.2. A recall procedure must be in place that includes a description of the responsibilities, actions to be taken within pre-defined periods of time and requirements for notification to the Health Authorities.

14.7.3. Recall actions must be taken within pre-defined periods of time and must include tracing all relevant tissues or cells and, where applicable, must include trace-back. The purpose of the investigation is to identify any donor who might have contributed to causing the adverse reaction and to retrieve available tissues or cells from that donor, as well as to notify consignees and recipients of components procured from the same donor in the event that they might have been put at risk.

14.7.4. The progress of the recall process must be recorded and a final report issued, including reconciliation of the delivered and recovered quantities of the tissues or cells.

14.7.5. The effectiveness of the arrangements for recalls should be regularly evaluated.

15. Documentation and records

15.1. **General principles**

15.1.1. Good documentation constitutes an essential part of the quality system and is key to operating in compliance with the requirements of good practice, which includes these guidelines. The various types of documents and media used must be defined fully in the QMS of the organisation.

15.1.2. Documentation may exist in various forms: paper-based, electronic or photographic. The main objective of the system of documentation used must be to establish, control, monitor and record all activities that directly or indirectly impact on all aspects of the quality and safety of tissues and cells as well as any derived medicinal products. The QMS must include sufficient instructional detail to facilitate common understanding of the requirements. It must also provide for adequate recording of the various processes and evaluation of any observations, so that ongoing application of the requirements may be demonstrated.

15.1.3. There are two primary types of documentation used to manage and record good practice compliance: instructions (directions, requirements) and records/reports. Appropriate practices should be applied to each type of document. Suitable controls must be implemented to ensure the accuracy, integrity, availability and legibility of documents. Instruction documents must be free from errors and available in writing. The term 'written' means recorded or documented on media from which data may be rendered in a readable form for humans.

15.2. **Required good practice documentation**

15.2.1. Documentation should include at least the following items:

15.2.1.1. a quality manual, if required by national legislation or other applicable quality standards;

15.2.1.2. specifications for materials, consumables and reagents;

15.2.1.3. approved SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;

15.2.1.4. identification and analysis of risks and a risk-mitigation plan;

15.2.1.5. records of the performance of operations, including processing records;

15.2.1.6. records of deviations, complaints, corrective and preventive actions and audits;

15.2.1.7. job descriptions, training and competency records of personnel;

15.2.1.8. qualitative and quantitative specifications for tissues and cells;

15.2.1.9. key quality indicators for tissues and cells.

15.2.2. **Instructions (directions or requirements)**

15.2.2.1. Specifications, based on policies and risk assessments, describe in detail the requirements to which the tissues and cells or other materials used or obtained during processing and distribution must conform.

15.2.2.2. Testing instructions detail all the starting materials, equipment and computerised systems (if any) to be used and specify all sampling and testing instructions. If applied, in-process controls must be specified, together with their acceptance criteria.

15.2.2.3. SOPs give directions for performing certain operations.

15.2.2.4. SOPs provide explicit instructions for performing certain discrete operations, and may also record the outcome (e.g. qualification and validation protocols).

15.2.2.5. Technical agreements are agreed between contract givers and acceptors for outsourced activities.

15.2.3. **Records/reports**

15.2.3.1. Records provide evidence of various actions taken to demonstrate compliance with instructions, e.g. activities, events, investigations and a history of all tissues and cells,

including their distribution. Records include the raw data that are used to generate other records. For electronic records, regulated users should define which data are to be used as raw data. All data on which quality decisions are based should be defined as 'raw data'.

15.2.3.2. Certificates of analysis provide a summary of testing results on samples of reagents, products or materials, together with the evaluation of compliance with a stated specification.

15.2.3.3. Reports document the carrying out of particular exercises, projects or investigations, together with the results, conclusions and recommendations.

15.3. **Generation and control of documentation**

15.3.1. Each type of document should be defined and its requirements should be followed, regardless of document forms or media. Complex systems must be understood, well documented and validated or verified, and adequate controls must be in place. Many documents (instructions and/or records) may exist in hybrid forms (i.e. some elements are electronic and others are paper-based). Relationships and control measures for master documents, official copies, data handling and records must be stated for both hybrid and homogeneous systems.

15.3.2. A document-control system, defined in a written procedure, must be established for the review, revision history and archiving of documents, including SOPs. Appropriate controls for electronic documents, such as templates, forms and master documents, must be implemented. Appropriate controls must be in place to ensure the integrity of the record throughout the retention period.

15.3.3. Documents should be designed, prepared, reviewed and distributed with care. Reproduction of working documents from master documents should not allow errors to be introduced through the reproduction process.

15.3.4. There must be a document-control procedure in place to ensure that only current versions are in use.

15.3.5. Documents containing instructions must be approved, and dated by appropriate and authorised persons. This may also be undertaken electronically. Documents should have unambiguous content and be uniquely identifiable. The effective date must be defined.

15.3.6. Documents containing instructions should be laid out in an orderly fashion, be clear and be easy to check. The style and language of documents should fit with their intended use.

15.3.7. Documents within the QMS must be regularly reviewed and kept up to date. A periodic review process should be established to ensure that the documentation for any given process, system or equipment is complete, current and accurate.

15.3.8. All changes to documents must be reviewed, dated, approved, documented and implemented promptly by authorised personnel.

15.3.9. Instructional documents should not be handwritten; however, where documents require the entry of data, sufficient space should be provided for such entries.

15.4. **Good documentation practices**

15.4.1. Records must be legible and may be handwritten, transferred to another medium such as microfilm, or documented in a computerised system.

15.4.2. Records should be made or completed at the time each action is taken and in such a way that all significant activities concerning the coding, donor eligibility, procurement, processing, preservation, storage, transport and distribution or disposal, including aspects relating to quality control and quality assurance of tissues and cells, are traceable.

15.4.3. For every critical activity, the materials, equipment and personnel involved must be identified and documented.

15.4.4. The record system must ensure continuous documentation of the procedures performed from the donor to the recipient. That is, each significant step must be recorded in a manner that permits tissue and cells or procedure to be traced, in either direction, from the first step to final use/disposal.

15.4.5. Any alteration made to the entry on a document must be signed and dated; the alteration should permit reading of the original information. Where appropriate, the reason for the alteration must be recorded. In the case of electronic records, there must be an audit trail, so that it is possible to trace what data have been altered, when the record was altered and who altered it.

15.4.6. Access to records (registers and data) must be restricted to persons authorised by the Responsible Person, and to the Health Authority for the purpose of inspection and control measures.

15.4.7. Data protection and confidentiality measures must be in place, in accordance with Article 14 of Directive 2004/23/EC.

15.5. **Retention of documents**

15.5.1. It should be clearly defined which record is related to each activity and where this record is located. Secure controls must be in place to ensure the integrity of the record throughout the retention period. These controls must be validated if appropriate.

15.5.2. Records encompassing identification, donor tests and clinical evaluation of the donor must be retained and must include at least the following details:

15.5.2.1. identification;

15.5.2.2. age;

15.5.2.3. sex;

15.5.2.4. medical and behavioural history;

15.5.2.5. relevant clinical data, laboratory test results and results of any other tests;

15.5.2.6. outcome of physical examination, results of autopsy (if carried out) or preliminary verbal report for deceased donors;

15.5.2.7. completed haemodilution algorithm (where applicable);

15.5.2.8. consent/authorisation forms;

15.5.2.9. for HPC donors, report of donor's suitability for intended recipient and, if donor is unrelated, relevant donor data to confirm suitability.

15.5.3. Donor-testing records must be accessible at the laboratory and must include date and time of sampling, date and time of sample receipt at the testing facility, record of test kits used to test donor sample and the results of donor testing, including repeat testing.

15.5.4. Records of procurement of tissues and cells must be retained. A procurement report should be available that includes:

15.5.4.1. identification of procurement organisation, and person responsible for procurement, including signature;

15.5.4.2. identification of TE receiving the tissue/cells;

15.5.4.3. donor identification data (including how and by whom the donor was identified);

15.5.4.4. donation unique number;

15.5.4.5. date, time and place of donation, and SOP used for procurement;

15.5.4.6. type of donation;

15.5.4.7. description of the procurement area, including environmental conditions;

15.5.4.8. storage conditions for deceased donors (including whether refrigeration was applied, and time of start and end of refrigeration);

15.5.4.9. details of materials, reagents and transport solutions;

15.5.4.10. any incidents during procurement.

15.5.5. Records of the processing of tissues and cells must be retained. A processing report should be available that at least includes the details of:

15.5.5.1. tissues and cells received, and evaluation of their suitability;

15.5.5.2. SOP used to process the tissues and cells;

15.5.5.3. equipment used during processing;

15.5.5.4. materials used during processing;

15.5.5.5. sterilisation or decontamination;

15.5.5.6. cryopreservation and freezing protocols;

15.5.5.7. environmental monitoring;

15.5.5.8. tissues and cells testing, including microbiological testing;

15.5.5.9. any incidents that occurred during processing.

15.5.6. Records of the storage and distribution of tissues and cells must be retained, and reports should be available that detail:

15.5.6.1. storage location (and transfer record if location is changed);

15.5.6.2. date placed in storage and date removed from storage;

15.5.6.3. storage temperature;

15.5.6.4. any incidents that occurred during storage;

15.5.6.5. name of party responsible for distribution;

15.5.6.6. identification of establishment, courier or individual who transported tissues/cells at any stage between procurement and end use;

15.5.6.7. packaging;

15.5.6.8. time and date of distribution and delivery;

15.5.6.9. identification of receiving establishment, clinician or ORHA;

15.5.6.10. any incidents that occurred during distribution.

15.5.6.11. identification code (in the EU, the SEC).

15.5.7. Records of the clinical application of tissues and cells should be retained by the ORHA and should include:

15.5.7.1. identification of supplier TE;

15.5.7.2. identification of clinician or ORHA;

15.5.7.3. type(s) of tissues and/or cells;

15.5.7.4. tissues and cells identification;

15.5.7.5. identification of the recipient;

15.5.7.6. date of clinical application;

15.5.7.7. any incidents that occurred during clinical applications;

15.5.7.8. any adverse reactions in the recipient;

15.5.7.9. health outcomes of children born following MAR.

15.5.8. Specific retention requirements for certain documentation apply.

15.5.8.1. Records must be retained for a period complying with local, national or EU requirements, as appropriate.

15.5.8.2. Traceability data (that allow tracing from donor to recipient and vice versa) must be retained for a minimum of 30 years.

15.5.8.3. All records (including raw data) that are critical to the safety and quality of the tissues and cells must be kept for at least 10 years after the expiry date, clinical use or disposal.

15.5.8.4. Quality system documentation and associated records should be retained for a minimum of 10 years.

15.5.8.5. For other types of documentation, the retention period should be defined on the basis of the business activity that the documentation supports. These retention periods should be specified.

16. Traceability

16.1. General principles

16.1.1. A robust system must be established and maintained to trace a specific tissue/cell during any step from donor/donation to recipient or child conceived as a result of MAR treatment, and vice versa. In the EU, tissues and cells are traceable from procurement to human application or disposal and vice versa through documentation and the use of the SEC (Directive 2006/86/EC as amended by Commission Directive EU 2015/565). Tissues and cells used for advanced therapy medicinal products must be traceable under this directive at least until transferred to the Advanced Therapy Medicinal Product manufacturer.

16.1.2. All relevant data relating to products and materials coming into contact with tissues and cells must also be traceable.

16.1.3. Each organisation holding tissues or cells must have effective and accurate procedures to uniquely identify and label cells/tissues collected, received, processed, distributed/disposed or used for human application. The application of an SEC does not preclude the additional application of other codes in accordance with member states' national requirements.

16.1.4. For accurate transcription of critical identification information, electronic transfer should be used. If manual transcription is used, double checking of data should be implemented.

16.1.5. Responsibility for traceability among the different organisations involved in procurement, processing, distribution and human application of cells/tissues must be clearly defined. Responsibility should be defined in a written technical and legal agreement.

16.1.6. Traceability data must be kept for the long term after clinical use to allow adequate biovigilance and follow-up. In the EU, information related to traceability, as described in Annex VI of the Directive 2006/86/EC, must be retained for at least 30 years after application or cell/tissue expiry date. Data that are critical to the safety and quality of cells/tissues should be maintained for at least 10 years.

16.1.7. Traceability data must be stored securely in an appropriate archive. In the case of change of storage location, a link between the previous location and the new location should be established.

16.1.8. Audits of traceability from donor to recipient and vice versa should be included in the quality-management plan.

17. Biovigilance

17.1. General principles

17.1.1. TEs must have documented procedures in place for the reporting of serious adverse reactions and events (SAREs) as defined in Directive 2004/23/EC.

17.1.2. There should be systems in place to ensure that adverse events, adverse reactions and near misses are documented, carefully investigated and, where necessary, followed up by the implementation of corrective actions to prevent recurrence.

17.1.3. Surveillance systems must be in place to assure the follow-up of living donor, tissue and cells recipients and children conceived after MAR treatment.

17.1.4. There should be procedures in place for reporting SAREs in a timely manner to the Responsible Person at the TE and to the Health Authorities. Adequate resources must be made available for their immediate investigation, alerts and resolution, and for the implementation of any corrective and preventive actions.

17.1.5. There should be a biovigilance co-ordinator, who has responsibility for vigilance and surveillance specified in their job description.

17.1.6. Vigilance programmes should include an activity of scanning for new risks that have not been recognised previously or have not yet occurred. Such new risks may be related to donors, emerging infectious diseases, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing, and new inheritable pathogenic DNA variants, that MAR donors have not been tested for.

17.1.7. Co-ordination between various systems of vigilance (organ and haemovigilance, material/device vigilance, pharmacovigilance) should be in place at the local level (TE) and at the Health Authority level.

17.1.8. Effective and regular communication to healthcare professionals of the results of vigilance systems is fundamental to ensuring that the benefits of these programmes are realised in practice to support continued notification of adverse reactions and events.

17.1.9. Programmes of training and awareness should be organised to encourage reporting. Professionals should be trained about what to communicate, when and to whom. The reporting system should be non-punitive and confidential.

17.1.10. TEs and clinicians should promote a culture that encourages reporting in a non-punitive context for the benefit of patients, children conceived after MAR treatment and donors.

17.2. **Adverse reactions**

17.2.1. Adverse reactions must be detected, reported, investigated and evaluated in terms of severity, imputability, probability and frequency of recurrence, and consequences.

17.2.2. Efficient systems for rapid quarantine and recall of unsafe tissues or cells must be in place, along with procedures for look-back where donors or recipients are found to have been exposed to a risk.

17.2.3. Important outcomes from each adverse reaction should be disseminated appropriately.

17.2.4. TEs that supply tissues and cells, including MAR, should provide organisations representing clinical users with clear instructions on how to report adverse reactions, preferably using standardised documentation in accordance with national and local requirements.

17.2.5. If serious adverse reactions (SARs) are detected in relation to tissues or cells that have entered international distribution channels, appropriate international collaboration should ensure that all those involved (clinicians, TEs and Health Authorities) in each of the countries concerned are informed and participate, if necessary, in the investigation and follow-up.

17.2.6. The following are examples of reportable adverse reactions [with abbreviated descriptions in square brackets]:

17.2.6.1. suspected harm in living donor related to procurement [donor harm];

17.2.6.2. unexpected primary infections possibly transferred from donor to recipient (e.g. viral, bacterial, parasitic, fungal, prion) [infection from donor]

17.2.6.3. suspected transmitted infection (viral bacterial, parasitic, fungal, prion) possibly due to contamination or cross-contamination by an infectious agent in the procured tissues, cells or associated materials, between procurement and their clinical application [infection from infected/contaminated tissues or cells];

17.2.6.4. immunological reactions, including allergic reactions, graft-*versus*-host disease, rejection, haemolytic reactions or other immunological reactions [immunology];

17.2.6.5. malignant disease possibly transferred by the tissues or cells (donor-derived, process-associated or other) [malignancy];

17.2.6.6. unexpected delayed or absent engraftment or graft failure (including mechanical failure) [failure];

17.2.6.7. toxic effects on tissues and cells or associated materials [toxicity];

17.2.6.8. unexplained immunological reactions due to tissue or cell mismatch [mismatch];

17.2.6.9. aborted procedure involving unnecessary exposure to risk (e.g. wrong tissue supplied, discovered after patient is anaesthetised and the surgical procedure has begun) [undue risk];

17.2.6.10. suspected transmission of genetic disease by transplantation or gamete/embryo donation [genetic abnormality];

17.2.6.11. suspected transmission of other (non-infectious) illness [other transmission];

17.2.6.12. transfusion-associated circulatory overload in haematopoietic progenitor cell transplantation [volume overload];

17.2.6.13. neurological reaction [insult];

17.2.6.14. severe febrile reaction [fever];

17.2.6.15. other [other].

17.3. **Adverse events**

17.3.1. Adverse events can occur at any moment from donor selection to clinical application. For effective detection of adverse events, all relevant parties must be aware of their responsibilities for identifying errors or unexpected results. This includes all staff at TEs and procurement organisations, those working in organisations such as testing laboratories that provide 'third party' services to TEs, and clinical users who may also detect errors at the point of clinical use. In EU Directive 2006/86/EC, the definition of a serious adverse event (SAE) includes those incidents often referred to as 'near misses', i.e. where an error or fault is detected and corrected without causing harm.

17.3.2. Non-compliances with the quality system should be documented and investigated as part of the internal QMS. On occasions, however, a particular non-compliance may be of such importance that it should be considered an SAE and reported through the vigilance system.

17.3.3. Deviations from SOPs in TEs (or other adverse events) that have implications for the quality and safety of tissues and cells, including MAR, should result in SAE reporting to the Health Authority if one or more of the following criteria apply:

17.3.3.1. inappropriate tissues or cells (including gametes, embryos or germinal tissues) have been distributed for clinical use, even if not used;

17.3.3.2. the event could have implications for other patients, including children conceived after MAR treatment, or donors because of shared practices, services, supplies or donors;

17.3.3.3. the event resulted in loss of any irreplaceable autologous tissues or cells (including gametes, embryos or germinal tissues) or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;

17.3.3.4. the event resulted in the loss of a significant quantity of unmatched allogeneic tissues or cells, including accidental loss of gametes, embryos or germinal tissues (e.g. break-down of incubators, accidental discard, manipulation errors), resulting in a total loss of chance of pregnancy for one cycle.

17.3.3.5. any type of gamete or embryo misidentification or mix-up of gametes or embryos;

17.3.3.6. the event resulted in a loss of traceability of gametes or embryos.

Part D. Tissue and cell monographs

Tissue and cell monographs

Introduction

In this 5th edition of the Guide to the quality and safety of tissues and cells for human application the *ad hoc* working group has elaborated a series of monographs. The role of a monograph is to provide information about individual preparations and clinical applications that are precisely defined and have been shown to be safe and effective when used in patients. Each tissue and cell monograph combines two aspects, a consolidated process and a consolidated use, in a single document that is complementary to other sections of the Guide. Part A of the Guide determines the generic requirements for donor selection, procurement, processing, storage, distribution (including import and export) and related matters, while Part B describes the specific criteria for each tissue and cell type.

Tissue and cell monographs complement and connect these parts, linking both aspects to define specific critical properties and quality-control requirements for specific substances of human origin, prepared in specific ways and to be used for specific clinical indications.

Data collected from EU member states in 2020 on the reporting of serious adverse reactions and events for tissues and cells (during 2019) indicated that more than 1 360 300 units of tissues and cells were distributed, to treat a wide range of clinical indications using various processing methods. These processes, which include freezing, cryopreservation and lyophilisation, are very well-established methods and have been consolidated through many years of experience with the support of scientific results and publications showing the safety, the quality and the effectiveness of the tissues and cells prepared and used in these validated conditions.

A common language is necessary for tissue establishments and Health Authorities when defining the commonly accepted, relevant, validated quality criteria for each consolidated processing method applied to each specific substance of human origin type and indication. Thus, tissue and cell monographs are intended to provide tissue establishments and Health Authorities with recommendations on the minimum controls necessary to ensure the quality of the substances of human origin that are processed by a tissue establishment. These monographs are also tools that allow tissue establishments to demonstrate to their Health Authorities that they have fulfilled appropriate quality requirements when producing their tissue and cell preparations. In those cases, Health Authorities can be confident and they need only check that the quality parameters for the intended clinical uses are being appropriately reached through the processes performed by the tissue establishments.

The field of tissues and cells is a dynamic one and approaches continually change with advances in the development of new processing methods and the emergence of new indications.

It is important that these new improvements are carefully evaluated by tissue establishments and Health Authorities, notably via the process of authorisation. Chapter 18 of this guide provides generic guidance on good practice for the introduction of new or changed procedures and their subsequent authorisation. By reference to these monographs, both tissue establishments and Health Authorities will know that products that do not have a matching monograph may need more attention (more risk assessment, validation, possibly clinical studies etc.) before they can be authorised and supplied for routine use.

In future editions of the Guide, new processes/products will eventually have their own tissue or cell monograph once they become consolidated by evidence and there is consensus on the release criteria and appropriate clinical indications.

19.1. Organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

Tissue/cell product	Human organ-cultured corneal donor tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Human organ-cultured corneal donor tissue to be used for anterior lamellar keratoplasty: cornea, full thickness (low endothelial cell density).
Established clinical indications	• Stromal disease caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal opacities. • Endothelial cell density measurement may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) when anterior chamber is penetrated. • No evidence of microbial growth.
Quality control requirements	• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2 at end of organ culture storage or ≥ 2 200 cells/mm^2 if only measured before organ culture). • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 7 days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density, if applicable – Diameter of the central clear zone – Expiration date.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

19.2. Cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)

Tissue/cell product	Human cold-stored corneal tissue for (deep) anterior lamellar keratoplasty (ALK/DALK)
Definition	Human cold-stored corneal donor tissue to be used for anterior lamellar keratoplasty: cornea, full thickness (low endothelial cell density).
Established clinical indications	• Stromal disease caused by keratoconus, corneal dystrophies, scars and keratitis, or similar diseases compromising corneal function or the integrity of the cornea, when the endothelium of the recipient is assumed to have normal function.
Critical properties	• Absence of stromal opacities. • Endothelial cell density measurement may be required for DALK if surgeon needs to switch to penetrating keratoplasty (PK) when anterior chamber is penetrated. • No evidence of microbial growth.
Quality control requirements	• Stroma is clear and without scars within a 7.50 mm diameter zone. • If applicable, endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2). • Not to be used if the preservation medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density, if applicable – Diameter of the central clear zone – Expiration date.
Special warnings	• Not to be used for penetrating keratoplasty unless surgeon needs to switch to PK and the endothelial cell density is as specified in the quality control requirements (above).

19.3. Organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

Tissue/cell product	Human organ-cultured corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Human organ-cultured corneal donor tissue to be used for DMEK: cornea for endothelial keratoplasty (precut/peeled in the tissue establishment).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with the endothelium attached, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy after organ culture but before Descemet membrane peeling (preferably ≥ 2 000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 8.00 mm). • Tears (damage) in Descemet membrane within 8.00 mm zone must be noted in the accompanying documentation. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of the medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with or without an osmotically active agent. • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 7 days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Endothelial cell density – Expiration date – Graft diameter and presence of tears (damage).
Special warnings	• Not to be used for penetrating keratoplasty.

19.4. Cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)

Tissue/cell product	Human cold-stored corneal tissue for Descemet membrane endothelial keratoplasty (DMEK)
Definition	Human cold-stored corneal donor tissue to be used for DMEK: cornea for endothelial keratoplasty (precut/peeled in the tissue establishment).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • No evidence of microbial growth.
Quality control requirements	• Descemet membrane is manually peeled off the corneal stroma with attached endothelium, either completely (free roll) or attached centrally or peripherally to the corneal stroma. • Endothelial cell density measurement by microscopy before Descemet membrane peeling (≥ 2 000 cells/mm^2). • Graft diameter measurement with calliper or trephine (peeled zone ≥ 8.00 mm). • Tears (damage) in Descemet membrane within 8.00 mm zone must be noted in accompanying documentation. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Endothelial cell density – Expiration date – Graft diameter and presence of tears (damage).
Special warnings	• Not to be used for penetrating keratoplasty.

19.5. Organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

Tissue/cell product	Human organ-cultured corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Human organ-cultured corneal donor tissue to be used for DSAEK: cornea for endothelial keratoplasty (precut/peeled) in the tissue establishment.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy after organ culture but before pre-cutting (≥ 2 000 cells/mm^2). • Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography). • Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness). • Graft diameter measurement with calliper (preferably cap diameter ≥ 9.00 mm). • Replaced anterior corneal cap after pre-cutting. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be kept at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 144 hours depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density – Expiration date – Central graft thickness – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

19.6. Cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)

Tissue/cell product	Human cold-stored corneal tissue for Descemet stripping automated endothelial keratoplasty (DSAEK)
Definition	Human cold-stored corneal donor tissue to be used for DSAEK: cornea for endothelial keratoplasty (precut/peeled) in the tissue establishment.
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Regraft for endothelial decompensation.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before pre-cutting (≥ 2 000 cells/mm^2). • No stromal opacities within a 6.00 mm central zone. • Central stromal thickness measurement of the graft (ultrasound or, preferably, optical coherence tomography). • Minimal variation in graft thickness from centre to periphery (≤ 50 % increase in thickness). • Graft diameter measurement with calliper (preferably cap diameter ≥ 9.00 mm). • Replaced anterior corneal cap after pre-cutting. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density – Expiration date – Central graft thickness – Cap diameter.
Special warnings	• Not to be used for penetrating keratoplasty.

19.7. Organ-cultured corneal tissue for penetrating keratoplasty (PK)

Tissue/cell product	Human organ-cultured corneal tissue for penetrating keratoplasty (PK)
Definition	Human organ-cultured corneal donor tissue to be used for PK: cornea, full thickness (high endothelial cell density).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, corneal dystrophies with stromal involvement, scars). • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy (≥ 2 000 cells/mm^2 at end of organ-culture storage). • No stromal scars/opacities within a 7.50 mm diameter central zone. • Not to be used if the organ-culture medium is turbid or becoming yellow, or if samples of medium are culture-positive for bacteria or fungi.
Storage and transport	• The graft is stored in organ-culture medium with an osmotically active agent to keep the tissue thin (Dextran or similar component). • The graft must not be refrigerated but should be stored at room temperature (15-25 °C) or in an incubator (28-37 °C). • The graft can be stored up to 7 days depending on the concentration and type of osmotic thinning agent used in the medium.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density – Expiration date – Diameter of the clear zone.
Special warnings	• None.

19.8. Cold-stored corneal tissue for penetrating keratoplasty (PK)

Tissue/cell product	Human cold-stored corneal tissue for penetrating keratoplasty (PK)
Definition	Human cold-stored corneal donor tissue to be used for PK: cornea, full thickness (high endothelial cell density).
Established clinical indications	• Primary endothelial failure (mainly Fuchs corneal dystrophy). • Secondary endothelial failure (mainly pseudophakic bullous keratopathy). • Stromal disease (keratoconus, corneal dystrophies with stromal involvement, scars) • Keratitis and similar diseases compromising corneal functions or the integrity of the eye. • Regraft.
Critical properties	• Endothelial cell density. • Absence of stromal opacities. • No evidence of microbial growth.
Quality control requirements	• Endothelial cell density measurement by microscopy before cold storage (≥ 2 000 cells/mm^2). • No stromal scars or opacities within a 7.50 mm diameter central zone. • Not to be used if the storage medium is turbid or becoming yellow.
Storage and transport	• The graft is stored in cold-storage medium. • The graft is refrigerated (2-8 °C). • The graft can be stored up to 14-21 days according to the manufacturer's instructions (precise duration of storage depends on the type of storage medium).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Endothelial cell density – Expiration date – Diameter of the clear zone.
Special warnings	• None.

19.9. Sclera

Tissue/cell product	Sclera for use as a patch graft in scleral reinforcement or as coverage of synthetic ocular draining devices
Definition	Human stored scleral tissue prepared in a tissue establishment.
Established clinical indications	• Scleral defects needing reinforcement. • Coverage of synthetic ocular draining devices. • Coverage of prosthetic eye globes after enucleation.
Critical properties	• Scleral tissue devoid of intraocular contents (vitreous, lens, iris, choroidal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). • No evidence of microbial growth.
Quality control requirements	• No signs of intraocular contents or adnexa. • No presence of the additional donor exclusion criteria as related to vascularised tissues.
Storage and transport	• Sclera may be stored – whole, or divided into smaller, individually packaged pieces in ethanol (≥ 70 % v/v) or glycerol, or fixed in formalin, freeze-dried, deep-frozen (– 80 °C) or kept in physiological solution or medium with antibiotics. • Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days). • The shelf life for sclera stored by ethanol or glycerol, or fixed in formalin, freeze-dried, deep-frozen is 1-2 years.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Size of the scleral graft, either whole or size of individually packaged pieces – Expiration date.
Special warnings	• None.

20.1. Amniotic membrane sheet

Tissue/cell product	Amniotic membrane (AM) sheet
Definition	Human amniotic membrane obtained from placenta processed in a tissue establishment and preserved for use as biological dressing and substrate for cell growth in different clinical applications.
Established clinical indications	• Ophthalmological indications (e.g. ophthalmic corneal ulcerations, persistent epithelial defects, conjunctival defects, limbal stem cell deficiency, chemical or thermal burns, traumatic macular hole). • Burns (as a temporary or permanent wound dressing). • Wound healing (e.g. vascular ulcers, epidermolysis bullosa, surgical wounds).
Critical properties	• Preserved structural integrity (barrier function). • No evidence of microbial growth. • Adequate graft size. • For freeze-dried AM: residual moisture.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • For freeze-dried AM: residual moisture of 5-10 % (w/w) or available water (aW) of < 0.5 is recommended.
Storage and transport	**Cryopreservation** • Cryopreserved AM grafts are stored in liquid or vapour phase of nitrogen at temperatures below – 140 °C. The shelf life of cryopreserved AM is up to 5 years. Distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. **Freezing** • Deep frozen AM (with or without cryoprotectant) should be stored at < – 60 °C and transported at a similar temperature as was used during storage. Transport temperatures of cryoprotected AM above – 60 °C must be avoided to ensure the stability of the product and maximum safety for the recipient. The shelf life ranges between 1 and 2 years and should be justified according to the storage temperature conditions that could impact on the critical properties of the graft. **Lyophilisation/freeze-drying** • Lyophilised/freeze-dried AM can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be validated to allow for used packaging system and residual water. **Glycerolisation** • Glycerolised AM (preserved in glycerol 85 % solution) should be both stored and transported at 2-8 °C. For glycerolised AM the shelf life is up to 2 years.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must be included in the accompanying documentation • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Key specifications (e.g. graft dimensions in cm2, appropriate radiation-sensitive labels) – Instructions for appropriate rehydration or thawing according to the different storage conditions – Information on the composition of antibiotic decontamination cocktail or other residues – Information on the composition of cryoprotective solution when/if used – Orientation of amnion (epithelial or stromal side on carrier material).
Special warnings	• Rehydration time of freeze-dried grafts or thawing time of frozen/cryopreserved grafts, additionally to expiry date after preparation, should be defined by originating tissue bank, and rehydration or thawing should be performed under sterile conditions. • For AM immersed in a storage solution, the liquid should be removed and the graft rinsed with sterile saline solution or equivalent. • Do not re-freeze thawed AM graft. • Information about possible residues from processing. • Single recipient use.

20.2. Derivatives of the amniotic membrane

Tissue/cell product	Amniotic membrane in liquid form
Definition	Human amniotic membrane derivates obtained from placental tissues, processed in a tissue establishment and preserved for use: liquid amniotic membrane suspension for drops or for injection; amniotic membrane homogenate and extract; injectable matrix/gel.
Established clinical indications	• Orthopaedic indications (e.g. osteoarthritis, plantar fasciitis, tendinopathy, cartilage and bone injuries). • Ophthalmological indications (e.g. corneal ulcerations, epithelial defects, chemical burns, dry eye disease). • Wound healing (e.g. ulcers).
Critical properties	• No evidence of microbial growth. • Suitable consistency/texture for each specific application.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • In cases of lyophilisation (freeze drying) a residual moisture of 5-10 % (w/w) or available water (aW) of < 0.5 is recommended.
Storage and transport	**Cryopreservation** • Cryopreserved AM derivates are stored in liquid or vapour phase of nitrogen at temperatures below – 140 °C. Distribution should be in dry ice (solid carbon dioxide) or in a liquid nitrogen dry-shipper. **Freezing** • Deep frozen AM derivates (with or without cryoprotectant) should be stored at < – 60 °C and transported at a similar temperature as was used during storage. Transport temperatures of cryoprotected products above – 60 °C must be avoided to ensure the stability of the product and maximum safety for the recipient. The shelf life ranges between 1 and 2 years and should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. **Lyophilisation/freeze-drying** • Lyophilised/freeze-dried AM derivates can be stored and transported at room temperature (15-25 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the known range of ambient variables that could affect the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels) – Instructions for dilution, reconstitution, rehydration or thawing (appropriate solutions, timing) – Volume to use – Instruction for injection or drop application – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Dried, lyophilised, frozen or cryopreserved allografts should be used within appropriate timing after rehydration or thawing following instructions. • Rehydration time of freeze-dried products or thawing time of frozen products should be defined by originating tissue bank and rehydration or thawing should be performed under sterile conditions. • Do not re-freeze thawed AM products. • Single recipient use.

21.1. Acellular dermal matrix (ADM)

Tissue/cell product	Acellular dermal matrix (ADM)
Definition	Human decellularised dermis from donor skin to be used for wound-healing procedures.
Established clinical indications	• Burn injuries. As dermal substitute in partial- or full-thickness burns when donor sites are insufficient for autograft or when patient condition is critical, to improve scar quality and to prevent post-burn joint contracture. • Reconstructive surgery. As a valid reconstructive tool for any surgical wound in general surgery (e.g. abdominal wall repair), orthopaedic (e.g. rotator cuff reconstruction), oncologic (e.g. breast-conserving surgery), ear nose and throat (ENT) surgery (e.g. myringoplasty, rhinoplasty) and bariatric surgery. • Ulcers. Uninfected, chronic ulcers and diabetic foot ulcers, to accelerate the closure and healing rate. • Full-thickness acute wounds. As a scaffold to support cell ingrowth and granulation tissue formation and to achieve durable coverage of exposed critical structures (bone, tendons). • Composite graft technique. To restore the dermal component by application of human dermis or dermal equivalents, and thin autologous grafts or keratinocytes.
Critical properties	• Absence of donor cells (and genetic material). • Graft thickness ranging 0.2-0.8 mm (thin), 0.8-1.2 mm (medium thickness) and 1.3 mm or higher (thick). • Preserved structural integrity. • Flexibility/pliability. • Resistance to mechanical stress and suturability. • No evidence of microbial growth.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • Graft thickness measurement. • Biocompatibility test (cytotoxicity tests). • Mechanical resistance test. • Morpho-structure evaluation by histological staining (e.g. orcein, Masson, haematoxylin-eosin) or electron microscopy (EM). • Decellularisation process by specific histological staining e.g. DAPI, Hoersch and DNA quantification assay (ng DNA/mg dry tissue). • Residual water test (≤ 5 %) – for lyophilised ADM.
Storage and transport	• Depending on the processing methods, most dermal matrices are stored and transported at room temperature (15-25 °C) – e.g. acellular glycerol-preserved, lyophilised and/or irradiated matrices – or refrigerated at 2-8 °C.
– Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Appropriate radiation-sensitive labels must be used for irradiated ADM – Thickness and size – Instructions for appropriate manipulation by end-users – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Do not sterilise irradiated dermal matrices. • Rehydration of glycerol-preserved or lyophilised allografts is recommended before use (e.g. by washing in saline solution for 20-30 min).

21.2. Deep-frozen skin allografts

Tissue/cell product	Deep-frozen skin allografts
Definition	Viable skin allografts preserved in a cryoprotective solution.
Established clinical indications	• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation).
Critical properties	• Cell viability: it is maintained by deep-frozen skin grafts to a lesser degree if compared to cryopreserved skin grafts; to consider a graft as viable, a minimum of 20 % of residual cell viability should be achieved. The mean percentage after 10-20 days of storage is reportedly 30-51 % (deep-frozen skin grafts) compared to that of the fresh skin, according to different viability assays. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft thickness ranging 0.2-0.8 mm. • Mechanical resistance. • Capable of engraftment.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi). • Cell viability assessment (if required, depending on the intended application).
Storage and transport	• Deep-frozen skin grafts are stored in ultra-low-temperature refrigerators, which generally maintain a temperature between – 60 ° and – 80 °C. • Transport in dry ice.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Graft area in cm^2 – Graft thickness – Number of sheets – Decontamination solution composition – Cryoprotective solution composition – Cell viability – Instructions for appropriate manipulation by end-users.
Special warnings	• Do not re-freeze thawed skin graft. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use. • Rinse with an appropriate solution (saline solution or balanced salt solution).

21.3. Glycerol-preserved skin allograft

Tissue/cell product	Glycerol-preserved skin allograft
Definition	Human split-thickness, glycerol-preserved, de-vitalised skin grafts, with epidermis and upper dermis components for the treatment of skin loss.
Established clinical indications	• Temporary biological dressing: – in partial-thickness burns, – on meshed autografts (sandwich technique), – on donor site, – after application of *in vitro* cultured keratinocytes. • Temporary wound coverage after excision in full-thickness burns. • Temporary coverage in toxic epidermolytic necrolysis. • Temporary biological dressing for difficult, non-healing wounds, to protect and preserve the viable granulation tissue from desiccation and necrosis (antalgic and antibacterial effect). • Wound-bed preparation (promoting wound healing).
Critical properties	• Graft thickness ranging 0.2-0.8 mm. • Plain or meshed. • No evidence of microbial growth.
Quality control requirements	• Intact epidermis and upper dermis (normal morphological structure). • Microbiological testing (aerobic and anaerobic bacteria, fungi).
Storage and transport	• The graft is stored in glycerol 85 % solution to keep the tissue preserved before use. • The graft is stored at refrigerator temperature (2-8 °C); storage at room temperature (15-25 °C) during transportation is possible. • Maximum time: storage at 2-8 °C for 5 years.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Size of graft, width and length – Graft thickness – Plain or meshed – Instructions for appropriate manipulation by end-users.
Special warnings	• Rinse out glycerol before use (incubation in a large volume of sterile 0.9 % NaCl solution for 10 min at room temperature). • Not to be used if the storage medium is opaque.

21.4. Cryopreserved skin allografts

Tissue/cell product	Cryopreserved skin allografts
Definition	Viable skin allografts preserved in a cryoprotective solution, for the treatment of skin loss.
Established clinical indications	• Temporary/semipermanent covering of burns. • Temporary covering of epidermolytic diseases (e.g. toxic epidermolytic necrosis, staphylococcal scalded skin syndrome). • Treatment of hard-to-heal ulcers. • Treatment of cutaneous loss due to meningococcal septicaemia and purpura fulminans. • Temporary covering of wounds with exposed bone and/or tendons. • Wound-bed preparation (promoting neovascularisation and providing dermal template for epidermal grafts).
Critical properties	• Cell viability: it is maintained by cryopreserved skin grafts; to consider a graft as viable, a minimum of 20 % of residual cell viability should be achieved. The mean percentage after 10-20 days of storage is reportedly 20-60 % for cryopreserved skin grafts, compared to that of the fresh skin, according to different viability assays. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure). • Graft thickness ranging 0.2-0.8 mm. • Mechanical resistance. • Capable of engraftment. • Long-term storage.
Quality control requirements	• Cell viability assessment (if required, depending on the intended application). • Microbiological testing (aerobic and anaerobic bacteria, fungi).
Storage and transport	• Cryopreserved skin grafts are stored in liquid or in the vapour phase of nitrogen at temperatures lower than – 140 °C. • Transport in a nitrogen dry-shipper or in dry ice (according to the TE procedures).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Graft area in cm^2 – Graft thickness in mm – Number of sheets – Decontamination solution composition – Cryoprotective solution composition – Cell viability – Instructions for appropriate manipulation by end-users.
Special warnings	• Do not re-freeze thawed skin graft. • Do not irradiate viable skin graft. • Rinse out cryoprotectants before use with an appropriate solution (saline solution or balanced salt solution).

