

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



European Committee
(Partial Agreement)
on Organ Transplantation
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Foreword

Founded in 1949, the Council of Europe is the oldest and largest of all European institutions and now numbers 47 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 & Health-Care (EDQM). This Directorate is a key European organisation involved in the harmonisation, co-ordination, standardisation, regulation and quality control of medicines, blood transfusion, organ transplantation, pharmaceuticals, pharmaceutical care, consumer health, cosmetics and food packaging.

Transplant medicine and transplantation have progressed during 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 transplanted material of human origin, they carry risks of disease transmission that must be controlled by application of scrupulous donor selection and testing criteria, as well as ensuring that comprehensive quality systems are in place.

The *Guide to the quality and safety of tissues and cells for human application*, published first in 2013 and now in its 3rd edition, collates the most up to date information to provide transplant 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 collection centres, fertility clinics, tissue establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing the 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.

This Guide includes recommendations considered to be ‘minimum standards’ that align with the

* 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, Norway, Poland, Portugal, Romania, Russian Federation, San Marino, Serbia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, ‘the former Yugoslav Republic of Macedonia’, Turkey, Ukraine, United Kingdom.

principles set out in the various relevant European Union (EU) directives. Thus, the Guide provides technical support both to EU member states that have implemented the directives and to those non-EU states that are considering their adoption. These minimum standards state ‘what must be done’; however, this Guide goes further by providing additional technical advice based on good manufacturing practice (GMP) guidelines and on best practice consistent with current scientific knowledge, expert opinion and the results of many EU-funded projects. It describes background information that should be considered in forming policy decisions and educational initiatives by explaining the ‘why and how’. It also refers to recent developments that may be reflected in future updates of EU legislation, where necessary and relevant, thereby providing advance information and recommendations regarding developments in the field. Throughout this Guide, use of the word ‘must’ indicates mandatory compliance in alignment with Council of Europe recommendations and resolutions as well as with EU directives, whereas the use of the word ‘should’ indicates recommended compliance in accordance with commonly accepted good practice.

Whereas tissue establishments in EU member states are required to comply with legislation derived from EU directives, this Guide is intended to facilitate ongoing improvements in the donation, procurement, testing, processing, preservation, storage and distribution 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. 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 may be in place.

Unless otherwise stated, the guidelines apply only to human tissues and cells intended for transplantation or clinical use (including insemination and fertilisation). Tissues and cells used for research, as well as tissues and cells procured and applied in the same medical procedure, do not fall under the scope of the present Guide.

This Guide has been divided into three parts:

- Part A (Chapters 1-15) contains general requirements applicable to all tissue establishments and organisations involved in the donation, procurement, testing, processing, preservation, storage and distribution of tissues and cells.
- Part B (Chapters 16-25) contains specific guidelines and requirements for the various tissue and cell types. The general guidelines of Part A also apply to tissues and cells that have not

been mentioned specifically in Part B of the present edition.

- The new Part C (Chapters 26-29) of this guide introduces novel therapeutic approaches, most of them under development, such as cell-based therapies, decellularisation techniques and the use of natural scaffolds for clinical application, or the human application of other human-derived substances such as breast milk, faecal microbiota, platelet-rich plasma or serum eye drops. This section includes not only tissues and cells that are already in routine use in patients but others that are in research and development and are currently undergoing clinical trials. It is worth noting that, in different countries, some of these products may be regulated under different regulatory frameworks. For example, in the EU, some of the activities described in this section are considered to be manufacturing of advanced therapy medicinal products (ATMPs) and professionals involved in the processing, distribution and clinical application of such products are required to comply with the regulatory framework for medicinal products.

In this 3rd edition, chapters have been updated and extended, and new chapters have been added. As a consequence of this revision, Chapter 1 (Introduction) has been updated to include important principles that must be respected in the field of new and developing therapies with cells or tissues for human application.

The most important change in Chapter 2 (Quality management, risk management and validation) is the expansion of the section on validation. This now includes more specific guidance on the validation and qualification of premises, materials and technical procedures and methods.

Another significant enhancement to the Guide has been the development of separate chapters dedicated to general quality and safety aspects of processing and storage (new Chapter 7) and a specific chapter on quality aspects of premises (new Chapter 8). Chapter 9 remains dedicated to the principles of microbiological testing of tissue and cell preparations and has been further expanded.

New Commission directives on imported tissues and cells (2015/566/EC) and requirements for the Single European Code (2015/565/EC) both have an implementation date for EU member states of 29 April 2017. Chapter 10 (Distribution and import/export) has accordingly been updated with important information on the importation of tissues and

cells, and a new chapter on coding, labelling and packaging (Chapter 13) has been added to the Guide. The importance of and requirements for traceability are now addressed separately in Chapter 14.

In Part B of the Guide each of the chapters for specific cells or tissues has been revised, updated and extended with additional information provided by experts from scientific and professional organisations, including the American Association of Tissue Banks (AATB), the European Association of Tissue Banks (EATB), the European Eye Bank Association (EEBA), the European Society for Human Reproduction and Embryology (ESHRE), the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee–ISCT & EBMT (JACIE). In particular, the chapter on haematopoietic progenitor cells (Chapter 21) has undergone a major review and revision, and the established use of pancreatic islets in the clinic justified a chapter of its own (Chapter 22).

The previous ‘place holder’ on adipose tissue has been expanded. Chapter 23 now contains a more complete guide to procurement, testing, storage and human application of adipose tissue. Likewise, the chapter on fertility preservation in the 2nd edition is now a more complete chapter covering the state of the art on how and when fertility preservation may be useful.

As the fields of donation and transplantation of tissues and cells evolve, new and more sophisticated technologies will provide opportunities to make tissues and cells safer and their engraftment more effective. Depending on the degree of complexity of the processing or the manner in which the tissues or cells are applied to the recipient, some of these tissues and cells are classified in the EU as ‘medicinal products’. In many non-EU European countries, this differentiation is not made at a regulatory level, or there is no regulation of the field. Even in EU member states, many tissue establishments are working with tissues or cells that are subsequently sent for manufacture as advanced therapy medicinal products (ATMP) or are manufacturing those products themselves under the ‘hospital exemption’ allowed by the EU Regulations on ATMP.

Hence, the new Part C in this Guide addresses the field of cell therapy in particular. Chapter 26 (General considerations for cell-based therapies) provides a didactic overview of the field by describing the different ways in which cells can be expanded, modified or combined with scaffolds to replace damaged or diseased tissues in the recipient. This chapter includes information about the regulatory framework governing the production and use of some of these

therapies in the EU. The new Chapter 27 (Decellularisation and preparation of natural scaffolds) provides technical guidance to any tissue establishment in decellularisation techniques and the preparation and potential use of scaffolds. Chapter 28 (Developing applications for somatic cells) focuses on developing applications for several types of cell (apart from haematopoietic progenitor cells) that are rapidly becoming important tools for the treatment of patients. The chapter does not attempt to give complete guidance on the processing of these cells since the field is expanding rapidly. Instead, the specific issues in donor selection, procurement and testing (including testing for quality/specificity if applicable) are covered, and the chapter includes an overview with references, as a table. Chapters of this Guide that relate to donor selection, consent, procurement and the testing of tissues and cells apply in full to the tissues and cells donated for any clinical application, including those covered in Part C.

Chapter 29 updates information about several other substances obtained from humans for autologous or allogeneic use, including breast milk, faecal microbiota, teeth, platelet-rich plasma, platelet-rich fibrin and serum eye drops. The regulatory status of these substances varies in most countries. However, the risks associated with their human origin and the processes applied to procure, process and preserve them are analogous to those of the rest of the tissues and cells described in this Guide. Therefore, this chapter provides a generic quality and safety framework for healthcare professionals treating patients with these substances.

A dedicated working group, composed of well-recognised international experts nominated by member states, was convened for the preparation of this Guide. This group was chaired by Mona Hansson (Health and Social Care Inspectorate, Sweden) and John Armitage (European Eye Bank Association). This expert group made an exceptional contribution by sharing their expertise, reviewing the literature in their respective specialist areas and extracting and distilling knowledge from numerous international guidelines, collaborative projects and diverse publications 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 ensured access to appropriate and relevant expertise 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. The final draft was submitted to open consultation, and

comments and suggestions received were carefully analysed by the working group. All the professionals who participated in the preparation of the Guide are listed in Appendix 24.

All those professionals who participated in the open consultation and provided extremely useful comments and suggestions should also be acknowledged.

The elaboration of this Guide was partly funded by the European Commission through a Direct Grant Agreement (contract number 2012 51 01) entitled 'Dissemination of best practices in tissues and cells donation/transplantation'. Special thanks are due to Ioana-Raluca Siska and Deirdre Fehily, from DG-SANTE, who ensured that the current text remained aligned with EU directives and who made available the results from EU-funded projects. In addition, Ana Mancho Rojo, from the Executive Agency for Health and Consumers (CHAFEA), provided tremendous assistance with the financial and administrative tasks.

Several professional associations – in particular, the AATB, EATB, EEBA, ESHRE, EBMT and JACIE, and the International Council for Com-

monality in Blood Banking Automation (ICCBBA) – should also be thanked for sharing their experience and knowledge.

The drafting and publication of the 3rd edition of the Guide was co-ordinated by Marta López Fraga (Scientific Officer in charge of the Council of Europe European Committee on Organ Transplantation [CD-P-TO]) and Mar Lomero (Scientific Assistant), with the assistance of Ahlem Sanchez, David Crowe, Gerard M.-F. Hill, Isabelle Vernay and Marie-Agnès André. An extended thank-you should also be given to Karl-Heinz Buchheit, Head of the Department of Biological Standardisation, OMCL Network & HealthCare (DBO), and Susanne Keitel, Director of the EDQM.

The entire project has been an exceptional combined effort, with extensive discussions dedicated to the common goal of increasing the safety, efficacy and quality of donated tissues and cells for human application. The final result is this 3rd edition of the Guide, which constitutes a common European standard, based on the long-standing expertise and knowledge of the EDQM.