22.1. Cryopreserved aortic heart valve allograft, antibiotic decontamination

Tissue/cell product	Cryopreserved aortic heart valve allograft, antibiotic decontamination
Definition	Human aortic heart valve, including the root of the aorta and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Congenital pulmonary or aortic valve malformation. • Aortic valve stenosis or insufficiency. • Aortic valve or pulmonary valve endocarditis. • Replacement of prosthetic valve (mechanical/bioprosthetic) in aortic position.
Critical properties	• There should be no visible (or minimal) calcification or malformations present in the valve or associated vessel. • There must be a rim of myocardium or mitral leaflet of at least 2 mm depth surrounding the base of the vessel. • The associated aortic conduit should not be cut below the level of 1-2 mm of the height above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test. • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi). • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• Grafts should be stored at < – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If graft is shipped in dry ice, it should not be returned to storage at < – 140 °C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions (annular diameter and length/diameter of associated vascular conduit) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.2. Cryopreserved pulmonary heart valve allograft, antibiotic decontamination

Tissue/cell product	Cryopreserved pulmonary heart valve allograft, antibiotic decontamination
Definition	Human pulmonary heart valve, including pulmonary trunk and variable length of associated artery. Decontaminated by incubation with one or more antibiotics and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Tetralogy of Fallot. • Double outlet right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Pulmonary valve atresia. • Absent pulmonary valve syndrome. • Ross procedure. • Pulmonary valve endocarditis. • Prosthetic valve degeneration in the pulmonary valve position. • Complications of trans-catheter pulmonary valve replacement.
Critical properties	• There should be no visible (or minimal) calcification or other malformations present in the valve or associated vessel. • There must be a rim of myocardium of at least 2 mm depth surrounding the root of the pulmonary trunk. • The associated pulmonary artery should not be cut below the level of 1-2 mm of the height above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decontamination and preservation protocols applied, as demonstrated by a functionality test. • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi). • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• Grafts should be stored at < – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft. • If the graft is shipped in dry ice, it should not be returned to storage at < – 140 °C unless this is supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions (annular diameter and length/diameter of associated vascular conduit) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze the thawed heart valve graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.3. Cryopreserved artery allograft, antibiotic decontamination

Tissue/cell product	Cryopreserved artery allograft, antibiotic decontamination
Definition	Human artery, either iliac or femoral artery or descending aorta. Decontaminated by incubation with one or more antibiotics, and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Replacement of infected prosthetic vascular allografts. • Mycotic arterial aneurysm. • Chronic limb ischaemia. • Critical acute limb ischaemia. • Vascular trauma. • Malignant infiltration of the vascular wall. • Vascular dissection.
Critical properties	• There should be no visible (or minimal) atheroma or calcification present. • There should be no visible stenosis or dilation present. • Branching arteries should be 2-3 mm in length. • There should be no cuts or significant haematomas in the vessel wall. • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi).
Storage and transport	• Grafts should be stored at or below – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • •Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions (proximal and distal annular diameter and length) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze the thawed artery graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.4. Cryopreserved non-valved patches and conduits, antibiotic decontamination

Tissue/cell product	Cryopreserved non-valved patches and conduits, antibiotic decontamination
Definition	Human artery wall, either patches or conduits. Decontaminated by incubation with one or more antibiotics, and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Tetralogy of Fallot. • Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Ventricular septal defect. • Pulmonary stenosis. • Hypoplastic heart (L/R).
Critical properties	• There should be no visible (or minimal) atheroma or calcification present. • There should be no visible stenosis or dilation present. • There should be no cuts or significant haematomas in the vessel wall. • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no pathogenic micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi).
Storage and transport	• Grafts should be stored at or below – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions (proximal and distal annular diameter and length) – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing and dilution of the cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze the thawed patch or conduit graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.5. Decellularised aortic heart valve allograft, antibiotic decontamination

Tissue/cell product	Decellularised aortic heart valve allograft, antibiotic decontamination
Definition	Human aortic heart valve, including the aortic root and variable length of associated ascending aorta. Decellularised according to validated procedures and decontaminated by incubation with one or more antibiotics.
Established clinical indications	• Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis or insufficiency. • Aortic valve stenosis or insufficiency. • Aortic valve or pulmonary valve endocarditis. • Congenital pulmonary or aortic valve malformation. • Replacement of prosthetic valve (mechanical/bioprosthetic) in aortic position.
Critical properties	• There should be no visible (or minimal) calcification present in the valve or associated vessel. • There must be a rim of myocardium or mitral leaflet of at least 2 mm depth surrounding the base of the vessel. • The associated aortic artery should not be cut below the level of 1-2 mm of the height above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by decellularisation, decontamination and preservation protocols applied, as demonstrated by a functionality test. • Post-decellularisation and decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no highly virulent micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi). • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• Grafts should be stored at 2-8 °C for long-term storage. The shelf life at this temperature is validated to be around 1-2 years, but needs to be validated by the distributing TE. • Grafts should be transported at 2-8 °C. • The grafts are to be stored at 2-8 °C after distribution; the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in the accompanying documentation: – Donor age – Key dimensions (annular diameter and length/diameter of associated conduit) – Identity of any residual processing chemicals (antibiotics and decellularisation agents) – Instructions for appropriate handling.
Special warnings	• Use as soon as possible after removing from cold storage. The maximum permissible period thereafter until transplantation should be defined, based on validation data or a documented rationale. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.6. Decellularised pulmonary heart valve allograft, antibiotic decontamination

Tissue/cell product	Decellularised pulmonary heart valve allograft, antibiotic decontamination
Definition	Human pulmonary heart valve, including pulmonary trunk and variable length of associated artery. Decellularised according to validated procedures and decontaminated by incubation with one or more antibiotics.
Established clinical indications	• Tetralogy of Fallot. • Double output right ventricle. • Truncus arteriosus. • Transposition of the great vessels. • Pulmonary valve stenosis. • Pulmonary valve atresia. • Absent pulmonary valve syndrome. • Ross procedure. • Endocarditis of the native or prosthetic pulmonary valve. • Prosthetic valve degeneration in the pulmonary valve position. • Complications of trans-catheter pulmonary valve replacement.
Critical properties	• There should be no visible (or minimal) calcification or leaflet malformation present in the valve or associated vessel. • There must be a rim of myocardium of at least 2 mm depth surrounding the base of the vessel. • The associated pulmonary artery should not be cut below the level of 1-2 mm of the height above the commissure. • The native biomechanical and hydrodynamic properties of the valve should not be altered by the decellularisation and decontamination and preservation protocols applied, as demonstrated by a functionality test. • Post-decellularisation and decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no highly virulent micro-organisms detectable on the graft.
Quality control requirements	• Pathological analysis on the remnants of the heart. • Microbiological testing (aerobic and anaerobic bacterial, fungi). • Functionality tests (such as competency) should be within the acceptable criteria.
Storage and transport	• Grafts should be stored at 2-8 °C for long-term storage. The shelf life at this temperature is validated to be around 1-2 years, but needs to be validated by the distributing TE. • Grafts should be transported at 2-8 °C. • The grafts are to be stored at 2-8 °C after distribution; the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions (annular diameter and length/diameter of associated conduit) – Identity of any residual processing chemicals (antibiotics and decellularisation agents) – Instructions for appropriate handling.
Special warnings	• Use as soon as possible after removing from cold storage. The maximum permissible period thereafter until transplantation should be defined, based on validation data or a documented rationale. • Rinse with an appropriate solution (saline solution or balanced salt solution).

22.7. Cryopreserved pericardium, antibiotic decontamination

Tissue/cell product	Cryopreserved pericardium, antibiotic decontamination
Definition	Human pericardium patches. Decontaminated by incubation with one or more antibiotics, and cryopreserved using controlled rate cooling in the presence of a cryoprotectant.
Established clinical indications	• Vascular patch plasty or reconstruction. • Ventricular septal defect (VSD) closure. • Atrial septal defect (ASD) closure. • Widening plasty during valve replacement. • Reconstruction of ventricular aneurysms. • Dural substitute.
Critical properties	• No visible tears or cuts to be present in the patch. • Mechanical properties should be preserved after the processing procedure (decontamination and cryopreservation). • Post-decontamination, no viable micro-organisms detectable on the graft. • Pre-decontamination, no highly virulent micro-organisms detectable on the graft.
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacterial, fungi).
Storage and transport	• Grafts should be stored at or below – 140 °C for long-term storage. The shelf life at this temperature should be justified by reference to the critical properties. • Grafts should be transported to the point of use using either liquid nitrogen cooled shippers (dry shipper) or solid carbon dioxide refrigeration (dry ice). • If grafts are to be stored at – 80 °C (or other temperature > – 140 °C) after distribution, the shelf life at this temperature should be supported by validation data or a documented rationale based on maintenance of the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Donor age – Key dimensions – Identity of any residual processing chemicals (antibiotics and cryoprotectants) – Instructions for appropriate thawing and dilution of cryoprotectant.
Special warnings	• Use as soon as possible after thawing. The maximum permissible period between thawing and transplantation should be defined, based on validation data or a documented rationale. • Do not re-freeze thawed pericardium graft. • Rinse with an appropriate solution (saline solution or balanced salt solution).

23.1. Cancellous/corticocancellous bone

Tissue/cell product	Cancellous / Corticocancellous bone
Definition	Cancellous bone, also referred to as trabecular bone or spongy bone, forms the inner part of the bone. Cancellous bone is the porous mineralised component of bone that has a honeycomb or sponge-like appearance. The bone matrix is organised into a three-dimensional lattice of bony processes (trabeculae) arranged along lines of stress. Cancellous bone provides an osteoconductive matrix for bone remodelling and healing. Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons) conferring an excellent structural integrity. Cancellous and corticocancellous bone grafts include different presentations ranging from chips sawn or ground to various sizes (generally 1-10 mm diameter/edge length); geometrical pieces (discs, wedges, blocks, dowels); customised bone grafts; particles; granules and powder.
Established clinical indications	• Bony defects filling • Bone loss in fractures • Prosthetic replacement surgery • Tumours • Spinal fusion • Malunion • Maxillofacial and periodontal surgery
Critical properties	• Cancellous bone free of muscle, periosteum and cortical bone. • Corticocancellous bone free of muscle. Cartilage can be preserved or removed in the case of geometrical pieces if necessary (femoral head, femoral condyle, tibial plateau …) • Reduction or removal of fat, blood cells and bone marrow • Absence of microbiological growth after processing • Adequate size and volume • For geometrical pieces supporting load constraints: the essential native biomechanical properties of the bone must not be significantly altered (including terminal sterilisation if applied) • Absence of cytotoxicity due to the processing protocol • In cases of dehydration (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • In cases of lyophilisation (freeze-drying): residual moisture or available water • Size and shape characterisation
Storage and transport	• There are different approaches to preserving cancellous grafts. The process could require a lyophilisation step, or frozen step or cryopreservation step, each of which requires particular storage and transport conditions. • Final product storage and transport conditions depend on product final preservation method: – Frozen grafts can be stored between – 15 °C and – 80 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. – Dehydrated/freeze-dried grafts can be stored and transported at room temperature (4-30 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the known range of ambient variables that could affect the critical properties of the graft. – Cryopreserved grafts can be stored between – 140 and – 196 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.

Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels) – Instructions for use, including reconstitution, rehydration or thawing (appropriate solutions, timing) – Volume, particle size, graft size if applicable – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use • Dehydrated, frozen or cryopreserved allografts should be used within appropriate timings after rehydration or thawing following instructions. Timings must be defined by originating tissue bank and rehydration/thawing operations must be performed under sterile conditions. • Do not re-freeze thawed grafts.

23.2. Cortical bone

Tissue/cell product	Cortical bone
Definition	Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons) conferring an excellent structural integrity. Cortical bone graft is obtained from long bone shafts (usually femur, tibia, humerus and fibula). It includes a range of different presentations: shaft segments; hemicylinders; plates; struts; particles; granules and powder.
Established clinical indications	• Bony defects filling • Bone loss in fractures • Prosthetic replacement surgery • Tumours • Spinal surgery • Malunion • Maxillofacial and periodontal surgery
Critical properties	• Free of muscle, periosteum and cancellous bone • Reduction or removal of fat, blood cells and bone marrow • Absence of microbiological growth after processing • Adequate size and volume • For grafts supporting load constraints: the essential native biomechanical properties of the bone must not be significantly altered • Absence of cytotoxicity due to the processing protocol • In cases of lyophilisation (freeze drying) a residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • In cases of dehydration (freeze drying): residual moisture or available water • Size and shape characterisation
Storage and transport	• There are different approaches to preserving cortical grafts. The process could require a lyophilisation step, or frozen step or cryopreservation step, each of which requires particular storage and transport conditions. • Final product storage and transport conditions depend on product final preservation method: – Frozen grafts can be stored between – 15 and – 80 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. – Dehydrated/freeze-dried grafts can be stored and transported at room temperature (4-30 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the known range of ambient variables that could affect the critical properties of the graft. – Cryopreserved grafts can be stored between – 140 and – 196 °C and transported at a similar temperature as was using during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels) – Instructions for use including reconstitution, rehydration or thawing (appropriate solutions, timing) – Volume, particle size, graft size if applicable – Information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Dehydrated, frozen or cryopreserved allografts should be used within appropriate timings after rehydration or thawing following instructions. Timings must be defined by originating tissue bank and rehydration/thawing operations must be performed under sterile conditions. • Do not re-freeze thawed grafts.

23.3. Structural bone

Tissue/cell product	Structural bone
Definition	Structural bone is a corticocancellous human graft; its main purpose is to replace a bone segment in the recipient. It can be processed with or without ligament or muscular attachments, and with or without articular cartilage. It can be a whole bone or a part from it (distal, proximal, half, third).
Established clinical indications	• Bone loss in fractures • Prosthetic replacement surgery • Tumours • Malunion • Maxillofacial surgery
Critical properties	• Free of periosteum. Ligament and muscular attachments as well as cartilage can be preserved if applicable • Absence of microbiological growth after processing • No morphology alterations (i.e. deformities, fracture callus …) • Absence of significant alteration of the native biomechanical properties(including terminal sterilisation if applied) • Absence of cytotoxicity due to the processing protocol
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • Side, size and shape characterisation
Storage and transport	• There are different approaches to preserving corticocancellous grafts. The process could require a frozen step or cryopreservation step, each of which requires particular storage and transport conditions. Lyophilisation is not recommended because it produces a significant reduction of graft biomechanical properties. • Final product storage and transport conditions depend on product final preservation method: – Frozen grafts can be stored between – 15 and – 80 ºC and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. – Cryopreserved grafts can be stored between – 140 and – 196 ºC and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Key specifications (e.g. appropriate radiation-sensitive labels) – Instructions for use including thawing (appropriate solutions, timing) – Side, size and/or shape characteristics – Information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use • Frozen or cryopreserved allografts should be used within an appropriate time after thawing, following instructions. Timings must be defined by originating tissue bank and operations must be performed under sterile conditions • Do not re-freeze thawed grafts.

23.4. Meniscus

Tissue/cell product	Meniscus
Definition	Meniscus is a crescent-shaped fibrocartilaginous structure located at the knee joint. Its main function is to provide stability to the femorotibial joint, distribute axial load and absorb shock. It can be processed with or without bone block from tibia plateau.
Established clinical indications	• Meniscus replacement
Critical properties	• Absence of meniscal surgery antecedent on medical history • Absence of blood straining and degenerative or traumatic lesions • Absence of microbiological growth after processing • Absence of significant alteration of the native biomechanical properties of meniscus (including terminal sterilisation if applied) • Processing steps should largely reduce or remove fat, blood cells and bone marrow from bone block if applicable • Absence of cytotoxicity due to the processing protocol • Donor–recipient matching: meniscus dimensions (recommended) or donor–recipient height/weight
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • Side and size characterisation (anterior-posterior diameter, medial-lateral diameter)
Storage and transport	• There are different approaches to preserving meniscal grafts. The process could require a frozen step or cryopreservation step, each of which requires particular storage and transport conditions. Lyophilisation is not recommended because it produces histological changes and a significant reduction of graft biomechanical properties. • Final product storage and transport conditions depend on product final preservation method: – Frozen grafts can be stored between – 15 and – 80 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. – Cryopreserved grafts can be stored between – 140 and – 196 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – key specifications (e.g. appropriate radiation-sensitive labels) – instructions for use, including thawing (appropriate solutions, timing) – side and size characteristics – information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within appropriate timing after thawing, following instructions. Timings must be defined by originating tissue bank and operations must be performed under sterile conditions. • Do not re-freeze thawed grafts.

23.5. Tendon

Tissue/cell product	Tendon
Definition	Ligament is a fibrous connective tissue that connects bones. The patellar ligament is considered the distal portion of the common tendon of the Quadriceps femoris muscle, which continues from the patella to the tibial tuberosity, and it is also called the patellar tendon. Tendon is a dense fibrous connective tissue that connects muscle to bone. Tendons can be processed with bone block (patellar ligament, Achilles tendon) or without it (i.e. anterior tibialis tendon, posterior tibialis tendon, peroneus longus tendon, semitendinosus tendon, gracilis tendon, Achilles tendon).
Established clinical indications	• Joint instability • Injuries of the extensor mechanism of the knee • Bridging a gap in tendon injuries • Repair of chronic muscle tendon ruptures • Muscle transpositions
Critical properties	• Absence of tendinous pathology antecedent on medical history • Absence of muscle and peritendon • Absence of blood staining, fibre separation, stiffness and degenerative or traumatic lesions • Absence of microbiological growth after processing • Absence of significant alteration of the native biomechanical properties (including terminal sterilisation if applied) • Processing steps should largely reduce or remove fat, blood cells and bone marrow from bone block, if applicable • Absence of cytotoxicity due to the processing protocol
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • Size characterisation (length, thickness, double loop thickness if applicable)
Storage and transport	• There are different approaches to preserving tendon grafts. The process could require a frozen step or cryopreservation step, each of which requires particular storage and transport conditions. Lyophilisation is not recommended because it produces a significant reduction of graft biomechanical properties. Final product storage and transport conditions depend on product final preservation method: – Frozen grafts can be stored between – 15 and – 80 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. – Cryopreserved grafts can be stored between – 140 and – 196 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation:: – key specifications (e.g. appropriate radiation-sensitive labels) – instructions for use, including thawing (appropriate solutions, timing) – side and size characteristics – information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Frozen or cryopreserved allografts should be used within appropriate timings after thawing, following instructions. Timings must be defined by originating tissue bank and operations must be performed under sterile conditions. • Do not re-freeze thawed grafts.

23.6. Osteochondral allograft

Tissue/cell product	Osteochondral allograft
Definition	Hyaline cartilage results from a combination of a semitranslucent matrix, mainly composed of randomly oriented collagen fibres and some elastin, and chondrocytes. It is normally found on joint surfaces. Osteochondral graft includes hyaline cartilage and subchondral bone. It is mainly obtained from knee (most common), ankle, shoulder or hip.
Established clinical indications	• Treatment of chondral or osteochondral lesions • Sequel of joint fractures
Critical properties	• Absence of chondral pathology antecedent on medical history • Absence of blood staining and degenerative or traumatic lesions • Absence of microbiological growth at the time of distribution (depends on shelf life, which is determined by preservation method) • Preservation of ≥ 70 % of viable chondrocytes (through validation) • Donor–recipient matching: either graft dimensions (recommended) or donor–recipient height/weight
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • Side and size characterisation
Storage and transport	• Fresh preservation (hypothermic or organ-cultured at 37 °C) is the best method to preserve chondrocyte viability. Cryopreservation is not recommended because it produces a drastic decrease in chondrocyte viability. Final product storage and transport conditions depend on product final preservation method: • Fresh hypothermic grafts can be stored between +2 and +8 °C and transported at a similar temperature as was used during storage. The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft. • Fresh organ-cultured grafts can be stored between 28 and 37 °C and transported at room temperature (15-25 °C). The shelf life should be validated according to the storage temperature conditions that could impact on the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – key specifications (e.g. storage temperature) – instructions for use, including rinsing (appropriate solutions, timing) – side and size characteristics – information on the composition of antibiotic decontamination cocktail or other residues.
Special warnings	• Single recipient use. • Fresh allografts should be used within appropriate timings, following instructions. Rinsing steps and timings must be defined by originating tissue bank and must be performed under sterile conditions. • Do not freeze grafts.

23.7. Demineralised bone matrix

Tissue/cell product	Demineralised bone matrix
Definition	Cancellous bone, also referred to as trabecular bone or spongy bone, forms the inner part of the bone. Cancellous bone is the porous mineralised component of bone that has a honeycomb or sponge-like appearance. Cortical bone, also referred to as compact bone or lamellar bone, forms the cortex of most bones and is much denser, harder and stiffer than cancellous bone. It consists of multiple microscopic columns (osteons) conferring an excellent structural integrity. Demineralised bone matrix (DBM) graft can be composed of cortical bone, cancellous bone or a combination of both, when they are submitted to a demineralisation process to reduce calcium content and activate bone morphogenetic proteins (BMP). This process provides the osteoinductive capability to the graft. DBM includes different presentations, ranging from chips sawn or ground to particles; granules, powder, putty and paste (putty and paste require the use of a carrier).
Established clinical indications	• Bony defects filling • Spinal fusion • Malunion • Maxillofacial and periodontal surgery
Critical properties	• Free of muscle, periosteum and cartilage • Reduction or removal of fat, blood cells and bone marrow • Absence of microbiological growth after processing • Calcium content ≤ 5 % • Osteoinductive capability • Adequate size and volume • Absence of cytotoxicity due to the processing protocol • Residual moisture of 1-6 % (w/w) or available water (aW) of < 0.5 is recommended
Quality control requirements	• Microbiological testing (aerobic and anaerobic bacteria, fungi) • Final calcium content • In vivo/in vitro osteoinduction test (recommended) • In case of lyophilisation (freeze-drying): residual moisture or available water • Volume characterisation
Storage and transport	• Lyophilisation is the preservation method. Lyophilised/freeze-dried grafts can be stored and transported at room temperature (4-30 °C). Freezing and high temperatures (> 30 °C) should be avoided. The shelf life should be justified according to the known range of ambient variables that could affect the critical properties of the graft.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – key specifications (e.g. storage temperature) – instructions for use, including reconstitution, rehydration or handling (appropriate solutions, timing) – volume and/or particle size if applicable – information on the composition of antibiotic decontamination cocktail or other residues if applicable.
Special warnings	• Single recipient use. • Lyophilised allografts should be used within appropriate timings after rehydration, following instructions. Timings must be defined by originating tissue bank and must be performed under sterile conditions. • Do not freeze or heat grafts.

24.1. Haematopoietic progenitor cells from bone marrow – HPC(M)

Tissue/cell product	Haematopoietic progenitor cells from bone marrow – HPC(M)
Definition	HPC are found in small numbers in bone marrow. The infused HPC(M) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or can be further processed (e.g. buffy-coat preparation, cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability *a.* for autologous transplantation: – nucleated cell dose: >1.0-2.0 × 10^8/kg recipient body weight, – viable $CD34^+$ cell dose: ≥ 2.0 × 10^6/kg recipient body weight; *b.* for allogeneic transplantation: – nucleated cell dose: ≥ 2.0-3.5 × 10^8/kg recipient body weight, – viable $CD34^+$ cell dose: ≥ 2.0-3.5 × 10^6/kg recipient body weight. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should limited to 0.2 to 0.4 mL/kg or 10-30 mL • In cases of cryopreserved HPC(M), DMSO volume should be less than 1 mL/kg recipient body weight.
Quality control requirements	• Nucleated cell count • Enumeration of viable $CD34^+$ cells • Microbiological testing • ABO Rh blood group for allogeneic products • Measurement of residual ABO-incompatible red cell volume
Storage and transport	• Fresh HPC(M) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C) as requested by the transplant centre. • Cryopreserved HPC(M) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved HPC(M) can be stored for up to 10 years or longer. • Thawed HPC(M) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – donor name (autologous or related donors) or donor ID (unrelated donors) – recipient name (if permitted), recipient ID (if applicable) – nucleated cell count and viable $CD34^+$ cell enumeration – ABO Rh blood group – volume – identity of the collection facility and/or donor registry – identity of processing and distribution facility – instructions for appropriate thawing, if applicable.
Special warnings (if needed)	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If presence of microbial contamination, consider antibiotic treatment in the recipient.

24.2. Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)

Tissue/cell product	Haematopoietic progenitor cells from peripheral blood apheresis – HPC(A)
Definition	HPC(A) are procured by apheresis from the mononuclear cell fraction of circulating blood after their mobilisation from the bone marrow. The infused HPC(A) can originate from the recipient (autologous) or from another individual (allogeneic). They can be used as fresh unmanipulated product or further processed (e.g. cell selection, cryopreservation).
Established clinical indications	• Restoration of haematopoiesis after chemo- and/or radiation therapy (autologous and allogeneic transplantation). • Establishment of donor chimerism (allogeneic transplantation).
Critical properties	• Cellularity/viability *a.* for autologous transplantation: – viable $CD34^+$ cell dose: ≥ 2.0×10^6/kg recipient body weight; *b.* for allogeneic transplantation: – target viable $CD34^+$ cell dose: approximately > 5.0×10^6/kg recipient body weight, – minimum viable $CD34^+$ cell dose: 1.5-3.5 × 10^6/kg body weight. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be limited to 0.2 to 0.4 mL/kg or 10-30 mL recipient weight. • In case of cryopreserved HPC(A), DMSO volume should be less than 1 mL/kg recipient body weight.
Quality control requirements	• Nucleated cell count • Viable $CD34^+$ cell enumeration • Microbiological testing • ABO Rh blood group for allogeneic products • Measurement of residual ABO-incompatible red cell volume
Storage and transport	• Fresh HPC(A) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C), as requested by the transplant centre. • Fresh HPC(A) can be stored up to 72 hours without cryopreservation. • Cryopreserved HPC(A) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved HPC(A) can be stored for up to 10 years or longer. • Thawed HPC(A) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC that must be included in accompanying documentation: – donor name (autologous or related donors) or donor ID (unrelated donors) – recipient name (if permitted), recipient ID (if applicable) – viable $CD34^+$ cell enumeration – ABO Rh blood group – volume – identity of the collection facility and /or donor registry – identity of processing and distribution facility – instructions for appropriate thawing (if applicable).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing. • If presence of microbial contamination, consider antibiotic treatment in the recipient.

24.3. Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)

Tissue/cell product	Mononuclear cells from unstimulated peripheral blood apheresis – MNC(A)
Definition	Unstimulated mononuclear cells are procured by apheresis from the circulating blood. The procured cells can originate from the recipient (autologous) or from another individual (allogeneic). Unstimulated mononuclear cells can be used as fresh non-manipulated products or further processed (e.g. cryopreservation, cell selection, starting material for ATMPs).
Established clinical indications	• MNC(A) after allogeneic stem cell transplantation from the original HPC donor are used in cases of relapse and mixed chimerism or as relapse prophylaxis to enhance the graft-*versus*-malignancy effect, to promote immune reconstitution and prevent infection complications. • MNC(A) for generation of cellular therapies and ATMPs (e.g. NK-cell therapy, virus-specific T-cells, CAR-T cells).
Critical properties	• Cellularity/viability *a.* After allogeneic transplantation to enhance immunity and graft-*versus*-malignancy effect: – escalating cell doses of $CD3^+$ cells, depending on the clinical situation and the transplant setting (e.g. in case of relapse from 1.0×10^6/kg to 1.0×10^8/kg body weight), – $CD3^+$ cell dose > 1.0×10^8/kg body weight per infusion should be avoided due to increased risk of graft-*versus*-host disease; *b.* As starting material for generation of cellular therapy and ATMPs: – required cell dose according to the specific protocol. • Absence of microbial contamination (the presence of microbial contamination may not preclude release but may indicate the need for antibiotic treatment in the recipient).
Quality control requirements	• Nucleated and mononuclear cell count • Viability • Viable CD_3^+ cells enumeration • Microbiological testing • ABO Rh blood group for allogeneic products
Storage and transport	• Fresh MNC(A) can be stored and transported up to 72 hours at room temperature (15-25 °C) or refrigerated (2-8 °C) as requested by the transplant centre. • Fresh MNC(A) can be stored up to 72 hours without cryopreservation. • Cryopreserved MNC(A) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved MNC(A) can be stored for up to 10 years or longer. • Thawed MNC(A) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC that must be included in accompanying documentation: – donor name (autologous or related donors) or donor ID (unrelated donors) – recipient name, recipient ID (if applicable) – total nucleated and mononuclear cell count – viable $CD3^+$ cell count – ABO Rh blood group (allogeneic products) – volume – identity of the collection facility and /or donor registry – identity of processing and distribution facility – instructions for appropriate thawing, if applicable.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing (if applicable). • If presence of microbial contamination, consider antibiotic treatment in the recipient.

25.1. Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)

Tissue/cell product	Haematopoietic progenitor cells from umbilical cord blood – HPC(CB)
Definition	HPC are found in umbilical cord blood (UCB). UCB banks collect, transport, process and store UCB and, after validation of the UCB, transfer the data to a stem cell registry. Upon request, UCB units are distributed cryopreserved as whole blood or buffy-coat enriched.
Established clinical indications	• Alternative source for haematopoietic progenitor cell transplantation when no sibling or family HLA-match donors are available.
Critical properties	• Cellularity/viability Cell dose will depend on clinical indication, age and patient's body-weight. It is suggested to increase cell dose if there is HLA mismatch increase or if the patient has a non-malignant disorder. Minimum recommended cell dose for a single UCB transplantation: TNC > 3E7/kg CD34 > 1.5E5/kg • Absence of microbial contamination (the presence of microbial contamination will not preclude release but may indicate the need for antibiotic treatment in the recipient). • In case of ABO incompatibility, red cell volume should be less than 1 mL/kg recipient body weight. If not, a washing step is recommended before infusion.
Quality control requirements	• Total nucleated cell count • Viable $CD34^+$ cell enumeration • Viability of $CD45^+$ and $CD34^+$ cells • Maternal IDM • Microbiological testing • ABO Rh blood group and verification HLA typing • Measurement of residual ABO incompatible red cell volume • CFU or other validated potency assay
Storage and transport	• Cryopreserved HPC(CB) are stored and transported at temperatures equal to or below – 140 °C. • Cryopreserved HPC(CB) can be stored for more than 10 years. • Fresh and thawed HPC(CB) are stored and transported refrigerated (2-8 °C).
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • If applicable: warning statements and/or biohazard label. • Specific information not coded in the SEC that must be included in accompanying documentation: – donor name (autologous or related donors) or donor ID (unrelated donors) – recipient name, recipient ID (if applicable) – nucleated cell count and viable $CD34^+$ cell enumeration – result of a potency assay – ABO Rh blood group – volume – identity of the collection facility and/or donor registry – identity of processing and distribution facility – instructions for appropriate thawing and washing if units have not been red cell reduced prior to cryopreservation – information brochure (including: handling instructions for the use of cellular therapy products, with indications, contraindications, side-effects and hazards, dosage and infusion recommendations).
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only, if applicable. • Do not use leukoreduction filters. • Use immediately after thawing.

29.1. Vitrified oocytes for non-partner donation

Tissue/cell product	Vitrified oocytes for non-partner donation
Definition	Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for non-partner oocyte donation in medically assisted reproduction (MAR). Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	Women needing a non-partner oocyte donation to achieve a pregnancy. The most common indications are: • Premature ovarian failure, either primary or secondary, including surgical oophorectomy, irreversible gonadal damage after chemotherapy or radiotherapy, Turner syndrome and other chromosomal disorders causing gonadal dysgenesis. • Ovaries inaccessible, or inaccessible for oocyte retrieval. • Start of natural menopause, or other age-related infertility. • Carriers of genetic diseases that cannot be treated by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre-implantation genetic diagnosis for chromosomal structural rearrangements (PGT-SR). • Carriers of mitochondrial diseases.
Critical properties	• Maturation status of the oocytes is metaphase II (MII). • Absence of giant oocytes.
Quality control requirements	• Oocytes should be vitrified within 2 hours after procurement. • Morphological assessment of oocyte size and maturation status.
Storage and transport	• Temperature below – 140 °C.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – number of shipped samples – type of medium used for storage, including batch information – type of storage device – number of oocytes per storage device – instructions for warming. • In cases when the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor file that may have implications for usage.

29.2. Vitrified oocytes for fertility preservation and/or partner donation

Tissue/cell product	Vitrified oocytes for fertility preservation and/or partner donation
Definition	Vitrified human oocytes obtained after controlled ovarian hyperstimulation, to be used for fertility preservation and/or partner oocyte donation in medically assisted reproduction (MAR). Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	Women using their own cryopreserved oocytes to achieve a pregnancy. The most common indications are: • Wish to defer childbearing years for social (non-medical) reasons (social egg freezing). • Fertility preservation (e.g. single women, transgender men). • Avoidance of surplus embryos in culture for legal/ethical reasons. • Oocyte accumulation for cycle optimisation.
Critical properties	• Maturation status of the oocytes is metaphase II (MII). • Absence of giant oocytes.
Quality control requirements	• Oocytes should be vitrified within 2 hours after procurement. • Morphological assessment of oocyte size and maturation status.
Storage and transport	• Temperature below – 140 °C.
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – number of shipped samples – type of medium used for storage, including batch information – type of storage device – number of oocytes per storage device – instructions for warming. • In cases when the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor file that may have implications for usage.

29.3. Cryopreserved sperm for non-partner donation

Tissue/cell product	Cryopreserved sperm for non- partner donation
Definition	Cryopreserved human spermatozoa obtained by ejaculation, to be used in non-partner sperm donation for medically assisted reproduction (MAR). Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately – 100 °C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of a non-partner sperm donation to achieve a pregnancy, either by intra-uterine insemination or by *in vitro* fertilisation of oocytes. The most common indications are: • Azoospermia. • Recurrent fertilisation failure. • Single women. • Carriers of genetic diseases that cannot be treated by pre-implantation genetic diagnosis for monogenic/single gene defects (PGT-M). • Carriers of structural abnormalities that cannot be treated by pre-implantation genetic diagnosis for chromosomal structural re-arrangements (PGT-SR).
Critical properties	• Presence of post-thaw viable and motile sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing must be adequate for the intended use: intra-uterine insemination (IUI), routine *in vitro* fertilisation (IVF) or microinjection (ICSI).
Storage and transport	• Cryopreserved sperm can be shipped in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – number of shipped samples – pre-cryopreservation sperm concentration and motility – number of motile spermatozoa per device – type of medium used for storage, including batch information – type of storage device – instructions for thawing. • In cases when the cryopreservation device is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered event and reactions in the donor file that may have implications for usage.

29.4. Cryopreserved sperm for partner donation

Tissue/cell product	Cryopreserved sperm for partner donation
Definition	Cryopreserved human spermatozoa obtained by ejaculation or lavage, to be used in partner sperm donation for intra-uterine insemination (IUI), *in vitro* fertilisation (IVF) or microinjection (ICSI). Processed by equilibrating the sperm sample with cryoprotectants (with or without previous washing) followed by controlled cooling rate down to a temperature of approximately – 100 °C and thereafter transferred to liquid nitrogen.
Established clinical indications	Couples or individuals in need of their own cryopreserved sperm to achieve a pregnancy, either by intra-uterine insemination, IVF or ICSI. The most common indications are: • Retrograde ejaculation. • Erectile dysfunction. • Regular absence of the male partner from the location of treatment. • Collection by electro-ejaculation. • Fertility preservation for transgender women.
Critical properties	• Presence of post-thaw viable and motile sperm.
Quality control requirements	• The number of motile spermatozoa after test thawing must be adequate for the intended use: IUI, IVF or ICSI.
Storage and transport	• Cryopreserved sperm can be shipped in liquid nitrogen or on carbon dioxide ice.
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – number of shipped samples – pre-cryopreservation sperm concentration and motility – number of motile spermatozoa per device – type of medium used for storage, including batch information – type of storage device – instructions for thawing. • In cases when the cryopreservation device is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered event and reactions in the donor file that may have implications for usage.

29.5. Vitrified embryos for fertility preservation, partner and/or non-partner donation

Tissue/cell product	Vitrified embryos for fertility preservation, partner and/or non-partner donation
Definition	Vitrification of human embryos obtained after *in vitro* fertilisation (IVF) or microinjection (ICSI), to be used for fertility preservation, partner and/or non-partner embryo donation. Vitrification is an ultra-rapid cooling method consisting of a very fast temperature drop (4 000-6 000 °C/s up to > 10 000 °C/s depending on the volume and device used) of the solution in which the specimen is cryopreserved without formation of ice crystals and where the potential toxicity of cryoprotectants is minimised due to shorter exposure in comparison with other cryopreservation approaches.
Established clinical indications	Women undergoing fertility preservation or needing partner or non-partner embryo donation to achieve a pregnancy. The most common indications are: • Women in existing relationships facing gonadotoxic treatment. • Freeze-all strategy in case of suboptimal endometrium. • Storage of supernumerary embryos for later usage. • PGT-cases of blastocyst biopsy. • Embryo donation.
Critical properties	• Embryo viability (e.g. 50 % intact cells at cleavage stage) and stage-appropriate development.
Quality control requirements	• Morphological and/or morphokinetical assessment of embryos.
Storage and transport	• Temperature below – 140 °C.
Special labelling and accompanying information	• Placed in a container, and when applicable the accompanying documentation, must be appropriately labelled with a uniquely identifying code. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – number of shipped samples – type of medium used for storage, including batch information – type of storage device – number of oocytes per storage device – instructions for warming. • In cases when the vitrification device is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Any registered events and reactions in the donor file that may have implications for usage.

30.1. Cryopreserved ovarian tissue for autologous transplantation

Tissue/cell product	Cryopreserved ovarian tissue for autologous transplantation
Definition	Fragments of ovarian cortical tissue preserved in a cryoprotective solution. An entire ovary, a semi-ovary or ovarian cortical biopsies can be procured. In cases of using the whole ovary, it will be bisected and the medulla discarded. Cortical pieces are cryopreserved in the presence of cryoprotectants, followed by controlled slow freezing down to a temperature of approximately – 100 °C and thereafter transferred to liquid nitrogen.
Established clinical indications	• Fertility preservation for pubertal patients and women in need of urgent gonadotoxic treatments like chemotherapy where ovarian stimulation and subsequent oocyte cryopreservation is not possible. • Fertility preservation for prepubertal and peripubertal girls in need of gonadotoxic treatments like chemotherapy.
Critical properties	• Cellularity/viability: – Presence of primordial follicles – Cortical thickness on average 1-2 mm, free of residual medulla.
Quality control requirements	• Primordial follicle count. • Microbiological testing on the preparation medium and freezing solution. • Testing for presence of malignant cells by standard haematoxylin-eosin staining. • Intact ovarian cortex free of signs of tissue necrosis. • Adequate size of the pieces (on average 5 mm × 5 mm). • After transplantation: the resumption of oestrogen production (within 2-4 months after transplantation).
Storage and transport	• The procured ovary is best processed immediately after procurement, but successful transplantations have been published with cooling and transport of the organ up to 20 h. Cryopreserved cortical pieces are stored and transported at temperatures ≤ – 140 °C.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • In the EU, when grafts are distributed for human application, they must be labelled with the Single European Code (SEC) as applicable. • Specific information not coded in the SEC that must be included in accompanying documentation: – Number of shipped vials – Results of the follicle count – Results of the microbiological testing – Results of the testing for presence of malignant cells – Number of cortical pieces per vial – Type of medium used for storage, including batch information – Type of storage device – Instructions for thawing – Any remarks concerning abnormalities of the ovary and/or characteristics of the cortex while preparing the tissue, not limited to the tightness or fragility of the cortex. • In cases when the container is too small to include the donation identification sequence (DIS) on the label, the DIS must be included in the accompanying documentation, permitting traceability between the sample code and the DIS.
Special warnings	• Do not irradiate. • Properly identify intended recipient and product. • For use by intended recipient only. • For autologous use only. • Use immediately after thawing. • If presence of microbial contamination is detected, consider prophylactic antibiotic treatment in the recipient. • Risk of metastases despite tissue sampling for search to find malignant cells. • Any registered event and reactions in the donor file that may have implications for usage.

31.1. Frozen pasteurised donor human milk

Tissue/cell product	Frozen pasteurised donor human milk
Definition	Human milk expressed by a donor mother, stored frozen and processed in a human milk bank, following specific recommendations, for use by a recipient that is not the mother's own infant.
Established clinical indications	• First feeding option when mother's own milk is not available. • Prevention of necrotising enterocolitis in premature infants. • Prevention of bronchopulmonary dysplasia in premature infants. • Prevention of feeding intolerance.
Critical properties	• Microbiological safety: no bacterial growth should be present
Quality control requirements	• Microbiological testing pre-pasteurisation (recommended) and post-pasteurisation (mandatory) • Macronutrient analysis (recommended)
Storage and transport	• Storage between – 18 °C and – 20 °C for a maximum duration of 3 to 6 months; and at – 80 °C for a maximum of 12 months. • Donor human milk should be transported frozen to the site of utilisation.
Special labelling and accompanying information	• Pasteurised human milk for nutritional use, clearly stated. • To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. Specific information that must be included in the accompanying documentation or on the label: volume, storage temperature, nutritional information (if available), instructions for appropriate thawing and date of expiry.
Special warnings	• After thawing it should be kept at 4 °C and administered within 24 hours. • Do not refreeze. • Do not heat with microwave oven.

31.2. Frozen raw donor human milk

Tissue/cell product	Frozen raw donor human milk
Definition	Human milk, expressed by a CMV-negative donor mother, that has been stored frozen in a human milk bank, following specific recommendations, for use by a recipient that is not the mother's own infant.
Established clinical indications	• First feeding option when mother's own milk is not available. • Prevention of necrotising enterocolitis in premature infants. • Prevention of bronchopulmonary dysplasia in premature infants. • Prevention of feeding intolerance.
Critical properties	• Microbiological safety: < 103 CFU/mL and no bacterial growth of potential pathogens allowed.
Quality control requirements	• Microbiological testing (mandatory) • Macronutrient analysis (recommended)
Storage and transport	• Storage between – 18 °C and – 20 °C for a maximum duration of 3 to 6 months; and at – 80 °C for a maximum of 12 months. • Donor human milk should be transported frozen to the site of utilisation.
Special labelling and accompanying information	• Raw human milk for nutritional use, clearly stated. • To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation. • Specific information that must be included in the accompanying documentation or on the label: volume, storage temperature, nutritional information (if available), instructions for appropriate thawing and date of expiry.
Special warnings	• After thawing it should be kept at 4 °C and administered within 24 hours. • Do not refreeze. • Do not heat with microwave oven.

32.1. Cryopreserved donor faeces capsules

Tissue/cell product	Cryopreserved donor faeces capsules
Definition	Glycerol-preserved and encapsulated, minimally processed human faeces from a single healthy donor, prepared in a tissue establishment to be used for faecal microbiota transplantation (FMT).
Established clinical indications	• Recurrent infection with *Clostridioides difficile* following standard antibiotics therapy • Refractory or fulminant infection with *Clostridioides difficile*
Critical properties	• Minimally processed and encapsulated donor faeces • Capsules
Quality control requirements	• Cryopreservation is to be documented according to standard protocols in the tissue establishment.
Storage and transport	• Storage at – 80 °C for up to 24 months • Storage at – 20 °C for up to 4 weeks • Storage at 2-8 °C for up to 24 hours • Storage at ambient temperature for up to 6 hours • Thawing may be done overnight at 2 °C to 8 °C or at ambient temperature for up to 6 hours. Thawing time from – 20 °C at ambient temperature is usually 15 minutes.
Special labelling and accompanying information	• To be placed in a container, which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation.
Special warnings	• Do only apply capsules to patients who do not have swallowing difficulties. • Capsules should be visually inspected before application, and all capsules should have intact shape. Capsules that have visible cracks should be discarded. In case of damaged packaging, capsules should not be used. • Do not refreeze thawed donor faeces capsules. • Capsules should not be administered to patients with a history of anaphylactic reaction secondary to food allergy.

32.2. Cryopreserved donor faeces suspension

Tissue/cell product	Cryopreserved donor faeces suspension
Definition	Liquid suspension of minimally processed human faeces from a single healthy donor, prepared in a tissue establishment to be used for faecal microbiota transplantation (FMT).
Established clinical indications	• Recurrent infection with *Clostridioides difficile* following standard antibiotics therapy • Refractory or fulminant infection with *Clostridioides difficile*
Critical properties	• Minimally processed donor faeces • Cryobag or syringes with connection to enteral feeding tube, endoscopy flushing channel or rectal catheter
Quality control requirements	• Cryopreservation is to be documented according to standard protocols in the tissue establishment.
Storage and transport	• Storage at – 80 °C for up to 24 months • Storage at – 20 °C for up to 4 weeks • Storage at 2-8 °C for up to 24 hours • Storage at ambient temperature for up to 6 hours • Thawing may be done overnight at 2 °C to 8 °C or at ambient temperature for up to 6 hours
Special labelling and accompanying information	• To be placed in a container (cryobag or syringes), which must be appropriately labelled with a uniquely identifying code. When applicable, this code must also be included in the accompanying documentation.
Special warnings	• Connector for infusion must NOT be compatible with Luer Lock. • Do not refreeze thawed donor faeces suspensions. • Donor faeces should not be administered to patients with a history of anaphylactic reaction secondary to food allergy.

Appendices

Appendix 1. **General reference documents used**

The experts who developed the chapters in this Guide incorporated principles and specific text from many regulatory, professional and scientific publications. The following are the principal reference documents used.

- American Association of Tissue Banks. *Standards for tissue banking of the American Association of Tissue Banks*. 14th edition. McLean VA, USA: American Association of Tissue Banks, 2014.
- Council of Europe (1950/2004), Convention for the Protection of Human Rights and Fundamental Freedoms, as amended by Protocols No. 11 and No. 14, available at http://conventions.coe.int/treaty/en/treaties/html/005.htm, accessed 15 March 2022.
- Council of Europe (1997), Convention for the protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, available at http://conventions.coe.int/Treaty/en/Treaties/Html/164.htm, accessed 15 March 2022.
- Council of Europe (2002), Additional Protocol to the Convention on Human Rights and Biomedicine concerning Transplantation of Organs and Tissues of Human Origin, available at http://conventions.coe.int/Treaty/en/Treaties/Html/186.htm, accessed 15 March 2022.
- Council of Europe (2005), Convention on Action against Trafficking in Human Beings, and its Explanatory Report, available at http://conventions.coe.int/treaty/en/Treaties/Html/197.htm, accessed 15 March 2022.
- Council of Europe (2014), Convention against Trafficking in Human Organs, available at https://rm.coe.int/16806dca3a, accessed 28 November 2022.
- Council of Europe (2017), Safety, quality and ethical matters related to the use of organs, tissues and cells of human origin: Council of Europe conventions, recommendations, resolutions and reports, 3rd edition, available at https://freepub.edqm.eu/publications/AUTOPUB_15/detail, accessed 28 November 2022.
- Council of Europe (2020), Recommendation CM/Rec (2020) 5 of the Committee of Ministers to member states on the quality and safety of tissues and cells for human application, available at https://search.coe.int/cm/Pages/result_details.aspx?ObjectId=0900001680 9fdcde, accessed 15 March 2022.
- Council of Europe (2020), Recommendation CM/Rec (2020) 6 of the Committee of Ministers to member states on establishing harmonised measures for the protection of haematopoietic progenitor cell donors at https://rm.coe.int/0900001680 9fdd5b, accessed 15 March 2022.
- Council of Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM) (2011). Technical Memorandum – TS057 Risk behaviours having an impact on blood donor management. Strasbourg: Council of Europe/EDQM.

- Council of Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM) (2020), *Ph. Eur. (European Pharmacopoeia)*, 10th edition. Strasbourg: Council of Europe.
- Council of Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM) (2020), *Guide to the preparation, use and quality assurance of blood components*, 20th edition, Council of Europe, available at https://freepub.edqm.eu/publications/AUTOPUB_48/detail, accessed 9 November 2022.
- Council of Europe, European Directorate for the Quality of Medicines & HealthCare (EDQM) (2022), *Guide to the quality and safety of organs for transplantation*, 8th edition, Council of Europe, available at https://freepub.edqm.eu/publications/PUBSD-88/detail, accessed 28 November 2022.
- Council of Europe/United Nations, Trafficking in organs, tissues and cells and trafficking in human beings for the purpose of the removal of organs, Joint Study, 2009, available at https://freepub.edqm.eu/publications/AUTOPUB_18/detail, accessed 28 November 2022.
- Eudralex Volume 4 – EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, available at https://health.ec.europa.eu/latest-updates/eudralex-volume-4-eu-guidelines-good-manufacturing-practice-medicinal-products-human-and-veterinary-2022-02-21_en, accessed 28 November 2022.
- EudraLex, Volume 4, EU Guidelines for Good Manufacturing Practice – Medicinal products for human and veterinary use, Annex 1, Manufacture of Sterile Medicinal Products (2008), available at https://health.ec.europa.eu/system/files/2022-08/20220825_gmp-an1_en_0.pdf, accessed 28 November 2022.
- European Centre for Disease Prevention and Control. Publication data, available at www.ecdc.europa.eu/en/publications-data, accessed 15 March 2022.
- European Commission, Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells, available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF, accessed 15 March 2022.
- European Commission, Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells, available at https://health.ec.europa.eu/publications/commission-directive-200686ec_en, accessed 28 November 2022.
- European Commission (2008), Inspection of tissue and cell procurement and tissue establishments, Operational manual for competent authorities version 1.0, available at https://ec.europa.eu/health/system/files/2016-11/manual_11_en_0.pdf, accessed 15 March 2022.
- European Commission, Decision 2010/453/EU of 3 August 2010 establishing guidelines concerning the conditions of inspections and control measures, and on the training and qualification of officials, in the field of human tissues and cells provided for in Directive 2004/23/EC of the European Parliament and of the Council, available at http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010D0453&from=EN, accessed 15 March 2022.
- European Commission, Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC as regards certain technical requirements for the testing of human tissues and cells [Internet], available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0024:0025:EN:PDF, accessed 15 March 2022.
- European Commission, Directive (EU) 2015/565 amending Directive 2006/86/EC as regards certain technical requirements for the coding of human tissues and cells, available at https://health.ec.europa.eu/publications/commission-directive-eu-2015565_en, accessed 28 November 2022.
- European Commission, Directive (EU) 2015/566 implementing Directive 2004/23/EC as regards the procedures for verifying the equivalent standards of quality and safety of imported tissues and cells, available at https://health.ec.europa.eu/publications/commission-directive-eu-2015566_en, accessed 28 November 2022.
- European Good Tissue Practices (EuroGTP I), including the guidance document (2011), avail-

able at www.goodtissuepractices.eu/index.php/euro-gtp-i, accessed 15 March 2022.

- European Good Tissue Practices (EuroGTP II), Outputs of the EU-funded project, including the guidance document (2011), available at www.goodtissuepractices.eu/index.php/project-outcomes, accessed 15 March 2022.
- European Parliament, Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data and on the free movement of such data. *Official Journal* L 281, 23/11/1995 P. 0031–0050.
- European Parliament, Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work, available at https://osha.europa.eu/en/legislation/directives/exposure-to-biological-agents/77, accessed 28 November 2022.
- European Parliament, Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:en:PDF, accessed 15 March 2022.
- European Parliament, Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32004L0023&from=EN, accessed 28 November 2022.
- European Commission, Directive 2006/17/EC, implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells, available at https://health.ec.europa.eu/publications/commission-directive-200617ec_en, accessed 28 November 2022.
- European Quality System for Tissue Banking, Guide of recommendations for tissue banking, EQSTB Project (co-funded by the European Commission), 2007, available at http://ec.europa.eu/health/archive/ph_projects/2003/action2/docs/2003_2_09_interim_report_2.pdf, accessed 15 March 2022.
- European Union Standards and Training for the Inspection of Tissue Establishments Project (Eustite), Outputs of the EU-funded project, including the Vigilance tools and guidance, final vigilance recommendations and inspection guide (2006 to 2009), available at www.notifylibrary.org/background-documents#European-Union-Standards-and-Training-for-the-Inspection-of-Tissue-Establishments-Project-(Eustite), accessed 15 March 2022.
- FACT-JACIE International standards for hematopoietic cellular therapy: product collection, processing, and administration, 8th edition (2021), Joint Accreditation Committee (JACIE) and Foundation for the Accreditation of Cellular Therapy (FACT), available at www.ebmt.org/8th-edition-fact-jacie-standards, accessed 15 March 2022.
- Fehily D, Brubaker SA, Kearney JN, Wolfinbarger Ll, editors (2012). *Tissue and cell processing: an essential guide*. London: Wiley-Blackwell.
- Gillan H, Pamphilon D, Brubaker S. Principles of cell collection and tissue recovery. In: Fehily D, Brubaker S, Kearney JN, Wolfinbarger Ll, editors (2012). *Tissue and cell processing: an essential guide*. London: Wiley-Blackwell.
- Harper J, Magli MC, Lundin K *et al.* When and how should new technology be introduced into the IVF laboratory? *Hum Reprod* 2012;27(2): 303-13.
- Human Tissue Authority (UK) Code of Practice, available at www.hta.gov.uk/hta-codes-practice-and-standards-0, accessed 15 March 2022.
- ISO 11137-1:2006. Sterilization of health care products – Radiation – Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices.
- ISO 11137-2:2013. Sterilization of health care products – Radiation – Part 2: Establishing the sterilization dose.
- ISO/FDIS 13022:2012. Medical products containing viable human cells – application of risk management and requirements for processing practices.
- Joint United Kingdom Blood Transfusion and Blood Transplantation Services Professional Advisory Committee, Guidelines

for the blood transfusion services in the UK [the 'Red Book'], 8th edition (2013), available at www.transfusionguidelines.org.uk/index.aspx?Publication=RB, accessed 15 March 2022.

- NetCord–FACT. *International cord blood standards*, 7th edition (2020), available at www.factwebsite.org/cbstandards/, accessed 15 March 2022.
- Notify. Exploring vigilance notification for organs, tissues and cells. Centro Nazionale Trapianti/*Organs Tiss Cells* 2011;14(3), available at www.notifylibrary.org/sites/default/files/Notify%20exploring%20vigilance%20notification%20for%20organs%2C%20tissues%20and%20cells%2C%20Notify%20Group%2C%20Bologna%2C%20p.133%2C%20%282011%29.pdf, accessed 15 March 2022.
- Nuffield Council on Bioethics, *Human bodies: donation for medicine and research*, Report available at http://nuffieldbioethics.org/wp-content/uploads/2014/07/Donation_full_report.pdf, accessed 15 March 2022.
- SoHO V&S (Vigilance and Surveillance of Substances of Human Origin), Outputs of the EU-funded project, including the draft Vigilance guides for competent authorities and for clinical users of tissues and cells (2010 to 2013), available at www.notifylibrary.org/background-documents#Vigilance-and-Surveillance-of-Substances-of-Human-Origin-Project-(SOHOV&S), accessed 15 March 2022.
- SoHO V&S (Vigilance and Surveillance of Substances of Human Origin), Outputs of the EU-funded project, Guidelines for healthcare professionals on vigilance and surveillance of human tissues and cells, Deliverable 10, Part 1 – Tissues (2014), available at www.notifylibrary.org/sites/default/files/SOHOV%26S%20Vigilance%20Guidance%20for%20Healthcare%20Professionals%20-%20Part%201%20Tissues_0.pdf, accessed 15 March 2022.
- United States Food and Drug Administration, HCT/Ps Good tissue practices, Code of Federal Regulations Title 21, available at www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271, accessed 15 March 2022.
- WHO (World Health Organization), *Aide-mémoire* on access to safe and effective cells and tissues for transplantation, available at www.who.int/entity/transplantation/AM-HCTTServices.pdf, accessed 15 March 2022.
- WHO (World Health Organization), *Aide-mémoire* on key safety requirements for essential minimally processed human cells and tissues for transplantation, available at https://apps.who.int/iris/handle/10665/342054, accessed 28 November 2022.
- WHO (World Health Organization), Department of Reproductive Health and Research. *WHO laboratory manual for the examination and processing of human semen*, 5th edition (2010). Geneva, Switzerland: World Health Organization.
- WHO (World Health Organization), Guiding principles on human cell, tissue and organ transplantation (2010), available at https://apps.who.int/iris/handle/10665/341814, accessed 15 March 2022.

Appendix 2. **Acronyms and other abbreviations**

3D	three-dimensional
AAS	ascending aorta
AATB	American Association of Tissue Banks
AAV	*adeno*-associated virus
Ab	antibodies
ACI	autologous chondrocyte implantation
AFC	antral follicle count
AIDS	acquired immunodeficiency syndrome
ALK	anterior lamellar keratoplasty
AMH	anti-Müllerian hormone
Anti-CMV	antibody to *Cytomegalovirus*
Anti-EBV	antibody to Epstein–Barr virus
Anti-HBc	antibody to hepatitis B core antigen
Anti-HCV	antibody to hepatitis C virus
Anti-HIV-1	antibody to HIV-1
Anti-HIV-2	antibody to HIV-2
ARE	adverse reactions and events
ART	assisted reproductive technology
ARTHIQS	Assisted Reproductive Technologies and Haematopoietic stem cells for transplantation Improvements for Quality and Safety throughout Europe [joint action]
ATMP	advanced therapy medicinal product
aW	available water
BET	bacterial endotoxin test
BFU-E	burst-forming units erythroblast
BM	bone marrow
BMDW	Bone Marrow Donors Worldwide [organisation: now part of WMDA]
BMP	bone morphogenetic proteins
BMSC	bone marrow stromal cells
BRCA	BReast CAncer gene
BSS	balanced salt solution
CAPA	corrective and preventive action
CAR	chimeric antigen receptor
CAT	Committee for Advanced Therapies
CBC	complete blood count
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* infection
CD-P-TO	European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe
CE (marked)	Conformité Européenne
CEA	cultured epithelial autografts
CFU	colony-forming units
CFU-GM	colony-forming units-granulocyte/monocyte
CHAPS	3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate
CJD	Creutzfeldt–Jakob disease
CLET	cultivated limbal epithelial transplantation
CMV	*Cytomegalovirus*
CNS	central nervous system
CNT	Centro Nazionale Trapianti (Italy)
CNV	copy number variation
COC	cumulus enclosed oocyte
COD	cause of death
COH	controlled ovarian hyperstimulation
COMET	cultivated oral mucosal epithelial transplantation
DALK	deep anterior lamellar keratoplasty
DBD	deceased by brain death
DBM	demineralised bone matrix

DBO	Department of Biological Standardisation, OMCL Network & HealthCare (at the European Directorate for the Quality of Medicines & HealthCare)
DC	dendritic cells
DCD	deceased by circulatory death
DH-BIO	Committee on Bioethics of the Council of Europe
DLI	donor lymphocyte infusions
DM	*Diabetes mellitus*
DMEK	Descemet membrane endothelial keratoplasty
DMSO	dimethyl sulfoxide
DQ	design qualification
DSAEK	Descemet stripping automated endothelial keratoplasty
DVT	deep-vein thrombosis
EATCB	European Association of Tissue and Cell Banks
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr virus
EC	European Commission
ECCTR	European Cornea and Cell Transplant Registry
ECDC	European Centre for Disease Prevention and Control
ECM	extracellular matrix
ECVAM	European Centre for the Validation of Alternative Methods
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDTA	ethylenediamine tetra-acetic acid
EEBA	European Eye Bank Association
EGF	endothelial growth factor
EGTA	ethylene glycol tetra-acetic acid
EIA	enzyme immunoassay
EK	endothelial keratoplasty
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EQSTB	European Union project 'European Quality System for Tissue Banking'
ESBL	extended-spectrum beta lactamases
ESC	embryonic stem cell
ESHRE	European Society for Human Reproduction and Embryology
EU	European Union *not* endotoxin units (*see* IU)
EUROCET	European Registry for Organs, Tissues and Cells
Eurocode IBLS	Eurocode International Blood Labelling Systems
EuroGTP	Euro Good Tissue Practices [EU project]
EuroGTP-II	Good [Tissue] Practices for demonstrating safety and quality through recipient follow-up [EU project]
Eustite	European Standards and Training in the Inspection of Tissue Establishments [EU project]
EUTC	European Code for Tissues and Cells
FACS	fluorescence-activated cell sorting
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration (USA)
FED	Fuchs endothelial dystrophy
FET	frozen embryo transfer
FIPS	fingerprints
FMEA	failure mode and effects analysis
FMECA	failure mode, effects and criticality analysis
FMT	faecal microbiota transplantation
FNHTR	febrile non-haemolytic reactions
FOS	fastidious organism supplement
FSH	follicle-stimulating hormone
GAG	glycosaminoglycans
G-CSF	granulocyte-colony stimulating factor
GEMM	granulocyte-erythrocyte-macrophage-megakaryocyte
GF	growth factors
GM	granulocytes and macrophages
GM-CSF	granulocyte macrophage-colony stimulating factor
GMP	Good manufacturing practice [EU document]
GPA	glycerol-preserved allografts
GTP	good tissue practice
GV	germinal vesicle
GvHD	graft-*versus*-host disease
GvT	graft-*versus*-tumour
HACCP	hazard analysis and critical control points
hAM	human amniotic membrane
HAV	hepatitis A virus
HBc	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEPA	high-efficiency particulate air
HES	hydroxyethyl starch
HHV	human *Herpes* virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMG	human menopausal gonadotrophin
HPC	haematopoietic progenitor cells
HPV	human *papilloma* virus
HRV	human *rotavirus*
HSC	haematopoietic stem cells
HSG	hysterosalpingography
HSV	*Herpes simplex* virus
HTLV	human T-lymphotrophic virus

HVAC	heating, ventilating, and air conditioning
HyCoSy	hysterosalpingo-contrast sonography
IATA	International Air Transport Association
ICCBBA	International Council for Commonality in Blood Banking Automation
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	intracytoplasmic sperm injection
ICU	intensive care unit
IDM	infectious disease marker
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IL	interleukin
iPSC	induced pluripotent stem cell
IQ	installation qualification
ISCT	International Society for Cellular Therapy
ISN	International Society for Nephrology
ISO	International Organization for Standardization
ISPE	International Society for Pharmaceutical Engineering
ISSCR	International Society for Stem Cell Research
IT	information technology
IU	international unit *(for endotoxins)*
IUI	intra-uterine insemination
IVF	*in vitro* fertilisation
IVM	*in vitro* maturation
JACIE	Joint Accreditation Committee–ISCT & EBMT
KIR	killer immunoglobulin-like receptors
KLAL	keratolimbal allograft
KPI	key performance indicator
LAL	limulus amoebocyte lysate
LH	luteinising hormone
LSC	limbal stem cells
MCM	metastatic cutaneous melanoma
MESA	microsurgical epididymal sperm aspiration
MII	metaphase II
MNC	mononuclear cells
MPHO	medical products of human origin
MRA	marrow re-populating ability
MRSA	methicillin-resistant *Staphylococcus aureus*
MRT	mitochondrial replacement therapy
MSC	mesenchymal stromal (stem) cells
mtDNA	mitochondrial DNA
MTT	tetrazolium salt assay
NAC	nipple–areola complex
NAT	nucleic acid amplification technique/ nucleic acid test
NEC	necrotising enterocolitis
NICE	National Institute of Health and Clinical Excellence [UK]
niPGT	non-invasive pre-implantation genetic testing
NK	natural killer
NPC	non-valved pulmonary conduit
NRT	neutral red test
NtPSC	nuclear-transfer pluripotent stem cells
OA	osteoarthritis
OECD	Organisation for Economic Co-operation and Development
OHSS	ovarian hyperstimulation syndrome
ONT	Organización Nacional de Trasplantes [Spain]
OQ	operational qualification
ORHA	organisation responsible for human application
Parvo-B19	*Parvovirus* B19
PBK	pseudophakic bullous keratopathy
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PESA	percutaneous epididymal sperm aspiration
PGD	pre-implantation genetic diagnosis, *see* PGT
PGS	pre-implantation genetic screening, *see* PGT
PGT	pre-implantation genetic testing (formerly known as PGD and PGS, *see above*)
PGT-A	pre-implantation genetic testing for aneuploidies
PGT-M	pre-implantation genetic testing for monogenic/single gene defects
PGT-SR	pre-implantation genetic testing for chromosomal structural re¬arrangements
Ph. Eur.	*European Pharmacopoeia*, 10th edn, Strasbourg: Council of Europe 2022
PIC/S	Pharmaceutical Inspection Co-operation Scheme
PK	penetrating keratoplasty
PN	ProNucleus
PO	procurement organisation
POI	premature ovarian insufficiency
POSEIDON	Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe [EU project]
PPA	preparation process authorisation
PQ	performance qualification
PRF	platelet-rich fibrin

PRIVILEGED	Privacy in Law, Ethics and Genetic Data [EU project]
PROH	1,3-propanedial
PRP	platelet-rich plasma
PVP	PolyVinylPyrrolidone
QC	quality control
QM	quality manager
QMS	quality management system
QRM	quality risk management
RABS	Restricted Access Barrier System
RANTES	regulated on activation, normal T-cell expressed and secreted
RATC	rapid alerts for tissues and cells
RBC	red blood cell
RCT	randomised control trial
RhD	Rhesus D antigen
rhG-CSF	recombinant granulocyte-colony stimulating factor
RP	Responsible Person
RPN	risk priority number
SAE	serious adverse event
SAL	sterility assurance level
SAR	serious adverse reaction
SARE	severe adverse reactions and events
SDS	sodium dodecyl sulfate
SEC	Single European Code
SIG	special interest group
SLA	service-level agreement
SNV	single nucleotide variation
SoHO V&S	Vigilance and Surveillance of Substances of Human Origin
SOP	standard operating procedure
SP-CTO	Council of Europe Committee of Experts on the Organisational Aspects of Co-operation in Organ Transplantation
S(P)EAR	serious (product) events and reactions
SSC	spermatogonial stem cell
SVF	stromal vascular fraction
T1DM	Type-1 *Diabetes mellitus*
TAMC	total aerobic microbial count
TBV	total blood volume
TCR	T-cell receptor
TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TGF	tumour growth factor/transforming growth factor
TLT	time-lapse technology
TNC	total nucleated cells
TNF	tumour necrosis factor
TPV	total plasma volume
TRALI	transfusion-related acute lung injury
TSB	tryptic soy broth
TSE	transmissible spongiform encephalopathy
TTS	The Transplantation Society
TYMC	total combined yeasts/moulds count
UCB	umbilical cord blood
UPS	uninterrupted power supply
V&S	vigilance and surveillance
vCJD	variant Creutzfeldt–Jakob disease
VEGF	vascular endothelial growth factor
VISTART	Vigilance and Inspection for the Safety of Transfusion, Assisted Reproduction and Transplantation [joint action]
VMP	validation master plan
VOC	volatile organic compound
VRE	Vancomycin-resistant *enterococci*
WHO	World Health Organization
WMDA	World Marrow Donor Association
WNV	West Nile virus

Appendix 3. **Glossary**

Acceptance criteria	Requirements needed to meet the relevant quality and safety standards in order to ensure an acceptable final product for human application.
Adipose tissue	Loose connective tissue, composed of adipocytes and stromal vascular fraction, which serves as energy storage and endocrine organ.
Advanced therapy medicinal product	A medicinal product that can be a gene therapy medicinal product, a somatic cell therapy medicinal product, a tissue-engineered product or a combined advanced therapy medicinal product (which is a medicinal product incorporating cells and medical devices or actively implantable medical devices).
Adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells. *See also:* Serious adverse event.
Adverse reaction	Any unintended response, including a communicable disease, in the donor or the recipient that is associated with the procurement or human application of tissues and cells. *See also:* Serious adverse reaction.
Agarose gel electrophoresis	Diagnostic tool to visualise DNA fragments.
Allogeneic	Refers to tissues and cells removed from one individual and applied to another of the same species.
Allograft	Tissues or cells transplanted between two genetically different individuals of the same species. The term is synonymous with 'homograft'.
Ambient temperature	The temperature of the surrounding environment. In temperature-controlled facilities, ambient temperature is usually 17-21 °C for thermal comfort. Referred to as 'room temperature' in this Guide.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the fetus during pregnancy.
Angiogenesis	Physiological process by which new blood vessels form from pre-existing vessels.
Antibiogram	*See:* Resistogram.
Antibiotic/antimicrobial	An agent that inhibits the growth of, or destroys, micro-organisms.
Apheresis	A medical technique in which peripheral blood of a donor or patient is passed through an apparatus that separates one particular constituent and returns the remaining constituents to the donor or patient.
Aseptic techniques	Procedures designed to prevent contamination from micro-organisms and spread of infection.
Assisted reproductive technology	All interventions that include *in vitro* handling of both human oocytes and sperm, or of embryos, for the purpose of reproduction. This includes, but is not limited to, *in vitro* fertilisation (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete and embryo cryopreservation, semen, oocyte or embryo donation and gestational carrier cycles. Thus, ART does not, and ART-only registries do not, include assisted insemination using sperm from a woman's partner or a sperm donor. *See also:* Medically assisted reproduction.

Audit	Periodic, independent and documented examination and verification of activities, records, processes and other elements of a quality system to determine their conformity with specific internal or external requirements. They may be conducted by professional peers, internal quality system auditors or auditors from certification or accreditation bodies.
Autologous	Refers to tissues or cells removed from and applied in the same individual.
Azoospermia	Absence of spermatozoa in the ejaculate.
Bacteraemia	The presence of viable bacteria in the circulating blood.
Banking	Processing, preservation, storage and distribution of tissues and cells for human application or other purposes, including research and training.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.
Batch	A defined quantity of starting material, packaging material or product processed in one process (or series of processes) so that it can be considered to be homogeneous.
Bioactivity	The effect of a substance upon a living organism.
Biobank	A collection of biological material and the associated data and information stored for research purposes. Also known as a bio-repository.
Bioburden	Total number of viable micro-organisms or total microbial count present, on or in tissues or cells or in the environment, usually measured before the application of a decontamination or sterilisation process.
Biochemical cue	Chemical signal that occurs in a biological organism and causes a biological response.
Biocompatibility	Property of a material being compatible with living tissue. Biocompatible materials do not produce a toxic or immunological response when exposed to the body or body fluids.
Biodegradability	Disintegration of materials by biological processes.
Biomechanical cue	Mechanical signal that occurs in a biological organism and causes a biological response.
Bionics	Biologically inspired engineering is the application of biological methods and systems found in nature to the study and design of engineering systems and modern technology.
Biophysical cue	Physical signal that occurs in a biological organism and causes a biological response.
Bioprinting	Combination of cells, growth factors and biomaterials using layer-by-layer deposition to fabricate biomedical parts that maximally imitate natural tissue characteristics, including structures that are later used in medical and tissue engineering fields.
Bisbenzimidine	Organic compound used as a fluorescent stain for DNA in molecular biology applications.
Blastocyst	An embryo, around 5-6 days after fertilisation, with an inner cell mass, an outer layer of trophectoderm and a fluid-filled blastocoele cavity.
Blastomere	A cell in a cleavage-stage embryo.
Blood groups	ABO or ABo. Both forms are widely used, but this Guide uses O. The O is from German *ohne* ('without') and means the same as o ('zero'): these are red blood cells without A or B antigens on the cell surface.
Bone	The hard, rigid, mineralised form of connective tissue constituting most of the skeleton of vertebrates and composed primarily of calcium salts. There are two types of osseous tissue that form bones: cortical bone (the compact bone of the shaft of a bone that surrounds the marrow cavity) and cancellous or trabecular bone (typically occurs at the ends of long bones, proximal to joints and within the interior of vertebrae). Cancellous bone is highly vascular and frequently contains bone marrow.
Bone marrow	Tissue at the centre of large bones. It is the place where new blood cells are produced. Bone marrow contains two types of stem cell: haematopoietic (which can produce blood cells) and stromal (which can produce fat, cartilage and bone).
Calibration	Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties; and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication.
Cell	The smallest transplantable and functional unit of life.
Cell culture	Growth of cells in a nutrient medium *in vitro*.
Cell migration	Movement of cells in particular directions, often in response to specific external signals, including chemical signals and mechanical signals.
Circulation	Transfer of tissues or cells from a tissue establishment to another operator for further processing.

Clean area, clean environment, clean-room	An area with defined environmental control of particulate and microbial contamination, and constructed and used in such a way as to reduce the introduction, generation and retention of contaminants within the area.
Cleavage-stage embryo	Embryo, beginning with the 2-cell stage and up to, but not including, the morula stage.
Clinical evaluation	Clinical follow-up studies for monitoring predefined clinical outcome indicators to evaluate quality, safety and effectiveness/efficacy of tissue or cell product for a defined number of patients.
Closed system	A system that employs a physical barrier that completely separates the internal environment from the external environment, and allows contact between them only through irreversible measures.
Coding	A system for unique identification of tissues and cells for human application, comprising a donation identifier and product identifier for the specific type of tissue or cell.
Collagen	Main structural protein.
Colonisation	The natural, biological presence or spread of micro-organisms.
Compatibility testing	Testing for the presence or absence of recipient antibodies to HLA and to blood group antigens present on the tissues or cells for transplantation.
Competent authority	*See:* Health Authority.
Computerised system	A system including the input of data, electronic processing and the output of information, to be used either for reporting or for automatic control.
Consent to donation	Lawful permission or authorisation for removal of human cells, tissues and organs for transplantation. *See also:* Opt-in donation; Opt-out donation.
Contained laboratory, contained area	According to EU GMP, an area constructed and operated in such a manner (and equipped with appropriate air handling and filtration) as to prevent contamination of the external environment by biological agents from within the area.
Contamination	Accidental inclusion or growth of harmful micro-organisms, such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and by-products. Contamination is different from colonisation, which is the natural, biological presence of micro-organisms.
Controlled ovarian stimulation	Pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes.
Cord blood	Blood collected from placental vessels and umbilical cord blood vessels after the umbilical cord is clamped and/or severed as a source of haematopoietic progenitor cells.
Cord blood bank	A specific type of tissue establishment in which haematopoietic progenitor cells collected from placental and umbilical cord blood vessels are processed, cryopreserved and stored. It may also be responsible for collection, testing or distribution.
Cornea	The transparent anterior part of the outer fibrous coat of the eye. A collagenous tissue bounded by an outer stratified epithelium and an inner monolayer of endothelial cells. The major refractive component of the eye.
Critical	Potentially having an effect on the quality and/or safety of (or having contact with) tissues and cells.
Cross-contamination	Unintentional transfer of micro organisms and/or other material from one donation or processing batch to another.
Cryopreservation	Preservation and storage at sub-zero temperature of viable tissues and cells (including gametes and embryos) using a cryoprotectant to preserve viability, either by controlled slow freezing or by vitrification.
Cryoprotectant	A chemical compound that is used to protect cells and tissues against freezing injury.
Cumulus cell	The multi-layered mass of granulosa cells surrounding the oocyte.
Cycle	The process (including medication or not) that leads to an unique collection of gametes (for donation or own use), to the single (at a unique time) use of gametes or to the transfer of one or more embryos in a single procedure.
Cytotoxicity	Quality of being toxic to cells.
DAPI	4',6-diamidino-2-phenylindole, a fluorescent stain that binds strongly to adenine–thymine-rich regions in DNA.
Deceased donor	A person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been procured for the purpose of human application. *See also:* Donor after brain death; Donor after circulatory death.
Decontamination	The process of removing or neutralising contaminants.
De-epidermisation	Process by which epidermis is removed from skin.
Delivery rate	Number of deliveries of neonates expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. It includes deliveries that resulted in the birth of one or more live and/or stillborn babies.

Denudation	The removal or stripping of the cumulus cells from the oocyte.
Design qualification	The first step in the qualification of new equipment or facilities.
Deviation	Departure from an approved instruction/protocol or established standard.
Differentiation	Process by which a less specialised cell becomes a more specialised cell type.
Diminished ovarian reserve	A term generally used to indicate a reduced number and/or reduced quality of oocytes, such that the ability to reproduce is decreased.
Direct use	Any procedure in which tissues and cells are donated and used without banking or storage.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on density.
Disinfection	A process that reduces the number of viable micro-organisms, but does not necessarily destroy all microbial forms, such as spores and viruses.
Disposal (of tissues/cells)	The act or means of discarding tissues and/or cells.
Distribution	Transportation and delivery of cells or tissues intended for human application.
Donor	An individual, living or deceased, who is a source of tissues or cells for human application and for other purposes including research.
Donor after brain death	A donor who is declared dead based on the irreversible loss of neurological functions. Also known as deceased heart-beating donor.
Donor after circulatory death	A donor who is declared dead based on circulatory criteria. Also known as deceased non-heart-beating donor.
Donor evaluation	The procedure for determining the suitability of an individual, living or deceased, as a donor of cells or tissues.
Donor human milk	The human milk expressed by a breastfeeding mother and donated voluntarily and freely to a Human Milk Bank.
Donor selection	*See:* Donor evaluation.
Double embryo transfer	Transfer of two embryos.
Effectiveness	Presence of functionality proven by *in vitro* analytics (e.g. potency assays) depending on the mode of action of the tissue or cell product.
Efficacy	Presence of desired clinical effects/patient outcome depending on the mode of action of the tissue or cell product.
Elastin	Highly elastic protein in connective tissue that allows many tissues in the body to resume their shape after stretching or contracting.
Electron beam irradiation	Use of beta irradiation, usually of high energy under elevated temperatures and nitrogen atmosphere, for sterilisation or cross-linking of polymers.
Embryo	The result of continued development of the zygote to 8 completed weeks after fertilisation, equivalent to 10 weeks of gestational age.
Embryo biopsy	The removal of cells (blastomeres or trophectoderm cells) from the embryo for the purpose of genetic analysis.
Embryo donation	Transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.
Embryo transfer	Procedure in which one or more embryos are placed in the uterus or Fallopian tube.
Emerging disease	A disease that has recently appeared in a population for the first time, or that may have existed previously but is rapidly increasing in incidence or geographic range.
End user	A healthcare practitioner who undertakes human application procedures.
Endotoxins	Large molecules consisting of a lipid and a polysaccharide, which are found in the outer membrane of Gram-negative bacteria.
Error	A mistake or failure to carry out a planned action as intended, or application of an incorrect plan that may or may not cause harm to patients.
Escharectomy	Surgical procedure based on removal of necrotic skin tissue from a full-thickness burn.
Ethylene oxide	Organic compound and toxic gas, being a surface disinfectant widely used in hospitals and the medical equipment industry for sterilisation.
Exceptional release	The distribution for clinical use of a unit of tissues and/or cells that does not fully comply with the defined safety and quality criteria for release. The release is justified by a specific clinical need in which the benefit outweighs the risk associated with the non-compliance *See also:* Negative-to-date release.
Expert	Individual with the appropriate qualifications and experience to provide technical advice to a Health Authority inspector.
Expiry date	The date after which tissues or cells are no longer suitable for use. Also known as 'expiration date'.
Export	Act of transporting a tissue or cell intended for human application to another country where it is to be processed further or used directly. In the EU, 'export' refers to transport to a third country (i.e. outside the EU).
Facility	A physical building or part of a building.

Faecal microbiota transplantation	Transfer of minimally processed donor faeces from one donor to a patient.
Fallopian tube	A long duct in the female abdomen that transports the oocytes that have been released from the ovary to the uterus.
Fascia	A layer of fibrous connective tissue that surrounds muscles, groups of muscles, blood vessels and nerves; it binds some structures together while permitting others to slide smoothly over each other.
Fertilisation	Entry of the oocyte by a spermatozoon followed by combination of their genetic material, resulting in the formation of a zygote.
Fertility	The capacity to establish a clinical pregnancy.
Fertility preservation	Cryopreservation of reproductive tissues or cells to preserve reproductive capacity.
Fibronectin	High-molecular glycoprotein of the extra-cellular matrix (ECM) that plays a major role in cell adhesion, growth, migration and differentiation.
Final product	Any tissue or cell preparation intended to be transplanted or administered after the final release step.
Follow-up	Subsequent evaluation of the health of a patient, living donor or recipient, for the purpose of monitoring the results of the donation or human application, maintaining care and initiating post-donation or post-application interventions.
Freeze drying	*See:* Lyophilisation.
Freezing	As used in this guide, it means storage of tissues at sub-zero temperatures, with or without cryoprotectant.
Full-thickness grafts (FTSG)	Graft composed of epidermis and full-thickness dermis (with adnexal structure).
Fungaemia	The presence of fungi in the circulating blood.
Gamma irradiation	Penetrating electromagnetic radiation arising from the radioactive decay of atomic nuclei. It is used for medical equipment sterilisation.
Gestational carrier	A woman who carries a pregnancy with an agreement that she will give the offspring to the intended parent(s). Gametes can originate from the intended parent(s) and/or a third party (or parties).
Glycosaminoglycans (GAG)	Long unbranched polysaccharides that are highly polar and thus attract water; useful as a lubricant or shock absorber.
Good laboratory practice	Set of principles that provides a framework within which studies are planned, carried out, monitored, recorded, reported and archived by laboratories conducting testing of all kinds.
Good Manufacturing Practice (GMP)	An EU standard applied internationally for the safe manufacture of medicinal products. Although the processing of tissues and cells is not normally regulated under medicinal manufacturing legislation, many of the principles of GMP can be applied usefully to tissues and cells for human application.
Good practice	A method or technique that has consistently shown results superior to those achieved by other means and which is currently used as a benchmark.
Graft	Part of the human body that is transplanted in the same or another person to replace a damaged part or to compensate for a defect.
Grafting	*See:* Transplantation.
Haematopoietic progenitor cells	Primitive haematopoietic cells capable of self-renewal as well as maturation into any of the haematopoietic lineages, including committed and lineage-restricted progenitor cells, unless otherwise specified and regardless of tissue source. Also known as 'haematopoietic stem cells'.
Haematoxylin-Eosin	Histology staining used in medical diagnosis.
Haemodilution	In reference to blood samples from a donor, a decrease in the concentration of the donor's plasma proteins, circulating antigens or antibodies and any infectious agent present, resulting from the transfusion of blood or blood components and/or infusion of fluids, e.g., colloid(s) and/or crystalloid(s). Also known as 'plasma dilution'.
Haemolysis	Damage to red cells resulting in the release of haemoglobin into serum/plasma.
Health Authority	In the context of this Guide, the body which has been delegated with the responsibility for ensuring that tissue and cell donation, banking and human application are appropriately promoted, regulated and monitored in the interests of donor and patient safety and public transparency on a national or regional basis by their government. Other terms, such as 'regulatory authority', 'regulatory agency' or, in the EU, 'competent authority', are equivalent to it.
Heart valve	One of the four structures within the heart that prevent the backflow of blood by opening and closing with each heartbeat. They include two semilunar valves (aortic and pulmonary), the mitral (or bicuspid) valve and the tricuspid valve. They permit blood flow in only one direction.
Homograft	*See:* Allograft.
Human application	Use of tissues or cells on or in a human recipient.