Part A. General requirements

Chapter 1. Introduction

1.1. Scope and purpose of this guide

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 in an increasing variety of new ways. Many of these developments, such as advances in transplantation therapy or in assisted reproductive technology (ART), 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 to cover major burns or 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 and craniofacial/maxillofacial, dental 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 relatives have 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 and placenta, parathyroid tissue and skull bone are donated by living persons. Additionally, 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, chondrocytes, hepatocytes), mesenchymal stromal cells and limbal stem cells. Oocytes, sperm, ovarian, testicular tissue and embryos can be used in ART procedures to achieve pregnancy.

This is the 3rd 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:

- a. 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. Human tissues and cells for clinical applications represent a special class of basic essential healthcare products, as well as being the potential starting material for much more complex products in the future. All material of human origin carries risks of disease transmission that must be controlled by application of scrupulous criteria of donor selection and testing, and comprehensive systems to assess quality. The idea behind this Guide is to help professionals on a practical level by providing generic guidance that will help improve the rate of successful clinical application of tissues and cells.
- b. 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), several directives describe the requirements and have been transposed into the national legislation of the 28 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. The 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 do-

nation, 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 and distribution, imports and exports;
- d. there are programmes for vigilance and surveillance of adverse outcomes;
- e. there is monitoring and reporting of donation, processing, storage, distribution and import and 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. Unless otherwise indicated, the term ‘member states’ applies to 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 should 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 procured and applied in 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, as well as by the members and observers of the European Committee of Experts on Organ Transplantation (CD-P-TO), for which see Appendix 25.

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 *Guide to the preparation, use and quality assurance of blood components* [2], both published by the Council of Europe.

1.2. Brief history of the human 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 BC, 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. Similarly, 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 by Gordon Murray in Toronto, 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 transplanted 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 leucocyte 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 immune 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 to treat a child with Fanconi's anaemia with cells from his healthy HLA-identical sibling (related) donor. The first unrelated bone-marrow registry was

established in London in 1973 by Shirley Nolan, whose son was diagnosed with Wiskott–Aldrich syndrome. After this first donor recruitment drive, the number of bone-marrow and peripheral haematopoietic progenitor cell donors has increased all over the world, with more than 25 million donors now registered, including more than 600 000 cord blood donors [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 and Robert Edwards. Since then, this 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 (PGD) [8] and clinical management (such as improving methods for ovarian stimulation and embryo culture conditions) – thereby leading to a considerable increase in the use of ART. To date, more than 5 million babies have been born worldwide through ART. Data from the International Committee Monitoring Assisted Reproductive Technologies (ICMART) show that around 1.5 million ART cycles are now performed globally each year, with around 350 000 babies born as a result [9]. This number continues to rise.

1.3. **European Committee on Organ Transplantation, the European Directorate for the Quality of Medicines & HealthCare and the 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 [10], 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 [11], and the newly appointed CD-P-TO took over as the steering committee [12].

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 (DH-BIO) and several 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.

1.4. Recommendations and regulations in the field

1.4.1. Council of Europe

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 [13]. 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) [14] 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) [15], 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) [16], signed in Strasbourg on 17 September 1974, lays the groundwork for development of mutual assistance in the supply of tissue-typing reagents and 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) [17], 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) [18], 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.

This latter 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) [19], 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 es-

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) [20], 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 [21], 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 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) [22], with its Explanatory Report [23], 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 legislations of member states relating to removal, grafting and transplantation of human substances [24];
- Recommendation No. R (94) 1 of the Committee of Ministers to member states on human tissue banks [25];
- Recommendation No. R (98) 2 of the Committee of Ministers to member states on provision of haematopoietic progenitor cells [26];

- Recommendation Rec(2004) 8 of the Committee of Ministers to member states on autologous cord blood banks [27];
- Recommendation Rec(2006) 4 of the Committee of Ministers to member states on research on biological materials of human origin [28].

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* [29], 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.

The Council of Europe also produces other guidelines, including this 3rd edition of the *Guide to the quality and safety of tissues and cells for human application*, the 6th edition of the *Guide to the quality and safety of organs for transplantation* and the 19th edition of the *Guide to the preparation, use and quality assurance of blood components*.

1.4.2. 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 [30]. 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 [31], which endorsed the updated WHO Guiding Principles on human cell, tissue and organ transplantation [32]

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 [33] 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 [34] was adopted in 2008, as an initiative of The Transplantation Society (TTS) and the International Society for Nephrology (ISN). 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. The declaration asserts that because transplant commercialism targets impoverished and otherwise vulnerable donors, it leads inexorably to inequity and injustice and should also be prohibited. Organ trafficking, transplant tourism and transplant commercialism were defined by the declaration by providing principles of practice based on those definitions. The Declaration of Istanbul distinguishes transplant tourism from travel for transplantation. Travel for transplantation is the movement of organs, donors, recipients or transplant professionals across jurisdictional borders for transplantation purposes. Travel for transplantation becomes transplant tourism if (1) it involves organ trafficking and/or transplant commercialism or (2) if the resources (organs, professionals and transplant centres) devoted to providing transplants to patients from outside a country undermine the country’s ability to provide transplant services for its own population.

Robust bi-directional donor–recipient traceability is a prerequisite to achieving effective vigilance and surveillance worldwide. For this reason, Resolution WHA 63.22 [31] 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 WHO to promote the sharing of informa-

tion on adverse incidents for improving safety and efficacy [35].

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 [36]. 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 [37, 38].

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.

1.4.3. European Union

1.4.3.1. EU tissues and cells legislation

Article 168 of the Treaty on the Functioning of the European Union [39] (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.

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 applications, including gametes and 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.

Directive 2004/23/EC [40] 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 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 [41] established specific technical requirements for each step in the human tissue and cell preparation process, in particular 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 tissue establishment and 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 [42].

Commission Directive 2006/86/EC [43] 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) [44] and a second one amending Directive 2006/86/EC, providing detailed requirements on the coding of human tissues and cells (Directive 2015/565) [45].

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 [46, 47].

The EU directives dictate that EU member states must encourage voluntary and unpaid donations of tissues and cells and must 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 mandates for the consent of donors and the anonymity of all 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 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'.

The European Commission has 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 [48]:

- 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) [49] developed guidance and training courses for EU competent authorities on the inspection of tissue establishments and on vigilance 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.
- EURO CET [50] 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) [51] developed a Good tissue practices guide 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 Banks (EATB), 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) [52] 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) [53] was launched in 2014. It is 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) [54] aims 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) [55] aims 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) aims 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 [56].
- 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.

1.4.3.2. *Other relevant EU legislation*

When human tissues and cells are used in the manufacture of medicinal products that are either a gene therapy medicinal product, a somatic cell therapy medicinal product or a tissue engineered product, Regulation (EC) No. 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products ('ATMP Regulation') applies [57, 58] (see Chapter 26). For such products derived from human tissues and cells, Directive 2004/23/EC and its implementing directives apply only to their donation, procurement and testing.

Clinical trials of medicinal products containing human tissues and cells must comply with the quality and safety standards laid down in Directive 2004/23/EC and its implementing directives, in addition to the EU legislation on clinical trials [59].

The EU Directives on active implantable medical devices (90/385/EEC) and on medical devices (93/42/EEC) are intended to be replaced by a Regulation on Medical Devices. The final text of the regulation was agreed in mid-2016 and final formal adoption by both the Council and the Parliament is expected during the first semester 2017. The revised requirements include medical devices combined with tissues and cells and medical devices incorporating non-viable derivatives of human tissues or cells, in particular human collagen [60].

Directive 95/46/EC on the protection of individuals with regard to the processing of personal

data and the free movement of such data [61] must be applied when processing personal data (e.g. data related to donors and recipients).

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

In practice, the decision to transplant 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

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 foetus; transfers oxygen and nutrients from mother to foetus.	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 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). Assisted reproductive technologies 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.

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 transplant 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 transplant 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 case 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 4. Specific criteria regarding tissues and cells are discussed in detail in the relevant chapters of Part B of this Guide.

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.

In summary, human application of tissues or cells can confer great benefit for a patient, but it is not without risk. In exceptional cases, donation of tissues or cells that does not meet all the neces-

sary 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 alternative options for treatment of that patient carry a poor prognosis. Similarly, couples undergoing ART 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 generic disease). 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.

1.6. 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 1.1.

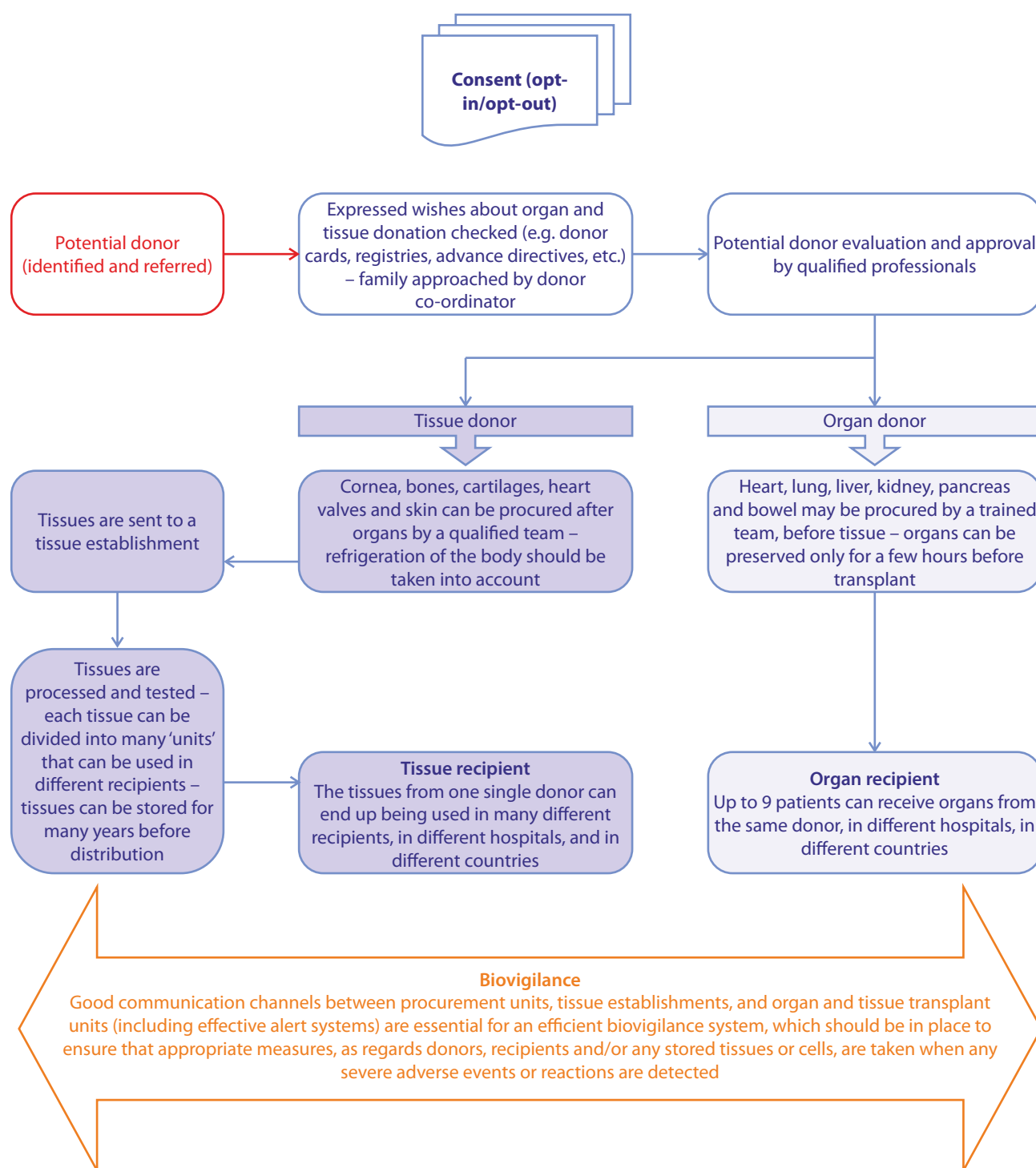
It is appropriate to conceptualise the entire process in terms of organisation and workflows. In the case of donation after death, transplantation can take place only if trained professionals are available to talk to the family of the deceased potential donor, if there is the necessary infrastructure to procure tissues within a given timeframe and process them, if appropriate transport services exist to transport tissues in an adequate manner and if surgeons are available to carry out tissue transplantation into the recipient. Similarly, for living donation to be made