Human error	A mistake made by a person rather than being caused by a poorly designed process or by the malfunctioning of a machine such as a computer.
Human Milk Bank	A facility established with the purpose of selecting, collecting, processing, storing and distributing human milk donated by lactating mothers who are not biologically related to the recipient infant, to meet the specific needs of individuals for whom donor human milk is prescribed by health care providers.
Human tissues and cells for human application	Material containing or consisting of human tissues and/or cells intended for implantation, transplantation, infusion or transfer into or onto a human recipient.
Hybrid scaffold	Scaffold obtained using different types of materials.
Hydroxiproline quantification	Hydroxiproline is a non-essential amino acid (proline derivative) which results from collagen acid hydrolysis.
Identification of tissues and cells	The labelling of tissues and cells to uniquely designate their origin, use or destination. *See also:* Labelling.
Immune-privileged niche	A certain site of the human body able to tolerate the introduction of antigens without eliciting an inflammatory immune response.
Implantation (in the context of assisted reproductive technologies)	Attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts 5-7 days after fertilisation. *See also:* Transplantation.
Import	In this context, the act of bringing tissues or cells into one country from another for the purpose of human application or further processing.
Importing tissue establishment	A tissue bank or a unit of a hospital or another body established within the EU which is a party to a contractual agreement with a third-country supplier for the import into the EU of tissues and cells coming from a third country and intended for human application.
Imputability	Assessment of the probability that a reaction in a donor or recipient may be attributable to the process of donation or clinical application or to an aspect of the safety or quality of the tissues or cells applied.
In-process control	Checks undertaken during processing to monitor and, if necessary, to adjust the process to ensure that a product conforms to its specification. Control of the environment or equipment may also be regarded as a part of in-process control.
In vitro fertilisation	Assisted reproductive technology procedure that involves extracorporeal fertilisation. It includes conventional *in vitro* insemination and ICSI, for which *see* Intracytoplasmic sperm injection.
In vitro maturation	The maturation of oocytes recovered from non-preovulatory follicles that have been primed or not with exogenous gonadotrophins. Also, the *in vitro* process of maturation from immature dendritic cells (DC) to mature DC.
Incident	A generic term for an adverse reaction or adverse event.
Incident reporting (adverse event reporting, serious/ critical incident reporting)	A system in a healthcare organisation for collecting, reporting and documenting adverse occurrences that affect patients and are inconsistent with planned care (e.g. medication errors, equipment failures, violations).
Informed consent	A person's voluntary agreement, based upon adequate knowledge and understanding of relevant information, to donate, to participate in research or to undergo a diagnostic, therapeutic or preventive procedure.
Inner cell mass	A group of cells in the blastocyst that give rise to the embryonic structures: the fetus, the yolk sac, the allantois and the amnion.
Inspection	On-site assessment of compliance with local/national regulations on tissues and cells, carried out by officials of the relevant Health Authority.
Installation qualification	The performance and documentation of tests to ensure that equipment (such as equipment, premises) used in a procurement or production process is appropriately selected, correctly installed and works in accordance with established specifications.
Intracytoplasmic sperm injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Intra-uterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.
Keratoplasty	Corneal transplantation.
Key performance indicator	A quantifiable measure or a set of quantifiable measures used to trace performance over time.
Labelling	Includes steps taken to identify packaged material by attaching the appropriate information to the container or package so it is clearly visible on or through the immediate carton, receptacle or packaging. *See also:* Identification of tissues and cells.
Laminine	High-molecular-weight protein of the extracellular matrix with important roles in cell differentiation, migration and adhesion.
Laparoscopy	A surgical procedure in which a small incision is made through which a viewing tube (laparoscope) is inserted.
Limbal stem cells	The population of stem cells residing in the basal epithelium of the limbus, giving rise to the corneal epithelium.

Limbal tissue	Tissue bridging the junction between the cornea and sclera. Site of the limbal stem cells that renew the corneal epithelium. Limbal stem cell deficiency causes ocular surface disease.
Limbus	The area bridging the junction between the cornea and sclera.
Live birth rate	Delivery of one or more infants with any signs of life expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles.
Living donor	A living person from whom cells or tissues have been removed for the purpose of human application.
Lyophilisation	A controlled freezing and dehydration process through the sublimation of water under vacuum from ice directly to vapour to a residual water content of < 5 %. Typically used to preserve a non-viable perishable material or to make the material more convenient for transport. Also known as freeze drying.
Malignancy	Presence of cancerous cells or tumours with a tendency to metastasise, potentially resulting in death.
Manipulation	Preparation of retrieved tissues or cells to make them suitable for human application. In the context of processing of haematopoietic progenitor cells, this is a laboratory procedure that selectively removes, enriches, expands or functionally alters the cells.
Masson trichrome	Staining protocol used in histology for distinguishing cells from the surrounding connective tissue.
Measurement accuracy	Closeness of agreement between a measured quantity value and a true quantity value of a measurand. The concept 'measurement accuracy' is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.
Measurement uncertainty	Non-negative parameter characterising the dispersion of the quantity values being attributed to a measurand, based on the information used.
Medically assisted reproduction (MAR)	Reproduction brought about through various interventions, procedures, surgeries and technologies to treat different forms of fertility impairment and infertility. These include ovulation induction, ovarian stimulation, ovulation triggering, all ART procedures, uterine transplantation and intra-uterine, intracervical and intravaginal insemination with semen of husband/partner or donor. *See also:* Assisted reproductive technology (ART).
Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or which may be used in or administered to human beings with a view to either making a medical diagnosis, or restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action.
Meiotic spindle	Spindle apparatus composed of microtubules that support and segregate chromosomes during meiotic division.
Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
MHC II antigen	Class of major histocompatibility complex (MHC) molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells and B cells.
Micromanipulation in ART	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is a colourimetric assay for assessing cell metabolic activity.
Musculoskeletal	Tissues that are part of the skeleton and muscular system, including muscles, bones, cartilage, tendons and ligaments, which function in the support and movement of the body.
Negative-to-date release	The release of tissues or cells for human application before completion of testing for bacterial or fungal cultures. The cultures are negative at the time of release.
Next of kin	A person's closest living blood relative or relatives.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.
Non-partner donation	Donation of reproductive cells between individuals who do not have an intimate physical relationship; also called 'third-party donation'.
Novelty	Any new tissue and cell preparation or change that could significantly affect the quality and/or safety of tissues and cells and/or the safety of recipients.
Oligozoospermia	Total concentration of spermatozoa $< 15 \times 10^6$/mL.

One-off import	Import of any specific type of tissue or cell that is for the personal use of an intended recipient or recipients known to both the importing tissue establishment and the third-country supplier before the importation occurs. Such an import of any specific type of tissue or cell must not normally occur more than once for any given recipient. In the EU imports from the same third-country supplier taking place on a regular or repeated basis must not be considered to be 'one-off imports' (Directive 2015/566/EC).
Oocyte	The female gamete (egg).
Oocyte cumulus complex	Oocyte surrounded by the granulosa and corona radiate cells.
Oocyte sharing	Refers to a female partner who enters ART treatment and decides to donate a specified number of her retrieved oocytes. Also known as egg sharing.
Operational qualification	Documented verification that the system or sub-system performs as intended throughout all anticipated operating ranges.
Opt-in donation	System for determining voluntary consent to donate where consent has been given by an individual during their lifetime or by an individual's family after their death. Also known as explicit or express consent.
Opt-out donation	System for determining voluntary consent to donate where donation may proceed unless an individual has expressed an objection during their lifetime. Also known as presumed or deemed consent.
Organ	Differentiated and vital part of the human body, formed by different tissues, that maintains its structure, vascularisation and capacity to develop physiological functions with a significant level of autonomy.
Organ culture	Culture of the whole or parts of an organ in medium *in vitro* to preserve cell–cell and cell–matrix interactions and to maintain structure and function.
Organisation responsible for human application	A healthcare establishment or unit of a hospital or another body that carries out human application of human tissues or cells.
Ovarian hyperstimulation syndrome	An exaggerated systemic response to ovarian stimulation characterised by a wide spectrum of clinical and laboratory manifestations. It is classified as 'mild', 'moderate' or 'severe' according to the degree of abdominal distension, ovarian enlargement and respiratory, haemodynamic and metabolic complications.
Package insert	A document included in the packaging of a distributed tissue or cell product that includes important information for the end users on handling, storage, traceability and adverse outcome reporting and, in some cases, on the product's properties or characteristics.
Packaging	Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (initial or in-process packaging) or to the clinical user (final packaging) in a suitable manner. Also refers to containers. *See also:* Primary packaging; Secondary packaging.
Packaging material	Any material employed in the packaging of tissues or cells, excluding any outer packaging used for transportation or shipment. Packaging materials are referred to as 'primary' or 'secondary' according to whether or not they are intended to be in direct contact with the product. Also refers to containers.
Paracetic acid	Organic compound used for the disinfection of medical supplies to prevent biofilm formation.
Partner donation	Donation of reproductive cells between a couple who declare that they have an intimate physical relationship.
Percutaneous epididymal sperm aspiration	Sperm aspiration by percutaneous puncture of the epididymis by a fine-needle technique.
Performance qualification	Documented verification that the integrated system functions as intended, in its normal operating environment; processes may be proven also by documented verification through appropriate testing that the finished product produced by a specified process meets all release requirements.
Pericardium	A double-walled sac that contains the heart and the roots of the great vessels.
PicoGreen assay	Selective dsDNA quantification of as little as 25 pg/mL.
Placenta	An organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
Plasma dilution	*See:* Haemodilution.
Polar body	A haploid cell with very little cytoplasm that is formed and is separated from the oocyte during meiosis and that contains a nucleus produced in the first or second meiotic division.
Pooling	Physical contact between, or any process that could result in cross-contamination between, tissues or cells from more than one procurement from the same donor, or from two or more donors.

Posthumous donation	The donation of tissue or cells after the donor's death with prior written consent of the donor.
Pre-implantation genetic testing	A test performed to analyse the DNA from oocytes (polar bodies) or embryos (cleavage stage or blastocyst) for HLA-typing or for determining genetic abnormalities. These include: PGT for aneuploidies (PGT-A); PGT for monogenic/single gene defects (PGT-M); and PGT for chromosomal structural rearrangements (PGT-SR).
Preservation	Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of tissues or cells.
Primary packaging	Any material employed in the packaging of tissues and cells that is intended to be in direct contact with the graft, excluding any outer packaging used for transportation or shipment. Also refers to containers.
Procedures	Description of all the tasks, operations and processes to be carried out, the precautions to be taken and measures to be applied to ensure the quality and safety of tissues and cells from procurement through processing, testing and storage to human application.
Processing	All operations involved in the preparation, manipulation, preservation, storage and packaging of tissues or cells intended for human application.
Procurement	A process by which tissues or cells are made available for banking or human application. This process includes donor identification, evaluation, obtaining consent for donation, donor maintenance and retrieval of tissues, cells or organs.
Procurement organisation	A healthcare establishment or a unit of a hospital or another body that undertakes the procurement of human tissues or cells.
Proliferation	Rapid reproduction of a cell.
Pronucleus	The nucleus of the sperm or the oocytes during the process of fertilisation, after the sperm has entered the oocytes but before they fuse.
Prophase I oocyte	Immature oocyte at the prophase of the first meiotic division.
Propidium iodide	Fluorescent intercalating agent used to evaluate cell viability or DNA content in cell cycle analysis.
Pyrogenic	Producing or produced by heat or fever.
Pyrogens	Substance, typically produced by a bacterium, which produces fever when introduced or released into the blood.
Qualification	According to EU GMP, the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as to operators and the relevant written procedures.
Quality	Fulfilment of a specific set of standards, characteristics and requirements.
Quality assurance	The actions planned and performed to provide confidence that all systems and elements that influence the quality of the product are working as expected, both individually and collectively.
Quality control	The part of quality management focused on fulfilling quality requirements. In terms of preparation, it concerns sampling specifications and testing; for an organisation, it relates to documentation and release procedures, which together ensure that the necessary and relevant tests have actually been carried out and that materials have not been released for use until their quality has been judged to be satisfactory.
Quality improvement	The actions planned and performed to develop a system to review and improve the quality of a product or process.
Quality management system	The organisational structure, with defined responsibilities, procedures, processes and resources, for implementing quality management, including all activities that contribute to quality, directly or indirectly.
Quarantine	The initial status of procured tissues or cells while awaiting a decision on their acceptance or rejection, or tissues or cells isolated physically or by other effective means from other donated material for other reasons until their suitability for use is established.
Randomised control trial	A study in which samples or subjects are allocated at random into groups, called the 'study' and 'control' groups, to receive or not receive an experimental therapeutic intervention.
Rapid alert	An urgent communication to relevant individuals/organisations to ensure the protection of donors or recipients when an unexpected risk has been identified.
Recall	Removal from use of specific stored or distributed tissues and cells that are suspected or known to be potentially harmful. *See also:* Return; Withdrawal.
Recipient	Person to whom human tissues, cells or reproductive cells and embryos are applied.
Recovery	*See:* Procurement.

Registry	A repository of data collected on tissue, cell and organ donors and/or recipients for the purpose of audit, clinical outcome assessment, quality assurance, validation, healthcare organisation and planning, research and surveillance.
Regulatory authority	*See:* Health Authority.
Release	The act of certifying compliance of a specific tissue or cells of batch of tissues or cells with the requirements and specifications.
Remodelling	Change of the micrometric structure.
Reproductive cells	Oocytes and spermatozoa – in this Guide, oocytes and spermatozoa collected to be used for the purpose of assisted reproduction or fertility preservation.
Resazurin	Indicator of cell viability based on oxidation-reduction reactions.
Resistogram	The result of a test for the sensitivity of an isolated bacterial strain to different antibiotics. Also known as an antibiogram.
Retrieval	*See:* Procurement.
Return	Sending back recalled tissues or cells to the tissue establishment that supplied them for human application.
Risk assessment	Identification of potential hazards with an estimation of the likelihood that they will cause harm and of the severity of the harm should it occur.
Risk mapping	Risk mapping is a way of representing and prioritising the risks of an organisation. It enables a diagnosis of the vulnerabilities of a system, an organisation or a process (major risks) to be established and to objectively assist in decision-making by ranking the risks according to their criticality. Finally, it enables the implementation of actions to manage the risk.
Root cause analysis	A structured approach to investigating and identifying the factors that resulted in the nature, magnitude, location and timing of a harmful or potentially harmful outcome.
Roughness	Quality or state of having an uneven or irregular surface.
Scaffold	A structure made using scaffolding.
Sclera	Fibrous white outer coat of the eye.
Secondary packaging	Any material employed in the packaging of tissues and cells that is not intended to be in direct contact with the graft, and excluding any outer packaging used for transportation or shipment. Also refers to containers.
Semen analysis	A description of the ejaculate to assess function of the male reproductive tract. Characteristic parameters may include volume and pH, the concentration, motility, vitality and morphology of spermatozoa, and the presence of other cells.
Septicaemia	A systemic disease caused by the spread of pathogenic microorganisms or their toxins via the circulating blood.
Serious adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or to life-threatening, disabling or incapacitating conditions for a patient, or which might result in, or prolong, hospitalisation or morbidity (Directive 2004/23/EC).
Serious adverse reaction	An unintended response, including a communicable disease, in the donor or in the recipient, associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling or incapacitating or which results in, or prolongs, hospitalisation or morbidity (Directive 2004/23/EC).
Shipment	A type of transport where the transfer of tissues or cells from the distributing to the receiving facilities is carried out by means of a contract with a third party, usually a specialised logistics company.
Skin	Thin layer of tissue forming the natural outer covering of the human body. Skin is composed of two primary layers: the epidermis and dermis. These layers are separated by a thin sheet of fibres, the basement membrane. Keratinocytes constitute 95 % of the epidermis. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils and elastic fibres, embedded in proteoglycans.
Somatic cells	Any cell of a living organism other than the reproductive.
Spermatozoon	The mature male reproductive cell.
Split-thickness skin graft (STSG)	Graft composed of epidermis and partial-thickness dermis.
Sporicidal	Refers to a substance, agent or product used for killing bacterial spores.
Standard operating procedure	Written instructions describing the steps in a specific process, including the materials and methods to be used and the expected result. *See also:* procedures.
Starting material	Any procured human cell or tissue that will constitute an integral part of an advanced therapy medicinal product active substance.

Sterilisation	Any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites or viruses, present on a surface, in a fluid, in medication or in a compound such as biological culture media. Sterilisation can be achieved by applying the proper combinations or conditions of heat, chemicals, irradiation, high pressure and filtration.
Sterility assurance level	Represents the expected probability of a micro-organism surviving on an individual unit of product after exposure to a sterilisation process. SAL 10^{-6} has been established as the standard for allografts and indicates a probability of one chance in a million that one unit of product will be contaminated with a single organism after a sterilisation process. If the product meets or exceeds this standard, it is then considered sterile.
Storage	Maintenance of a product under appropriate controlled conditions until distribution.
Storage temperature	Temperature at which tissues and cells must be stored to maintain their required properties.
Supercritical carbon dioxide	Fluid state of carbon dioxide where it is held at or above its critical temperature and critical pressure. It is an alternative for terminal sterilisation of biological materials and medical devices in combination with peracetic acid.
Supernumerary embryos	Excess embryos after embryo transfer.
Surveillance	Systematic collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health responses, as necessary.
Swim up	A preparation technique based on the ability of spermatozoa to swim in the culture medium.
Tendon	A tough band of fibrous connective tissue that usually connects muscle to bone and which can withstand tension.
Terminal sterilisation	A method for achieving the sterility of a product in its sealed container and with a sterility assurance level of 10^{-6} or better.
Testicular sperm extraction/aspiration	A surgical procedure involving testicular biopsies or needle aspirations to obtain sperm for use in IVF and/or ICSI.
Third countries	Term used within the EU to refer to countries that are not members of the EU.
Third party	Any organisation that provides a service to a procurement organisation or tissue establishment on the basis of a contract or written agreement.
Time-lapse imaging	The photographic recording of microscope image sequences. In this Guide, used for documentation of gametes, zygotes, cleavage-stage embryos or blastocysts at regular intervals.
Tissue	An aggregate of cells joined together by, for example, connective structures and performing a particular function.
Tissue bank	*See:* Tissue establishment.
Tissue establishment	A facility or a unit of a hospital or another organisation where the activities of processing, preservation, storage or distribution of human tissues and cells for human application are undertaken. It may also be responsible for procurement and/or testing of tissues and cells.
Toxicity	Degree to which a substance can damage an organism.
Traceability	Ability to locate and identify a specific tissue/cell during any step from procurement, through processing, testing and storage, to distribution to the recipient or disposal. This implies the ability to identify: the donor; the tissue establishment or processing facility that receives, processes or stores the tissue and cells; and the recipient(s) at the medical facility/facilities applying the tissues and cells to the recipient(s). Traceability also covers the ability to locate and identify all relevant data relating to products and materials coming into contact with those tissues and cells.
Transmissible disease	Comprises all clinically evident illnesses (i.e. characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of micro-organisms in an individual or the transmission of genetic conditions to the offspring. In the context of transplantation, malignancies and autoimmune diseases may also be transmitted from donor to recipient.
Transplantation, implantation or grafting	Transfer (engraftment) of human tissues or cells from a donor to a recipient with the aim of restoring function(s) in the body. *See also:* Implantation (in the context of assisted reproductive technologies).
Transport	The act of transferring a tissue or cellular product between distributing or receiving facilities under the control of trained personnel.
Trophectoderm	Outer layer of cells in a blastocyst (composed of trophectoderm and inner cell mass cells). A group of cells in the blastocyst that do not produce any embryonic structures but give rise to the chorion, the embryonic portion of the placenta.

Unique identification code	A code that unambiguously identifies a particular donor and donation (e.g. a unique donation + tissue product code). *See also:* Coding.
Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use (EU Directive 2006/86/EC). or Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (ISO9000:2015).
Vas deferens, vasa deferentia	Tube(s) that transport(s) sperm from the epididymis to the ejaculatory ducts.
Verification	Preferred term for the validation or qualification of IT systems/software. or Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (ISO9000:2015).
Vigilance	Alertness to and/or awareness of serious adverse events, serious adverse reactions or complications related to donation and human application of tissues, cells and organs, involving an established process for reporting at local, regional, national or international level. *See also:* Surveillance.
Viraemia	The presence of viruses in the blood.
Vitrification	Method of ice-free cryopreservation achieved through an extreme elevation in solution viscosity sufficient to suppress the crystallisation of water. Requires rapid cooling and/or high concentrations of solutes, such as the conventional cryoprotectants, to reach the glass transition temperature without ice formation.
Wettability	Tendency of one fluid to spread on, or adhere to, a solid surface in the presence of other immiscible fluids. Wettability refers to the interaction between fluid and solid phases.
Window period	Period of time before infection can be detected by a specific testing method.
Withdrawal	Process instigated by a tissue establishment to recall tissues or cells that have been distributed.
Xenograft	Graft of tissue taken from a donor of one species and grafted into a recipient of another species.
Xenotransplantation	Any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live tissues, cells or organs from a non-human animal source, or (b) human body fluids, tissues, cells or organs that have had *ex vivo* contact with live non-human animal cells, tissues or organs.
Zygote	A diploid cell resulting from the fertilisation of an oocyte by a spermatozoon, before completion of the first mitotic division.

Appendix 4. **Example of cleanroom qualification**

Short description of equipment or process being validated.

Qualification of cleanrooms for use in regulated environments.

Details of equipment used in the validation.

An active Environmental Monitoring System (EMS)

Settle plates

Contact/air sampling plates

Particle counter

Active air sampler

Details of testing levels and methods used in validation.

Eudralex Volume 4, "The rules governing medicinal products in the European Union", Annex 1: Manufacture of sterile medicinal products

SOP254 – Environmental monitoring using contact plates

SOP975 – Environmental monitoring using the active air sampling

SOP978 – Environmental monitoring using settle plates

SOP2382 – Environmental monitoring equipment

SOP4007 – SCI cleanroom cleaning

ISO14644 – BSEN14644 and EU GMP cleanroom standards

Installation qualification

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
1.	Cleanroom designed in accordance with required operating specifications.	Appropriate specifications available and cleanroom designed to meet specifications. Current drawings for cleanroom layout and air handling unit(s) are available.				
2.	Cleanroom layout, fixtures and finishes are installed according to the current drawings and are of an appropriate standard.	Cleanroom finishes are smooth, impervious, non-shedding and also crack- and crevice-free. Floor to wall, wall to wall and wall to ceiling junctions are coved and finished in vinyl and are defect-free. All wall and ceiling penetrations are fully sealed with silicone sealant and are defect-free. Light fittings and filter housings are surface-mounted, are fully sealed with silicone sealant and are defect-free. There are no uncleanable recesses and minimal projecting ledges, shelves, cupboards and equipment. Fixtures, fittings and cleanroom furniture are all present, secure and free of rust and defects. Cleanroom entry/exit doors and pass-through hatch doors are interlocked or otherwise controlled to prevent both doors being opened simultaneously.				
3.	Confirm access to the EMS system data is available.	Records must be accessible during the validation process.				
4.	Confirm that particle counters and differential pressure monitoring systems are calibrated and available.	In-date calibration certificates must be available and equipment free for use during the entire validation period.				
5.	Ensure cleanroom and associated air handling unit(s) is registered as an asset in QPulse.	QPulse asset number must be generated.				

Operational qualification

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
1.	Particle Challenge Leak Test for testing of each installed HEPA filter using dispersed oil particulate (DOP).	External contractor to perform DOP testing of facilities in accordance with ISO 14644-3. Aerosol concentrations must be ≤ 0.01 % of the upstream concentration.				
2.	Air exchange rate testing. Tested in accordance with BSEN 14644-3-2005.	External contractor to measure airflow volume or airflow velocity. Air change rate in compliance with design specification and should achieve > 20 air changes per hour.				
3.	Particle Counting for classification of the cleanroom.	External contractor to perform particle counting in cleanroom to meet EU GMP Annex 1 "at rest" limits for particulates (working to ISO 14644-1).				
4.	Air flow distribution testing using smoke visualisation.	External contractor to perform smoke visualisation test in accordance with ISO 14644-3 demonstrating: • flow distribution is satisfactory within each room; • any dead spots within each room have been identified; • no areas of excessive turbulence below working height exist (that could lead to particulate contamination).				
5.	Perform a weekly clean of the cleanroom as per SOP 4007.	Cleanroom cleaning must be easy to facilitate and unobstructed.				
6.	Perform weekly at rest environmental monitoring as per SOP 254, SOP 975 and SOP 978.	Full set of plates must be exposed and results shown to not exceed EU GMP Annex 1 limits for microbial contamination.				
7.	Perform routine weekly at rest particle counting monitoring as per SOP 2382.	Full set of counts must be obtained in accordance with SOP 2382, and checked for compliance with EU GMP Annex 1 "at rest" limits.				

No.	Description	Acceptance criteria	Results	Pass/ Fail	Comments	Signature and date
8.	Record the differential pressures for cleanroom facilities during "at rest" monitoring.	Daily records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades at rest).				
9.	Perform simulated operation environmental monitoring as per SOP 254, SOP 975 and SOP 978.	Full set of plates must be exposed while non-clinical cleanroom work is performed, and results shown not to exceed EU GMP Annex 1 limits for microbial contamination.				
10.	Perform simulated operation particle counting monitoring.	Full set of counts must be obtained while non-clinical cleanroom work is performed and checked to ensure compliance with EU GMP Annex 1 "in operation" limits.				
11.	Record the differential pressures for cleanroom facilities "simulated operation" monitoring.	Records must be obtained for differential pressures, and compliance with the design specification and EU GMP confirmed (10-15 Pa between adjacent rooms of different grades during working).				
12.	Review at rest and simulated operation results and data.	Results should not highlight any problems or trends. All results must not exceed the upper limits for the relevant grades of room, in compliance with EU GMP Annex 1.				
13.	Particulate clean-up rate within stipulated limits.	Particulate air recovery/ clean-up rate must be achieved within 20 minutes in the "at rest" state and after operators/ workers have left the room (and after simulated operation tests).				

Performance qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Perform a weekly clean of the cleanroom as per SOP 4007 for a minimum of an 8-week period.	Cleanroom cleaning must be easy to facilitate and unobstructed.				
2.	Perform environmental monitoring as per SOP 254, SOP 975 and SOP 978 for a minimum of 8 consecutive weeks.	Full set of plates must be exposed at least weekly (in either the at rest or in use state) and results shown not to exceed EU GMP Annex 1 limits.				
3.	Perform particle counting monitoring for a minimum of 8 consecutive weeks.	Full set of counts must be obtained at least once per week and in accordance with SOP 2382, and checked for compliance with EU GMP Annex 1 limits (at rest or in operation, as appropriate for time of monitoring).				
4.	Record the differential pressures for cleanroom facilities for a minimum of 8 weeks of continued monitoring.	Records must be obtained for differential pressures for each day that environmental monitoring is performed, and checked for compliance with the design specification and EU GMP (10-15 Pa between adjacent rooms of different grades).				
5.	Review results and data. Identify any issues and trends.	Results should not highlight any problems or trends. All results must not exceed the action limits for the relevant grades of room, in compliance with EU GMP Annex 1.				

Note: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live.

Deviations and adverse events

QPulse No.	Details	Date raised	Date closed

Further testing details (if applicable)

Source: National Health Service (NHS), United Kingdom.

Appendix 5. **Example of incubator qualification**

Short description of equipment or process being validated.

Cleanroom incubators are used within processing to incubate samples at a set temperature for culture. Temperature of each incubator needs to be assessed prior to use in order to evaluate suitability of incubator for use and position of the temperature monitoring system probe.

Details of equipment used in the validation.

Calibrated temperature monitoring devices.

Supplier: _____

Model: _____

Serial No. _____

Details of testing levels and methods used in validation.

See IQ, OQ, PQ description and acceptance criteria.

Temperature mapping carried out as per SOP XXX

Recorded on FRM XXX

Installation qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Site incubator	Undamaged on delivery and fits designated area satisfactorily.				
2.	Ensure that cleanroom air flow is not affected	Air flow is satisfactory				
3.	Instruction manual	Manual present				
4.	Certificate of conformance	Certificate of conformance				
5.	Register warranty	Register warranty				
6.	Add to asset register	Add to asset register				
7.	Cleaning instructions provided by supplier	Instructions supplied				

Operational qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Incubator functions	Switches on				
2.	Ensure shelves fitted correctly	Shelves fitted correctly				
3.	Create SOP and FRM for incubator use, cleaning and maintenance.	FRM and SOP created				
4.	Clean incubator as per instructions provided	Batch numbers/expiry of cleaning products recorded				
5.	Swab each shelf onto TSA and SABC agar plates and send for incubation	Swab results clear and appended				
6.	Set temperature to required level	Set temperature to required setting.	Temperature setting: __ °C			
7.	Set CO_2 % level to required level	CO_2 level set to required setting	CO_2 level setting: __ %			

Performance qualification

No.	Description	Acceptance criteria	Results	Pass/Fail	Comments	Signature and date
1.	Perform initial temperature mapping (EMPTY)	Satisfactory as per SOP XXX				
2.	Site temperature mapping probe	As informed by step 1				
3.	Connected to Environmental monitoring system (EMS) and ensure temperature alarms are set	Connected for both high and low alarms Low alarm limit: High alarm limit: Delay time:	EMS alarm name: Low alarm limit: High alarm limit:		Append EMS record	
4.	Set CO_2 levels on Environmental monitoring system (EMS)	Connected for both high and low alarms. Delay time for alarms calculated by comparing readings on incubator with EMS. Low Alarm Limit: High Alarm limit: Delay Time:	EMS alarm name: Low alarm limit: High alarm limit: Delay time:		Append EMS record	
5.	Perform empty but humidified temperature mapping	Satisfactory as per SOP XXX				
6.	Perform simulated/ full load, humidified temperature mapping	Satisfactory as per SOP XXX			Simulated load details recorded in mapping record	
7.	Enable EMS	EMS alarms enabled				

Note: Each Validation Phase must be signed off before commencing the next phase of testing and before go-live.

Deviations and adverse events

QPulse No.	Details	Date raised	Date closed

Further testing details (if applicable)

_____

Source: National Health Service (NHS), United Kingdom.

Appendix 6. Example of process validation – tissue transportation

The example of a process validation outlined below describes a process that will be common to most, if not all, tissue establishments. That is, the need to transport tissues from one place to another (for example, from the site of procurement to the processing facility or from the tissue bank to the end user). Control of the conditions of transportation is critical for ensuring tissue quality. The example provided below refers specifically to the transportation of skin allografts from the procurement site to a tissue establishment at refrigerated temperatures. However, the principles are identical for all types of transportation.

The first stage was to define the process in detail. This was achieved by addressing the following questions:

- Which type of tissue and what maximum volume will be transported?
- How is the tissue contained? What is the nature, volume and temperature of any transport solution to be used? Which type of packaging has been used?
- Which refrigerant has been used and what is its specification and volume?
- What are the specifications of the transport container (i.e. dimensions, insulation, etc.)?
- What are the most extreme transportation conditions allowable in terms of transport time and ambient temperature?

Once the process had been defined, the acceptance criteria needed to be defined. In our example, they were that the:

- Temperature of the skin allograft must remain at 0-10 °C for the duration of the transit.
- Integrity of the tissue packaging must be maintained during transit.
- Integrity of the transport container must be maintained during transit.
- pH of the transportation fluid must be 7.0-7.5 at the end of the transportation.

For some tissues, it may be advisable to go further and validate the quality of the tissue after transit (e.g. by assessment of its viability or histological structure).

It was determined that the maximum amount of skin that would be transported would be 6 000 cm^2, immersed in a minimum volume of 300 mL of transport fluid. Specifications of the packaging, transport container, and refrigerant were also documented. The most extreme acceptable transportation conditions were defined as an ambient temperature of 40 °C (e.g. a hot summer day in a vehicle) for a maximum of 12 h, with the minimum volume of refrigerant and transport solution, and the maximum volume of tissue.

A protocol was written and a model prepared using skin obtained from donors unsuitable for clinical donation. This protocol was based on the defined transport solution, refrigerant, packaging and container specifications. A calibrated data-logging thermometer was used to record the temperature on the external surface of the tissue packaging. The container was placed into a shaking incubator, set at an ambient temperature of 40 °C. A shaking incubator was used to model the agitation of the container

during vehicular transit (the model should approximate real-life conditions as closely as possible).

The study was repeated in triplicate. Acceptable results were obtained on each occasion. All results were well within the pre-defined acceptance criteria, so the process was accepted based on the results of the three replicates.

Note, however, that it may be necessary to find a compromise between an 'ideal' validation and some operational practicalities that cannot be avoided. For example, it may not be possible or ethical to obtain and sacrifice large amounts of tissue for validation studies. In these cases, an acceptable compromise should be reached using risk-assessment principles (e.g. use of animal tissue as a substitute).

Application of sufficiently robust process validations (e.g. by challenging a transport process with extremes of time and temperature) obviates the need for routine temperature monitoring of the process. Thus, if the physical conditions identified by the validation study are complied with (e.g. the correct container, containing at least the minimum amount of refrigerant, in transit for less than the maximum modelled time), then it can be reliably concluded that the process itself has been carried out correctly. Therefore, to demonstrate compliance with the validated process, all that the operatives need to do is confirm that they have complied with the relevant standard operating procedures.

Appendix 7. Example of method validation – oocyte vitrification

Preservation of tissues and cells is used where cell viability must be maintained for a long period of time. However, the survival rate after thawing represents a critical step affecting the success of the treatment. The following example refers to the validation of a new method of cryopreservation in human oocytes. For prospective validation, a randomised clinical study with specific endpoints (primary and secondary) and acceptance criteria should be performed. See Figure A7.1.

As an alternative approach, the validation can be performed in parallel by comparing the new method with the established one, as follows. See Figure A7.2.

If and when a validation is performed in a randomised clinical study with defined endpoints (see Figure A7.1), usually a multicentre study is needed and the endpoints (primary and secondary) should be defined and agreed upon between the ART centres.

Figure A7.1. **Validation of a new oocyte vitrification method in a multicentre study**

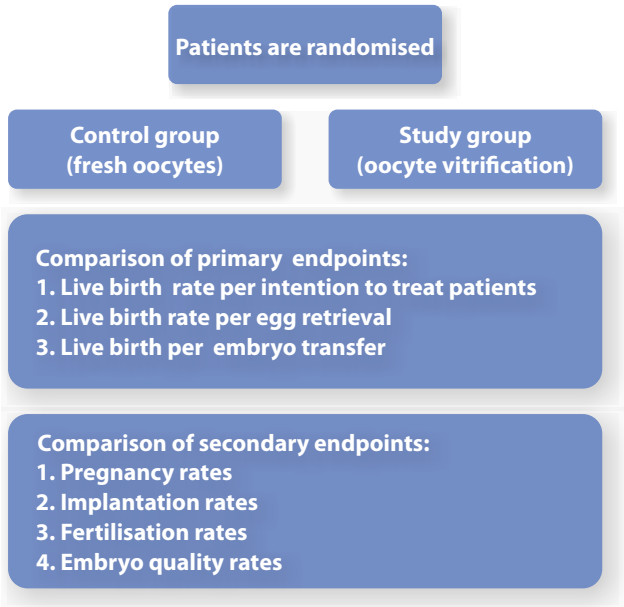
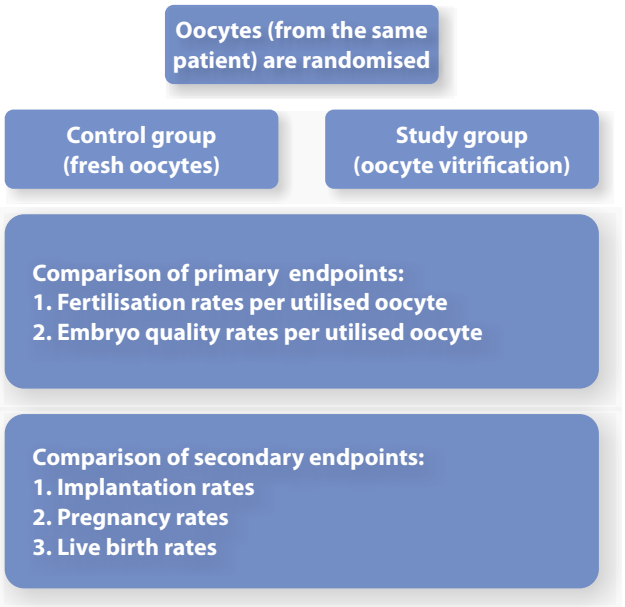
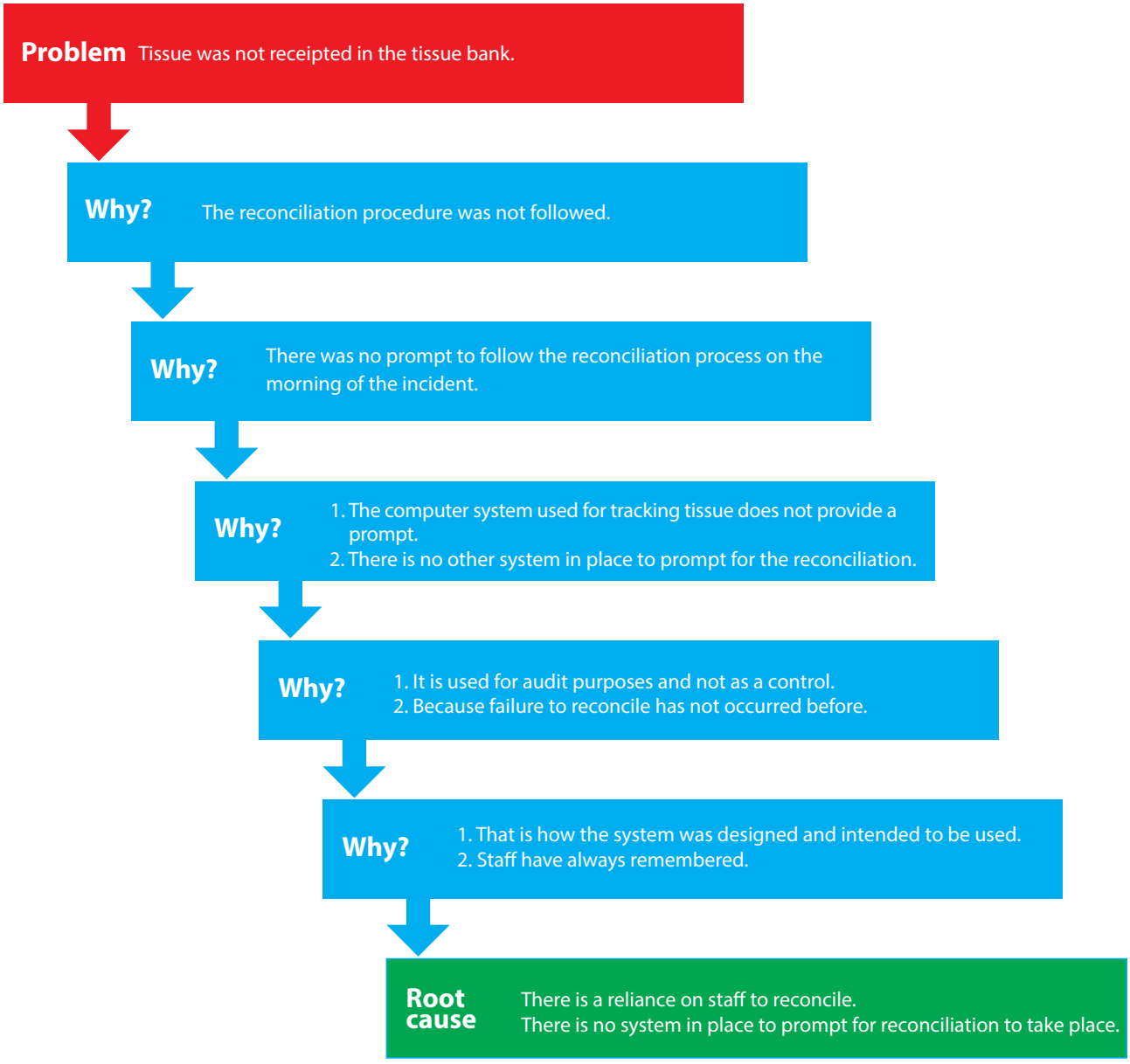


Figure A7.2. **Validation of a new oocyte vitrification method in a single centre**



Appendix 8. Example of root cause analysis: 5 whys – receipt of tissue

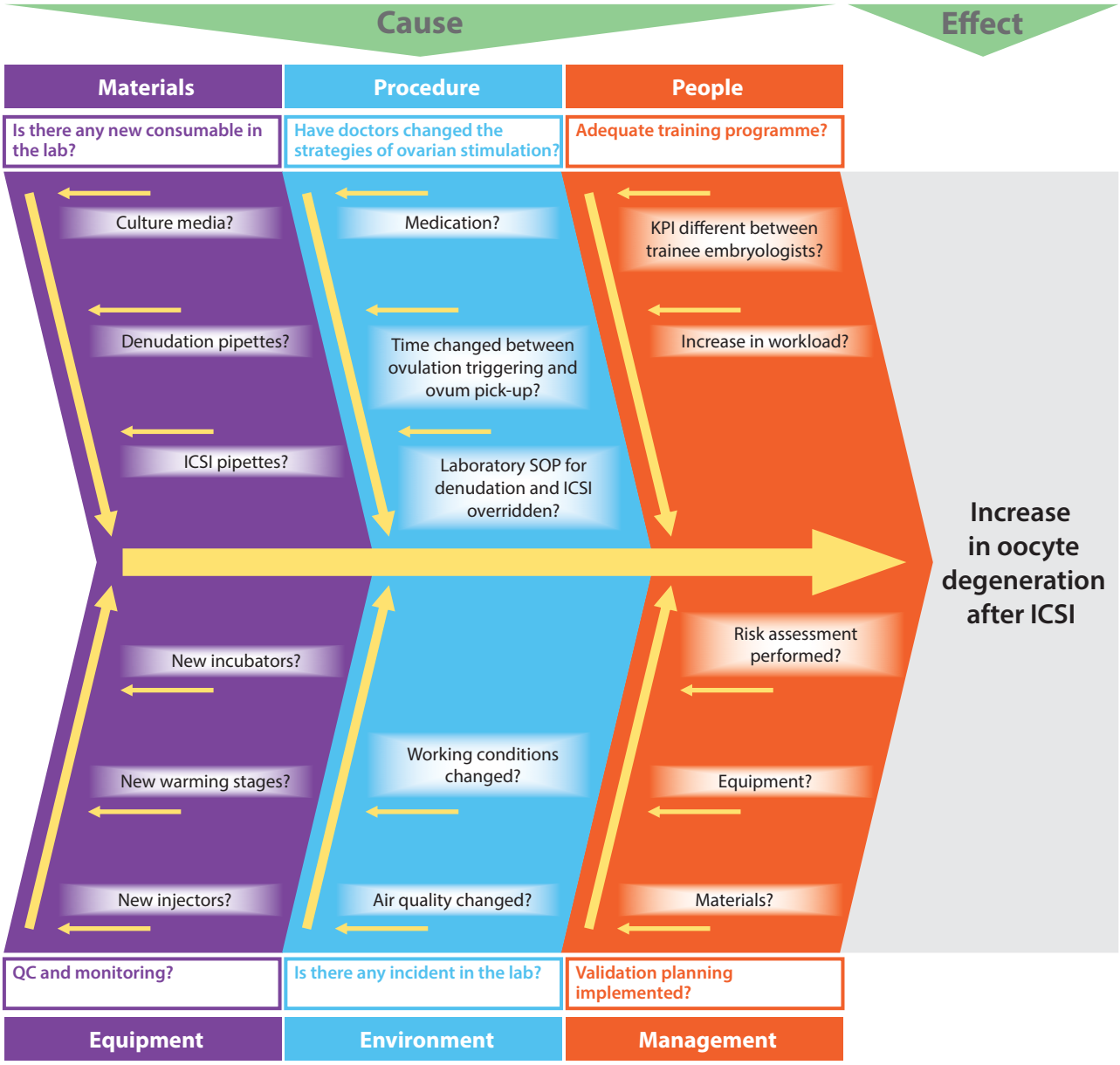


Appendix 9. Example of root cause analysis: fishbone diagram – receipt of tissue

Root causes

1. Reconciliation procedure not followed by tissue bank staff following the receipt and storage of tissue by the porters.
2. Computer system does not alert if reconciliation is not completed.