possible, professionals need to recruit and evaluate potential donors, and adequately trained personnel must carry out the complex processes that will lead to generation of the medical products needed to treat patients.

The histories of the many forms of tissue banking and cell banking highlight the increasingly complicated and interconnected ways in which one

person's tissues and cells may be used to help others or themselves. The central role of tissue establishments in modern medicine, in providing material for treatment and research, highlights the complicated networks that may now connect the sources and recipients of donated bodily material, and the many intermediaries involved in processing the material, to facilitate its use by the clinical user.

Figure 1.1. **Complex links between donors and recipients in the context of donation after death**



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 the complex flows and multiple intermediaries involved in the process [62]. Awareness highlights the central part that must be played by organisations and organisational structures in the donation and subsequent use of bodily material. The process includes, for example, the creation of professional roles such as ‘donor co-ordinators’ and the extent to which they are expected to maximise opportunities for donation, how these professionals approach 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 possibility of 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 maintain 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 upward 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 phenotypic similarities 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.

1.7. Tissue banks, biobanks and tissue establishments

A ‘tissue bank’ is a term commonly used to describe an establishment that collects and stores human tissues or cells for either medical research or human application.

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. Within Europe, the terms currently in use are tissue establishments and biobanks, respectively.

The term ‘biobank’ is widely used for repositories storing human biological samples for use in research. Presently, there is not an internationally agreed definition of a biobank, but the term is generally used for organised collections of human biological material (blood, tissues, cells, other body fluids, DNA, RNA, etc.) and associated information stored for one or more research purposes. 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’ [63]. Several other definitions, as used in EU legislation/guidelines, are available on the website of the EU-funded project PRIVILEGED (Privacy in Law, Ethics and Genetic Data) [64].

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. [65]

The term ‘tissue establishment’ became widely used in Europe following publication of the 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.

In the ART field, the term ‘tissue establishment’ refers to the laboratories in ART 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 the context of this Guide, the term ‘tissue establishment’ will be used and refer to all of the banks, units, centres and clinics mentioned above. 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 the clinical application and practices undertaken in the clinical units of ART centres.

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, labeling, and distribution of tissue’ [66].

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 was agreed to use the term ‘tissue establishment’ and its definition in accordance with Directive 2004/23/EC.

1.8. Quality and safety

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 14) and vigilance (see Chapter 15), 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 ART 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 ART 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 13).

In the specific case of ART, 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 oocyte 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. All gamete donors should be recorded in national registers, and all centres should participate in the collection of national and international data. ART centres and Health Authorities should collect data on a systematic basis to follow up the long-term

health effects of ART activity, including the health of the donor, recipient and newborn. Good-quality evidence on these effects is essential for appropriate concern to be given to the welfare of oocyte donors in future policies. In addition, there should be a limit to the number of times a woman may donate, and a minimum interval between donations should be established. Ultimately, the welfare of oocyte donors should underpin any consideration about donation.

1.9. 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) [18] and the Additional Protocol on transplantation of organs and tissues of human origin (2002) [19]. 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 [24], the WHO Guiding Principles on human cell, tissue and organ transplantation [32] and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [34].

Any action in the field of tissues and cells for human application should be carried out in accordance with professional obligations and standard procedures.

A very wide range of tissues and cells may be donated. Many of these tissues and cells may be donated only after death, but some may be provided by living donors. Tissues donated for transplantation after death are governed by the same ethical principles as organs since they enter a common pool to be used according to need and their use cannot be directed to a particular individual. Cells such as those from the bone marrow can be donated by a living person and directed for transplantation to another specific person. Similarly, gametes may be donated

for use within the couple but may also be donated to unrelated recipients for altruistic reasons. 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 [67].

1.9.1. 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 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.

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 3. Specific cases related to consent in ART procedures are outlined in Chapter 24.

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 may be abused (for example, by using the donated material in a manner which is not in accordance with consent) and that additional material may 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 adequate, the person or official body providing authorisation for the human application, must be given appropriate information beforehand on the purpose and nature of the proce-

ture, 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, physicians will reduce the risk of nefarious trading and potential physical harm from the use of transplantable tissue for human application.

1.9.2. 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.

It is of the utmost importance that patients undergoing ART treatment should be counselled appropriately for their conditions and given realistic estimates of the prospects of success of their treatment, based on their age and specific medical circumstances. Similarly, the welfare of potential donors (especially with respect to oocyte donors) should be central in determining what constitutes acceptable practice. Gamete scarcity or financial profit 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 women 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 oocytes.

1.9.3. 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. 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 to carry 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 [68]. 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 (especially oocyte 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 the reasonable amount of 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, transportation, infrastructure and administration, and the need to incorporate state-of-the-art processes and equipment, among others.

The allocation of tissues and cells should 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.

1.9.4. **Equitable access to transplantation or to assisted reproductive technology (ART) treatment**

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 a decent minimum of 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 according to medical criteria. The persons or official bodies responsible for the allocation decision must be designated within this framework.

With regard to access to ART, 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 ART are heterogeneous and, in some countries, still under development. The debate on ethical and social issues (including access to ART 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 as a limited availability of non-

partner gametes and embryos), access cannot be expected to be unlimited. Ultimately, access to ART should be considered in a structured way to include efficiency, safety and equity to avoid discrimination [69]. 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 treatment have to pay for it themselves.

Cross-border reproductive care refers to a widespread phenomenon whereby patients seeking ART 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 law evasion if the technique is forbidden *per se* or if a particular group is excluded from treatment. There may be other limitations to access at home (e.g. long waiting lists). Other reasons for travelling are better quality of care and less expensive treatment [70, 71].

The ideal situation is fair access to fertility treatment at home for all patients. This ideal should be promoted at all levels [71]. However, if for some reason treatment at home is not possible or not available, cross-border reproductive care may provide a solution for patients. Furthermore, it is in accordance with the principle of freedom of movement of patients within Europe [72]. 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.

1.9.5. Equity in unpaid 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, or 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/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 renders certain social groups (and especially women because they are oocyte donors) in the field of ART particularly susceptible to disparities based on social and economic status.

With respect to cross-border reproductive 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.

1.9.6. 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 ART, 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. Other countries allow the possibility of the offspring to gain access to non-identifying information about the donor (e.g. hair colour, ethnicity). Other countries even 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).

1.9.7. 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 allocation, transplant activities and outcomes for both recipients and living donors, 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 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.

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Chapter 2. **Quality management, validation and risk management**

2.1. **Quality management**

2.1.1. **Introduction**

This chapter outlines the general principles of quality management systems (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 and import/export activities for tissues and cells are performed.
- b.* The quality management system, which is a tool 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 of this Guide.
- d.* The authorisations in place for the specific activities, from specific competent authorities.

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

- The International Organization for Standardization (ISO) requirements, as addressed in the ISO 9000 Quality Management System 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.
- 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.
- The EU Guidelines for Good Manufacturing Practices (GMP) [1] provide specific guidance for the preparation of medicinal products. However, much of their content is also relevant for 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 (ATMP), then the full requirements of GMP must be applied.
- Directive 2004/23/EC – 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.

- 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].

2.2. Applying quality management 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 the entire process. A good system addresses quality management under the following headings:

- a. Personnel and organisation;
- b. Premises, equipment and materials;
- c. Contractual arrangements;
- d. Documentation and record-keeping;
- e. Quality control;
- f. Quarantine and release;
- g. Process validation;
- h. Traceability;
- i. Complaints;
- j. Investigation and reporting of non-conformance, adverse events and reactions;
- k. Recall;
- l. Self-assessment, internal and external audit;
- m. Quality risk management;
- n. Fiscal and continuity planning;
- o. 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, signed by them. 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. 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. Each relevant organisation must also have an identified medical specialist/adviser who may or may not be the RP. The processing and quality-control functions should be independent to ensure the effective and reliable evaluation of processes. Tissue and cell manipulation should be carried out by appropriately qualified personnel. An adequate and independent audit system should be implemented.

2.3.2. Training

Personnel must receive initial and continued training appropriate to the duties assigned to them. Criteria should be defined and satisfied before declaring personnel qualified for a specific task or processing tissue and or cells. Training methods must be documented and training records maintained. The effectiveness of training programmes should be monitored by regular assessment of the competence of personnel. Personnel should also be trained in quality principles relevant to their duties and in the broad ethical and regulatory framework in which they work. When applicable, personnel should have relevant knowledge of microbiology and hygiene, and should be constantly aware that microbial contamination of themselves, donors, recipients and 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.

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. Standard universal precautions 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.

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, equipment and materials**

Premises and equipment 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 maintenance to avoid contamination and cross-contamination.

2.4.1. **Premises**

Suitable, quiet 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 clean-

liness. 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 facility, such as mortuaries, may also be adequate for the procurement of certain types of tissues, but they should be assessed for suitability on a case-by-case basis. Further guidance on facilities for tissue and cell procurement is given in Chapters 6, 7 and 8, 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. In this context, ‘critical’ means those consumables and reagents 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). These areas should allow for adequate (physical or electronic) segregation of those materials in quarantine from those released for use. They should be temperature-mapped and monitored when necessary.

Storage conditions for tissues and cells must be controlled and monitored. If certain conditions are critical to maintenance of the required properties of tissues or cells, appropriate alarms 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 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 7 and in Part B of this Guide.

Processing facilities should be dedicated to this activity and should be designed, validated and monitored to ensure that air quality is appropriate for the process being carried out. An international standard should be followed in full to achieve the appropriate air quality (e.g. rules governing medicinal products in the EU, *Volume 4: EU guidelines to good manufacturing practice*, or ISO 8573-1, ISO 14644 and ISO 14698). For tissue establishments in the EU, the zone in which the tissues or cells are exposed to the air must be Grade A, with a surrounding environment of at least Grade D (GMP classification), unless specifically defined criteria for exemption from this

requirement are met; the latter applies notably in the field of assisted reproductive technology (ART). Some national requirements are more stringent, requiring Grade B and C backgrounds for certain processes or tissue or cell types. Processing and storage facilities should be cleaned according to a schedule and procedure that has been validated to achieve the required level of cleanliness and all cleaning procedures should be documented. Where products containing tissues and cells are classified as ATMPs in the EU, fully GMP-compliant facilities are required.

More specific guidance on requirements for processing facilities is given in Chapter 8 and in Part B of this Guide.

2.4.2. Equipment

A list or register of equipment that might influence the quality or safety of the tissues or cells should be maintained (a validation plan). All equipment on this list must be designed, validated and maintained to suit its intended purpose and minimise any hazard to donors, recipients, operators or to the quality and safety of the tissues and cells. The validation plan should be designed through a risk-assessment exercise and should indicate when and how critical pieces of equipment should be validated and re-validated as necessary (see section 2.15). Equipment should be selected that permits effective cleaning. Maintenance, monitoring and cleaning must also be carried out according to a schedule and documented in equipment logbooks.

Trending and analyses of calibration and monitoring results (e.g. via statistical process control) 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 status of all equipment that requires calibration must be readily available.

To ensure appropriate performance of a system or equipment, a monitoring plan must be developed and implemented. The plan should take into account the criticality of the 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.

All equipment with a critical measurement function must be calibrated according to a planned schedule. Calibration is a procedure that confirms, under defined conditions, the relationship between values obtained from an instrument or system and those obtained using an appropriate certified standard. Calibration addresses accuracy and precision. ‘Measurement accuracy’ refers to the closeness of agreement between a measured quantity value and the true quantity value of what is being measured. ‘Measurement precision’ refers to the closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Hence, if the measured value is close to the true value, the measuring system has high accuracy and if the spread of the values is small when measurements are repeated, the measuring system has high precision. The acceptable tolerance should be set according to the critical quality attributes of a tissue/cells and these tolerance limits of the same equipment may have different needs depending on the tissues/cells subject of the process.

In practice, each piece of critical measuring equipment must be traceable. There must be an unbroken chain of calibration back to a recognised standard. Hence, the equipment 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 device used must be provided together with the calibration report. There must be an SOP that provides specific requirements for the calibration of each measuring device, such as defining the frequency of calibration, the number of measurement repeats, and the expectations and interpretation of obtained results which define acceptable limits for accuracy and precision. If the limits of accuracy and precision are not met, there must be provisions for remedial action to re-establish 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 or precisely, risk assessment should be applied to decide on appropriate corrective or preventive actions regarding the fate of the tissues and cells.

A periodic review process should be established to ensure that the documentation for system or equipment is complete, current and accurate. If

deviations or problems are found, actions should be identified, prioritised and planned.

2.4.3. Materials, consumables and reagents

A controlled list should be constructed of all materials and consumables 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 reagents and consumables must be documented. Only materials from qualified suppliers that meet the documented specifications should be used. When indicated, 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. Equipment and materials should conform to international standards and EU and national licensing arrangements, where these exist.

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

Apparent deviations in the quality and/or performance of equipment and materials must be investigated and documented 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, reported to the Health Authority.

Further guidance on reagents and materials used in tissue and cell processing is provided in Chapter 7.

2.5. 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 describes the roles and responsibilities of all parties for maintaining the quality chain and the quality requirements for the service provided. Agreements should allow for on-site audits of contracted third parties to confirm their compliance to expectations. An example of an expectation is that when a supplier changes specifications for equipment or reagents provided to a tissue establishment, or they provided a substitute

for an ordered item, they must first ensure that these changes are acceptable to the tissue establishment.

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 processed in co-operation with a third party. They must keep a complete list of these agreements and make them available at the request of Competent Authorities.

Agreements must 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 software applications.

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 storage conditions, appropriate handling and preparation before use, and full traceability must be maintained. Maintenance of quality and traceability is usually achieved by providing users with clear and detailed written instructions. The 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. However, this is not necessary for partner donation treatment in an ART centre or autologous haematopoietic progenitor cell transplantation where all steps (including clinical application) are carried out in the same facility.

Distribution direct to the patient for use without supervision of a health professional (e.g. autologous serum drops) requires particular attention to instructions for storage and use, and should be carried out only if it is the only available option. Direct distribution of sperm to individuals for use without supervision of a health professional should be avoided for quality and safety reasons.

2.6. Documentation and record-keeping

Documentation must enable all steps and all 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. In ART, traceability also involves the follow-up of the outcome from these treatments, including the children (see Chapter 24). 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 documentation in general, and donor referral records, in particular must be subject to the same controls.

Documentation must be version-controlled and include at least the following items:

- a. a quality manual;
- b. specifications for materials and reagents;
- c. approved SOPs for all activities that influence the quality or safety of the tissues or cells, including the management of the quality system itself;
- d. identification and analysis of risks and a risk-mitigation plan;
- e. records of the performance of operations, including processing records;
- f. records of complaints, audits and non-compliances;
- g. training and competency records of personnel;
- h. qualitative and quantitative specifications for tissues and cells;
- i. key quality indicators for tissues and cells.

Documents, including SOPs and forms, must be approved by appropriate and authorised persons and be part of a document control system that ensures only the current version of the document is in use. The system for distribution of controlled documents must ensure that all relevant personnel have access to the correct version.

A documented system for change control should be in place that controls changes to premises, equipment, processes, personnel and any item that may impact the quality and safety of the tissues and cells. This change control system should link the rationale for change with the approval/rejection of the proposed change, criticality of the change with respect to the quality and safety of the tissues and cells; impact of the change on the tissue establishment as a whole, validation requirements of the proposed change and associated training requirements.

Records must be legible and indelible and should not be handwritten, except for those situations where data can be recorded only in this way. Any alterations made to a record must be dated and signed. Documentation must be retained according to national requirements. Processing records must be maintained for all critical steps, and they must be dated and signed by the personnel responsible for carrying out the activity. All quality-control tests and checks must be documented. Any deviations from the standard documented procedures must be recorded and reviewed, and corrective action must be documented.

The QMS must define the period of time for which documents 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, must be retained for 10 years and traceability documentation for 30 years after use or expiry of the tissues and cells. Data can be stored on paper, electronically or on microfilm. International and national regulations on data protection have to be respected. Personnel should only have access to those categories of data for which they are authorised. See Chapter 12 for further guidance on computerised systems (including requirements for their validation).

Quality specifications should be prepared for each type of tissue and cell graft; these should be the basis for quality-control testing and product release.

2.7. 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 also 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 (see also 7.6.1).

Detailed guidance on microbiological testing is provided in Chapter 9. Guidance on specific quality-control tests for specific types of tissues and cells is provided in Part B of this Guide.

2.8. Quarantine and release

All tissues and cells must be stored with an unambiguous quarantine status until all quality-control tests and checks have been conducted and

the results reviewed by the individual responsible for release. Release of tissues and cells may be conducted in two steps. The first step confirms compliance of the donor with defined acceptance criteria (which is usually carried out by clinical personnel). The second step confirms compliance of the tissues or cells themselves, their characteristics, processing and storage, with those criteria defined in the product specification. The latter is usually carried out by quality assurance personnel. The concept of 'quarantine and release' is not applicable to partner donation in ART and to some types of autologous or direct donation (see Chapter 11 for guidance on Exceptional Release). Tissues and cells that cannot be categorised as 'released' during storage must be stored with an unambiguous quarantine status.

2.9. 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 and consistent with the approved specifications. Written procedures should be in place to describe the actions to be taken if a change is proposed to a 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, documented and accepted formally. The likely impact of the change of 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 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) must 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 regulatory authority.

2.10. Traceability

Full traceability of donations from donor to recipient and of all materials, reagents and equipment that come into contact with tissues and cells is fundamental to recipient safety. Detailed guidance is provided in Chapter 14.

2.11. 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 the assessment of whether the complaint is justified and related to a potential non-compliance. The latter should then be investigated thoroughly, including root-cause analysis and identification of corrective measures (see section 2.12 for details).

2.12. Investigation and reporting of non-conformance, adverse events and adverse reactions

Examples of non-conformance include deviations from SOPs, errors and accidents. Non-conformance might result in an adverse reaction in a living donor or in a recipient and must, therefore, be linked to the vigilance reporting system. There must be an SOP in place that defines how the organisation manages non-conformance and includes a log of all the instances of non-conformance that are investigated, including detailed documentation of the investigation, root-cause analysis and corrective/preventive actions taken. A categorisation of cases of non-conformance depending on criticality with respect to the quality and safety of tissues and cells is a useful tool for prioritising corrective actions. Procedures should be in place to identify appropriate corrective and preventive actions to be taken and to inform the relevant authorities as appropriate. Reporting of errors and incidents in a non-punitive context should be encouraged to help achieve improvements in practice. Tracking and trending of non-conformance should be carried out to identify common failures and identify areas for concern.