Appendix 10. **Example of root cause analysis: fishbone diagram – medically assisted reproduction**



Appendix 11. **Example of consent form: female (NHS, UK)**

Human Fertilisation & Embryology Authority

For clinic use only

Record of information provided before obtaining consent – female or egg provider

We recommend you use this form to record the information you have given to egg providers, female patients or female donors giving consent. There is also a version of this form for sperm providers, male patients or male donors. It is designed to help you demonstrate that you have met the requirements of the Human Fertilisation and Embryology Act (1990 (as amended) and 2008) before asking people to give consent. A completed copy of this form should be retained in the relevant medical records.

What information should be recorded?

First record the personal details of the person giving consent. You should then tick the relevant consent forms that this person will need to complete and add notes about any verbal information that was given before obtaining their consent. If information was provided in any other way (eg, at an information evening or through information leaflets) then it is a good idea to note this too.

Personal details

First name(s)

Surname

Date of birth

Other patient identifiers (optional)

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Treatment and storage:		
WT Your consent to your eggs and embryos created using your eggs being used in treatment (IVF and ICSI) or stored		

› Continues on next page

Version 4, 1 July 2022

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
WPT Your consent to providing eggs or embryos created with your eggs for your partner's treatment		
WGI Your consent to the use of your eggs in GIFT		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Storage only:		
GS Your consent to the storage of your eggs or sperm		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Renewal:		
RE Renewal of consent to storage of your embryos for treatment		

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
RG Renewal of consent to storage of your eggs or sperm for treatment		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Donation:		
WD Your consent to donating your eggs		
ED Your consent to donating embryos		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Disclosure of information:		
CD Your consent to disclosing identifying information **(State if only 'part one – general purposes' or 'part two – research purposes' was provided instead of the full version)**		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Name of consent form	**Tick**	
Parenthood:		
PBR Your consent to being registered as the legal parent in the event of your death		
PP or PP (gender neutral) Your consent to being the legal parent		
WP or WP (gender neutral) Your consent to your partner being the legal parent		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Surrogacy:		
WSG Your consent to the use and storage of eggs or embryos for surrogacy		
SPP or SPP (gender neutral) Your consent to being the legal parent in surrogacy		
SWP or SWP (gender neutral) Your consent (as a surrogate) nominating an intended parent to be the legal parent		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Withdrawal:		
WCS Withdrawing your consent to the storage of your own eggs, sperm and embryos		
WSU Withdrawing your consent to use of your eggs, sperm or embryos in someone else's treatment		

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
WCP Withdrawing your consent to legal parenthood		
LC Stating your spouse or civil partner's lack of consent		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Mitochondrial donation:		
WDM Mitochondrial donation: consent to donating your eggs		
WMT Mitochondrial donation: consent to use your eggs in treatment and storage		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Statutory notices:		
RNG Request to renew consent to storage of eggs or sperm within the renewal period		
RNE Request to renew consent to storage of embryos within the renewal period		
NDG Notification that eggs or sperm will be removed from storage and disposed of if the patient does not renew consent to storage before the end of the renewal period		
NDE Notification that embryo(s) may be removed from storage and disposed of if patients do not renew their consent to storage before the end of the renewal period		
NWC Notification to each person whose eggs or sperm were used to create embryo(s) that consent to storage has been withdrawn		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Record of the offer of counselling

Has counselling been offered? Yes ☐ No ☐

Date(s) counselling was offered	Who offered counselling?

Additional information (eg, the type of counselling and the person's response to the offer)

Source: Human Fertilisation and Embryology Authority (HFEA), United Kingdom.

Appendix 12. **Example of consent form: female (CNPMA, Portugal)**

cnpma conselho nacional de procriação medicamente assistida

CRYOPRESERVATION OF OOCYTES AND/OR OVARIAN TISSUE

Informed Consent

Oocytes are female reproductive cells that, in their immature form, are already present in the ovaries of the newborn. From puberty onwards, during each normal menstrual cycle, groups of oocytes will undergo maturation that will culminate in the release by the ovary of a mature oocyte that can be fertilised.

In certain clinical situations – for example, when required therapies may compromise the survival of reproductive cells - cryopreservation of oocytes and/or ovarian tissue has been proposed in an attempt to preserve future fertility

Cryopreservation of oocytes may also be considered whenever a woman considers carrying out a reproductive project at an advanced reproductive age, when oocyte production/viability will likely be very low or absent.

A number of key points should be highlighted:

- Cryopreservation of oocytes does not guarantee a pregnancy; it only ensures a stock of female reproductive cells that may be used in the future through In Vitro Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI).
- IVF and ICSI include stimulating the development and maturation of ovarian follicles containing the oocytes through the use of injectable drugs; the response of the ovaries to these drugs varies from woman to woman and will be monitored with blood tests and/or ultrasound at intervals to be defined on a case-by-case basis.
- Puncture of the ovaries for collection (aspiration) of oocytes is performed vaginally, under local anesthesia and/or sedation.
- For various reasons, most often a deficient response of the ovaries to the medication, the treatment cycle may need to be cancelled before the puncture to obtain the oocytes
- In rare situations, stimulation of the ovaries may trigger an excessive response, giving rise to the so-called “ovarian hyperstimulation syndrome”, which, in certain circumstances, will require specific treatment in an inpatient setting; in truly exceptional circumstances, ovarian hyperstimulation syndrome can be life-threatening.
- The administration of drugs for sedation or anesthesia may cause unexpected individual reactions, allergic or otherwise.
- As the collection of oocytes is a surgical procedure, in very rare cases, complications (for example, bleeding or infection) may require hospital admission; in truly exceptional circumstances, these complications can be life-threatening.
- The rate of pregnancy achieved with *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) of cryopreserved oocytes varies and will depend on the clinical situation of the patient to be treated
- Although no increase in foetal abnormalities has been reported, a possible increased risk of foetal abnormalities or regarding children development cannot be excluded at this time.
- Cryopreservation of oocytes does not entail any obligation on this centre concerning the future use of those cells, nor does it grant the person from whom they originated any type of preferential treatment; clinical criteria will determine the appropriate clinical conduct at all times.
- The collection of ovarian tissue for cryopreservation and its possible subsequent transplantation require surgical procedures with the inherent risks of the respective procedures.
- Unforeseen accidents, such as fires or calamities of other types, may, despite the safety precautions taken, lead to loss or destruction of the cryopreserved oocytes.

cnpma conselho nacional de procriação medicamente assistida

CONSENT

I, the undersigned, declare that:

- I have read and understood this document and the additional information provided.
- The queries and questions I have raised have been answered.
- I recognise that this text cannot describe exhaustively all situations that can occur in the future.
- I understand and accept that oocytes and/or ovarian tissue will be cryopreserved for a maximum period of five years and that I may revoke this authorisation at any time during this period.
- I understand and accept the conditions, risks and limitations of these techniques, namely that no guarantee can be given as far as the development and outcome of a pregnancy achieved with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
- I am aware that I alone have the right to use these frozen oocytes and/or ovarian tissue.
- I understand that, under the current legislation, until the end of this five-year period, I will have to sign a consent form in the centre for this cryostorage to continue. In the absence of a signed declaration requesting a further period of cryopreservation, I declare that I have been clearly informed that the oocytes and/or ovarian tissue will be thawed and destroyed, unless I have granted express authorisation for their use for scientific purposes. In these circumstances (write Yes or No):

  - I consent to the use of my oocytes in scientific research projects ☐

  - I consent to the donation of my oocytes to other patients I fully understand and accept the conditions, risks and limitations enumerated above. ☐

I declare that in case of donation to other patients, people born from one of these oocytes may obtain from the competent health services information regarding their genetic nature, and from CNPMA the civil identification of the donor, as from the age of 18.

Therefore, having been fully informed, I freely acknowledge the obligations implied in this agreement and give my consent for the use of these techniques for the preservation of my oocytes/ovarian tissue.

NAME _____

SIGNATURE _____

CIVIL ID/PASSPORT NO. _____

Medical Doctor: _____ ___/___/_____

cnpma conselho nacional de procriação medicamente assistida

PROTECTION OF PERSONAL DATA

CNPMA is responsible for the processing of personal data collected within the scope of the treatment for which consent is expressed through this document. These data will be processed within the scope of the CNPMA's attributions to regulate, supervise and monitor the practice of Medically Assisted Reproduction in Portugal, established by Law No. 32/2006, of 26 July, in its current wording. Your personal data will be treated securely, subject to technical and organizational measures aimed at safeguarding its confidentiality, integrity and availability. The data will be kept in compliance with the applicable legal retention period. The CNPMA guarantees data subjects the exercise of legally enshrined rights. To exercise these rights or to clarify doubts regarding the processing of your personal data, you must submit a written request to the CNPMA, namely by email to dpo.cnpma@ar.parlamento.pt or by mail to the National Council for Medical Assisted Reproduction, Assembleia da República, Palácio de São Bento, 1249-068 Lisboa.

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Source: Cryopreservation of oocytes and/or ovarian tissue, Portugal (CNPMA).

Appendix 13. **Example of consent form: male (NHS, UK)**

Human Fertilisation & Embryology Authority

For clinic use only

Record of information provided before obtaining consent – male or sperm provider

We recommend you use this form to record the information you have given to sperm providers, male patients or male donors giving consent. There is also a version of this form for egg providers, female patients or female donors. It is designed to help you demonstrate that you have met the requirements of the Human Fertilisation and Embryology Act (1990 (as amended) and 2008) before asking people to give consent. A completed copy of this form should be retained in the relevant medical records

What information should be recorded?

First record the personal details of the person giving consent. You should then tick the relevant consent forms that this person will need to complete and add notes about any verbal information that was given before obtaining their consent. If information was provided in any other way (eg, at an information evening or through information leaflets) then it is a good idea to note this too.

Personal details

First name(s)

Surname

Date of birth

Other patient identifiers (optional)

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Treatment and storage:		
MT Your consent to your sperm and embryos created outside the body using your sperm being used in treatment (IVF and ICSI) or stored		

› Continues on next page

Version 4, 1 July 2022

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
MGI Your consent to the use of your sperm in artificial insemination		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Storage only:		
GS Your consent to the storage of your eggs or sperm		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Renewal:		
RE Renewal of consent to storage of your embryos for treatment		
RG Renewal of consent to storage of your eggs or sperm for treatment		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Donation:		
MD Your consent to donating your sperm		
ED Your consent to donating embryos		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Disclosure of information:		
CD Your consent to disclosing identifying information **(State if only 'part one – general purposes' or 'part two – research purposes' was provided instead of the full version)**		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Parenthood:		
PBR Your consent to being registered as the legal parent in the event of your death		

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
PP or PP (gender neutral) Your consent to being the legal parent		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Surrogacy:		
MSG Your consent to the use and storage of sperm or embryos for surrogacy		
SPP or SPP (gender neutral) Your consent to being the legal parent in surrogacy		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Withdrawal:		
WCS Withdrawing your consent to the storage of your own eggs, sperm and embryos		
WSU Withdrawing your consent to use of your eggs, sperm or embryos in someone else's treatment		

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
WCP Withdrawing your consent to legal parenthood		
LC Stating your spouse or civil partner's lack of consent		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:
Mitochondrial donation:		
MD (including PNT) Consent to donating your sperm (including for use in pronuclear transfer)		
MMT Mitochondrial donation: consent to use your sperm in treatment and storage		
MD (PNT only) Mitochondrial donation: consent to sperm donation (PNT only)		Date information was provided: ☐☐ ☐☐ ☐☐ By whom:

› Continues on next page

Name of consent form	Tick	What relevant information was provided in relation to each consent and how?
Statutory notices:		
RNG Request to renew consent to storage of eggs or sperm within the renewal period		
RNE Request to renew consent to storage of embryos within the renewal period		
NDG Notification that eggs or sperm will be removed from storage and disposed of if the patient does not renew consent to storage before the end of the renewal period		
NDE Notification that embryo(s) may be removed from storage and disposed of if patients do not renew their consent to storage before the end of the renewal period		
NWC Notification to each person whose eggs or sperm were used to create embryo(s) that consent to storage has been withdrawn		
		Date information was provided: ☐☐ ☐☐ ☐☐ By whom

› Continues on next page

Record of the offer of counselling

Has counselling been offered? Yes ☐ No ☐

Date(s) counselling was offered	Who offered counselling?

Additional information (eg, the type of counselling and the person's response to the offer)

Source: Human Fertilisation and Embryology Authority (HFEA), United Kingdom.

Appendix 14. **Medical and social history questionnaire (NHS, UK)**

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | |

ODT Donor Number | | | | | | |

Medical and Social History Questionnaire

Directions for completion

1. This form must be completed in **black or dark blue ink** by the Specialist Requestor (SR)/Specialist Nurse – Organ Donation (SNOD)/Specialist Nurse – Tissue Donation (SNTD)/Tissue Donor Co-ordinator (TDC) and signed where required.

2. The original copy should be retained by the **SR/SNOD/SNTD/TDC** for the donor file.

3. In the event of organ and tissue donation, a legible copy should be sent to the relevant **Tissue Establishment**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.

The term relative is used throughout the form to refer to the relationship between the patient and the interviewee.

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

In order to ensure the safety of organs and tissue for transplant I will need to ask you some questions about *(name of patient)* medical and lifestyle history. Some of the questions are of a sensitive and personal nature. They are similar questions to those asked when someone donates blood. I will read and discuss each question with you and ask that you answer to the best of your knowledge with either a "Yes" or "No."

PATIENT INFORMATION

Patient's Forename(s) Please Print

Patient's Surname Please Print

Donating Hospital

NHS/CHI Number

Hospital Number

Cause of Death

Date of Birth (dd/mm/yyyy)

Occupation

Country of Birth

Country of residence

INTERVIEWEE INFORMATION

Information discussed with

Name Please Print

Relationship Please Print

For patients under the age of 18 months, or those who have been breast-fed or fed breast milk by a donor in the last 12 months, the mother is required to answer these questions with regard to her own and her child's health.

For children: has your child been breast-fed in the past 12 months? N/A ☐ No ☐ Yes ☐ Unknown ☐

NOTE: for all patients under the age of 18 months and any child who has been breast-fed in the last 12 months, a blood sample for microbiological testing is required from the mother, as well as from the patient.

For ALL female patients between 12 and 55 years of age*: Is there a possibility that your relative could be pregnant? N/A ☐ No ☐ Yes ☐ Unknown ☐

*DonorPath currently displays age range 13-53 years. This does not reflect current clinical guidance of 12-55 years documented in **MPD891**

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

GENERAL HEALTH INFORMATION

1) Did your relative visit a general practitioner in the last two years? No ☐ Yes ☐ Unknown ☐

If YES, give details

2) Was your relative currently seeing or waiting to see a general practitioner or any other healthcare professional? No ☐ Yes ☐ Unknown ☐

If YES, give details

3) Did your relative ever take regular medication? No ☐ Yes ☐ Unknown ☐

If YES, give details of any current or previous medication including any medication for acne, prostate or psoriasis

4a) Did your relative have a history of allergies to medication, food or other substances No ☐ Yes ☐ Unknown ☐

If YES, please provide details of the substance they were allergic to and describe the reaction

4b) Did your relative have any health problems due to exposure to toxic substances such as pesticides, lead, mercury, gold, asbestos, cyanide, agent orange etc? No ☐ Yes ☐ Unknown ☐

If YES, please provide details of the toxic substance and treatment

5a) Was your relative a diabetic? No ☐ Yes ☐ Unknown ☐

If YES, were they on insulin? N/A ☐ No ☐ Yes ☐ Unknown ☐

5b) Is there a family history of diabetes? No ☐ Yes ☐ Unknown ☐

If YES, is it insulin-dependent diabetes? N/A ☐ No ☐ Yes ☐ Unknown ☐

6. Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

7. Did your relative ever suffer from any bone, joint, skin or heart disease? No ☐ Yes ☐ Unknown ☐

If YES, specify which and give details

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | | ODT Donor Number | | | | | | |

8) Did your relative ever have hepatitis, jaundice or liver disease? No ☐ Yes ☐ Unknown ☐

If YES, give dates, diagnosis, treatment and hospital /clinic name if known

9) Did your relative recently suffer from significant unplanned weight loss? No ☐ Yes ☐ Unknown ☐

If YES, give details

10) Did your relative ever undergo any investigations for cancer or were they ever diagnosed with cancer? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

11) Did your relative have a history of eye disease, receive any medications for eye problems (e.g. eye drops), or undergo eye surgery or laser treatment? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

12) Did your relative ever have any operations? ***If NO go to question 15*** No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment, if possible

13) Did your relative ever have any surgery on the brain or spine? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible. **Surgery before 1993** is particularly significant

14) Did your relative ever have an organ or tissue transplant? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if known

15) Was your relative ever told not to donate blood? No ☐ Yes ☐ Unknown ☐

If YES, give details of where, when and the reason

16) Did your relative receive a transfusion of blood or blood product(s) at any time? No ☐ Yes ☐ Unknown ☐

If YES, give details including country, hospital name, dates and reason for transfusion

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17) Did your relative suffer from any type of brain disease such as Parkinson or Alzheimer disease or dementia? No ☐ Yes ☐ Unknown ☐

If YES, give details including hospital name and dates of treatment if possible

18) Did your relative suffer from any one or more of the following problems: memory problems or confusion, change in personality or behaviour, or were they unsteady on their feet? ***If NO go to Question 19, if YES*** No ☐ Yes ☐ Unknown ☐

18a) When did these symptoms start?

Please give details

18b) Did they worsen noticeably over time? No ☐ Yes ☐ Unknown ☐

Please give details

18c) Was your relative able to live independently? No ☐ Yes ☐ Unknown ☐

Please give details

18d) Were you aware of a condition causing these symptoms? No ☐ Yes ☐ Unknown ☐

If YES, please specify condition

19) Did your relative have a family history of prion disease, such as CJD, or were they ever told that they were at risk of prion disease? No ☐ Yes ☐ Unknown ☐

If YES, give details

20) Did your relative ever receive human pituitary extracts, e.g. growth hormones or fertility treatment or test injections for hormone imbalance? No ☐ Yes ☐ Unknown ☐

If YES, give details including dates and hospital/clinic name if known

21) Did your relative ever have any significant infection? No ☐ Yes ☐ Unknown ☐

If YES, give details, and any treatment received and hospital/clinic name if known

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

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Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

RECENT HISTORY

22) Did your relative come into contact with an individual with an infectious disease within the last month? No ☐ Yes ☐ Unknown ☐

If YES, please specify details, dates, symptoms, diagnosis, and treatment

23) Did your relative have any signs of infection, e.g. colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash within the last month? No ☐ Yes ☐ Unknown ☐

If YES, please specify dates, symptoms, diagnosis, and treatment

24) Did your relative have any immunisations within the last 2 months? No ☐ Yes ☐ Unknown ☐

If YES, give details including travel vaccinations and flu vaccination or flu nasal spray

25) Did your relative have tattooing, body piercing, botox injections, acupuncture, colonic irrigation, faecal transplantation, or any other cosmetic treatments or injuries that involve piercing the skin in the last 3 months? No ☐ Yes ☐ Unknown ☐

If YES, give details including where and when including unlicensed clinics in UK or abroad

26) In the last 12 months has your relative been bitten or scratched by any animal (strays, pets, wild, farm or ticks) or been bitten by a human. Or, has your relative ever been bitten or in close contact with bats anywhere in the world or been bitten by a mammal outside the UK? No ☐ Yes ☐ Unknown ☐

If YES, give details of incident, circumstances, animal, place, dates and treatment

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

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Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

TRAVEL HISTORY

27) Did your relative ever travel or live outside the UK (including business trips)? No ☐ Yes ☐ Unknown ☐

*If **NO go to question 33***

28) In the last 12 months did your relative go outside the UK (including business trips)? N/A ☐ No ☐ Yes ☐ Unknown ☐

Give details of dates and destinations visited

29) Did your relative ever have malaria or an unexplained fever which they could have picked up whilst abroad? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give date of fever/illness, places visited, duration and dates

30) Was your relative ever unwell whilst abroad or in the first month of their return to the UK? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details

31) Did your relative ever live or travel outside the UK for a continuous period of 6 months or more? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details of dates and destinations

32) Did your relative ever go to Central America, Mexico or South America for a continuous period of 1 month or more? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details of dates, places (remote/rural/urban areas), nature of visit

33) Was your relative's mother born in Central America, Mexico or South America? N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, give details (including date)

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Tissue Donor Number | | | | | | | | | | | ODT Donor Number | | | | | | |

BEHAVIOURAL RISK ASSESSMENT

34) Did your relative

a) Consume alcohol? No ☐ Yes ☐ Unknown ☐

If YES, give details (including type and amount)

b) Smoke tobacco or any other substance? No ☐ Yes ☐ Unknown ☐

If YES, give details of substance, frequency, history of smoking time and time elapsed since giving up.

c) Take any recreational drugs? No ☐ Yes ☐ Unknown ☐

If YES, give details including route of administration and dates

35) Is it possible that any of the following apply to your relative?

a) Was, or may have been infected with HIV, hepatitis or HTLV? No ☐ Yes ☐ Unknown ☐

b) Within the last 12 months have they injected, or been injected, with non-prescription drugs, including performance enhancing drugs or injectable tanning agents? No ☐ Yes ☐ Unknown ☐

c) Been in prison or a juvenile detention centre for more than 3 consecutive days in the last 12 months? No ☐ Yes ☐ Unknown ☐

d) Taken medication to prevent HIV infection e.g. (PrEP Pre/Post exposure prophylaxis)? No ☐ Yes ☐ Unknown ☐

If YES to any of the above questions a-d, give details, including dates.

36) Has your relative **ever** had sex – consensual or otherwise? N/A ☐ No ☐ Yes ☐ Unknown ☐

If NO, go to question 38.

If **YES**, is it possible that your relative:

a) Was given payment for sex with money or drugs in the last 3 months? N/A ☐ No ☐ Yes ☐ Unknown ☐

b) Ever had a sexually transmitted disease? N/A ☐ No ☐ Yes ☐ Unknown ☐

c) Ever had sex with anyone who was diagnosed with a Viral Haemorrhagic Fever N/A ☐ No ☐ Yes ☐ Unknown ☐

If YES, to any of the above, give details, including dates and where appropriate hospital/clinics, treatments.

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37)	Did your relative have sex, consensual or otherwise in the last 3 months?	N/A		No		Yes		Unknown	

If no, go to question 38.
If yes, is it possible that in the last 3 months your relative had sex with

a)	*(for male patients only)* another man?	N/A		No		Yes		Unknown	
b)	*(for female patients only)* a man who has ever had sex with another man?	N/A		No		Yes		Unknown	
c)	Anyone who is HIV or HTLV positive?	N/A		No		Yes		Unknown	
d)	Anyone who has hepatitis?	N/A		No		Yes		Unknown	
e)	Anyone who had a sexually transmitted disease?	N/A		No		Yes		Unknown	
f)	Anyone who has ever been given payment for sex with money or drugs?	N/A		No		Yes		Unknown	
g)	Anyone who in the last 12 months has injected or been injected with non-prescription drugs including performance enhancing drugs or injectable tanning agents?	N/A		No		Yes		Unknown	
h)	Anyone who could have had sex, in any part of the world, where AIDS/HIV is very common (this includes most countries in Africa)?	N/A		No		Yes		Unknown	
i)	Anyone who has developed an illness related to travel such as Zika?	N/A		No		Yes		Unknown	

If YES, give details

38)	Having answered all the previous questions, is there anyone else who you think may provide more information?	N/A		No		Yes		Unknown	

If YES, please specify

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Tissue Donor Number | | | | | | | | | |

ODT Donor Number | | | | | | |

Question number	Relevant additional information. If any questions have been answered as unknown, give an explanation

Signature of healthcare professional obtaining information

Please Print Name

Designation of healthcare professional obtaining information

Date of interview

Time of Interview

Source: National Health Service, United Kingdom.

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | |

ODT Donor Number | | | | | | |

CALCULATION FOR PLASMA DILUTION:		
Record ALL fluid administered in the 48 hours prior to taking sample for microbiology screening		
Fluid given. (Please give name, not just colloid or crystalloid)	Date and time administered	Vol. in millilitres

FRM4211/5.1 – Medical and Social History Questionnaire

NHS Blood and Transplant

Effective date: 18/03/2021

Tissue Donor Number | | | | | | | | | | ODT Donor Number | | | | | | |

Does a haemodilution calculation need to be carried out?	Yes		No		Reason	

A haemodilution calculation must be performed if the patient has:

1. Been transfused with blood or blood products.
2. Received infusions of synthetic colloids and/or crystalloids following blood loss*.

A - If the donor has received blood products/components, a pre-transfusion sample must be sought irrespective of volume of blood or blood product. This sample must be submitted for microbiological characterisation of the donor.

B - If haemodilution calculation is ≥50%, a pre-dilution sample must be sought.
If pre-transfusion or pre-dilution samples cannot be found, the Microbiology laboratory and all recipient centres/ tissue establishments must be informed and this documented.
* Decision on significance of blood loss should be discussed with clinical ITU team and made on a case by case basis.

CALCULATION FOR PLASMA DILUTION:

CRYSTALLOID INFUSED:

INTERVAL PRIOR TO SAMPLING	VOLUME INFUSED (ml)	% RETAINED	VOLUME RETAINED (ml)
>24 HOURS		0	None
2-24 HOURS		25	
1-2 HOURS		50	
<1 HOUR		75	
		TOTAL CRYSTALLOID RETAINED:	

BLOOD / COLLOID INFUSED:

INTERVAL PRIOR TO SAMPLING	VOLUME INFUSED (ml)	% RETAINED	VOLUME RETAINED (ml)
24-48 HOURS		100 (Blood) 50 (Colloid)	
0-24 HOURS		100	
TOTAL BLOOD / COLLOID RETAINED:			

Patient's weight: _____

ESTIMATED TOTAL BLOOD VOLUME:
70ml per kilogram of body weight
50ml per kilogram of body weight in ICU

% HAEMODILUTION

$$\frac{\text{(CRYSTALLOID RETAINED + BLOOD/COLLOID RETAINED) X 100}}{\text{BLOOD VOLUME}}$$

ACCEPT (<49%)/REJECT (≥50%)

Signature of healthcare professional obtaining information

Please Print Name

Date of interview

Time of Interview

Source: National Health Service, United Kingdom.

Appendix 15. **Physical assessment form (Dutch Transplant Foundation)**

Donor identification:

Donor number:

Date of birth:

Gender: M ☐ F ☐

Date recovery:

Identification verification: No ☐ Yes ☐

Consent: No ☐ Yes ☐

Recovery team members:

Start time recovery:

Eye tissue		Skin	
Heart valves		MS tissue	
Thoracic aorta		Femoral arteries	

Complications during procedure: No ☐ Yes ☐

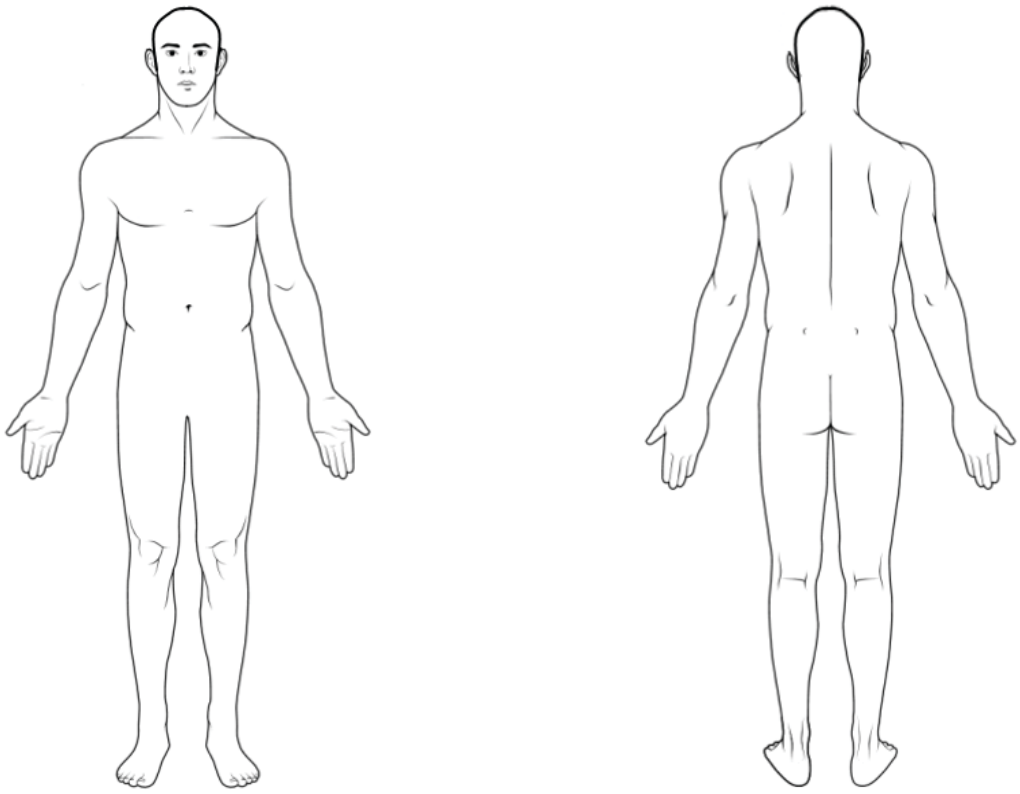
General appearance: Good ☐ Moderate ☐ Poor ☐

Height: cm Weight: kg

(O)	Ocular abnormalities	No ☐	Yes ☐	Unable to visualise ☐	
(WS)	White spots in the mouth	No ☐	Yes ☐	Unable to visualise ☐	
(J)	Jaundice	No ☐	Yes ☐		
(LN)	Abnormal lymph node(s)	No ☐	Yes ☐	Location? Size? Consistency?	
(L)	Enlarged liver	No ☐	Yes ☐		

(H)	Haematoma/bruises	No ☐	Yes ☐				
(GL/PL)	Genital and/or perianal lesions	No ☐	Yes ☐				
(NMI)	Non-medical injection sites	No ☐	Yes ☐				
(SL)	Skin lesions	No ☐	Yes ☐	Requires description			
(S)	Scars	No ☐	Yes ☐	Old ☐	Recent ☐		
(Ta/Pi)	Tattoos/piercings	No ☐	Yes ☐	Old ☐	Recent ☐		

(IV)	IV/Arterial line	(P)	Pacemaker/ICD	(BN)	Bone needle
(MP)	Needle entry site (medical procedures)	(D)	Drainage	(St)	Stoma
(BC)	Needle site blood collection	(C)	Cast	(Ca)	Catheter
(B)	Bandage	(I)	Autopsy/organ recovery incision	(De)	Decubitus



Describe findings/tattoos

Consultation No ☐ Yes ☐

Photos taken? No ☐ Yes ☐

Notes

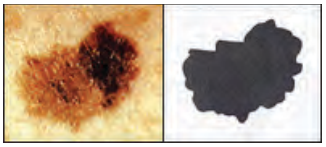
Appendix 16. **Evaluation of pigmented skin lesions**

A careful physical examination of the donor should be conducted, paying particular attention to the skin, looking for potential neoplasms or scars of previous surgical procedures (see Chapter 5).

The 'ABCDE rule' is an easy guide to detecting usual signs that may be indicative of melanoma [1-3].

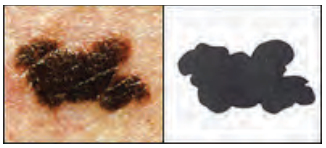
A. Asymmetry

If one half is not identical to the other half, suspect melanoma.



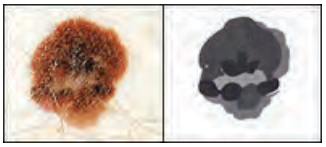
B. Border irregularity

Notched, scalloped, ragged or poorly defined borders should lead us to suspect melanoma.



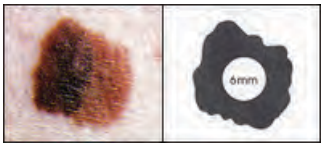
C. Colour

Naevi usually have a uniform colour; if there is colour variability from black-brown to red-blue-grey or white suspect melanoma.



D. Diameter/Difference

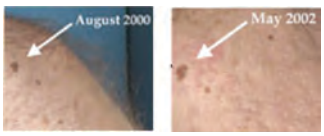
If the diameter is > 6 mm, suspect melanoma. Small lesions with some of the previous characteristics should also lead us to suspect melanoma.



If there are multiple lesions with a more or less regular aspect but among them there is one that has a very "ugly" aspect compared to the rest (ugly duckling sign), suspect melanoma.

E. Evolution

If there has been an evolution or change in appearance of a lesion, suspect melanoma. Any change – in size, shape, colour, elevation or another trait, or any new symptom such as bleeding, itching or crusting – points to danger.



Source of the images: www.skincancer.org/skin-cancer-information/melanoma/melanoma-warning-signs-and-images/#abcde.

Other warning signs are:

- Naevi are the most powerful predictor of risk of melanoma. An individual with more than 100 common naevi or more than two atypical naevi has a 5- to 20-fold increased risk of melanoma.
- People with a first-degree relative with melanoma are at increased risk of developing

melanoma; and 5-10 % of individuals with melanoma have a family history of melanoma. If there is a suspicious lesion and there is family history of melanoma, suspect melanoma.

References

1. Friedman RJ, Rigel DS, Kopf AW. Early detection of malignant melanoma: the role of physician examination and self-examination of the skin. *CA Cancer J Clin* 1985;35(3):130-51.
2. Whited JD, Grichnik JM. The rational clinical examination. Does this patient have a mole or a melanoma? *JAMA* 1998;279(9):696-701.
3. Gachon J, Beaulieu P, Sei JF *et al.* First prospective study of the recognition process of melanoma in dermatological practice. *Arch Dermatol* 2005;141(4):434-8.

Appendix 17. Evaluation of malignancies for risk assessment in tissue and cell donors

Table A17.1. **Grading of selected central nervous system tumours***

Diffuse astrocytic and oligodendroglial tumours	**1**	**2**	**3**	**4**
Astrocytoma, IDH-mutant		■	■	■
Diffuse astrocytoma, MYB- or MYBL1-altered	■			
Anaplastic astrocytoma, IDH-mutant			■	
Glioblastoma, IDH-wildtype				■
Glioblastoma, IDH-mutant				■
Diffuse midline glioma, H3K27M-mutant				■
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted		■	■	
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted			■	

Other astrocytic tumours	**1**	**2**	**3**	**4**
Pilocytic astrocytoma	■			
Subependymal giant cell astrocytoma	■			
Pleomorphic xanthoastrocytoma		■		
Anaplastic pleomorphic xanthoastrocytoma			■	

* *Source*: adapted from: Louis DN, Ohgaki H, Wiestler OD *et al.* (2016) World Health Organization histological classification of tumours of the central nervous system. Geneva: WHO Press/Lyon: International Agency for Research on Cancer; and Louis DN, Wesseling P, Brat DJ *et al.* The 2021 WHO classification of tumors of the Central Nervous System: a summary. *Neuro-Oncology* 2021;23(8):1231-51.