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 15. If products containing tissues or cells are classified in the EU as ATMPs, adverse occurrences should be reported through pharmacovigilance systems for process events and through biovigilance systems for donor reactions.

2.13. 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 may be necessary. Also, 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 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 15.

2.14. 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.

External audits are undertaken by independent bodies (often designated as approved/competent authorities or ISO certifying bodies) and are required for certification, accreditation and licensing 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 their completion should be recorded.

2.15. Validation

2.15.1. General principles

Validation is the part of the quality management system concerned with proving that all critical

aspects of the establishment's operations are sufficiently controlled to provide continual assurance that tissues and cells will remain safe for patients and fit for purpose. The critical aspects subject to validation include:

- the facilities and equipment used in procurement, processing, storage, testing and distribution, and any software used to manage their operation and data;
- materials and reagents used which come into contact with cells and tissues;
- labelling and tracking materials, equipment and software;
- operational staff and the written procedures that they use to instruct their work;
- process stages from collection 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;
- analytical test methods used to assess and confirm the safety and quality of donors, donations, tissues and cells;
- other auxiliary processes such as the transport and cleaning processes.

Validation is 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. Small 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 to confirm that the method has the intended outcome. Such small establishments should still document their validation policy explaining their approach on the basis of risk.

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 process or test method validation. 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 re-qualified at predetermined intervals, or when significant changes are made. Each individual item should be qualified separately to demonstrate consistent performance.

Process or test method validation should only be performed once all the items used have been qualified. Process validation 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 validation (retrospective validation). Any process or method changes should be assessed for impact and risk in accordance with quality risk management principles (Section 2.16) and re-validation considered where there is unacceptable risk.

The objective of validation is to challenge the critical aspects through a series of controlled tests representative of the conditions under which they are expected to operate to demonstrate that they achieve predefined quality and safety acceptance criteria. 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 test methods to be used and the acceptance criteria should be documented and approved by the establishment management before qualification or process validation commences. This document is commonly called the Validation Plan. The validation should be performed by trained and competent persons. The results of the validation 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, 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 and which are in the process of validation. Where anything is not in a fully validated state, there must be controls to prevent its use.

2.15.2. Validation planning

The Validation Policy should consider a process design phase where deep knowledge of the process is achieved. 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, transport, etc. 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 establishment operational, quality, regulatory and medical experts. Where necessary, for example the validation of new cleanroom facilities, external experts may be employed to advise.

Effective validation is not possible unless the establishment management is completely clear what their specific technical and quality requirements are. Establishments should use Quality Risk Management procedures to help determine their expectations for their processes and items used, which must address any significant risks to donors, recipients or quality of products and 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, 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, product characteristics etc. 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 Responsible Person in accordance with EU legislation, then that person should supervise all validation activity and be responsible for approving or rejecting the outcome of validation.

2.15.3. 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:

- a. Validation policy
- b. Organisational structure of validation activities
- c. Summary of facilities, systems, equipment and processes to be validated
- d. Documentation format
- e. Planning and scheduling
- f. Change control
- g. Reference to existing documents

2.15.4. 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. The usual approach is to conduct simulated processes using culture medium

in place of, or added to, tissues or cells. Each operator should be qualified by performing, for example, 3 consecutive medium simulation processes.

Before written procedures (SOPs and Work Instructions) are approved as part of the document control system (see 2.6) they should be read and qualified by an experienced operator to confirm that they are clear, understandable, accurate and practical.

2.15.5. Qualification of materials and suppliers

Detailed User Requirements Specifications (URS) should be available for materials (see 2.4.3 and 2.15). 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 process qualification of the material. Process 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 system. This should be confirmed through audit (see 2.14) of their operations and quality system. It is possible to conduct the audit through a questionnaire supported by copies of relevant certification from a recognised independent body or regulatory authority. Otherwise an on-site audit should be performed. The information gathered through the audit should be formally documented and assessed before the supplier is considered qualified.

2.15.6. Qualification of facilities and equipment

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

Facilities and equipment should be qualified following the four steps shown below. Each step should be completed and verification obtained that acceptance criteria have been met, before proceeding to the next step.

2.15.6.1. Design qualification

The first element of the validation of new facilities, systems or equipment can be considered ‘design qualification’ (DQ). This validation involves demonstration and documentation of the compliance of the design with Good Practice (i.e. the design is suitable for the intended purpose). DQ is not required for off-the-shelf equipment and systems as these have already been designed and built for specific uses. Only new facilities and equipment being designed or built specifically for the establishment require DQ. DQ should be complete before equipment, system or facilities fabrication starts.

2.15.6.2. 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. verification of construction materials.

IQ for new 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.

2.15.6.3. Operational qualification

Operational qualification (OQ) should follow IQ. OQ should include (but is not limited to) the following:

- a. tests that have been developed from knowledge of processes, systems and equipment;
- b. tests to include a condition or a set of conditions encompassing upper and lower operating limits (sometimes referred to as ‘worst-case’ conditions).

Completion of a successful OQ should allow calibration, operating and cleaning procedures, operator training and preventative maintenance requirements to be finalised. It should permit a formal ‘release’ of the facilities, systems and equipment.

2.15.6.4. Performance qualification

Performance qualification (PQ) should follow successful completion of IQ and OQ. PQ should include (but is not limited to) the following:

- a. tests, using production materials, qualified substitutes or simulated products, 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 of the process, the less testing during the PQ may be needed.

2.15.7. 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 collection, 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 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 it is put into service. At the very least this 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 UAT to verify

that existing functionality continues to operate as expected (regression testing) as well as testing new functionality.

2.15.8. Test method validation

The approach to test method validation will depend on whether the test is quantitative or qualitative. 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. For quantitative assays the acceptance criteria should consider accuracy, reproducibility, linearity, limits of detection and required range of measurement. The uncertainty of measurement should be established and quoted with subsequent results. For qualitative tests then specificity and sensitivity are the key criteria. It may be considered unnecessary to perform in-house method validation if test systems and kits certified as compliant with the EU *In-vitro* Diagnostic Medical Device Directive are used along with qualified equipment in accordance with the manufacturer's instructions. However, in-house verification studies should be done to demonstrate that the performance of the kit or test system, as used in the establishment, meets the expected specification. If using *Pharmacopeia* methods, e.g. for sterility testing, the methods must be validated in accordance with the method monograph.

2.15.9. Process validation

Process risk-assessment methods should be used to identify what processing stages require validation. Most processing of tissues and cells involves the removal, exclusion or reduction of unwanted or undesirable substances, while maintaining the functionality of the required tissue or cells. As a minimum, validation will focus on demonstrating that the desired characteristics are achieved in performing consecutive processes (usually 3) and confirming that the purity specifications have been reproducibly met in all cases. Process validation may also include *in vitro* and *in vivo* tests of functionality, where there is a risk that this may be affected. However, because of the inherent variability of human cells and tissues, establishments should consider supplementing prospective or concurrent validation with an ongoing process-verification programme of quality-control testing before release and of quality monitoring.

The processes for removing undesirable substances and in particular potentially pathogenic micro-organisms should be validated with a safety margin, or 'worse case' scenario. This will usually

involve spiking the material with a larger-than-normal level of the undesirable substance and demonstrating its effective removal, or reduction to safe levels, by the process. In the case of micro-organisms, strains that are known to be resistant to antimicrobial treatment, e.g. spore-forming, heat-resistant bacteria, may be used for spiking and to validate sterilisation processes, at sufficient levels to demonstrate at least a 6 log reduction. For safety-critical process validation it is recommended that published guideline methods are consulted where available.

More specific guidance on approaches to validation is given in other sections of this Guide. Some examples of qualification are given in Appendices 4 and 5, and examples of validation are in Appendices 6 and 7.

2.16. Risk management

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 assessment exercise. Risk assessment should include an estimation of the severity of any identified hazard (source of harm) and an estimation of the probability that the hazard will result in harm. Probability should be based on evidence and experience whenever possible.

Risk-mitigation strategies should be developed to protect the tissues and cells, the donor and recipient, 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. The level of effort, standardisation and documentation of the risk control process should be aligned with the estimated risk level.

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, particularly when departures from standard procedures and non-

conformance with standards and specifications are under consideration. Examples would 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 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, 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 5 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 Failure Mode and Effects Analysis (FMEA), Failure Mode, Effects and Criticality Analysis (FMECA) and Hazard Analysis and Critical Control Points (HACCP). These methods estimate severity and probability, but FMECA also includes a factor for detectability, taking into consideration that those hazards that are more easily detected represent a lower overall risk. FMECA allows the estimation of a risk priority number (RPN) for the ranking of identified risks. 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.

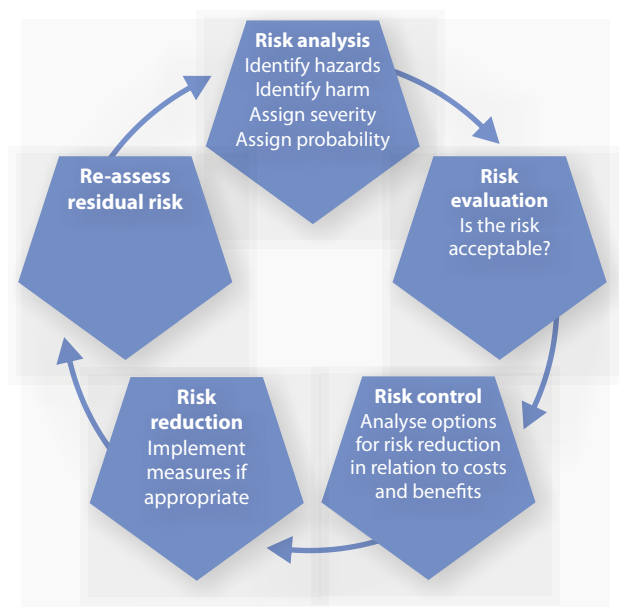
Undertaking risk assessment at various stages helps to define requirements and alternatives, aids in the process of supplier selection and helps to determine the scope and extent of validation. The methodology has been used for tissue and cell banking for

specific process steps [3] and for reviews of an entire process [4].