Ependymal tumours	**1**	**2**	**3**	**4**
Supratentorial ependymoma		■	■	
Posterior fossa ependymoma		■	■	
Myxopapillary ependymoma		■		
Ependymoma, RELA fusion-positive		■	■	
Anaplastic ependymoma			■	

Other gliomas	**1**	**2**	**3**	**4**
Angiocentric glioma	■			
Chordoid glioma of third ventricle		■		

Choroid plexus tumours	**1**	**2**	**3**	**4**
Choroid plexus papilloma	■			
Atypical choroid plexus papilloma		■		
Choroid plexus carcinoma			■	

Tumours of the pineal region	**1**	**2**	**3**	**4**
Pineocytoma	■			
Pineal parenchymal tumour of intermediate differentiation		■	■	
Pineoblastoma				■
Papillary tumour of the pineal region		■	■	

Meningiomas	**1**	**2**	**3**	**4**
Meningioma	■	■	■	
Atypical meningioma		■		
Anaplastic (malignant) meningioma			■	

Embryonal tumours	**1**	**2**	**3**	**4**
Medulloblastoma (all subtypes)				■
Embryonal tumour with multi-layered rosettes, C19MC-altered				■
Medulloepithelioma				■
CNS embryonal tumour, NOS				■
Atypical teratoid/rhabdoid tumour				■
CNS embryonal tumour with rhabdoid features				■

Neuronal and mixed neuronal-glial tumours	**1**	**2**	**3**	**4**
Dysembryoplastic neuroepithelial tumour	■			
Gangliocytoma	■			
Ganglioglioma	■			
Anaplastic ganglioglioma			■	
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	■			
Desmoplastic infantile astrocytoma and ganglioglioma	■			
Papillary glioneuronal tumour	■			
Rosette-forming glioneuronal tumour	■			
Central neurocytoma		■		
Extraventricular neurocytoma		■		
Cerebellar liponeurocytoma		■		

Tumours of the cranial and paraspinal nerves	**1**	**2**	**3**	**4**
Schwannoma	■			
Neurofibroma	■			
Perineurioma	■			
Malignant peripheral nerve sheath tumour (MPNST)		■	■	■

Mesenchymal, non-meningothelial tumours	**1**	**2**	**3**	**4**
Solitary fibrous tumour/haemangiopericytoma	■	■	■	
Haemangioblastoma	■			

Tumours of the sellar region	**1**	**2**	**3**	**4**
Craniopharyngioma	■			
Granular cell tumour	■			
Pituicytoma	■			
Spindle cell oncocytoma	■			

Table A17.2. **Recommendations on the use of organs from donors with CNS tumours***

Absolute contraindications
• Primary cerebral lymphoma
• All secondary intracranial tumours • Any cancer with metastasic spread

Intracranial tumours with an intermediate risk of cancer transmission
(2.2 % with an upper 95 % CI of 6.4 %) include WHO grade 4 tumours and equivalents:
• Glioblastoma (IDH wild type and IDH mutant)
• Giant cell glioblastoma
• Gliosarcoma
• Pineoblastoma
• Medulloblastoma (all subtypes)
• Medulloepithelioma
• Atypical teratoid/rhabdoid tumour
• Malignant peripheral nerve sheath tumour (MPNST) – grade 4
• Embryonal tumour (all subtypes)
• Choriocarcinoma
• Diffuse midline glioma, H3K27 M-mutant

Intracranial tumours with a low risk of transmission
(< 2 %) include WHO Grade 3 and equivalents:
• Anaplastic astrocytoma, IDH mutant
• Anaplastic oligodendroglioma, IDH mutant and 1p/19q deleted
• Ependymoma, RELA fusion-positive
• Choroid plexus carcinoma
• Anaplastic ganglioglioma
• Pineal parenchymal tumour of intermediate differentiation
• Papillary tumour of the pineal region
• Malignant peripheral sheath tumour grade 3
• Anaplastic/malignant meningioma
• Papillary tumour of the pineal region
• Haemangiopericytoma/ solitary fibrous tumour
• Anasplastic ependymoma
• Anaplastic pleomorphic xanthoastrocytoma

* *Source*: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). Transplantation of organs from deceased donors with cancer or a history of cancer. London: Department of Health and Social Care, December 2020.

Table A17.3. **Recommendations on the use of organs from donors with non-CNS cancers***

Absolute contraindications
• Active cancer with spread outside the organ
• Active haematological malignancy
High risk (> 10 % risk of transmission)
• Melanoma: without spread (except as below)
• Breast: cancer other than those identified below
• Colon: cancer other than those identified below
• Kidney: renal cell cancer > 7 cm or stages 2-4
• Sarcoma: > 5 years previously and resected
• Small cell cancer: lung/neuroendocrine
• Lung cancer: stage 1
Intermediate risk (2-10 % risk of transmission)
Kidney: renal cell carcinoma stage 1b (pT1b) (4-7 cm, Fuhrman/nucleolar grade 1 or 2) with curative surgery and cancer-free period of > 5 years
Breast: stage 1A (pT1, No; < 20 mm tumour size) with curative surgery and cancer-free period of between 5 and 10 years
Low risk (0.1-2 % risk of transmission)
• Melanoma: superficial spreading type, non-ulcerated, with tumour thickness < 0.8 mm with curative surgery and cancer-free period of > 5 years
• Breast: stage 1A (pT1 No; < 20 mm tumour size), with curative surgery and cancer-free period of > 10 years
• Colon: stage 1A (pT1 or pT2, No) adenocarcinoma with curative surgery and cancer-free period of > 5 years
• Thyroid: solitary papillary carcinoma 0.5-2.0 cm
• Thyroid: minimally invasive follicular carcinoma 1.0-2.0 cm
• Kidney: resected solitary renal cell carcinoma > 1.0cm and < 4 cm and Fuhrman (nucleolar) grade 1 or 2 with curative treatment and cancer free > 3 years
• Prostate: Gleason =7, with curative treatment and cancer free > 5 years
• Treated gastrointestinal stromal cancers: < 2 cm, < 5 % mitotic count, in stomach or duodenum
Minimal risk (< 0.1 % risk of transmission)
• Skin: basal cell carcinoma
• Skin: squamous cell carcinoma with no metastases
• Skin: non-melanoma skin cancer *in situ*
• Uterine cervix: *in situ* cancer
• Thyroid: solitary papillary carcinoma (< 0.5 cm)
• Thyroid: minimally invasive follicular carcinoma (< 1.0 cm)
• Bladder: superficial non-invasive papillary carcinoma (pTa, G1/G2)
• Kidney: resected solitary renal cell carcinoma < 1.0 cm and Fuhrman (nucleolar) grade 1/2
• Prostate: Gleason < 6, or < 7 with curative treatment and cancer free > 5 years

Note. Only those cancers where evidence is available for analysis have been classified. Cancers not included in this guidance must be considered on a case-by-case basis following appropriate professional consultation.

* *Source*: Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO). Transplantation of organs from deceased donors with cancer or a history of cancer. London: Department of Health and Social Care, December 2020.

Table A17.4. **Carcinoma *in situ* denomination depends on the location***

Organ	Type of tumour	Carcinoma *in situ*
Oesophagus		High-grade dysplasia, defined as malignant cells confined to the epithelium by the basement membrane
Small intestine		High-grade dysplasia/carcinoma *in situ*
Appendix	Carcinoma	Intramucosal carcinoma, invasion of the lamina propria into but not through the muscularis mucosa
	(LAMN)	• Low-grade appendiceal mucinous neoplasm confined by the muscularis propria • Acellular mucin or mucinous epithelium may invade into the muscularis propia
Anus		High-grade squamous intraepithelial lesion (previously termed carcinoma *in situ*, Bowen disease, anal intraepithelial neoplasia II-III, high-grade intraepithelial neoplasia)
Exocrine pancreas		• High-grade intraepithelial neoplasia (Panin-3) • Intraductal papillary mucinous neoplasm with high-grade dysplasia • Intraductal tubulopapillary neoplasm with high-grade dysplasia • Mucinous cystic neoplasm with high grade dysplasia
Lung		• Squamous cell carcinoma *in situ* (SCIS) • Adenocarcinoma *in situ* (AIS): adenocarcinoma with pure lepidic pattern, ≤ 3 cm in greatest dimension
Breast	LCIS	Lobular carcinoma *in situ* is considered a benign entity
	DCIS	Ductal carcinoma *in situ*
	Paget	Paget disease of the nipple not associated with carcinoma or DCIS in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorised based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted
Penis	Tis	Carcinoma *in situ* (penile intraepithelial neoplasia PeIN)
	Ta	Non-invasive located squamous cell carcinoma
Renal pelvis and ureter/urinary bladder/urethra/prostatic urethra	Ta	Papillary non-invasive carcinoma
	Tis	Urothelial carcinoma *in situ* "flat tumour"
Parathyroid		Atypical parathyroid neoplasm (neoplasm of uncertain malignancy)

* Source: American Joint Committee on Cancer. AJCC Cancer Staging Manuals, 8th edition, 2016. Last update 5 June 2018. See www.cancerstaging.org. Last accessed 5 April 2022.

Table A17.5. **Risk assessment in the case of a donor's heart for heart valves with diagnosed CNS tumour in the donor**
See Figure 5.1 (Chapter 5) Decision tree algorithm; for non-haematological and non-CNS tumour in deceased tissue donors.

In general, the tissues that can be procured from deceased donors are corneas, heart valves, arteries, musculoskeletal and skin. A previous history or a presence of malignancy requires a careful donor evaluation to avoid any risk to recipients.

Due to the special characteristics of corneas, they are excluded from this risk assessment, but see Chapter 19 for more information.

Because haematological malignancies are a general contraindication for the acceptance of these tissues, the focus here is on non-haematological malignancies and specifically those that are not carcinoma *in situ*.

For this risk assessment, it is important to know in detail the exact histology of the tumour entity, its stage and grade, its previous treatment and the cancer-free period. Another point that could influence the final decision is whether there is a scarcity of the donated tissue that could increase the benefit to the recipient, putting this benefit in front of the risk of waiting more time for the next available tissue.

When there is a tumour with dissemination (metastasis) it is considered an absolute contraindication for tissue donation. In the case of Central Nervous System (CNS) tumours, those grade 3 or 4 tumours (such as glioblastoma multiform) have low or intermediate risk in the case of organ transplantation. In the past, the general idea discussed among the scientific community has been that CNS tumours did not produce metastasis outside the brain. At present, although metastasis is a rare phenomenon, it has been documented from different brain tumours

and numerous paediatric brain tumours in different locations [1].

Therefore, the next question would be: In the case of a tissue donor with any CNS tumour, could the heart be donated for heart valves? Is there a risk of finding metastases in the heart from a CNS tumour?

Several aspects should be taken into consideration during this risk assessment:

- Metastases to the heart from other primary tumours are 30 times more common than primary cardiac tumours [2].
- In patients with known malignancies, the frequency of cardiac metastases in the general population in autopsy is 0.7 %-3.5 % and up to 9.1 % [3].
- Cardiac metastases mostly appear in patients with a disseminated tumour, and isolated metastases to the heart are very rare [4].
- The dissemination routes are: haematogenous spread; lymphatic spread; transvenous extension and direct extension [3].
- Two thirds of all cardiac metastases affect the pericardium, one third affect the epicardium or the myocardium and only 5 % affect the endocardium [5].
- The most frequent metastases come from malignant melanoma, mesothelioma, malignant germ cell tumours, lung cancer, undifferentiated carcinoma, breast carcinoma, ovarian carcinoma, lymphoproliferative neoplasm (lymphoma, leukaemia), gastric carcinomas, renal carcinomas, pancreatic carcinoma and oesophageal cancer [2][3][4][5][6][7][8][9].
- Malignant cells can be identified in the majority of malignant pericardial effusions drained by pericardiocentesis, and the cytology of malignant cells has an extraordinary correlation with the primary histological diagnosis [3][5].
- Tumour nodules may be found within the myocardium and on the endocardial surface. Individual masses may range from 1-2 mm to > 2 cm [7].
- In all previous references, there is no case of CNS tumour metastases to the heart; in some publications, this issue is remarked upon [10] [11].
- In paediatric donors, the epidemiology of cardiac metastases is very similar to that in adults. Although it is not a CNS tumour, one case of cardiac metastases with neuroblastoma has been described [12].
- Cardiac or pericardial metastases should be considered whenever a patient with a known malignancy develops cardiovascular symptoms [4].

Using this information, a risk assessment will proceed. The first question to answer is:

- Is there any risk of tumour transmission through the use of heart valves from a donor with CNS tumours (including glioblastoma multiforme)? See Table A17.6 Four steps to risk assessment.

Table A17.6. **Four steps to risk assessment**

The first step is to assess the probability of this situation happening.

With the previous information the score is: 1.
In the case of paediatric donors with neuroblastoma, the score could be 2.

Probability score	Description	Criteria
1	improbable	unlikely but possible
2	remote	unlikely but can be reasonably expected to occur
3	occasional	will occur from time to time
4	probable	occurs often
5	frequent	continually experienced

The second step is to assess severity.

In the case that there are unknown malignant cells in the heart valves and a tumour transmission has occurred, what is the severity of this in the recipient?
If the transmission occurred, the score could be 3 or 4.

Severity score	Impact	Criteria
1	negligible	temporary discomfort or inconvenience to user or patient
2	minor	temporary injury to patient or user (e.g. minor temporary irritation) not requiring medical intervention
3	serious	significant injury or side effect requiring unanticipated medical intervention
4	critical	permanent impairment, life-threatening injury
5	catastrophic	death

The next step is the classification of the risk.

With a probability of 1 and severity of 3 or 4, the risk is low. In the case of a paediatric patient with neuroblastoma, probability of 2 and severity 3 or 4, the risk is medium.

	Severity 1	Severity 2	Severity 3	Severity 4	Severity 5
Probability 1	1 Low	2 Low	3 Low	4 Low	5 Medium
Probability 2	2 Low	4 Low	6 Medium	8 Medium	10 High
Probability 3	3 Low	6 Medium	9 Medium	12 High	15 Very high
Probability 4	4 Low	8 Medium	12 High	16 Very high	20 Extreme
Probability 5	5 Medium	10 High	15 Very high	20 Extreme	25 Extreme

The risk management action by risk category is shown on the right.

Risk category	Action
Low	Acceptable if there is no cost-effective option to reduce further the risks
Medium	Make efforts to reduce risks within defined timescales
High/Very High	Unacceptable, unless huge benefit and no safer and equally effective option is available. Must take all measures to decrease risk if activity is to proceed
Extreme	Stop activity or do not start

To calculate the Priority Risk Number (PRN) in the FMECA mode we need detectability:

- Obvious
- Easy to detect
- Difficult to detect
- Very difficult to detect
- Impossible to detect

If only a macroscopic examination is performed, small metastases cannot be appreciated. In that case, the detectability could be 4. The PRN would be:

Probability of 1 × severity of 4 × detectability 4 = 16

If we examine the pericardium liquid and a pathologist does a whole heart examination, the detectability could be 2. The PRN would be:

Probability of 1 × severity of 4 × detectability 2 = 8

Applying the same criteria with children, with only macroscopic examination, the PRN would be:

Probability of 2 × severity of 4 × detectability 4 = 32

With macroscopic examination, the PRN would be:

Probability of 2 × severity of 4 × detectability 2 = 16

In conclusion, after performing the risk assessment explained above, accepting any donor with a CNS tumour has a low risk for tumour transmission when considering the heart valves, and the donation can be accepted but, to reduce the risk, a whole examination by a pathologist is recommended.

References

1. Rajagopalan V, El Kamar FG, Thayaparan R. Bone marrow metastases from glioblastoma multiforme—A case report and review of the literature. *J Neurooncol* 2005;72(2):157-61.
2. Taguchi S. Comprehensive review of the epidemiology and treatments for malignant adult cardiac tumors. *Gen Thorac Cardiovasc Surg* [Internet] 2018;66(5):257-62. Available at https://doi.org/10.1007/s11748-018-0912-3.
3. Goldberg AD, Blankstein R, Padera RF. Tumors metastatic to the heart. *Circulation* 2013;128(16):1790-4.
4. Lamba G, Frishman WH. Cardiac and pericardial tumors. *Cardiol Rev* 2012;20(5):237-52.
5. Bussani R, De-Giorgio F, Abbate A, Silvestri F. Cardiac metastases. *J Clin Pathol* 2007;60(1):27-34.

6. Hudzik B, Miszalski-Jamka K, Glowacki J *et al.* Malignant tumors of the heart. *Cancer Epidemiol* 2015;39(5):665-72.
7. Sarjeant JM, Butany J, Cusimano RJ. Cancer of the heart: epidemiology and management of primary tumors and metastases. *Am J Cardiovasc Drugs* 2003;3(6):407-21.
8. Catton C. The management of malignant cardiac tumors: clinical considerations. *Semin Diagn Pathol* 2008;25(1):69-75.
9. Skhvatsabaja. Secondary malignant lesions of the heart and pericardium in neoplastic disease. *Oncology* 1986;43:103-6.
10. McAllister HA. *Tumors of the heart and pericardium.* 2nd edn. Churchill Livingstone, 1991, pp. 1297-1334.
11. Katalinic D, Stern-Padovan R, Ivanac I *et al.* Symptomatic cardiac metastases of breast cancer 27 years after mastectomy: a case report with literature review – pathophysiology of molecular mechanisms and metastatic pathways, clinical aspects, diagnostic procedures and treatment modalities. *World J Surg Oncol* 2013;11:1-10.
12. Faingold R, Babyn PS, Yoo SJ *et al.* Neuroblastoma with atypical metastases to cardiac and skeletal muscles: MRI features. *Pediatr Radiol* 2003;33(8):584-6. https://doi.org/10.1007/s00247-002-0858-5.

Appendix 18. **Sample haemodilution algorithm**

This sample haemodilution algorithm is based on the US Food and Drug Administration Guidance document cited below. Note: This guidance gives 'colloid' a different definition from that used in Europe; note particularly point 2 (2) below.

The following definitions, as used in this sample haemodilution algorithm, are adapted from the US FDA Guidance, Chapter V, p. 31.

1. Blood component means a product containing a part of human blood separated by physical or mechanical means (§1271.3i).
2. Colloid means: (1) a protein or polysaccharide solution, such as albumin, dextran or hetastarch, that can be used to increase or maintain osmotic (oncotic) pressure in the intravascular compartment; *or* (2) blood components such as plasma and platelets (§1271.3j).
3. Crystalloid means an isotonic salt and/or glucose solution used for electrolyte replacement or to increase intravascular volume, such as saline solution, Ringer's lactate solution, 5 % dextrose in water (§1271.3k), or total parenteral nutrition.
4. Plasma dilution means a decrease in the concentration of the donor's plasma proteins and circulating antigens or antibodies resulting from the transfusion of blood or blood components and/or infusion of fluids (§1271.3p).

DONOR ID # _

Date and Time of Specimen Collection _ _ _ _ _ _ _

Donor's weight in kg _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

A = Total volume of blood transfused in the 48 hours before death or sample collection, whichever comes first

B = Total volume of colloid infused in the 48 hours before death or sample collection, whichever comes first

C = Total volume of crystalloid infused in the 1 hour before death or sample collection, whichever comes first

BV = donor's blood volume

Calculated blood volume = donor's weight (kg)/0.015 *or* donor's weight (kg) × 70 mL/kg

PV = donor's plasma volume

Calculated plasma volume = donor's weight (kg)/0.025 *or* donor's weight (kg) × 40 mL/kg

Calculate both:

1. Is B + C > PV?

2. Is A + B + C > BV?

[Enter a zero if a category (A, B, or C) was not transfused/infused.]

Determination of sample acceptability for infectious disease tests

- If the answers to both 1 and 2 are NO, the post-transfusion/infusion sample is acceptable.
- If the answer to either 1 or 2 is YES, the post-transfusion/infusion sample is not acceptable; use a pre-transfusion/infusion sample or reject the donor.

When calculating haemodilution of donors that have received plasma, the end result might differ according to the definition used:
For example: a donor 70 kg:
Donor plasma volume (PV): 2 800 mL
Donor blood volume (BV): 4 667 mL
Total volume of blood transfused (48 h): 600 mL = A
Total volume of plasma transfused (48h): 600 mL
Total volume of colloid transfused (48h): 1500 mL
Total volume of crystalloid infused (1h): 1000 mL =C

FDA guidance

1. (600 + 1 500) + 1 000 mL = 3 100 mL > PV
2. 600 + (600 + 1 500) + 1 000 mL = 3 700 mL < BV

European interpretation

1. 1 500 + 1 000 mL = 2 500 mL < PV
2. (600 + 600) + 1 500 + 1 000 mL = 3 700 mL < BV

Source: US Food and Drug Administration. Guidance for industry: eligibility determination for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps), August 2007, Appendix 2 at www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/tissue/ucm091345.pdf, accessed 6 April 2022.

Appendix 19. Example of validation of screening: infectious disease assays of blood from deceased donors

The reliability of the results of screening for infectious diseases in blood samples from deceased individuals is critical and can be enhanced substantially if appropriately validated assays are used. Assays for infectious diseases specifically labelled for use in screening blood donors are considered suitable for use in screening living donors of tissues and/or cells. However, few of these assays have been validated specifically for use with blood collected after the donor's heart has stopped beating. If an assay's manufacturer has not validated the assay for use with blood samples from deceased donors, no claims are made in regard to the performance or reliability of the test results generated with such samples. Consequently, screening laboratories are expected to specifically validate these assays with such blood samples to support performance of deceased donor screening in their laboratory.

Before any validation work, potential issues associated with screening blood from deceased individuals must be understood. There are three key issues:

a. occurrence of *post mortem* degradation, or fall in detectable level, of a screening target (a marker of infectious disease such as an antigen or antibody related to the infectious agent);

b. inhibition of the assay by substances accumulating from *post mortem* changes in blood;

c. the potential for a blood sample from a deceased donor to be haemodiluted.

In all three cases there is a possibility for a false-negative test result. Although sensitivity and specificity are important for any screening assay, whether samples from living or deceased individuals are tested, sensitivity is the more important of the two because a false-negative result is the major threat to the safety of the tissue or cell transplant. In general, specificity is not as important because algorithms can be employed to effectively discriminate between non-specific and specific reactivities in screening assays.

To validate assay performance when using blood samples from deceased donors, the following recommendations apply:

a. the collection times for blood samples from deceased donors used for assay validation must be representative of the full range of time points typically encountered during tissue procurement, specifically from immediately after death up to 24 h after death (see §6.3.1.1);

b. all information about storage and handling conditions for blood tubes from time of blood collection to time of testing must be documented and meet any assay sample-handling requirements stated;

c. each blood sample from a deceased donor used for validation must be evaluated for haemodilution using an approved algorithm;

d. use a dilution series prepared in deceased donor material; or use spiked specimens inoculated with the relevant infectious-disease marker at a potency near the assay's cutoff and vary sources used for spiking. In both cases, test in parallel with the same material diluted in serum or plasma from a living individual;

e. test a sufficient number of samples from different deceased donors (≥ 20);

f. include haemolysed samples;

g. the sample storage methods (e.g. refrigerated, frozen) used for validation should mimic the method of storage that is routine for that laboratory.

Assay evaluation is undertaken to determine the overall performance of an assay, specifically including its core sensitivity and specificity.

Validation is done to determine the suitability of an already evaluated assay for use for a specific purpose in a laboratory; for example, for use with a blood specimen type (in this case, from a deceased donor) not specifically stated as known to be acceptable by the manufacturer. Evaluations are undertaken using panels of provenanced samples of known status, and importantly include seroconversion panels (i.e. sets of sequential samples from infected individuals following the course of infection from pre-exposure to late infection). However, such samples are almost all obtained from living patients. Suitable comparable 'positive' specimens in a series from deceased individuals are not available, making this specific type of evaluation not possible. An alternative, less realistic approach but one that has been accepted by licensing authorities is to spike non-reactive blood specimens from deceased donors with known and varying levels of virus to attempt to mimic a 'true' positive specimen [1, 2, 3].

Nonetheless, before using assays with blood specimens from deceased individuals, the performance of the assays must be assessed in some way to make attempts to demonstrate there is no loss of, or other change in, the expected performance of the assay.

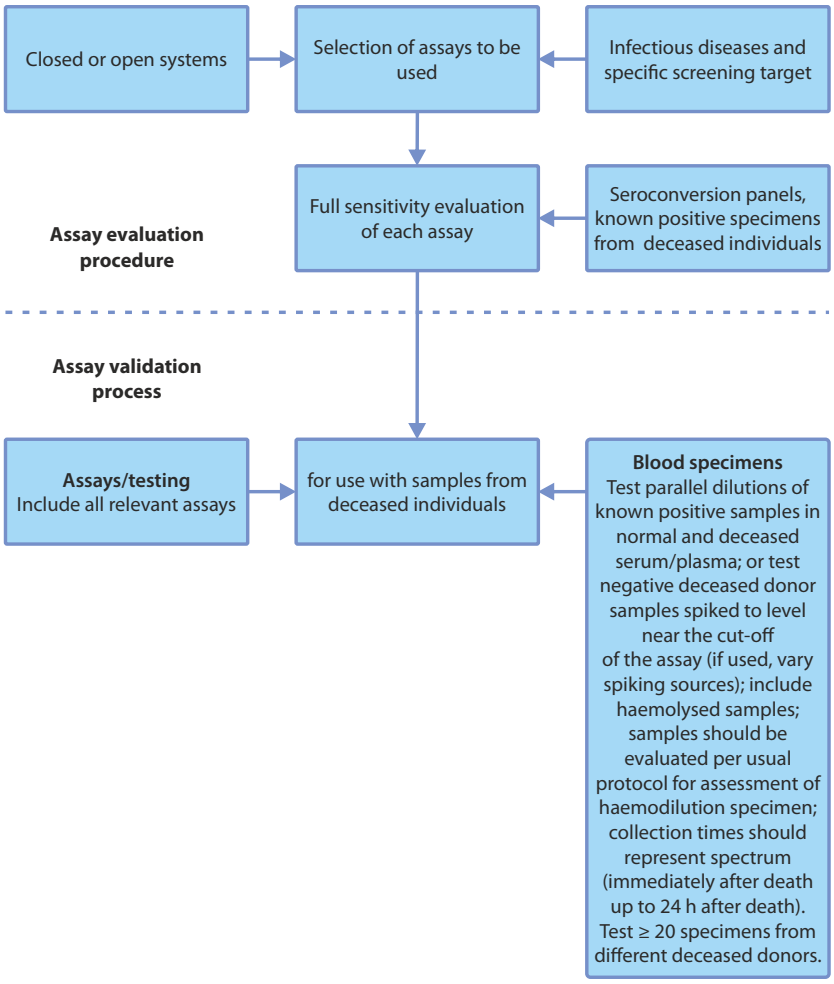
Validation of assays for use with blood from deceased individuals is critical, both for serological assays that detect antigens or antibodies and for molecular assays that detect viral nucleic acid. However, simultaneously, this is problematic due to the key issues stated above and a lack of suitable blood samples from deceased individuals to support such work. The issue of the degradation of any screening target that may be present is the hardest to deal with, due to lack of suitable published studies. However, if the timeframe for sample collection *post mortem* is limited by those carrying out tissue procurement, it can be theorised that it would be unlikely for any markers of a previously unidentified and relevant infection to have been degraded to a level that is undetectable using the high-quality assays available from major international diagnostics manufacturers.

If this aspect is controlled, the next issue to be considered is the potential 'inhibitory effect' of the sample as a result of any *post mortem* changes. To some degree the same argument applies in regard to degradation of the screening target *post mortem* because specimen collection within a suitable timeframe minimises the extent of any *post mortem* changes, whatever they are (e.g. red cell haemolysis, precipitants/byproducts of cell death, etc.). There are ways in which an inhibitory effect can be examined in serological and molecular assays. Known positive serum/plasma samples can be diluted in serum plasma from living individuals in parallel with serum/plasma from deceased individuals and from living individuals, either to a fixed point or to extinction, and the outcomes compared. In this aspect, validation of molecular assays is slightly easier than that of serological assays because the inclusion of an internal control in molecular assays validates each test result with respect to the presence of any 'inhibitory substance' that may be present in a sample. If the internal control is not amplified, the test result for the sample is deemed to be invalid. Therefore, although not definitive, such approaches would identify any gross effects, which would most likely result in false-negative results.

To meet donor/donation screening expectations, validation of infectious-disease assays for use with blood from deceased individuals must be undertaken. If the assays have been evaluated appropriately by the testing laboratory, then there is clear understanding of the baseline performance of each assay, and this can be used as the basis of additional assay validation work for use with blood samples from deceased donors. Lack of suitable samples to mirror evaluation panels is a particular problem, leaving comparative dilutional studies and/or spiked sample studies as feasible approaches to determine any problems associated with testing blood from deceased individuals.

Validation scheme

Flowchart courtesy of Alan Kitchen with input from Susan Best.



References

1. Edler C, Wulff B, Schroeder AS *et al.* A prospective time course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 hours after death. *J Med Microbiol* 2011;60:920-6.
2. Meyer T, Polywka S, Wulff B *et al.* Virus NAT for HIV, HBV, and HCV in post-mortal blood specimens over 48 h after death of infected patients – first results. *Transfus Med Hemother* 2012 Dec;39(6):376-80.
3. Commission Directive 2012/39/EU of 26 November 2012 amending Directive 2006/17/EC with regard to certain technical requirements for the testing of human tissues and cells, available at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012L0039, accessed 6 April 2022.

Appendix 20. *Treponema pallidum* testing

The algorithms that are recommended for the serological diagnosis of syphilis are challenging due to the inherent complexity of these methods. Thus, these tests are subdivided into treponemal and non-treponemal tests and the interpretation of the results obtained is often particularly difficult, hence the need for further confirmatory testing (see Table A20.1). See also the website of the European Centre for Disease Prevention and Control at www.ecdc.europa.eu.

Non-treponemal tests

Non-treponemal tests are tests that search for IgG and IgM directed against the lipids that are released from the damaged human cells during an early stage of the disease. The goal of these tests is therefore to search for antibodies to antigens that are not specific to an infection with species of the genus *Treponema*, as reflected in the term reaginic antibodies. The non-specific nature of this category of serological tests is also reflected in the fact that many other causes, such as advanced age, pregnancy, various types of malignant tumours, autoimmune diseases and other unrelated infections may result in the formation of anti-lipoid antibodies, thus generating false-positive results.

Consequently, a positive result obtained with a non-treponemal test should always be confirmed by means of a treponemal test. Moreover, non-treponemal tests usually display a low sensitivity in the detection of early syphilis, and the first positive results are not obtained until some 4-8 weeks after infection. The tests belonging to this category have mainly a diagnostic purpose as part of the therapeutic follow-up of patients with syphilis. Thus, a declining titre over a certain period of time is indicative of a favourable response to treatment. As a rule, successful treatment leads to negative results for these tests. The Venereal Diseases Research Laboratory (VDRL) test and Rapid Plasma Reagin (RPR) test belong to this group of non-treponemal tests used for serological syphilis screening.

Treponemal tests

Treponemal tests are serological screening tests that search for specific antibodies directed against species of the genus *Treponema*. No distinction can be made between the different treponematoses due to immunological cross-reactions. These tests usually remain positive after the initial infection, which means that they cannot be used to monitor the response to treatment or diagnose reinfections. Treponemal serological tests include the *T. pallidum* haemagglutination (TPHA) test, the *T. pallidum* particle agglutination (TPPA) test, treponemal enzyme immunoassays (EIA), chemiluminescence immunoassays (CLIA) and immunoblotting.

Algorithms in use

New developments, especially in the optimisation of treponemal immunoassays, offer new possibilities due to the earlier detection of syphilis and the shorter diagnostic window, but do not necessarily simplify the assessment of the overall serological picture. According to recent international recommendations the following screening algorithms can be used for serological syphilis screening.

Only the treponemal screening test

This screening strategy is commonly used in European blood banks and laboratories due to its potential for large-scale automation. This algorithm identifies both individuals in whom syphilis has been treated successfully as well as those who have not received any treatment. It is better suited to detect the early stages of infection than the sole use of a non-treponemal test. Given the fact that this strategy is mainly used for populations with a low prevalence of syphilis, it suffers from a considerable number of false-positive results.

Only the non-treponemal screening test

Ideally, a non-treponemal test carried out for screening purposes should be quantitative in nature in order to rule out the prozone effect when using undiluted blood samples. (This concerns < 2 % of samples, usually during the secondary phase of syphilis. These patients display extremely high titres of antibodies that interfere with the formation of antigen–antibody complexes, which are necessary to visualise flocculation when interpreting the non-treponemal test.) This algorithm can only detect active (infectious) syphilis, which means that it can miss the early stage of syphilis.

Treponemal and non-treponemal tests

This algorithm is especially useful to screen high-risk populations as well as to screen for the early stages of syphilis.

In the serological diagnosis of syphilis and independently of the screening algorithm used, a confirmatory test will always need to be carried out, regardless of which of the screening tests turned out positive.

a. If the initial screening test only included a treponemal test, the results should be confirmed by means of a second treponemal test based on a different analytical method as well as a quantitative non-treponemal test if this second treponemal test also turns out positive.

b. If the initial screening test only included a non-treponemal test, the positive result needs to be confirmed by means of a treponemal test, whereas the non-treponemal test should be performed in a quantitative manner if this was not initially the case.

c. If the initial screening was performed using a treponemal test as well as a non-treponemal test, the non-treponemal test should be performed in a quantitative manner. A second treponemal test based on a different analytical method may be used to rule out a false-positive result for the initial treponemal test only if the non-treponemal test is negative.

Table A20.1. **Interpretation of the results of biological screening tests for syphilis**

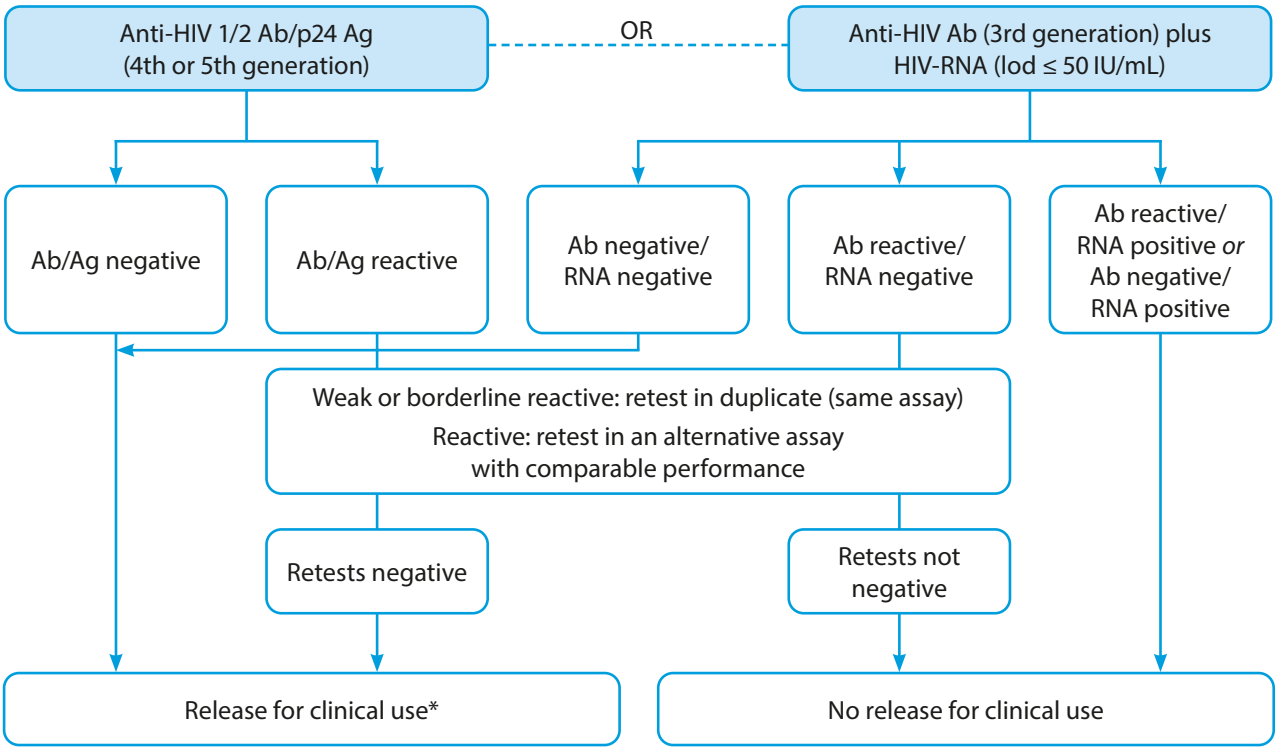
Treponemal test	Non-treponemal test	Interpretation	Consequence for the donation	Further management
positive	positive[1]	active infection	reject	contact the attending physician
positive	negative	treated (past) infection *or* early stage of infection *or* false-positive[2]	reject	contact the attending physician[2]
negative	not carried out or negative	no infection	release possible	no further action
negative	positive	false-positive result for the non-treponemal test *or* false-negative result for the treponemal test	release potentially possible[3]	no further action

1 Given the fact that in the vast majority of cases in which the non-treponemal test yielded false-positive results the titres were ≤ ¼, a 'positive treponemal test' is considered to be with a titre ≥ ⅛.

2 In such a case (positive treponemal, negative non-treponemal) a confirmatory treponemal test needs to be carried out. If this confirmatory treponemal test yields a negative result, the initially positive result of the treponemal test is not confirmed and therefore looked upon as false-positive, which justifies releasing of tissues and cells intended for donation and requires no contact with the attending physician concerned.

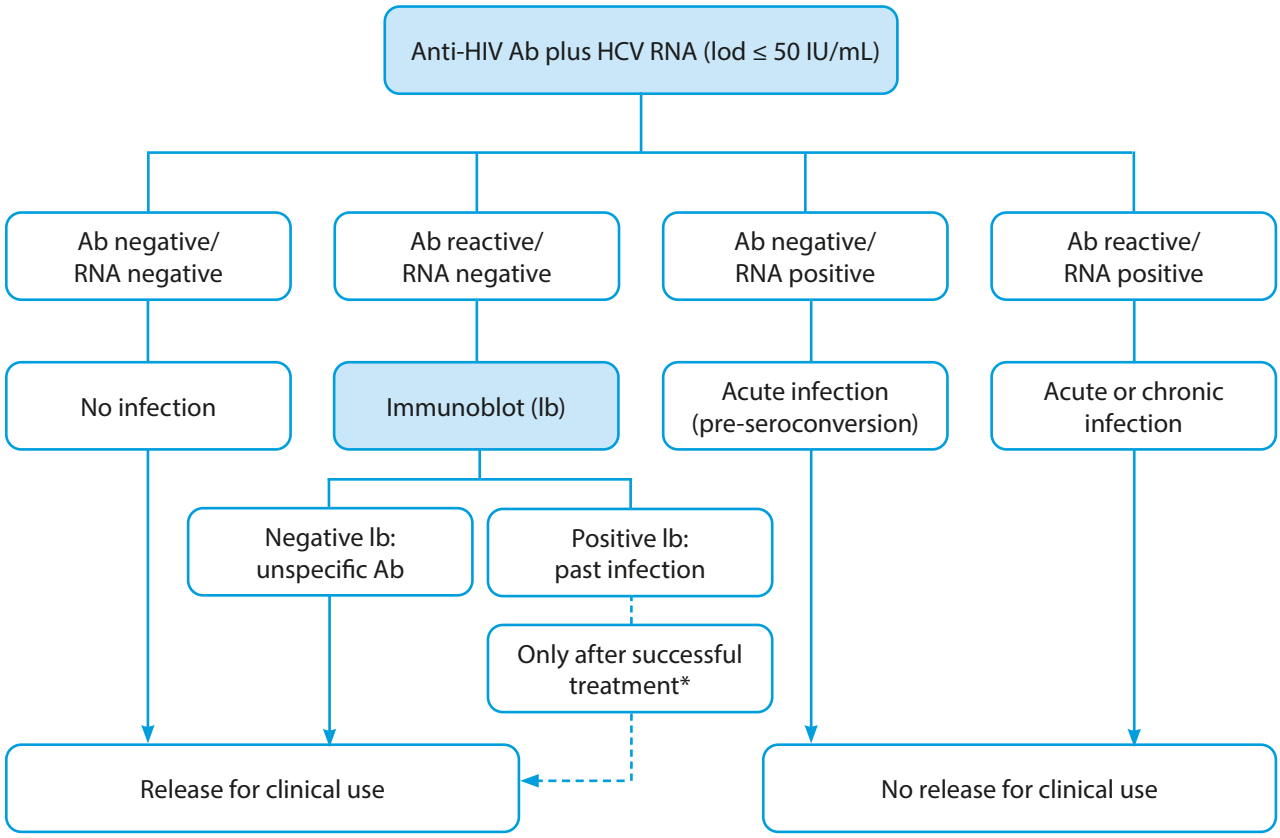
3 The TE administrator can still accept the tissues and cells after having consulted the clinical biologist, possibly after carrying out additional tests, and having received the informed consent of the recipient and the medical transplant team.