Risk assessment is not a 'one-off' process but a cyclical one (Figure 2.1). Risk assessment should be followed by risk avoidance and reduction (if possible) and continuous re-evaluation of residual risk.

Guidance on quality risk management is provided in Part III Q9 of the Rules governing medicinal products in the EU, Volume 4: *EU Guidelines for good manufacturing practice for medicinal products for human and veterinary use* [1], 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.

Figure 2.1. Cycle of risk assessment



2.17. 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 – should 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.

2.18. References

1. EudraLex – Volume 4, EU Guidelines for Good Manufacturing Practices – Medicinal products for human and veterinary use, available at ec.europa.eu/health/documents/eudralex/vol-4/, accessed 13 January 2017.
2. De los Santos MJ, Apter S, Coticchio G, Debrock S, Lundin K, *et al.* Revised guidelines for good practice in IVF laboratories (2015). Human reproduction. 2016;31(4):685-6.
3. 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.
4. 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.

Related documents:

Appendix 4. Example of cleanroom qualification;

Appendix 5. Example of incubator qualification;

Appendix 6. Example of a process validation;

Appendix 7. Examples of method validation (oocyte vitrification) in assisted reproductive technology.

Chapter 3. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate

3.1. Introduction

Developments in medical and biotechnological sciences include the use of human tissues and cells in transplantation therapy or for assisted reproductive technologies (ART). Some human tissues can only be obtained from deceased donors. Some other tissues and cells can be provided only by living donors, as long as this procedure does not endanger the donor's life or health. 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.

Ultimately, in order to ensure the success of any transplantation or human application programme, potential tissue and cell donors need to be recruited, in the case of unrelated living donors, or identified and referred. In any case (living or deceased donors), screening must be performed to exclude any contraindications to donate.

Successful donation programmes should at least include [1]:

- a. Adequate public awareness strategies, addressing not only organ donation but also tissues and cell donation;
- b. Effective systems to facilitate the identification and referral of all potential deceased tissue donors (e.g. routine medical chart reviews in

every case of in-hospital death) and the recruitment of living donors in an ethical manner and ensuring their safety and well-being;

- c. Adequate training of professionals involved in the recruitment of living donors, and on the identification and referral of deceased donors.

Once potential donors are recruited, or identified and referred, and before procurement can take place, consent or authorisation to donation is needed, given either by the donor before procurement if alive, or for deceased donors either by the donor before death (e.g. donor registries, donor card, advanced directives) or by the family. The way in which consent or authorisation is obtained will vary depending on the type of donor, the specific circumstances and the different legal systems for consent. In the case of deceased donors, communication with bereaved family members will require clear and sensitive procedures or protocols with consent obtained by appropriately trained specialists in donation (see Appendix 8).

Although the term 'consent' will be used throughout the 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.

3.2. Living donors

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

Some tissues can be collected as surgical residues (e.g. placenta, femoral heads removed during an operation to replace a hip joint, and heart valves from patients receiving a heart transplant). In some cases, tissues from a patient must be processed and stored for his/her own treatment in the future (e.g. skull bone obtained from a craniotomy, parathyroid tissue).

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;
- 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 an:
 - i. intended recipient, who can be related or unrelated to the donor (e.g. HPC);
 - ii. undirected recipient, unrelated to the donor (e.g. amnion).

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

- iii. partner donation (donation between a couple who declare that they have an intimate physical relationship);
- iv. non-partner donation.

The decision to be a living donor must be weighed carefully: benefits *versus* risks, for both donor and the recipient. In the case of surgical residues, there is no risk for the donor derived from the donation itself.

In some instances, donation may take years to happen or even never be fulfilled (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).

3.2.1. Donor recruitment

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

One way to increase awareness is to explain the benefits for the recipients of donated tissues and cells. Success stories describing patients' experiences and testimonials of family members may drive people to consider whether tissue donation is right for them. However, public campaigns should aim to increase undirected donations and registration on living donor registries. Publicising and/or advertising the need of donated tissues or cells for a given patient should be discouraged (in some countries such behaviour is 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 ART procedures.

3.2.1.1. Recruitment of haematopoietic progenitor cell donors

Successful HPC transplantation depends on matching the donor and recipient for human leucocyte 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 compatible related donor is under the age of 18, special consideration is needed to make sure that no harm is done to the donor (see Chapter 21). For those without a matching family member, there is a hope that a volunteer can be found who, by chance, has a matching HLA type. Therefore, it is of crucial importance that volunteer donors are recruited and registered on HPC registries around the world, particularly from diverse ethnic communities. National HPC registries are linked internationally using tissue-typing to establish rapid identification of potential donors.

When a person makes the decision to volunteer as an HPC donor, an initial evaluation of the potential donor will be performed, they will be HLA typed and their details placed on a donor registry. The potential donor will be requested to contact the registry if there are changes in their health status that could contraindicate donation. They will not be contacted again unless they are identified as the best possible match with a particular patient. If that is the case, the potential donor will be invited to the donation centre for an explanation of the procedures from one

of the clinical staff. The potential donor will also have a thorough medical examination by a doctor and will be asked to give consent for a number of blood tests, to ensure there are no medical contraindications to donation.

As with any other tissue or cells, donation of HPC should be voluntary and unpaid, and informed consent must be given in advance.

Further information on recruitment of HPC donors and HPC registries can be found in Chapter 21.

3.2.1.2. *Recruitment of donors in assisted reproductive technologies*

With the development of ART 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. 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 explained earlier, depending on the origin of the gametes and embryos, the donation can be classified as partner or non-partner donation. For the purpose of this chapter, we will only be addressing 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 factors such as:

- a. the reason for the donation, whether treatment of others or research;
- b. the type of donor, because the donation process for oocytes and sperm or embryos are very different in complexity, the medical interventions required and the risk of harm;
- c. the anonymity of those involved, whether known, identifiable or anonymous, and different compensation schemes for donors;
- d. the number of times a donor can donate and the number of offspring that may derive 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 voluntary and unpaid donation. Any type of reward, benefit or incentive may be a threat to voluntariness (see Chapter 24).

The donor recruitment activity, whether performed by 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. Unlike HPC registries, the purpose of these is not to have a database of potential donors but to allow for transparency of practices, traceability (see Chapter 14) and 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 15).

Further information on recruitment of gamete and embryo donors can be found in Chapter 24.

3.2.2. **Consent for living donation**

Since in most cases the individuals who donate tissues and cells for the benefit of others accept risks and inconvenience they would not otherwise face, the donation process is substantially different from undergoing a medical intervention for their own benefit. Donation therefore 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, which is why obtaining individual consent is crucial to assure that donation 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 before an official body. 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 they can understand. Some examples of forms to obtain consent for ART may be found in Appendices 9 and 10.

Recruitment of persons not able to consent should never be done through public registries. In addition, in some countries, specific regulations exist limiting 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).

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 or, in some cases, a person who has a very close relationship with the donor;
- c. the donation has the potential to be life-saving for the recipient;
- d. the authorisation of their representative, 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.g. expert committee or ethical committee);
- e. the potential donor concerned does not object and their opinion should be taken into consideration in proportion to their age and level of maturity.

Potential donors must beforehand be given appropriate information as to the types of tissues and cells to be procured, the purpose and nature of the intervention, its consequences 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 before they give consent. Full understanding of the consent is particularly important when practices may be controversial (e.g. the use of gametes or embryos for research purposes). 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 for a relative in need should have a donor advocate, who must not be the same physician responsible for the recipient. Information about potential risks for the donor and risks and benefit for the recipient must be clearly understood

before consent is given. In the case of HPC donors, they must also be informed that the results of the transplant are uncertain because they depend on many factors, including the recipient's situation. 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 must 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. In the specific case of gametes and embryos, some countries have regulations on the duration of storage.

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, should consider the donor's needs and should be able to answer questions about donation and transplantation processes.

The potential donor should be informed that tests will be performed to evaluate the possible existence of any transmissible diseases that would pose a risk to the recipient, and what would happen in the event of a positive result. In that case, they must be informed and receive adequate counselling. All results of the medical evaluation should be made 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 donor who is a carrier of a haemoglobinopathy) then the transplant centre must also be informed.

In the case of HPC donors, consent must be obtained at several stages: HLA typing, testing, stimulation and donation, including the collection method and the need to administer growth factors when peripheral HPC will be collected. In the case of cord blood donation, consent is usually obtained months before the delivery and should be obtained at least before the mother goes into labour to avoid interfering during delivery.

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

Donors must be informed that they may freely withdraw consent at any time. In case of HPC donors, they should 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 the case of autologous donation, the patient must be informed about options and the balance of risks to 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 final product or if the patient's condition contraindicated application). In such instances, the consent should indicate whether the tissues/cells could be used for the treatment of others, for research or disposed of.

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

In the case of planned surgical procedures where it may be possible to donate residues, 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 and viability of the final product and consent should include information about the processing, storage and intended use of the donated material.

3.3. 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, meniscus, fascia, cartilage;
- b. cardiovascular: heart valves, blood vessels, pericardium;
- c. skin: split thickness (typically, only the epidermis) and full thickness (epidermis and dermis);
- d. ocular: corneas, sclera;
- e. specific cell types from certain tissues (e.g. limbal stem cells).

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 identify and refer potential donors, obtain consent from potential donor families and refer donors to the appropriate procurement agencies or tissue establishments.

3.3.1. 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 for the elderly.

It is recommended that any potential donors considered for organ donation are also referred for potential tissue donation. 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, the cause of death or the known wishes of the donor or their family to become a tissue donor. 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.

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. Some key parties are summarised in Table 3.1.

The medical suitability of potential donors should be investigated 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, family doctor or other persons who have information about the donor's behavioural and medical history. Selection criteria must be taken into consideration before a donor is accepted, and risk factors such as sexual behaviour, travel and exposure to sources of infection must be evaluated. These criteria may vary depending on the type of tissue to be procured for human application (see Chapter 4 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 hospital or procurement centre.

Table 3.1. Key parties in 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.	- Lack of knowledge about donation; - 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; - 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 his/her family; - Increase availability of tissues for patients in need; - Contribute to public awareness of donation and transplantation.	- Discomfort or lack of knowledge about donation; - 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 to organ donation; - 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 home director	- 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 and nursing homes	- Support wishes of the donor and his/her 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.