Appendix 21. **Algorithm for HIV testing**



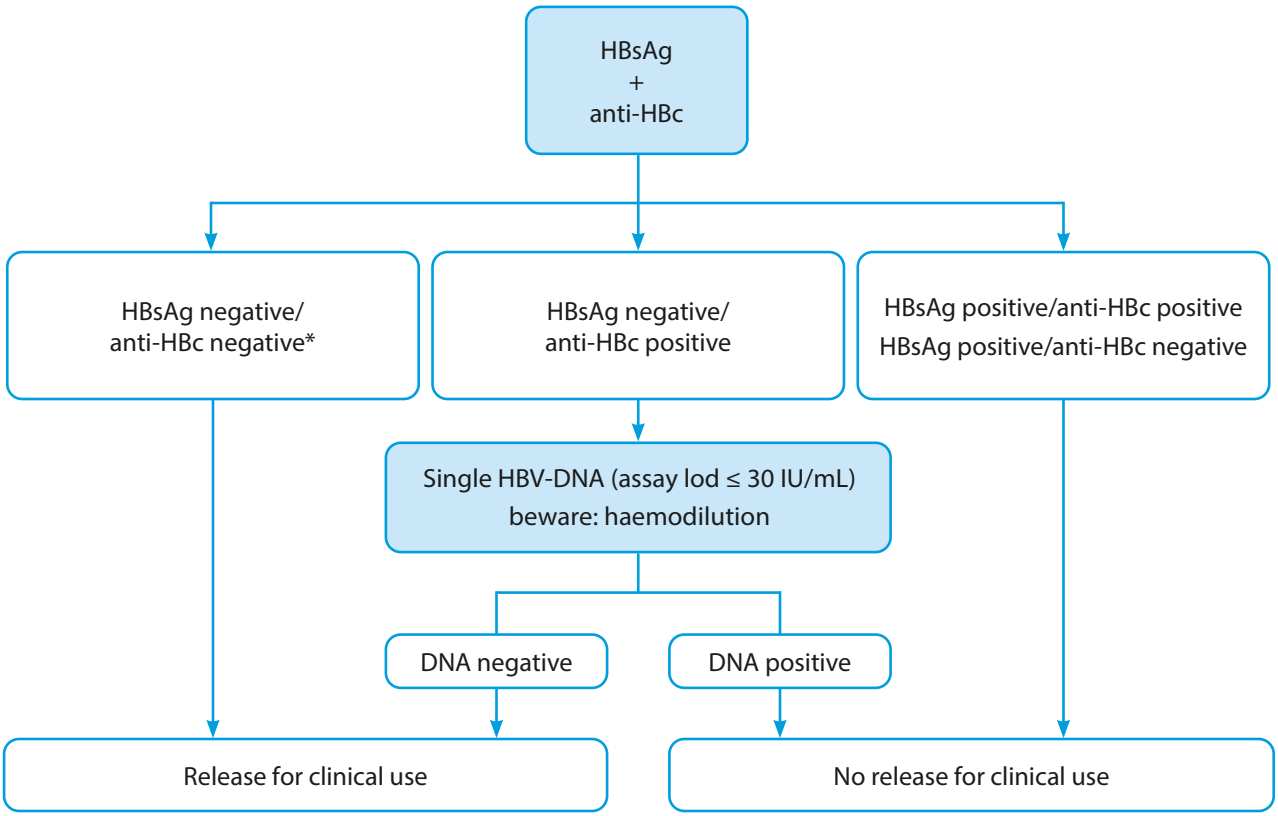
* Please note that when HIV NAT is done as mandatory test, it must also be negative in anti-HIV/p24Ag negative donors, to release tissues for clinical use.

Appendix 22. **Algorithm for HCV testing**



* Record of two negative HCV RNA results six months apart as evidence for a finished successful HCV treatment under medical supervision.

Appendix 23. **Algorithm for HBV testing**



* Please note that when HBV NAT is done as mandatory test, it must also be negative in HBsAg/anti-HBc negative donors, to release tissues for clinical use.

Appendix 24. **Sample form to assess working environment (NHS, UK)**

(extracted from NHS FRM3831/5.1) Effective: 16/12/13

Tissue Services Tissue Donation From Deceased Donors

Venue:	Donation Number and Donor Number:

The intent of the below is to assess the suitability of the working environment for safety to both staff and tissue.

Minimum Requirements:

	Yes	No	Comments
Are there a minimum 2 authorised people present?			
Is there good lighting and a sink with running water available?			
Do you know the evacuation procedure?			
Is there suitable access to minimise carrying and handling e.g. parking.			
Is the support equipment working and used where applicable? e.g. trolleys, control panels, 'in use' signs etc.			
Can unauthorised people view the donation? e.g. doors open, blinds open, clear glassed windows			
Is there unauthorised filming / photography equipment in use?			

Site Assessment:

	Yes	No	Comments / Action
Are the floors wet?			
Do any surfaces appear dirty / contaminated?			
Are there any sharp objects/dangerous equipment/clutter around i.e. hazards to avoid/move?			
Is the donation area a clean environment (if necessary clean with detergent prior to use)?			
Is a post mortem being carried out at the same time as the donation?			
Do you believe that tissue can be retrieved with minimal or no environmental contamination?			
Are there specific donor related risks and actions taken to mitigate these risks e.g. large donor?			

	Yes	No	Comments / Action
Was the Donors face protected prior to moving to donation area?			If No, explain.
Person Responsible for moving donor to / from donation area:	Delete as applicable: **Porters / APTs / TS Staff / Others** Others please specify:		

Authorisation to proceed:

Are you satisfied that this is a safe / clean working environment? Yes / No If No, contact the Duty Manager ASAP Name of Manager contacted:

(Template Version 07/10/08)

Cross-Referenced in Primary Document: MPD557

Source: National Health Service (NHS), United Kingdom.

Appendix 25. **Sample donor-identification form (NHS, UK)**

(extracted from NHS FORM FRM3831/5.1) Effective: 16/12/13

Tissue Services Tissue Donation From Deceased Donors

Donation Number:		
Confirm correct donor by transcribing identification details directly from identity band/label. You **must** have 3 points of matched ID (e.g. name, dob, hospital number, address, or (exceptionally) circumstances of death). Method of ID: Toe tag/Identity band/Mortuary label/Other……  **COPY TAG**		
Identification and Examination performed by (PRINT NAME)	Sign:	Date:
Identification Double checked by (PRINT NAME)	Sign:	Date:

Check Authorisation. Do you have 3 points of matched ID that correspond with the referral information and donor identification?			
Yes / No	Print name:	Sign:	Date:
If No, can you justify your rationale for proceeding with the donation below? **Yes / No / NA** **If No, contact Duty Manager ASAP** Name of Manager contacted:			

ADDITIONAL INFORMATION	SIGNATURE AND DATE

(Template Version 07/10/08)

Cross-Referenced in Primary Document: MPD557

Source: National Health Service, United Kingdom.

Appendix 26. **Checklist for revision of computerised systems**

Establishment . . . . . .

Date . . . . . .

Signature of person responsible for the revision . . . . . .

1. Identification of the system and function		Comments
Name of the system		
Version		
Supplier		
Platform		
Function		
Connected with other computerised systems		

2. Organisation	Comments
☐ Is the organisation for function and maintenance described in the QM system (system owner, system manager, person responsible)? ☐ Are these functions placed in an organisation scheme?	
☐ Is the responsibility of the supplier described in QM system and in written agreements? ☐ Is it clear that the user is responsible for validation when data are transferred between different systems?	
☐ Have the responsible persons (functions) received enough and documented training in case of malfunction of the system?	
☐ Is the computerised system included in the scheme for internal revision?	

3. Written agreements	Comments
☐ Are responsibilities for support (software and hardware) clearly defined? ☐ Are functions/responsibilities of subcontractors included?	
☐ Are instructions for documentation of unexpected events included? ☐ Is time-limit for corrective actions by responsible support defined?	
If data are transferred between different computerised systems: ☐ Are platforms and protocols described? ☐ Are obligations to inform each other about changes and events that may influence information transfer included? ☐ Are responsibilities for the different parts in the chain between the systems clearly defined?	

4. Documentation of the system	Comments
☐ Is complete and updated documentation of the system accessible? ☐ Does the documentation contain measures for managing malfunctions and fallbacks? Is a user guide with version number accessible: ☐ in paper copy? ☐ as electronic 'help-function'?	

5. Maintenance	Comments
☐ Are standard operating procedures available for measures in case of malfunction/total downtime? ☐ Back-up system? ☐ Reset of data? ☐ Are back-up system and read-back functions tested?	

6. Changes	Comments
☐ Is a test environment available? ☐ Are validation procedures defined and performed before updates, changes, new versions in the system?	

7. Information security	Comments
☐ Is access to the computers protected by locked doors (physical data protection)?	
☐ Is a virus protection system active (if applicable)?	
Is access to the computerised system protected by personal login? ☐ Single-level login ☐ Double-level login	
☐ Is access to the system (and login) associated with a certificate of authorisation? ☐ Who decides on, and keep records of, access to the system? ☐ Are records for access to the system updated (i.e. access removed when no longer needed)?	
☐ Does the system provide traceability of the user? ☐ Does the system provide traceability of changes in manually added data/text (with the original text still readable)?	
If data are manually inserted/transferred from another system: How is correctness of the data verified: ☐ by data insert of two individuals independently? ☐ by saving the original (paper) result? ☐ by signature(s) of the individual(s) inserting the data?	
If data are automatically transferred from another computerised system: ☐ Are 'check points' to verify the correctness of data transfer available in the system or as standard operating procedures?	

Adapted from SWEDAC DOC 10:5, 2010 Guidance for information security managers (available from www.isaca.org/Knowledge-Center/Research/ResearchDeliverables/Pages/Information-Security-Governance-Guidance-for-Information-Security-Managers.aspx) with interpretation of ISO/IEC 17025, 'General requirements for the competence of testing and calibration laboratories' (available from www.iso.org/iso/catalogue_detail?csnumber=39883) and ISO/IEC 27007:2011 (available from www.iso.org/obp/ui/#iso:std:iso-iec:27007:ed-1:v1:en).

Appendix 27. Serious adverse reaction or event: impact assessment form

This impact assessment tool assists practitioners and regulators in planning their response to a given serious adverse reaction or event (SARE), taking into account the broader consequences beyond the individual patient affected or potentially affected. The assessment should be based on available data, past experience and scientific expertise.

Although the assessment is common to all types of tissues and cells, it can be divided into non-reproductive and reproductive.

Assessment for non-reproductive tissues and cells

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On tissue/cell supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

Step 3: Application of the impact matrix

Likelihood of recurrence → / Impact of recurrence ↓	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/ almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Step 4

The response of a tissue establishment or health authority to a specific SARE should be proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White The tissue establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale shading Requires interaction between the tissue establishment and the Health Authority, which may request an inspection that focuses on the SARE and the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark shading The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication should be done as at the previous level; and possibly notification of health authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it should recur.

Source: Adapted from SoHO V&S Guidance for Competent Authorities.

Assessment for reproductive tissues and cells

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On tissue/cell supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/embryos for all couples

Step 3: Application of the impact matrix

Likelihood of recurrence → / Impact of recurrence ↓	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/ almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Step 4

The response of a tissue establishment or health authority to a specific SARE should be proportionate to the potential impact, as assessed by the matrix shown in Step 3 above and described below.

White The tissue establishment is to manage the corrective and preventive actions; the Health Authority is to file the report and keep a 'watching brief'.

Pale shading Requires interaction between the tissue establishment and the Health Authority, which may request an inspection that focuses on the SARE and the corrective and preventive actions to be followed up, including evidence of effective recall, where necessary. Written communication to professionals working in the field might be appropriate.

Dark shading The Health Authority will, in general, designate representatives to participate in developing or approving the corrective and preventive action plan (possibly a task force to address broader implications). Inspection, follow-up and written communication should be done as at the previous level; and possibly notification of health authorities in other countries where relevant.

Effectiveness of the response can be assessed by re-applying the impact matrix following implementation of corrective and preventive actions. The impact can be reduced by:

- reducing the probability of recurrence through preventive measures;
- increasing the detectability of the risk; or
- reducing the severity of the consequences, if it should recur.

Source: Adapted from SoHO V&S Deliverables 5 and 8.

Appendix 28. Example of serious adverse reaction (SAR) for non-reproductive tissues and cells

Vigilance notification to the tissue establishment (TE)

An orthopaedic surgeon calls the TE because the recipient of a peroneal longus tendon has an infection in the surgical site. The culture of the surgical site is positive for *Staphilococcus epidermidis* sensible to oxacilin, ceftaroline, amikacyn, clindamicyn, rifampicyn, quinupristin-Dalfoprostin. The orthopaedic surgeon suspects that the infection originates from the graft because the recipient had no previous infection or risk factors to develop an infection.

The recipient is receiving antibiotic treatment: theicoplanin 800 mg 1c/24 h and ceftazidime 2gc/8 h.

After 3 days the infection required wound debridement and graft removal.

The TE sends the notification of the adverse reaction to the biovigilance officer and starts the investigation as a potential SAR.

History of donor

A 55-year-old tissue donor. Admitted at the hospital due to myocardial infarction and died of cardiac arrest 48 hours after admission. Medical antecedents: high blood pressure and smoker. Family accepted tissue donation (corneas, musculoskeletal and skin). Procurement took place 8 hours after asystole.

Immediate actions and investigation

Immediately after receiving the notification, the TE puts into quarantine all the tissues still available in the tissue bank from the same donor.

The TE investigation/review

1. Assessment of information about the donor and procurement.

- The medical records of the donor: two blood cultures obtained at the procurement time negative.
- Musculoskeletal procurement: 12 cultures were negative.
- Skin procurement: 2 positive cultures (non-pathogenic sp.) out of 6 at the time of procurement.

- Microbiological and quality control of the clean room during the processing of the musculoskeletal tissue: no deviation found.
- Swab culture of the graft at the moment of implant performed by the orthopaedic surgeon in the hospital laboratory: negative.

2. Assessment of the outcome of other transplantations with tissues from the same donor.

- Check the clinical outcome of the transplantation procedure in other recipients that received tissues from the same donor (6 tissues and 5 recipients):

– two cornea recipients: no incident, in particular no infectious complications;
– two tendons to one recipient: tissue culture negative at the moment of transplant and the recipient without signs of infection;
– two femoral heads to two recipients: tissue cultures negative at the moment of transplant and both recipients without signs of infection.

Conclusions

After investigation the case is considered a serious adverse reaction (SAR). The rationale used for the analysis of this SAR has been performed based on the information provided in section 17.4.3 and Appendix 27:

- Imputability: was judged as 1 (possible) because the evidence is indeterminate. There are no clear alternative causes (0. Excluded) but neither is there evidence in favour of attributing the reaction to the tissue (2. Probable); see Table 17.2.
- The probability of the likelihood of occurrence/recurrence was judged as 3 (possible), i.e. may occur occasionally.

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

The impact or the consequence was judged as follows:

- On the individual level the impact was 3 (major) for the recipient/individual (the graft had to be removed).
- On the system level the impact was 1 (minor), based on the follow-up of other recipients of a graft from this donor.
- On tissues and cell supply level, the impact was 2 (moderate) because this event had a negative impact on tissues availability and assignment because the tissues of this donor were in quarantine for a period of time while investigation was ongoing.

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

Using the impact matrix gave a total score of 6, resulting in classification of this reaction in the pale shading on the table. This meant that the TE and Health Authority had to interact and analyse

potential corrective and preventive measures. Also required was a written communication of the investigation to the orthopaedic surgeon who instigated the notification (please see Appendix 27 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence → **Impact of recurrence ↓**	**1 Rare**	**2 Unlikely**	**3 Possible**	**4 Likely**	**5 Certain/ almost certain**
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive measures and corrective measures implemented

- Initially tissues were quarantined as a preventive measure.
- Once investigation was finished, the TE decided to unblock the tissue still in the TE.
- The TE will reinforce the need to notify any incident or reaction among final users and authorised centres.
- The TE has not been able to establish a corrective measure because there were no findings of protocol deviations or uncontrolled risk during the donation process.

Appendix 29. Example of serious adverse reaction (SAR) for reproductive tissues and cells

Vigilance notification to the tissue establishment (TE)

A few months after birth, Leigh Syndrome (autosomal recessive/Ndufv1 mutation) was detected in a baby born following a double non-partner gamete donation with ICSI.

The paediatrician who diagnosed the genetic abnormality considered that it might be linked to the donors. Reporting this case might prevent further distribution of gametes/embryos from that donor and the transmission of this genetic disease to other potential children born by using the gametes. The investigation started and the case was also communicated to the Health Authority.

History of donor

- The oocyte donor was 28 years of age. She had a normal karyotype and was asymptomatic. A total of five healthy babies were born from previous donations to other couples.
- The sperm donor was 27 years of age. He had a normal karyotype. A total of three healthy babies were born from his previous donations.

Investigation and immediate actions

It was the first time that this particular combination of oocyte donor and sperm donor was used.

The specific Ndufv1 gene mutation was not included in the expanded carrier genetic test at that time.

Analysis of the incidence of the SAR

The respective tissue banks were informed about the potential SAR, and advised to hold the remaining sperm cells and oocytes in quarantine pending investigation.

There was no remaining sperm nor oocytes stored, but there were three embryos that had been created with the donated gametes.

A repeated analysis of germline DNA of both donors for this specific gene mutation showed that both donors were carrier of the Ndufv1 gene mutation c.248 C> T, Ser 83 Leu.

Conclusions

After investigation and confirmation of the genetic link to the donated gametes, the case was considered a serious adverse reaction (SAR). The rationale used for the analysis of this SAR was based on the information provided in section 17.4.3 of Chapter 17 and Appendix 27:

- Imputability: Definitive/certain (3), as the expanded genetic test confirms that the transmission is linked to the donors, see Table 17.2.
- The probability of the likelihood of occurrence/recurrence was judged as 2 (unlikely).

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

- The impact of the consequences: a recent multicentre study on Leigh syndrome survival shows that the mean age before death occurs is 2.4 years, and only 20 % survived, with a maximum age of 20 years old. Thus, the impact was judged as 4 (catastrophic or extreme).

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/embryos for all couples

Note for the reader: This first approach was confirmed after the investigation as the child died at 2 years of age.

The impact matrix shows that a total score of 8 (2 × 4) locates this SAR in the pale shading of the table. This means that interaction between the TE and the Health Authority would be required (please see Appendix 27 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence → / **Impact of recurrence ↓**	**1 Rare**	**2 Unlikely**	**3 Possible**	**4 Likely**	**5 Certain/almost certain**
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive measures and corrective measures implemented

- All the donations were performed within the same tissue establishment, so there was no need to alert other centres involved.
- Despite very low incidence of this recessive genetic disorder, the tissue establishment decided to expand the carrier genetic screening tests to include Ndufv1 gene mutation.
- Donors were informed about the new gene carrier condition.
- These gamete donors would be eligible for donation only under the following premises:
  - Only one of the donors tested positive.
  - Both donors are positive; but, in that case, the already existing supernumerary cryopreserved embryos must be tested for Ndufv1 gene mutation using PGT-M technique.
- The case was closed.

Appendix 30. Example of serious adverse event (SAE) for non-reproductive tissues and cells

Vigilance notification

A surgical team asks for a left humerus for a planned surgery.

The tissue prescribed is sent to the hospital by the TE.

During the surgical operation, the surgeon discovers that the humerus received is a right humerus.

Immediate action

The incident was considered a potential SAE. The surgery was cancelled, the incident was seen during the preparation of the surgery room, before patient anaesthesia.

Investigation

First step: History of the organ/tissue removals

Organs and tissues were removed simultaneously from two donors in two different surgery rooms, in the same institution during the same night. Two nurses were in charge of these procurements.

- In the first surgery room, organs and tissues, including a right humerus, were removed (donor 1, nurse 1).
- In the second surgery room, organs and tissues, including a left tibia with extensor items, were removed (donor 2, nurse 2).

Second step: Context of the organ/tissue removals

The organ and tissue removals happened in a busy summer weekend during which 5 organ and tissue donors were managed in 4 days. During this period it was necessary to increase the duty staff.

Third step: Details of the organ/tissue removals management

Room 1: The organ and tissue removal process was finished before the removal process in room 2 was finished.

Nurse 1 (from room 1) offered to help her colleague nurse 2 (room 2) and performed the conditioning and packaging of all tissues from both room 1 (donor 1) and room 2 (donor 2).

Four containers were prepared following these two different donor retrievals:

- a container with the heart for valves collection from donor 1;
- a container with the heart for valves collection from donor 2;
- a container with corneas from donor 1 and corneas from donor 2;

- a container with the bones from donor 1 (right humerus) and from donor 2 (left tibia with extensor items) together.

(Not enough containers were available at that moment for multiple tissue removals.)

Only one file from each donor, including medical history, and clinical and biological parameters, was sent with all the containers. Normally, one file is sent with each container with tissues and organs retrieved from one donor. (The packaging of organs or tissue of different donors in one container is unusual.)

Moreover, a specific sheet explaining the details of the tissues removals was also sent with each tissue retrieved and put in the container with the tissues concerned. No sheet was not included in the various containers.

All tissues were labelled with the unique donor number for traceability. The laterality was also mentioned for the tibia, but the laterality of the humerus was not mentioned.

At the end of the tissue packaging steps, the nurses' services were finished and the nurses were replaced by a new one (nurse 3).

Fourth step: at the TE

A technician received the various containers, and mistakenly believed that both the left tibia and right humerus had come from the same donor (donor 2). As the laterality of the humerus was not mentioned, he called the duty nurse (nurse 3). She was busy on a new organs and tissues retrieval from another donor and she answered that the humerus was a left humerus.

The conversation was by phone, and no written document was sent to confirm this information.

Fifth step: discovery of the incident

Seven months later, a left humerus was requested from the TE by a surgeon for planned surgery.

In the surgery room, the surgeon noticed that the donor humerus sent by the TE was a right humerus instead of a left humerus (as was requested).

He contacted the TE, which subsequently contacted the procurement team, and confirmed that no humerus was retrieved from donor 2 and that a right humerus was retrieved from donor 1.

Following the confirmation of the incident, a biovigilance notification for an SAE was sent to the Health Authority (HA) by the TE.

Sixth step: analysis and conclusions

The incident is considered an SAE. The rationale used for the analysis of this SAE has been performed based on the information provided in section 17.5.3 and Appendix 27.

Root causes analysis

Immediate causes (human factors)

- work overload,
- tiredness,
- difference in practice for tissues packaging and labelling between the two nurses on the same organs and tissues procurement site,
- no adherence to protocol?

Root causes (organisational factors)

- absence of accurate standard operational procedures for simultaneous organ and tissue removals,
- no organisation defined when two nurses are together on organ and tissue removals and packaging/labelling;
- no common practices on the conditioning and labelling of tissues sent to the TE;
- not enough containers available for multiple tissue removals.

The probability or likelihood of occurrence/recurrence is considered as 3. Possibly, because corrective measures have been put in place. However, all human factors that led to this incident cannot be totally avoided, notably work overload and tiredness.

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

The worst impact consequences expected could be: 1. Minor damage because the surgery was postponed until a new adapted bone was provided by the tissues bank.

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		On individual(s)		On the system		On tissue/cell supply
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage	OR	Some applications postponed
2	Moderate	Serious	OR	Damage for short period	OR	Many cancellations or postponements
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair	OR	Significant cancellations – importation required
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild	OR	All allogeneic applications cancelled

The impact matrix gave a total score of 3 (3 × 1) locating the incident in the blank area.

This means that an interaction was not required between TE and the HA (please see Appendix 27 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence → / Impact of recurrence ↓	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/ almost certain
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive and corrective measures implemented

- Communication with all healthcare teams implicated in organs and tissues removals, about the new practices,
- Update of tissues removals procedures,
- Creation of labels for each tissue removed,
- Purchase of containers,
- Use of label sheets of different colour when tissues from different donors are simultaneously removed.

Appendix 31. Example of serious adverse event (SAE) for reproductive tissues and cells

Vigilance notification

At inspection on day 1 post-fertilisation, the staff of the laboratory noticed that all the oocytes of three women undergoing ART treatment on sequential days showed no signs of fertilisation. Hence, the full treatment cycle was lost because no embryos were obtained.

Immediate action

- The incident was considered a potential SAE and the laboratory informed the head practitioner, who in turn informed the patients about the case.
- The laboratory initiated an internal investigation to analyse this case and its potential causes and informed the Health Authority.

Investigation

- The laboratory investigated fertilisation rates of the oocytes that were ICSI-ed on the same microscope on the days before the incident. The percentage of fertilisation was within the normal range on the days before the incident. This is considered a look-back procedure to identify potential other patients that may have suffered from a possible technical failure of the heating device.
- They reviewed the temperature records of the heating device on the ICSI stations, and the recorded temperatures were as defined previously.
- The maintenance and verification records of the heating device were reviewed and showed no abnormalities.
- Because oocytes of different women were affected, the problem could potentially originate from a step common to all patients. One of the problems described widely in the literature is that fertilisation rate is affected by temperature.
- Although verification suggested that the heating device had worked correctly, the TE checked the heating device with a calibrated temperature probe to verify if there were discrepancies. The second device recorded on average a temperature that was 4 °C higher, implying that the temperature recorded by the initial heating device was not correct due to a malfunction of the temperature sensor.

Conclusions

The incident was considered a serious adverse event (SAE). The rationale used for the analysis of this SAE was based on the information provided in section 17.5.3 and Appendix 27:

- In the situation of an SAE, it is not possible to assess "imputability".
- The probability or likelihood of occurrence/recurrence: the decision is considered as 2 (unlikely).

Step 1: Assessment of the likelihood of occurrence/recurrence of the SARE

1	Rare	Difficult to believe it could happen again
2	Unlikely	Not expected to happen again
3	Possible	May occur occasionally
4	Likely	Expected to happen again, but not persistently
5	Probable	Expected to happen again on many occasions

- The worst impact consequences appeared on the system level, because the malfunction of one of the heating devices resulted in damage for a short period of time, which results in a score of 2 (moderate).

Step 2: Assessment of the impact/consequences of the SARE should it recur

Impact level		**On individual(s)**		**On the system**		**On tissue/cell supply**
0	Insignificant	Nil	OR	No effect	OR	Insignificant
1	Minor	Non-serious	OR	Minor damage or some procedures postponed	OR	Partial loss of gametes/embryos for one couple
2	Moderate	Serious	OR	Damage for short period. Many procedures cancelled or postponed	OR	Partial loss of gametes/embryos for one couple
3	Major	Life-threatening	OR	Major damage to system – significant delay to repair. Significant numbers of procedures cancelled	OR	Partial loss of gametes/embryos for one couple
4	Catastrophic or extreme	Death	OR	System destroyed – need to rebuild. All procedures cancelled	OR	Total loss of gametes/embryos for all couples

The impact matrix gave a total score of 4 (2 × 2), locating the incident in the pale shading area of the table.

This means that interaction between the tissue establishment and the Health Authority is required (please see Appendix 27 for more detailed information).

Step 3: Application of the impact matrix

Likelihood of recurrence → / **Impact of recurrence ↓**	**1 Rare**	**2 Unlikely**	**3 Possible**	**4 Likely**	**5 Certain/almost certain**
0 Insignificant	0	0	0	0	0
1 Minor	1	2	3	4	5
2 Moderate	2	4	6	8	10
3 Major	3	6	9	12	15
4 Catastrophic/extreme	4	8	12	16	20

Preventive and corrective measures implemented

- Update the information to the head practitioner with the results of the investigation.
- Take the heating device out of service.
- Replacement of faulty sensor and new implementation validation of heating device before taking it back into service.
- Safety check of the three similar heating devices that were in use in the lab (nothing abnormal detected).
- Update of the heating device maintenance SOP, increasing the frequency of quality control.
- Contact the manufacturer to inform them about the defect in the heating device.
- Send out warning about technical problem with heating device to the other IVF centres in the country through the national embryology society.

Appendix 32. **Serious adverse reaction: notification form – for ocular tissues (Agence de la Biomédecine, France)**

Notification form for SAR on ocular tissues

SEC | Recipient code | Date of birth *(dd/mm/yyyy)*

Date of transplant *(dd/mm/yyyy)* | Sex ☐ M ☐ F | Eye involved ☐ Right ☐ Left

Type of graft ☐ DALK ☐ DSAEK ☐ DMEK ☐ PK

SAR

1. Short term (pre-graft until 1 month post-graft)

- ☐ Primary graft failure (endothelial decompensation)
- ☐ Ocular infection (from bacterial, fungal, parasitic or viral origin including endophthalmitis)
- ☐ Irreversible rejection (specific immunologic response)
- ☐ Systemic infection (compatible with a donor-recipient transmission)
- ☐ Persistent ulceration or corneal perforation

2. Mid- to long term	1 month to 1 year	> 1 year
☐ Any ocular pathology that could suggest transmission from the donor (for example: tumoral pathologies of the anterior segment)	☐	☐
☐ Any systemic pathology that could suggest transmission from the donor (transmissible viral diseases (rabies, hepatitis, etc.), malignant diseases, prion disease …)	☐	☐
☐ Unrecognised donor corneal diseases (history of refractive surgery, corneal dystrophy including FUCHS, keratoconus, scars …)	☐	☐
☐ Endothelial cell decompensation including cornea guttata	☐	☐
☐ Chronic endophthalmitis	☐	☐
☐ Late-onset local infection including bacterial, fungal, viral, or parasitic keratitis or kerato-endotheliitis	☐	☐

☐ Failure (leading to re-graft). Specify		☐
☐ Persistence of complicated epithelial defects (epithelial ulcerations) (visual decline, infection, rejection, stromal ulceration, perforation …) or development of epithelial tumour, dysplasia or epithelial hyperplasia	☐	☐
☐ Defect of corneal transparency (corneal opacification, calcifications, corneal transparency delay, inflammatory infiltrate …)	☐	☐
☐ Astigmatism induced by the graft or giant astigmatism (> 8 D) linked or likely to be linked to recurrence or appearance of keratoconus on the graft	☐	☐
☐ Loss of eyeball (anatomical or functional)	☐	☐
☐ Death of patient linked to the ocular graft	☐	☐

Risk factors

☐ No risk factor identified

Risk factor identified (tick the one concerned)

☐ Neovascularisation – History of autoimmune disease (scarring pemphigoid)	☐ Neurotrophic history	☐ Atopic ground
☐ Graft size outside the range of 8.5 diameter	☐ Palpebral alterations (statics/kinetics)	☐ Paediatric patient or less than 16 years old
☐ Hypertonia/glaucoma	☐ Emergency cornea grafting or therapeutic keratoplasty	☐ Re-graft – how many? ☐
☐ Ocular inflammation/uveitis	☐ History of rejection on either ipsi or contralateral eye	☐ Herpes virus infection (HSV, VZV)
		☐ Dry eye syndrome
		☐ Chemical burn

☐ Other – give details

Appendix 33. **Serious adverse reaction or event: notification form – for ocular tissues (NHS, UK)**

FRM4159/4.2 – Ocular Tissue Transplantation - Serious Adverse Reactions/Events

NHS Blood and Transplant

Effective date: 30/04/2020

Part A – NOTIFICATION OF ADVERSE REACTIONS AND EVENTS

(See reverse side of this page for important additional information)

Please complete this Part A form to notify NHSBT of an Adverse Reaction/Event:

- Contact Tissue and Eye Services Customer Care on Tel: 0845 607 6820
- Send one copy of this form to Tissue and Eye Services Customer Care either by e-mail to tscustserv@nhsbt.nhs.uk, or by post to Tissue and Eye Services Customer Care Dept., NHSBT, 14 Estuary Banks, Speke, Liverpool L248RB;
- Retain original in the patient's notes
- **Please return this form as soon as possible, but within 24 hours of the incident being identified or confirmed.**

TISSUE REFERENCE No: (eg G170...)……**LEFT / RIGHT**

DATE OF TRANSPLANT: ……..**RECIPIENT HOSPITAL:**……..

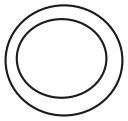
DATE OF EVENT: ……**CONSULTANT** (please print)**:**……..

DATE OF REPORTING:……**CONTACT DETAILS: Email**……..**Tel:**……..

TRANSPLANT TYPE (please tick): □ PK □ DALK □ SALK □ DMEK □ DS(A)EK □ KLAL □ Other
INDICATION/URGENCY OF TRANSPLANT (please tick): □ Urgent □ Elective

EYE BANK SUPPLYING THE TISSUE please tick)
□ NHSBT Filton □ NHSBT TES □ Moorfields □ East Grinstead

TYPE OF TISSUE OR CELLS (please tick)
□ Cornea (Whole) □ Cornea (Pre-Cut) □ Sclera □ Cornea in Ethanol □ Limbal
□ Other - please specify……

ADVERSE EVENT		ADVERSE REACTION	
Wrong material supplied	**YES / NO**	**Primary graft failure (corneal transplant never cleared)**	**YES / NO**
Tissue supplied is out of date	**YES / NO**	**Endophthalmitis or other serious ophthalmic infection**	**YES / NO**
Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery. (Please indicate on Diagram)	**YES / NO** 	**Graft failure due to donor tissue which was out of date, scarred or had evidence of previous surgery.**	**YES / NO**
Other, please Specify:		**Malignancy likely to be attributable to the transplanted tissue**	**YES / NO**
Had the patient already been anaesthetised?	**YES / NO**	**Systemic infection possibly attributable to the transplanted tissue**	**YES / NO**
Any other adverse impact on the patient (If yes please specify)	**YES / NO**	**Other, please specify (e.g. surgical complications):**	
Was the Tissue Used	**YES / NO**		

PLEASE CONTACT TES CUSTOMER CARE TO ARRANGE FOR RETURN OF ALL UNUSED TISSUE IN THE ORIGINAL CONTAINER TO THE ISSUING EYE BANK FOR INVESTIGATION.

FRM4159/4.2 – Ocular Tissue Transplantation - Serious Adverse Reactions/Events

NHS Blood and Transplant

Effective date: 30/04/2020

Ocular Tissue Transplantation - Adverse Reactions/Events

NHSBT SERIOUS ADVERSE REACTIONS/EVENTS REPORTING MECHANISM
Part A (i.e., this form) – To be completed by the person notifying NHSBT of an Adverse Reaction/Event
Serious Adverse Reactions/Events will be reviewed by NHSBT and OTAG[1]

DEFINITIONS[2]
'Serious adverse reaction'– means an unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening,
disabling, incapacitating or which results in, or prolongs, hospitalisation or morbidity

'Serious adverse event'– means any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalisation or morbidity.

Serious Adverse Reactions
Corneal Transplantation
1. Primary graft failure (graft never cleared)
2. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue[3]
3. Graft failure due to donor tissue which was out of date, damaged, scarred or had evidence of previous surgery
4. Malignancy likely to be attributable to the transplanted tissue
5. Systemic infection likely to be attributable to the transplant tissue
6. Other[4]

Ocular Tissue Stem Cell Graft
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue/cells[3]
2. Graft failure due to donor tissue/cells that were out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue/cells
4. Systemic infection likely to be attributable to the transplanted tissue/cells
5. Other[4]

Scleral Grafts
1. Endophthalmitis or other serious ophthalmic infection likely to be attributable to the transplanted tissue
2. Graft failure due to donor tissue which was out of date or damaged
3. Malignancy likely to be attributable to the transplanted tissue
4. Systemic infection likely to be attributable to the transplanted tissue
5. Other

Serious Adverse Events
There are potentially numerous serious adverse events. Specific serious adverse events that are peculiar to ophthalmology include
1. Wrong material supplied
2. Tissue supplied is out of date
3. Tissue supplied is damaged, scarred or has evidence of previous donor eye surgery
4. Other[5]

Notes:
1. OTAG Adverse Reactions and Events Reporting subgroup
2. EU Tissues and Cells Directive 2004/23/EC
3. This relates to the development of a severe infection likely to be attributable to the transplanted tissue. It does not include for example the occurrence of a microbial keratitis related to a suture abscess.
4. Other refers to any unexpected adverse reaction which is considered by the surgeon to be serious and possibly attributable to the transplanted tissue
5. Other refers to any adverse event which is considered to be serious and could potentially lead to a serious adverse reaction if the tissue is used in a patient.

Source: National Health Service, United Kingdom.

Appendix 34. **Decellularisation**

Several procedures can be applied to decellularise human material for clinical application, but the chosen method should take into account the final specific clinical use of the scaffold and the specific properties needed for this application, since the preserved properties may differ, depending on the method used [1, 2]. Clinical-grade extra-cellular matrix (ECM) may be used to preserve the intrinsic structural and biological properties of the substances of human origin (SoHO) while removing cells, cellular debris and alloantigen (to minimise immunogenicity). It is also important to achieve the elimination of toxins. However, it is clear that any decellularisation process brings some ECM denaturation.

Decellularisation agents

As ECM properties vary between tissues and organs, the main properties to safeguard must be clearly identified and determined in order to choose the correct decellularisation protocol. There are three general methods used to decellularise tissues or organs: physical, biological (enzymatic) and chemical [3, 4]. Each of these methods has a different mode of action and effects on the ECM [1, 5] (see Table A34.1), which can be critical for the functionality of the final decellularised ECM. These methods are usually combined to ensure complete decellularisation and can be also combined with different techniques to improve their effectiveness (see Table A34.2).

Aside from the selected decellularisation agents, the efficiency of tissues or organs decellularisation will depend on the intrinsic tissue properties, such as the specific cell density, thickness, compaction and lipid content. The maintenance of each scaffold's mechanical properties is directly related to the maintenance of one or more ECM components. For example, structural 3D maintenance is related to structural molecules such as collagens, elasticity depends on elastic fibres [6-9] and tensile strength is associated with structural proteins and chondroitin sulphate, while the ability to resist compressions is also associated with structural proteins conformation and hyaluronic acid [10].

Table A34.1. **Techniques used to apply decellularisation agents**

Technique	Advantages	Disadvantages
Perfusion	Facilitates chemical exposure and removal of cellular material	Pressure associated with perfusion can disrupt ECM
Pressure gradient across tissue	Facilitates chemical exposure and removal of cellular material	Pressure gradient can disrupt ECM
Supercritical fluid	Pressure can burst cells – facilitates chemical exposure and removal of cellular material	Pressure necessary for supercritical phase can disrupt ECM
Agitation	Can lyse cells – facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM

Table A34.2. **Modes of action and the effect of different decellularisation agents**

Physical			
Method		***Mode of action***	***Effects on ECM***
Snap freezing		Intracellular ice crystals disrupt cell membranes	Rapid freezing can disrupt or fracture ECM
Mechanical force		Pressure can burst cells, and tissue removal eliminates cells	Mechanical force can damage the ECM
Mechanical agitation		Cell membrane lysis; facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Non-thermal irreversible electroporation		Selective damage on cell membrane while sparing the other tissue components.	ECM preservation and produces a functional recellularisable scaffold.
Biological (enzymatic)			
Method		***Mode of action***	***Effects on ECM***
Trypsin		Cleaves peptide bonds on the C-side of Arg and Lys	Prolonged exposure can disrupt ECM structure; removes laminin, fibronectin, elastin and glycosaminoglycans (GAG)
Endonucleases		Catalyse the hydrolysis of the interior bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Exonucleases		Catalyse the hydrolysis of the terminal bonds of ribonucleotide and deoxyribonucleotide chains	Difficult to remove from the tissue and could invoke an immune response
Chemical			
Method		***Mode of action***	***Effects on ECM***
Alkaline/acid		Solubilises cytoplasmic components of cells; disrupts nucleic acids	Removes GAG
Hypo/hyperosmotic solutions		Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove cellular remnants
Alcohols		Dehydrate and lyse cells. Lipid removal	Fixative properties, protein precipitation and ECM ultrastructure damage.
Other solvents (acetone)		Lipid removal	Fixative properties and ECM ultrastructure damage
EDTA/EGTA		Chelating agents that bind divalent metallic ions, thereby disrupting cell adhesion to ECM	Typically used with enzymatic methods
Non-ionic detergents	Triton X-100	Disrupt lipid–lipid and lipid–protein interactions, while leaving protein–protein interactions intact	Mixed results; efficiency dependent on tissues; removes GAG
Ionic detergents	Sodium dodecyl sulfate (SDS)	Solubilise cytoplasmic and nuclear cellular membranes; tend to denature proteins	Removes nuclear remnants and cytoplasmic proteins; tends to disrupt native tissue structure, remove GAG and damage collagen
	Sodium deoxycolate		More disruptive to tissue structure than SDS and GAG removal
	Triton X-200		Achieves efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS)	Exhibit properties of non-ionic and ionic detergents	Efficient cell removal with ECM disruption similar to that of Triton X-100
	Sulfobetaine-10 and -16		Achieves cell removal and mild ECM disruption with Triton X-200
	Tri(*n*-butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties is minimal

Physical methods (such as agitation, pressure, freeze/thaw steps, sonication etc.) can be applied but have limited efficacy and should be carefully evaluated to assess any possible damage to the ECM.

Usually, they are combined with chemical and enzymatic methods. For the maintenance of ECM structure, ionic detergents could be the optimal choice, and enzymatic or alkaline/acid methods should be avoided, because the damage to the collagen can be limited with time and temperature using an ionic detergent; but the disruption provoked by enzymatic or alkaline/acid methods is highly aggressive. This consideration is valid for the preservation of any protein.

On the other hand, osmotic buffers are a milder method to obtain decellularised ECM but they are slower and cannot penetrate into thicker, compact organs. For dense tissues or intact organs, detergents can help buffers to penetrate, but they will affect the protein ultra-structure due to disruption of protein–protein interactions. Furthermore, any residual detergents can have cytotoxic effects and it is imperative to assure their elimination.

With the aim of removing specific proteins, enzymatic treatment should be used. However, it may cause the unspecific digestion of desired constituents and may not be sufficient for complete decellularisation of the whole tissue. Alcohols and other solvents can remove lipids very efficiently, but in the process they also crosslink proteins and modify the ECM ultrastructure. A compromise between lipid removal and crosslinking should be achieved to decide time and temperature of the treatment with these solvents.