3.3.2. 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 if donation was in accordance with the person's wishes, values and

beliefs, and whether the deceased had expressed an objection to donation during their lifetime, and give consent accordingly.

As explained before for living donors, it is important to emphasise that consent must be specific. Therefore, donors 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, for example, when the donated tissues may be sent to a biobank for research or used as starting material for advanced therapies by commercial organisations (see Chapter 2).

3.3.2.1. *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: one is an opting-in system, where consent to donation has to be obtained explicitly from the donor during their lifetime or from an authorised individual (usually the next of kin); the other is an opting-out system, in which consent to donation is presumed where no objection to donation has been registered by an individual during their lifetime or is known to the donor's family. In practice, variations exist within both systems, and the family still plays a prominent role in the decision.

An example of an opt-in system that allows the donors themselves, or their relatives after their death, to give consent is the Human Tissue Act 2004, which applies to England and Northern Ireland. According to this 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'. The Act prescribes a hierarchy of qualifying relationships (ranked from highest to lowest: spouse or partner, parent or child, brother or sister, grandparent or grandchild, niece or nephew, stepfather or stepmother, half-brother or half-sister, long-standing friend) and states that 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 material.

Table 3.2 gives an overview of national consent systems in Europe. The information is reproduced from a survey by the European Commission in August 2014 (Directive 2010/53/EU Implementation Survey). Of the 29 answering countries, it appears that the majority (18 countries) have an 'opt-out' system, 7 countries have an 'opt-in' system and 4 a mixed system. For example, in the United Kingdom there is an 'opt-in' system in three of the four UK administrations (England, Scotland and Northern Ireland), but Wales introduced an 'opt-out' system from December 2015. Other countries combine elements of both 'opt-in' and 'opt-out'.

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 it 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 may contain detailed information, e.g. consent 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 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 doctor or a donor co-ordinator involved in the donation process.

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

In other circumstances, when the expressed wish of a person was to become a donor but the relatives are absent or cannot be 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 doctor or friends). If this information necessary to establish the safety of the donor cannot be obtained, donation should not proceed.

Table 3.2. Legal provisions in European Union countries (and Norway) for consent to/authorisation of organ donation from deceased persons

Country	National consent system	Donor registry	Non-donor registry
Austria	opting-out		✓
Belgium	opting-out	✓	✓
Bulgaria	opting-out		✓
Croatia	opting-out		✓
Cyprus	opting-in	✓	
Czech Republic	opting-out		✓
Denmark	opting-in	✓	✓
Germany	opting-in, other*		
Estonia	mixed system	✓	✓
Finland	opting-out		
France	opting-out		✓
Greece	opting-out		✓
Hungary	opting-out		✓
Ireland	opting-in		
Italy	mixed system	✓**	✓**
Latvia	opting-out	✓	✓
Lithuania	opting-in	✓	
Luxembourg	opting-out		
Malta	opting out		
The Netherlands	opting-in	✓	✓
Norway	opting-out		
Poland	opting-out		✓
Portugal	opting-out		✓
Romania	opting-in	✓	
Slovakia	opting-out		✓
Slovenia	opting-out	✓	✓
Spain	opting-out	✓**	✓**
Sweden	mixed system	✓	✓
United Kingdom	mixed system	✓	✓

Notes: *"other" in survey; "opting-in" according to personal information. **Advanced Directive registry allows for registering willingness to donate or not to donate organs after death.

European Commission's Implementation Survey regarding Directive 2010/53/EU, status: October 2015.

3.3.3. 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 for the donation of tissue, 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 assist the family in their mourning and provide some solace in the knowledge that the wishes of the loved one are being fulfilled and that some good will come out of their death.

Conversations with a family about organ donation do not generally differ from those related to tissue donation. Therefore, 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.

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, so that they can support the family and champion a favourable attitude towards donation; they should not be limited to making a simple translation. The conversation should be planned, carried out at the right time, in the right place and by the right people. Proper preparation for the conversation reduces the likelihood of errors and the need for improvisation [3, 4, 5]. 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, some food).

In certain circumstances, families of potential tissue donors may be interviewed over the telephone. 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 and this can increase emotional distance. However, appropriately trained personnel should be able to accompany the family and provide support under these circumstances.

The doctor 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.

Conversation about tissue donation aims 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. Acceptance to donate 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.

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

- 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.
- 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 giver to charity, and how donation could help many people to benefit from a transplant.

The experience of interviews with families suggests that some difficulties and possible opposi-

tion 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 will not notice anything if they see the deceased person after procurement, albeit there can be rare problems such as bruising or bleeding.

- In the case of religious concerns, offer a consultation with a religious leader or representative.
- In cases of dissatisfaction with the healthcare provided, record the complaints but explain that the issue of donation should be kept separate.
- 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.
- 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.

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 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, etc. 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 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. All questions posed by the potential donor family should be answered and, beyond that, professionals should apply their own judgement to decide how much information the family would want or need to obtain. While providing the information, they may observe agitation, frustration or irrita-

bility in a family member; this may signal unwanted 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 with the type of consent being provided and should be increased if the potential application of the donated material is controversial.

It is helpful to ensure that, following donation, the family receives the appropriate care 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. Finally, establish whether the family wishes to assist with the final preparation of the body following donation, such as washing or dressing in certain items of clothing.

3.4. Conclusions

The continuing development of transplantation 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 the tissue and cell donation programmes.

Since tissues and cells come from a human being, either living or deceased, it is necessary to ensure that donors have the autonomy to decide freely about matters that are essentially 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.

3.5. References

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Related documents:

Appendix 8. Sample consent form;

Appendix 9. Sample consent form (women);

Appendix 10. Sample consent form (women);

Appendix 11. Sample consent form (men).

Chapter 4. Donor evaluation

4.1. Introduction

The reason for donor screening is twofold: first, to minimise the risk of transmitting disease to a recipient and, second, to exclude any tissues or cells whose quality may be adversely affected by a characteristic, including medical or other conditions, of the donor. An important part of the evaluation of living donors should be to assess whether the procurement process itself could be harmful to the donor and to ensure that arrangements are in place for long-term follow-up after procurement. Special attention is required for donors under 18 years old. These aspects of living donation are detailed in Chapter 21 on haematopoietic progenitor cells (HPC), Chapter 24 on assisted reproductive technology (ART) and Chapter 25 on fertility preservation. The screening of potential donors is critically important for the quality and safety of tissues and cells for human application or assisted reproduction.

There are autologous and allogeneic donors: the latter may be living or deceased. Accordingly, the evaluation criteria may vary for these different types of donor. The evaluation of autologous donors is a special situation as the donor may be a person being treated for a disease. Moreover, autologous donation may provide the starting material for a manipulated product. A thorough evaluation of the autologous donor is recommended.

In general, allogeneic donor evaluation consists of:

- a. obtaining information about the donor to identify absolute and relative contraindications to human application that may pose risks for a recipient:
 - i. medical history (including genetic disease and a family history of disease);
 - ii. social history (personal and behavioural information, including travel history);
 - iii. physical examination;
 - iv. psychological examination (living donors) – see Chapters 21 and 24;
 - v. tests for markers of transmissible disease by serology, microbiological testing and further complementary tests, such as nucleic acid technology (NAT) testing, which may be helpful in resolving the problem with specific donors (e.g. donors with steroid or immunosuppressive treatment), as detailed in Chapters 5 and 9;
- b. ensuring that the donation will not cause harm to a healthy living donor.

During donor evaluation, confirmations of the consent and of donor identity are essential steps (see Chapter 3).

For European Union (EU) member states, the selection criteria for deceased donors (including additional exclusion criteria for deceased child donors) and living donors of tissues and cells are specified in Annex I/III of Directive 2006/17/EC.

4.2. General evaluation of potential donors

4.2.1. Autologous donors

The evaluation of autologous donors is based on the disease/condition being treated. The clinician caring for the donor is making the decision on autologous donation/application according to guidelines and relevant scientific data. All relevant medical information should be available to enable the clinician to make the correct decision.

As the autologous donor is being treated for the disease/condition in question, the relevant medical history, results of laboratory tests and physical examination results are all available to the clinician. Eligibility for donation is evaluated on an individual basis, taking into consideration the possible complications and benefits. It is a general rule that harm to the patient should be minimal during the donation process.

If the procured tissues or cells will be processed and/or stored, screening for the same biological testing must apply as for an allogeneic living donor (see Chapter 5), although the results are not necessarily a contraindication for autologous donation as long as procedures are in place to avoid cross-contamination with other processed/stored tissues or cells. This applies to HPC and ART, and also if the tissues or cells will be used as starting materials for advanced therapy medicinal products (ATMP). In those cases the safety refers not only to donation (i.e. apheresis, see Chapter 21), but also to the safety of application of the cells (i.e. risks for transfer of living tumour cells). The expanding field of immunotherapies for malignancies, either with dendritic cells or tumour-specific T-cells (see Chapter 28) will utilise donors/patients that otherwise would be excluded as donors due to their malignancy. Safety aspects for the donor, but also the risk of transmission of infection to personnel during processing and storage of these cells, should be considered.

4.2.2. Allogeneic donors

Allogeneic donors can be living donors related to the intended recipient or unrelated (HPC donors who came from registry). Procurement of tissues from deceased donors is after circulatory arrest. Thus the time available for full donor evaluation is limited.

Once a potential donor has been identified, the priority is to establish their suitability by appropriate donor evaluation. To do so, several sources of information should be used:

- a. interviews with the family and/or other relevant sources;
- b. interview with the attending clinician and nurse, as well as the healthcare provider, general practitioner and others;
- c. detailed review of the medical notes;
- d. assessment of the donor's medical and behavioural history;
- e. physical examination;
- f. autopsy findings (for deceased donors), which must be communicated as soon as possible after procurement;
- g. laboratory tests, including all microbiologic testing (see Chapters 5 and 9).

Information obtained during this evaluation must be included in the donor's medical record. The medical and social history of a potential donor must be investigated for all kinds of transmissible diseases and any other conditions that may affect tissue quality and safety. An interview with relatives of deceased donors should be undertaken, bearing in mind that, under emotional stress, some details might be forgotten. Contact with the general practitioner of the donor and reviewing, where available, hospital records for historic data or other sources of information (e.g. tumour registry if available) are important to supplement and/or confirm information provided by the family.