In conclusion, specific combinations of mild physical, biological and chemical methods, along with the type of administration, should be tested, validated and controlled to obtain the best results for each tissue or organ with the aim to obtain a scaffold which presents the desired characteristics needed for a specific clinical application. Moreover, if the scaffold is a future starting material for a medicinal product, the manufacturer should refer to pharmaceutical guidelines for the development, validation and controls of its products.

References

1. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011;32(12):3233-43.
2. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*, General notices. 10th edition. Strasbourg, France: Council of Europe, 2022.
3. Gilpin A, Yang Y. Decellularization strategies for regenerative medicine: from processing techniques to applications, *Biomed Res Int* 2017, Article 9831534.
4. Badylak S, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds, *Annu Rev Biomed Eng* 2011;13:27-53.
5. Gilbert TW. Strategies for tissue and organ decellularization, *J Cell Biochem* 2012;113(7):2217-22.
6. Moroni F, Mirabella T. Decellularized matrices for cardiovascular tissue engineering. *Am J Stem Cells* 2014;3(1):1-20.
7. Tracy LE, Minasian RA, Caterson EJ. Extracellular matrix and dermal fibroblast function in the healing wound. *Adv Wound Care* 2016;5(3):119-36.
8. Hasan A, Ragaert K, Swieszkowski W *et al.* Biomechanical properties of native and tissue engineered heart valve constructs. *J Biomech* 2014;47(9):1949-63.
9. Montoya CV and McFetridge PS. Preparation of *ex vivo*-based biomaterials using convective flow decellularization, *Tissue Eng Part C. Methods* 2009;15(2):191-200.
10. Gavard Molliard S, Albert S, Mondon K. Key importance of compression properties in the biophysical characteristics of hyaluronic acid soft-tissue fillers, *J Mech Behav Biomed Mater* 2016;61:290-98.

Appendix 35. **Sample forms for the evaluation of heart valves**

Pulmonary Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number(if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph Available on request: Y / N

L R R L

Pathology noted on cusps:

Key (please annotate the diagram above)
Atheroma = …….
Fenestration = 000
Fibrosis = XXXX
Other:

Condition: Excellent / Good / Fair

Comments:

Pulmonary Inner Annular Diameter	mm	Left artery inner annular diameter	mm
Length of Vessel	mm	Left artery length	mm
Length of muscle skirt (Min / max)	mm	Right artery inner annular diameter	mm
Total Length	mm	Right artery length	mm

STERILITY REPORT	
Hepatitis B:	HCV PCR:
Hepatitis B Core Antibody:	HIV PCR:
Hepatitis C:	HBC PCR :
HIV I and II:	Microbiology Culture:
Syphillis:	Mycobacteria:
HTLV :	Other :

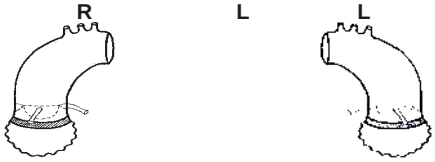
DONOR INFORMATION	
Age:	Sex:
Date of Death:	Cause of Death:

Information Entered By:	Date:	Signature:

Aortic Heart Valve Information

GRAFT INFORMATION	
Heart Valve Bank:	Donor Number:
ODT Number (if applicable):	Valve Number:
Date Dissected:	Date Cryopreserved:
Expiry Date of Valve:	Photograph available on request: Y / N

R L L R



Pathology noted on cusps:

NCC
LCC RCC

Aorta Inner Annular Diameter	mm
Length of Aorta	mm
Length of muscle skirt (Min / max)	mm
Total Length	mm

Key (annotate diagram above)	
Atheroma = ……	LCC = Left Coronary Cusp
Fenestration = 000	RCC = Right Coronary Cusp
Fibrosis = XXXX	NCC = Non Coronary Cusp
Other:	

Condition: Excellent / Good / Fair

Comments:

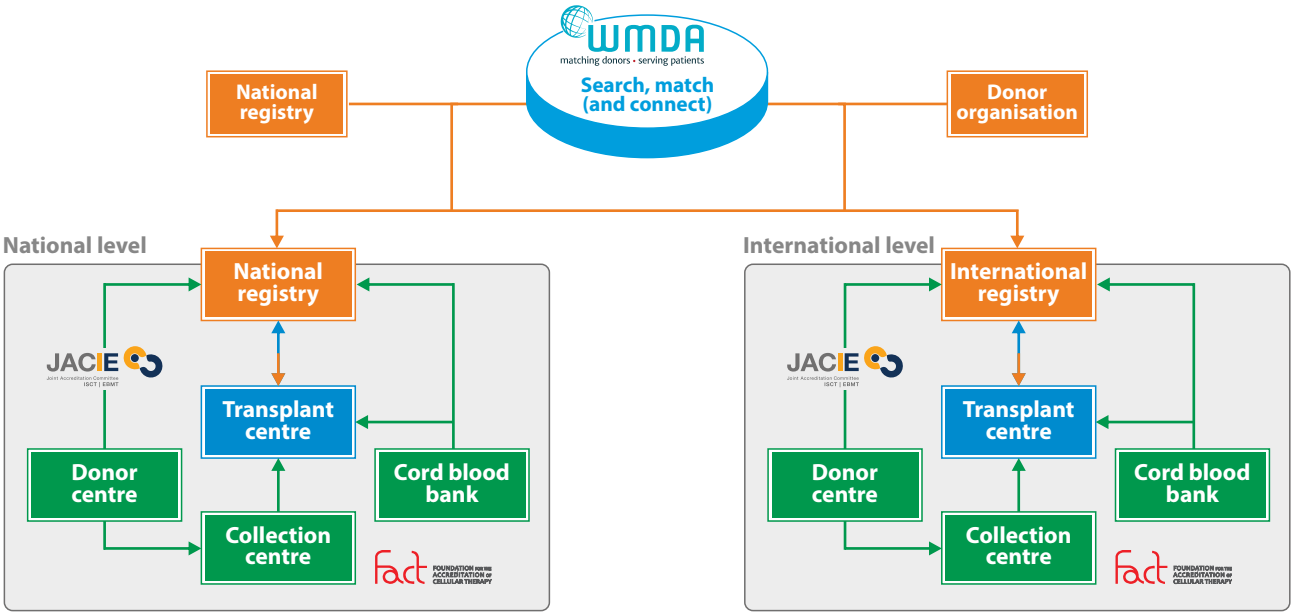
SEROLOGY / BACTERIOLOGY STATUS		
Hepatitis B:	Hepatitis B Core Antibody:	Hepatitis C:
HIV I and II:	HIV PCR:	HCV PCR:
HBC PCR:	Syphilis:	HTLV:
Mycobacteria:	Microbiology Culture:	
ABO Blood group (if known):	Other:	

DONOR INFORMATION	
Age	Sex:
Date of Death:	Cause of Death

Information Entered By:	Date	Signature

Source: National Health Service, United Kingdom.

Appendix 36. **Donor search through registries for haematopoietic progenitor cell transplantation**



WMDA: World Marrow Donation Association.
JACIE: Joint Accreditation Committee ISCT-Europe & European Society for Blood and Marrow Transplantation.
FACT: Foundation for the Accreditation of Cellular Therapy.
Search, Match & Connect Service: global database of unrelated donors, operated by WMDA.

A national registry is usually the organisation responsible for national co-ordination of the search for haematopoietic progenitor cells (HPC) (including cord blood) from donors unrelated to the potential recipient. The transplant centres, through the national registry, can activate a search for their patients. The registry, depending on the national organisation, can rely on donor centres, collection centres and cord blood banks for donor recruitment, consenting, testing, management and collection of HPC. The worldwide registries share their donors and cord blood units data in the Search, Match & Connect Service, the global database operated by WMDA, in order to facilitate the worldwide unrelated donor (UD) search.

The majority of registries are also reciprocally connected to exchange information on donors and to activate further test and selection steps.

Appendix 37. **Informed consent form for cord blood donors**

UHC Zagreb **TST**	**CORD BLOOD BANK ANA RUKAVINA** **Informed Consent Form for Cord Blood Donors**	**OZ-BAR/14** **Version: 2** **1/4**

Dear Madam,

We asked you to donate cord blood, which is rich in haematopoietic stem cells. Haematopoietic stem cells are used to treat patients with leukaemia, bone marrow diseases and some rare inherited diseases (e.g. inherent immune system, metabolism and enzyme disorders). Before you agree to donate cord blood, you should understand the purpose of donation and what is expected of you.

This is an informed consent form listing all the details of the process of cord blood collection and storage. If you decide to donate cord blood after reading this information and speaking to your doctor, please sign this document. The informed consent form for cord blood donation shall be signed in three copies; one remains with you, the second is permanently stored in the “Ana Rukavina” Cord Blood Bank and the third is filed in your patient history in the maternity ward.

Purpose

Haematopoietic stem cells are procured from bone marrow, peripheral blood and cord blood. They have been successfully used for transplantation in the treatment of various diseases for years. Bone marrow is the most frequent source of allogeneic (from another person) haematopoietic stem cells. In peripheral blood, haematopoietic stem cells can be found in larger numbers only after stimulating bone marrow cells with certain medication. Only then can they be collected in a quantity sufficient for transplantation. Unlike collecting haematopoietic stem cells from cord blood, the collection of bone marrow and the collection of haematopoietic stem cells from peripheral blood are procedures that carry a certain risk for healthy donors.

After delivery, blood containing a large number of haematopoietic cells similar to the cells from bone marrow remains in the blood vessels of the placenta and umbilical cord. These cells can generate all types of blood cells (red blood cells, leukocytes, platelets). Since they can regenerate bone marrow, these cells can be transplanted instead of bone marrow cells to treat haematological diseases and severe immune system deficiencies. The placenta is destroyed after delivery. Due to its biological capacity, the blood remaining in the placenta started to be procured and stored on a long-term basis on very low temperatures. Up until today, over 800,000 cord blood units have been stored worldwide in public banks, with numbers growing each day. Thanks to that, over 55,000 haematopoietic cord blood stem cell transplantations have been performed.

The “Ana Rukavina” Cord Blood Bank is a public bank that collects cord blood on the basis of voluntary, anonymous and free donations. If for some reason the collected blood does not meet the criteria for clinical use, it can be used for laboratory research limited to the improvement of the methods of storing haematopoietic stem cells and treatment with cord blood transplantation and cellular therapy.

The process of cord blood collection

Cord blood is collected after an uncomplicated birth (vaginal or caesarean section) using a simple process, which is not harmful to the newborn or the mother. The collection process does not require the doctor and midwife to change the regular procedure of delivery management.
As rare as it may be, complications can arise during birth, which will make it necessary to abandon the process of cord blood collection. Your health and the health of your child come first to the doctor in charge of your delivery. Collection will only be performed if the doctor assesses that it will not endanger your safety or the safety of your child.

UHC Zagreb **TST**	**CORD BLOOD BANK ANA RUKAVINA** **Informed Consent Form for Cord Blood Donors**	**OZ-BAR/14** **Version: 2** **2/4**

Your participation

By signing this document, you agree to the following:

1. You consent to the collection, processing, testing, freezing and storage of the blood remaining in the umbilical cord and placenta after birth. If the product meets the requirements for clinical use, the blood shall be stored until it becomes necessary for the treatment of a patient, or as long as it is suitable for storage.

If tests determine that the cord blood does not meet the quality requirements necessary for clinical use, you consent to it being used for research in the area of haematopoietic stem cell transplantation and cellular therapy, which has been approved by the ethical committee according to legislation in force in the Republic of Croatia. If the cord blood is used for research, the information on you and your child shall be protected.
The cord blood will under no circumstances be used for cloning or commercial purposes.

2. You consent to the determination of human leukocyte antigens (HLA antigens) in a blood sample. HLA typing shall be determined by DNA based typing. This is necessary in order to determine compatibility between the cells from your child's cord blood and the patient's cells.

3. You consent to the storage of a sample of the cord blood and, should it be chosen for transplantation, it being additionally tested for inherited diseases, such as Gaucher disease, adrenoleukodystrophy (ALD) and haemoglobinopathy. These tests are performed in order to prevent the transplantation of haematopoietic stem cells with an inherited disease.

All test results are confidential. The tests are performed to protect the patients who could be treated with the donated blood. We shall notify you on all confirmed positive test results that could affect your health or the health of your child. If you wish, we shall organize a counselling session with a doctor who is an expert in the specific area. If you do not want to be notified on test results, you should not sign this consent form or donate cord blood.

4. You consent to us taking a sample of your blood for testing for blood borne diseases after delivery (+/- 48 hours). The blood shall be tested for hepatitis B and C viruses, HIV 1/2 (the virus causing AIDS), cytomegalovirus (CMV), human T-lymphotropic virus type 1 and 2 (HTLV 1 and 2) and syphilis. If any of the tests are positive, the cord blood shall not be stored in the bank. Testing can also include tissue antigen determination. Part of the blood shall be frozen and stored for future testing for currently unknown diseases.

According to the legislation of the Republic of Croatia on the identity of persons with positive communicable disease test results, including HIV, hepatitis and syphilis, we must notify competent public health institutions. If test results are confirmed to be positive, these institutions may contact you in order to protect your health and the health of your family.

5. Truthfully fill in the medical questionnaire inquiring about your health status and that of your immediate family, including questions on your pregnancy, any medication you may be taking, health issues in your family and the family of the child's father. A part of the questions refers to your current and previous lifestyles and includes questions on your sexual activity and drug abuse. These questions are similar to those posed to voluntary blood donors. The answers are confidential and used to assess the quality of the cord blood for the Bank.

6. You consent to the doctors of the Bank having access to your medical history and the medical history of your child, if they assess it to be necessary, to obtain additional information on the course of the pregnancy and delivery and the tests done at the maternity ward.

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We may ask for your consent to contact your paediatrician or family doctor for additional notifications.

Possible risks and discomfort

Taking blood samples necessary for performing tests can lead to common side effects of drawing blood: discomfort, pain and hematomas or, very rarely, infection and loss of consciousness. We will take all necessary measures to prevent this.

Expected benefit for you and your child

Donating cord blood will not bring direct benefit to you or your child. The main benefit comes from the knowledge that this act can save the lives of people suffering from serious diseases.

However, it can happen that the tests reveal an infection or genetic disease that affects you and your child, which would otherwise remain undetected. An early detection of these diseases can lead to timely treatment and a better outcome.

If your child or a member of your family will need treatment with haematopoietic stem cell transplantation in the future and your child's cord blood is stored and available, it could be used for their treatment. Still, you should be aware of the fact that if your child's cord blood was used to treat another patient, it will no longer be available for your child or family.

Expected social benefit

There is currently a large number of patients worldwide who need a haematopoietic stem cell transplantation, but cannot find a matching donor. The cord blood stored in the *"Ana Rukavina" Cord Blood Bank* increases the chance of finding grafts that can save the lives of those patients.

Other possibilities of storing cord blood

Some banks collect, process and store cord blood for personal, family needs. This service is charged. If you decide to store cord blood for the needs of your family, it cannot be donated to the "Ana Rukavina" Bank.

Financial obligations

Donation is voluntary and your signature on this document binds you not to ask financial compensation from UHC Zagreb. UHC Zagreb will not charge you or your health insurance provider for the costs of collection, processing, testing and storing cord blood.

Privacy and confidentiality

Information on your identity and the identity of your child shall not be revealed to anyone except in cases of legal obligation to do so, or your request for the supply of information. We shall do everything necessary to protect your privacy and the privacy of your child.

Giving up on donation

If you decide not to donate cord blood, your and your child's right to health protection in health institutions will not be affected.
You are free to give up on donation at any time without consequences for the quality of future medical care in health institutions.
Still, if the donated blood has already been processed, tested and stored for use in unrelated haematopoietic stem cell transplantation, giving up on donation will no longer be possible. This especially refers to the situation in which your child's cord blood has already been used in a transplantation to treat a patient.

UHC Zagreb TST	CORD BLOOD BANK ANA RUKAVINA Informed Consent Form for Cord Blood Donors	OZ-BAR/14 Version: 2 4/4

Collection and storage of donated blood

It is possible that your child's cord blood will not be collected and stored. This could happen due to several reasons.

We shall not collect the blood if the medical collection team is not available or if complications arise during delivery, making collection impossible.

Cord blood shall not be stored if the collected volume is too small, if the blood contains a small number of cells, if an infection is present or if technical problems occur during processing and freezing.

There is also the possibility of unforeseen events preventing the collection and storing of cord blood.

If after donating cord blood you or your child develop a serious disease, which could affect the quality of the donated cord blood for haematopoietic stem cell transplantation, please notify the "Ana Rukavina" Cord Blood Bank of this.

If you have additional questions on donating cord blood, please feel free to contact the "Ana Rukavina" Cord Blood Bank, Clinical Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb, via phone (01-2388-708) or e-mail (banka.ana.rukavina@kbc-zagreb.hr).

The mother's consent

I have read and understood the information from the informed consent form. I had the opportunity to ask questions and get satisfactory answers. I received a copy of the informed consent form.

By signing this form, I give my consent for the collection, processing and testing of my child's cord blood after delivery and its storage into the "Ana Rukavina" Cord Blood Bank.

Mother's full name	Mother's date of birth
Mother's signature	Date

Signature of the person who notified the mother

I have explained the process of cord blood donation and storage to the mother and answered all of her questions. I believe that she understood the information from this document and that she willingly agrees to donate cord blood.

Full name of person notifying the mother	Signature of person notifying the mother	Date

Source: Ana Rukavina Cord Blood Bank, Clinical Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb.

Appendix 38. **Cord blood donor medical history questionnaire**

1. **Are you currently taking antibiotics?** ☐ Yes ☐ No

   Specify: _____ Why? _____

2. **Are you currently taking any other medication?** ☐ Yes ☐ No

   Specify _____ Why? _____

3. **Within the past few days have you …**

   a. … had ☐ fever, ☐ cold, ☐ cough, ☐ diarrhoea, ☐ vomiting, ☐ abscess, ☐ dental procedure, ☐ dental extraction, ☐ open wounds, ☐ any other sickness or infection?

   Specify _____

   b. … taken antibiotics? ☐ Yes ☐ No

   Specify _____ Why? _____

   c. … taken any other medication? ☐ Yes ☐ No

   Specify _____ Why? _____

4. **In the past 2 months have you …**

   a. … received any shots or vaccinations? ☐ Yes ☐ No

   Specify _____

   b. … been in contact with a person vaccinated for smallpox? ☐ Yes ☐ No

5. **In the past 6 months have you had …**

   ☐ an unexplained weight loss? ☐ an unexplained fever? ☐ frequent diarrhoea? ☐ swollen lymph nodes?

6. **Have you had any of the following diseases?**

   a. ☐ rheumatic fever, ☐ malaria, ☐ tuberculosis

   b. ☐ diabetes, ☐ renal disease, ☐ heart disease ☐ liver disease

   c. ☐ inflammatory bowel disease, ☐ ulcerative colitis, ☐ Chron's disease

   d. ☐ autoimmune disease Specify _____

   e. ☐ blood clotting problems

f. ☐ epileptic seizures

g. ☐ tumour ☐ carcinoma Specify

h. ☐ contagious jaundice ☐ hepatititis A ☐ hepatititis B ☐ hepatititis C

i. ☐ psychiatric disorder ☐ neurologic disease

7. **Are there any hereditary diseases in your family?**

a. ☐ enzymatic/metabolic disorders

b. ☐ immune system disorders

c. ☐ blood clotting problems/coagulopathy

d. ☐ haematological disorders

e. ☐ any other serious disease

Please specify who has it and which disease.

8. **Have you ever had any serious disease or sugery?** ☐ Yes ☐ No

Specify what exactly and when.

9. **In the past 12 months have you …**

a. … ☐ received a blood transfusion ☐ had acupuncture ☐ had a tattoo ☐ had a piercing?

Specify when:

b. … ☐ had a medical needle stick incident ☐ had contact with someone else's blood?

Specify when:

c. … been in contact with: ☐ a person who has hepatitis ☐ a person who had received a hepatitis B hyperimmune globulin?

Please specify: ☐ household member ☐ your sexual partner ☐ professional exposure ☐ other:

d. … used retinoids: ☐ Tegison ☐ Soriatane ☐ Accutane?

10. **Have you ever …**

a. … had a positive blood test for ☐ HIV/AIDS virus ☐ hepatitis B virus ☐ hepatitis C virus?

b. … been rejected as a blood donor? ☐ Yes ☐ No

Why? ☐ low body weight ☐ anaemia ☐ risk behaviour ☐ other:

c. … been treated with blood clotting factors concentrate?

Which?

When?

d. … received a transplant: ☐ tissue ☐ organ? Specify which and when.

e. … received a *dura mater* transplant? When?

f. … *(or members of your household)* had a transplant or a medical procedure that involves exposure to animal cells, organs or tissues? ☐ Yes ☐ No

Specify who and which procedure:

11. **Have you …**

a. … stayed in the United Kingdom between 1986 and 1997 for a period longer than 6 months? ☐ Yes ☐ No

b. … stayed in an area with HIV subtype O virus (West and Central Africa)? ☐ Yes ☐ No

c. … or your partner stayed in an area endemic for the HTLV virus (Japan, Caribbean, South America, West and Central Africa)? ☐ Yes ☐ No

d. … in the past 12 months stayed in an area where you could have contracted West Nile Virus (North America, Italy, Austria, Hungary, Serbia, Romania)? ☐ Yes ☐ No

… in the past 12 months travelled outside of Croatia? ☐ Yes ☐ No

If yes, when and where?

12. **Do you belong in any of the risk-behaviour groups listed below?**

a. Addiction to alcohol or drugs ☐ Yes ☐ No

b. Consumer of intravenous drugs ☐ Yes ☐ No

c. Persons who frequently change sexual partners ☐ Yes ☐ No

d. Persons who take drugs or money in exchange for sex ☐ Yes ☐ No

e. Sexual partners of persons from risk behaviour groups ☐ Yes ☐ No

f. Sexual intercourse with a man who has had sex with another man ☐ Yes ☐ No

g. Sexual intercourse with a person who has taken intravenous drugs ☐ Yes ☐ No

h. Sexual intercourse with a person who has haemophilia and has received human derived clotting factor concentrates ☐ Yes ☐ No

i. Person who has syphilis or has been treated for syphilis ☐ Yes ☐ No

j. Sexual intercourse with an HIV-positive person ☐ Yes ☐ No

k. persons who have been in jail longer than 72 hours ☐ Yes ☐ No

If you answered yes to any of questions 12.a-k, please give additional explanations:

13. **Does the child's father belong to any of the risk-behaviour groups?**
Specify:

14. **Has anyone in your family had Creutzfeldt–Jakob disease?**
Who? When?

15. **Has anyone in the child's immediate family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters)
had a: ☐ chromosome disorder, ☐ genetic disease?
Specify:

16. **Has anyone in the child's immediate family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters)
had: ☐ cancer, ☐ leukaemia, ☐ haematological disorder?

17. **Has anyone in the child's extended family**
(☐ mother, ☐ father, ☐ brothers, ☐ sisters ☐ grandparents) had …

a. … any genetic disease that can influence the lymphatic or haematopoietic system:
☐ spherocytosis ☐ thalassaemia ☐ sickle cell anaemia ☐ elliptocytosis ☐ Fanconi anaemia ☐ Diamond-Blackfan sy. ☐ SCID ☐ leukodystrophy ☐ chronic granulomatosis ☐ DiGeorge sy. ☐ Wiskott-Aldrich ☐ Nezelhof sy. ☐ hereditary thrombocytopaenia ☐ Glanzmann Alport ☐ Tay-Sachs ☐ Ataxia-Telangiectasia ☐ Gaucher ☐ Hurler ☐ Hunter?

b. … ☐ autoimmune diseases, ☐ muscular dystrophy, ☐ multiple sclerosis or ☐ other severe hereditary skin disease (e.g. neurofibromatosis)?

18. **Is the child´s father your blood relative?** ☐ Yes ☐ No

19. **Are you the child's biological mother?** ☐ Yes ☐ No

20. **Have you had previous pregnancies?** ☐ Yes ☐ No

Number of: Caesarean sections Vaginal births

a. **Have you had any complications in previous pregnancies?** Specify:	
b. **Have you had preterm deliveries?** Which week?	

c. **Have you had a positive blood test in this pregnancy?**
☐ HIV/AIDS virus, ☐ hepatitis B virus, ☐ hepatitis C virus, ☐ syphilis

I have understood the questions and responded to the best of my knowledge. My answers are truthful.

Mother's full name		**Mother's signature**		Date:	
Health questionnaire checked by		**Signature**		Date:	

Source: Ana Rukavina Cord Blood Bank, Clinical Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb.

Appendix 39. **Covid-19 infection risk assessment**

a. **Have you ever been tested for SARS-CoV-2? (If yes, please provide test results.)** ☐ Yes ☐ No

b. **In the past 28 days, have you had any clinical signs or symptoms of COVID-19? (If yes, which?)** ☐ Yes ☐ No

- ☐ Fever > 37 °C
- ☐ Runny nose
- ☐ Diarrhoea
- ☐ Shortness of breath/difficulty breathing
- ☐ Tiredness/excessive drowsiness
- ☐ Sore throat
- ☐ Dry cough
- ☐ Headache
- ☐ Pneumonia
- ☐ Dry cough with mucus production/coughing up mucus
- ☐ Muscle or body aches
- ☐ Chest pain
- ☐ Loss of smell and taste

c. **In the past 28 days, have you been in close contact* with a person with known or suspected Covid-19?** ☐ Yes ☐ No

If yes, when? _____

d. **In the past 28 days, have you been in close contact* with a person experiencing symptoms of upper respiratory tract infection or fever?** ☐ Yes ☐ No

If yes, when? _____

Type of contact: ☐ joint household, ☐ working place, ☐ hospital, ☐ other.

e. **In the past 14 days, have you been told to self-isolate due to possible Covid-19 infection?** ☐ Yes ☐ No

If yes, since when? _____

f. **In the past 14 days, have you had any close contact with a person in self-isolation who had symptoms of Covid-19 but tested negative for SARS-CoV-2?** ☐ Yes ☐ No

If yes, when was your last contact? _____

g. **In the last 28 days, have you or a member of your family travelled/stayed† in high-risk areas for COVID-19 ☐ in Croatia ☐ abroad?**

Where? _____

* Close contact includes the following (source www.hzjz.hr): direct physical contact with an infected person; spending more than 15 minutes within 2 m of an infected person; exposure in a health institution, including direct care for Covid-19 patients, work with health professionals infected with the new coronavirus, visiting patients or spending time in close proximity to a person with Covid-19 without personal protective equipment; working in close proximity to or sharing a closed space (such as classrooms, offices, waiting rooms, conference rooms and other closed spaces) with a person with Covid-19 for more than 15 minutes; travelling with a person with Covid-19 in any means of transport; sharing a household with a person with Covid-19.

† Including flight changes

Return date

Were you tested upon return? ☐ Yes ☐ No

What were you tested for?

h. **Have you ever received a dose of COVID-19 vaccine?** ☐ Yes ☐ No

If yes, when? 1st dose

2nd dose

Vaccine produced by technology:
- ☐ mRNA (Pfizer/BioNTech, Moderna, CureVac)
- ☐ viral vector-based vaccines (AstraZeneca/Oxford, Johnson&Johnson/Jansen, Sputnik V)
- ☐ attenuated vaccine (Chinese vaccine Sinopharm and Sinovac)

Maternal donor name and surname

Date of birth

Signature

Date

I have understood the questions and responded to the best of my knowledge. My answers are truthful.

Source: Ana Rukavina Cord Blood Bank, Clinical Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb.

Appendix 40. **Informed legal consent for cryopreserving and storing semen from a minor**

as a precautionary measure under medical circumstances with a risk of reduction of fertility

Informed consent declaration (Spanish Fertility Society – SEF) model

		Date	dd mm yyyy	History no.	
Mr	Name and surname of father				
ID/Passport no.				Age	
Resident at					

		Date	dd mm yyyy	History no.	
Mrs	Name and surname of mother				
ID/Passport no.				Age	
Resident at					

		Date	dd mm yyyy	History no.	
Mr	Name and surname of child				
ID/Passport no.				Age	
Resident at					

Due to the present circumstances of the under-age patient (illness, intervention or medical treatment that may cause sterility, and/or the advisability of preserving a sample of his semen for use in an assisted reproduction treatment), we wish to cryopreserve (freeze) a sample of his semen, which, through this act, we put at the disposal of the clinic so that it may be used at a later date.

For these purposes, we, and in particular the minor, who is intellectually and emotionally capable of understanding the scope of the circumstances, have been informed of the following dispositions: Law 14/2006 of 26 May on Assisted Reproduction Techniques and Law 41/2002 of 14 November, governing the independence of the patient and the rights and obligations to clinical information and documentation.

The cryopreserved semen may be kept in authorised gamete banks during the lifetime of the male to whom it belongs.

The present commitment to preserve the cryopreserved semen is agreed upon for a period of ☐ years, during which Instituto Valenciano de Infertilidad (IVI) is committed to adequately maintaining the samples. On expiry of the period agreed upon, we agree to pay the annual amount that we have been informed of through the corresponding financial information, in order to cover maintenance and preservation costs.

Should we be interested in possessing the cryopreserved samples, we are committed to contacting IVI to inform them of our intentions. If we do not inform them of our desire to maintain the samples longer, IVI will understand that we are no longer interested in maintaining them, the cryopreservation and storage commitment, as well as all the other obligations that IVI has assumed, then being automatically dissolved, so resulting in the destruction of the sample.

Furthermore, we are committed to informing IVI of any change of address that we, or the minor, may make in the future for the appropriate purposes.

Finally, we have been informed of the absence of risk to the gametes, resulting from their cryopreservation (with the exception of catastrophe or other justified causes outside the control of this centre) and of the economic cost of their cryopreservation and storage.

We (and in particular the minor) have understood the explanations that have been provided to us in clear and simple language and the physician who has attended to us has allowed us to ask as many questions as we like and has clarified all the doubts that we have expressed.

The information has been provided to us in sufficient time for us to think it over calmly and to reach a decision freely and responsibly, and it has been communicated to us 24 hours before the standard procedure.

We have been informed that pursuant to Royal Decree 9/2014, following blood collection, serum shall be stored for a specific period of time as provided in the applicable regulations.

We also understand that at any moment and without the need for any explanation, we may revoke the consent which we are now presenting.

We, therefore, declare ourselves to be satisfied with the information received and that we understand the scope and risks of the treatment.

Authorisation

We authorise the Reproduction Unit staff to cryopreserve the semen sample that, through this act, we put at the disposal of the clinic, from our son Mr ☐, ☐ years of age, ID no. ☐ for the purposes referred to and during the maximum time stated.

Signed at . . . . . .

Date . . . . . .

Parents' signatures . . . . . .

. . . . . .

Patient's signature . . . . . .

Laboratory director's signature . . . . . .

This consent form must be signed by the patients on **all** pages and on both sides.

Consent revocation

For the exercise of patients' rights and for the revocation of this consent, patients may fill in a form requiring the enforcement of such rights at any time during the procedure. This fact will be reflected in the medical history.

Source: *Reproducción humana asistida, protocolos de consentimiento informado de la Sociedad española de fertilidad*, Editorial Comares año 2002. (Human assisted reproduction – informed consent models, Spanish Fertility Society Model, 2002.)

Appendix 41. Legal framework for the development of advanced therapy medicinal products in the European Union

To provide a common framework for the marketing of ATMPs in the EU, Regulation No. 1394/2007/EC of the European Parliament and of the Council on advanced therapy medicinal products (hereafter 'the ATMP Regulation') was adopted in 2007. Specifically, cells used in human application that have been subject to substantial manipulation, and/or cells that are used for an essential function or functions in the recipient different from their function in the donor, are regulated as medicinal products in the EU.

According to Article 1 (a) of the ATMP Regulation, an ATMP is any of the following medicinal products for human use:

a. a gene therapy medicinal product;
b. a somatic cell therapy medicinal product;
c. a tissue-engineered product.

A gene therapy medicinal product is a biological medicinal product that fulfils these two characteristics:

a. it contains an active substance that contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;
b. its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.

Gene therapy medicinal products must not include vaccines against infectious diseases.

A somatic cell therapy medicinal product is a biological medicinal product that has two characteristics:

a. it contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;
b. it is presented as having properties for (or is used in humans with a view to) treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its tissues or cells.

For the purposes of point (a), the manipulations listed in Annex I to Regulation 1394/2007/EC are not considered as substantial: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing, cryopreservation and vitrification. Note that this list is non-exhaustive and that other processes may also fall outside the scope of Regulation 1394/2007/EC. Thus, based on scientific considerations, other manipulations may also be judged 'non-substantial' (e.g. manipulations that have been used in clinical practice in a hospital setting over many years).

A tissue-engineered product is a product that:

a. contains or consists of tissues or cells that have been subject to substantial manipulation, or tissues or cells that are not intended to be used for the same essential function(s) in the recipient and the donor;

b. is presented as having properties for (or it is used in humans with a view to) regenerating, repairing or replacing human tissue.

A tissue-engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, bio-molecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition.

A combined advanced therapy medicinal product means an ATMP product that fulfils the following conditions:

a. It must incorporate, as an integral part of the product, one or more medical devices within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of the Article 1(2)(c) of Directive 90/385/EEC, and

b. Its cellular or tissue part must contain viable cells or tissues, or

c. Its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be considered as primary to that of the devices referred to.

Marketing authorisation for ATMP can only be granted if, after a scientific assessment of the product's quality, efficacy and safety profile, it is demonstrated that the benefits outweigh the risks. The Committee for Advanced Therapies (CAT) is a specialised and multidisciplinary committee at the European Medicines Agency (EMA) responsible for assessing the quality, safety and efficacy of ATMPs that follow the centralised procedure for marketing authorisation and it assists in the preparation of any documents related to fulfilment of the objectives of Regulation 1394/2007, where relevant. The application for a marketing authorisation must be submitted to the EMA and the final decision is taken by the European Commission.

Developers of products based on genes, tissues or cells can seek advice from the CAT on whether a specific product is an ATMP. The procedure is intended as an incentive for developers, who can ascertain at an early stage of development if their product must comply with the requirements that apply to ATMPs [1].

Where ATMPs contain human cells or tissues, Directive 2004/23/EC applies to the donation, procurement and testing of the tissues and cells.

The ATMP Regulation gives member states the power to authorise the use of custom-made ATMPs prepared on a non-routine basis in the absence of a centralised marketing authorisation, provided that the product is used for individual patients in a hospital and under the professional responsibility of a medical practitioner. This 'hospital exemption' requires the application of national requirements on quality, traceability and pharmacovigilance equivalent to those required for authorised medicinal products.

It is important to stress that, in the EU, ATMPs are medicinal products. It follows that the overall regulatory framework governing medicines (including, but not limited to, rules on manufacture, distribution, packaging, labelling, evaluation of risks and benefits, determination of the data needed to demonstrate efficacy and safety, pharmacovigilance and advertising of medicines) apply to ATMPs. Furthermore, use of ATMPs in an investigational setting is also subject to EU rules on clinical trials.

However, flexibility in the development of ATMPs is important to anticipate the rapid evolution of science and technology in the field. To facilitate the process, the EU introduced the risk-based approach [2], which is based on the identification of various risks associated with the clinical use of an ATMP and risk factors inherent to the ATMP with respect to quality, safety and efficacy.

Additional information about the EU regulatory framework for ATMPs can be found at the EMA and EC Internet websites [3].

This guidance is not intended to affect the scope of the EU rules on medicines, and any operator who intends to process, store, distribute or use cells in humans should first seek advice from national authorities on the appropriate, applicable legal framework.

National competent authorities

In EU member states, human cells for human application can be subject to different regulatory frameworks (depending on the intended use, mode of action and degree of manipulation). Advice on the

classification of a specific cell therapy can be sought from the national competent authorities or from the CAT [4]. In some countries, the regulatory body is the same for all cell therapy products whereas, in others, those classified as ATMP are regulated by a different body/agency from those classified as cells for transplantation.

The ethical and legal position on the use of human stem cells or progenitor cells, as well as the regulatory oversight, differs in countries around the world [5]. In the EU, each member state is able to make decisions on the use of progenitor stem cells for basic research. However, they must be compliant with the requirements stated in the ATMP Regulation if they are to be used for the manufacture of ATMP for treating patients.

Some countries have national legislation on paediatric donors that should be taken into account when cells from children are considered.

Independent ethics committees

Local/regional/national independent ethics committees (IECs) are important bodies designated to approve and review biomedical and behavioural research involving humans, including the scientific rationale for the clinical application of a new therapy. For the latter, IECs should consider the irreversible nature of some gene/cell therapies and address the acceptability of exposing a donor to a research protocol for the benefit of the recipient, in particular where the donor is a relative of the subject, especially a parent or a sibling, to be included in the trial. IECs should also check appropriate traceability and guarantees regarding subject data protection and confidentiality. Written informed consent for receiving a cellular therapy is considered a prerequisite, as in any clinical trial.

The International Society for Stem Cell Research (ISSCR) guidelines recommend that special emphasis be placed on the risks of stem cell-based clinical research during the informed consent process. The risks include tumour formation, immunological reactions, unexpected behaviour of cells, unknown long-term health effects and sensitivities around the source of cellular products [6].

Considerations on conduct of clinical research

Clinical research, including trials of experimental interventions, is essential in translating cell-based applications and it requires the participation of human subjects, whose rights and welfare must be protected [3]. All people involved, including sponsors, investigators, oversight bodies and regulators, must be responsible for ensuring the ethical conduct of clinical trials. As with all clinical research, clinical trials must follow internationally accepted principles governing the ethical design and conduct of clinical research and the protection of human subjects [7, 8]. In the EU, a specific directive regulates clinical trials [9]. The EMA published a guideline in order to help professionals. This guideline is currently under revision [10]. The EU published GCP on Good Clinical Practice specific to Advanced Therapy Medicinal Products [11].

References

1. European Medicines Agency. Reflection paper on classification of Advanced Therapy Medicinal Products EMA/CAT/600280/2010 rev.1 4/19, available at www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-classification-advanced-therapy-medicinal-products_en-0.pdf, accessed 23 April 2022.
2. European Medicines Agency. Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC applied to Advanced therapy medicinal products, available at www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500139748.pdf, accessed 23 April 2022.
3. European Union. [actions and legislation], available at https://european-union.europa.eu/priorities-and-actions/actions-topic/health_en and http://ec.europa.eu/health/human-use/advanced-therapies/index_en.htm, both accessed 23 April 2022.
4. European Medicines Agency. Advanced therapy [medicinal products] classification, available at www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000296.jsp&mid=WC0b01ac058007f4bc, accessed 23 April 2022.
5. Food and Drug Administration. [Guidance on] Regulatory considerations for human cells, tissues, and cellular and tissue-based products: minimal manipulation and homologous use, available at www.fda.gov/media/109176/download, accessed 23 April 2022.
6. ISSCR [International Society for Stem Cell Research], Guidelines Updates Task Force. Guidelines for stem cell research and clinical translation, 2016; available at www.isscr.org/docs/default-source/guidelines/isscr-guidelines-for-stem-cell-research-and-clinical-translation.pdf?sfvrsn=2, accessed 23 April 2022.
7. Department of Health, and Education and Welfare. Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (The Belmont Report), 1979. 44 Fed Reg 23, 192.

8. World Medical Association. Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* 2013 Nov 27;310(20):2191-4.
9. European Commission. Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use.
10. European Medicines Agency. Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials. Draft 2019, available at www.ema.europa.eu/documents/scientific-guideline/draft-guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy_en.pdf, accessed 23 April 2022.
11. European Commission. Guidelines on Good Clinical Practice specific to Advanced therapy medicinal products, issued 10/10/2019, and available at https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/atmp_guidelines_en.pdf, accessed 23 April 2022.

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Human tissues and cells are being used in an increasing variety of ways, and advances in transplantation therapy have unquestionable benefits. Human cells and tissues for transplantation can save lives or restore essential functions, but the use of human tissues and cells also raises questions of safety and quality. Only tissues and cells recovered, processed and stored following strict quality and safety standards are likely to function satisfactorily. Careful evaluation of donors is essential to minimise the risk of transmission of infections or malignancies. Furthermore, since human tissues and cells can currently only be derived from the body of a person, strong ethical principles need to be associated with their donation and use. The Council of Europe approaches tissue and cell transplantation in compliance with the principles of non-commercialisation and voluntary donation of materials of human origin. This 5th Edition of the *Guide to the quality and safety of tissues and cells for human application* contains information and guidance for all professionals involved in donation, banking, transplantation and other clinical applications of these medical products of exceptional nature. For matters dealing with the use of organs and blood or blood products, see the Council of Europe *Guide to the quality and safety of organs for transplantation* and *Guide to the preparation, use and quality assurance of blood components*, respectively.

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