The donor medical record should be documented with details of hospital admission (if the donor died in a health facility); cause of death; medical and behavioural history, including 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, scars, skin or mucosal lesions.

Standardised questionnaires should be used for interviews to ensure all the above information is obtained (see Appendix 12). The interviews should be done, documented and signed by an adequately trained and competent authorised person. They should be held in private and carried out ideally before donation (see Chapter 3).

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 from the procurement organisation or tissue establishment.

Careful review of all the collected donor information will ensure an appropriate donor evaluation and assessment of the risks, including the identification of any potential absolute and tissue-specific contraindications for donation (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.

4.2.2.1. Medical history

The medical history of a donor should include:

- a. current clinical information, e.g. diseases/diagnoses, transfusions/infusions, medication/vaccinations, cause of death (COD) for deceased donors;
- b. previous diagnosis of disease, surgeries, vaccinations, genetic disease, chronic diseases; family history. For living allogeneic donation, special attention should be given in cases of family adoption or conception by donated gametes/embryos as it may not be possible to trace the genetic family history.

4.2.2.1.1. Current clinical information

The attending clinician or other medical staff caring for the potential donor should be interviewed, with the aid of standardised questionnaires, in order to obtain information about the current clinical history and, in the case of a deceased donor, the events leading to death.

If a potential donor appears eligible after initial screening, available records should be obtained, reviewed and evaluated as soon as possible. The types and extent of records to be obtained should vary depending on the type of donor in the following manner:

a. Living donors

The information to be collected will depend on the type of donation and the tissue donated:

- i. The clinical information to be obtained in order to ensure safety for the recipient should be the same as that obtained for deceased donors (see below);
- ii. In addition, the health risks for the donor must also be considered by a clinician not involved in the treatment of the potential recipient to avoid conflicts of interest (except in the case of surgical residues).

b. Deceased donors

The following records, where available, should be obtained and reviewed:

- i. emergency room and emergency medical transport (ambulance) records;
- ii. admission records, progress notes, clinician's orders/notes and nursing observations;
- iii. results of laboratory tests (microbiology, chemistry, haematology, virology, urinalysis, toxicology, genetic screening, pathology);
- iv. information relating to transfusions and infusions (to be used for evaluation of haemodilution). If haemodilution is $> 50\%$, serology testing on blood samples drawn at the time of procurement may not be reliable (see Chapter 5 and Appendix 15). Potential haemodilution should be considered in donors with massive trauma, intra-operative blood loss or ruptured abdominal aneurysms. When haemodilution is suspected/confirmed, blood samples taken before haemodilution should be used for serology testing. If pre-transfusion/infusion samples are not available, haemodiluted samples must be tested using only validated methods;
- v. radiography/magnetic resonance imaging/computed tomography;
- vi. surgical records (review for additional transfusions and infusions that may have taken place, materials used during surgery, such as xenografts, and any biopsy reports);
- vii. general practitioner records;
- viii. records of consultations (e.g. psychiatry, infectious disease, neurological, orthopaedic, oncology, rheumatology, counselling);
- ix. discharge summary or death certificate (and determine whether an autopsy is planned).
In addition, when death occurred outside a healthcare facility, the following records may be available and, if so, they should be located and reviewed:
 - x. police records;
 - xi. primary care records (general practitioner);
 - xii. records from the medical examiner or coroner;
 - xiii. records from the extended care facility (assisted living facility);
 - xiv. records from a funeral home.

4.2.2.1.2. Previous diagnosis of disease, surgical procedures, vaccinations, chronic diseases and family history

Thorough investigation of the previous diseases of the potential donor must be carried out. The general practitioner is probably the most direct source for this information, but also family and hos-

pitals could be consulted. All medical events, diseases, surgical procedures and medications that the donor used should be evaluated, including assessment of the effect on the quality and safety of the tissues and cells to be procured, to ascertain if potential donors qualify for donation. This evaluation should include any past history related to:

- a. chronic/previous disease, e.g.
 - i. chronic persistent infection
 - ii. malignancy
 - iii. autoimmune disease
 - iv. neurological disease
 - v. genetic disease
- b. medication
- c. vaccinations
 - i. recent vaccinations that indicate travel risks
 - ii. vaccinations with live attenuated virus
- d. family history, for instance
 - i. 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)[1]
 - ii. because the risk associated with human T-cell leukaemia virus type 1 (HTLV-1) in a donor may be higher if the donor or his/her parents originated from a high-prevalence area.

4.2.2.2. *Social history, evaluation of behavioural and personal risk*

Behavioural and personal risk (including travel history) must be evaluated as they may completely exclude a donor, indicate that certain tissues may be compromised or suggest an increased risk of infectious diseases [2].

It is necessary to ask about sexual behaviour (e.g. prostitution, frequently changing partners regardless of their gender, not only for men having sex with men). Also the use of intravenous drugs, cocaine, lifestyle or imprisonment. Even when donor relatives trust the interviewer, they may neglect or not disclose this information or may not know the entire truth.

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. Emerging, non-tropical diseases also exist in some European regions, e.g. West Nile virus, chikungunya virus.

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.europa.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).

Any history of travel or residence abroad must be expanded with information about living conditions, migration background, refugee status and workplaces (e.g. sewage plant, woodlands, farm, airport, hospital, foreign countries). This helps to identify risks in respect of places/countries with inferior hygienic standards or a high prevalence of certain infections. Information about hobbies (e.g. home, garden, animals, woodlands) should also be obtained with the same intention.

Asking about contact with fauna, especially bites from pets, domestic or wild animals, bats and birds, is essential, but it cannot rule out all infection risks.

4.2.2.3. *Physical evaluation of donors*

Physical evaluation is required for each donor, and should be designed to look for evidence of high-risk behaviour, unexplained jaundice, hepatomegaly, hepatitis or other infection, neoplastic disease or trauma (e.g. check for scars, old or new, healed or purulent wounds, exanthema, rash, injections).

It should be carried out before procurement and must be documented. The information obtained through physical examination is complementary to the comprehensive summary of clinical data.

An example of a tissue donor physical assessment form provided by the American Association of Tissue Banks can be found in Appendix 13.

In the case of abnormal findings, each tissue establishment should establish – following their standard operating procedures (SOPs) – whether further investigations should be carried out. The limited sensitivity and specificity of physical examination for discovering pathologies must be taken into account in the donor risk assessment.

4.2.2.3.1. *Deceased donor*

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. Indications that imply risk related to donor social behaviour and lesions or fractures related to the cause of death that can totally or partially contraindicate the donation (e.g. open fractures) must be recorded.

Risks to look for should include signs of

- a. systemic disease:
 - i. active malignancy (suspicious skin lesions; see Appendix 14);

- ii. malnutrition, multiple deformities;
- b. bacterial or viral infection:
 - i. recent receipt of a live vaccination (vaccination site infection, scabs, lesions);
 - ii. recent receipt of a tattoo, body piercing or acupuncture where non-sterile instruments were used (shaved area, redness, swelling or scabbing may require further investigation to assess risk);
 - iii. skin lesions such as a rash, petechiae, skin ulcers, blue/purple or grey/black lesions, shingles;
 - iv. oral lesions such as ulcers or thrush (not always possible for rigor mortis);
 - v. enlarged lymph node(s);
 - vi. icterus, hepatomegaly;
- c. high-risk behaviour (related to HIV infection or viral hepatitis):
 - i. injected drug abuse (non-medical injection sites);
 - ii. inspection of tattoos for hidden injection sites or for any additional information (e.g. some tattoos may suggest imprisonment or high-risk sexual behaviours);
 - iii. genital or skin lesions or trauma indicative of a sexually-transmitted disease (e.g. evidence of anal intercourse, insertion trauma, peri-anal or herpetic lesions, syphilitic chancres or other lesions);
- d. trauma:
 - i. fractures, avulsions, lacerations or abrasions that may affect (contaminate, compromise integrity of) the tissue to be procured;
 - ii. internal trauma that can cause cross-contamination between cavities (e.g. injury to the bowel, penetrating or crushing injuries);
- e. cleanliness of the body, the condition in which the body was found (this can also relate to increased risk for contamination/cross-contamination);
- f. scars (surgical, scarification); if findings do not match the donor's history, further investigation may be required.

Each donor (adult or child) must be thoroughly examined following established protocols, covering the anterior and posterior aspects of the body as well as an inspection of body cavities. Excessive weight of the donor cannot compromise the requirement to carry out a thorough assessment. No finding suggestive of possible risk should be left unresolved.

Child donors must be screened with as much diligence as adult donors. Physical assessment must not be overlooked or shortened simply because the

donor is a child. Although risk associated with sexual activity may not seem relevant, infectious disease associated with child abuse (sexual) is possible, so examination of the genital and peri-anal regions is recommended.

Visual examination of the body by procurement staff may be necessary during early, initial screening if adequate information regarding the condition of the body cannot reliably be obtained verbally.

The physical examination may include taking a picture of suspicious lesions that may indicate a risk or taking a sample for histology.

The physical examination can result in rejection of a donor before procurement, or the tissues or cells after procurement, thereby demonstrating its importance [3].

4.2.2.3.2. Living donors

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 for allogeneic use.

More detailed information about HPC and ART donors, including paediatric donors, is given in Chapters 21 and 24.

4.2.2.4. *Special considerations for paediatric donors*

Special screening considerations are required for paediatric donors. If the child is 18 months old or younger, or has been breastfed in the 12 months before death, the birth mother should be tested and evaluated for risks associated with HIV, HBV, HCV, HTLV and syphilis, as with any other allogeneic donor. Other diseases that can be transmitted vertically from mother to foetus may also be relevant, such as malaria or Chagas disease. A child's immune system is not fully developed, so protective antibodies may not yet have been produced against infection, thereby increasing the risk of undetectable infection with serologic screening.

In the EU, Directive 2006/17/EC stipulates that children aged under 18 months born to mother with infection by HIV, HBV, HCV or HTLV, or who are 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 analytical tests.

4.3. Generic contraindications for donation for donors

4.3.1. Autologous donors

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 (e.g. autotransplantation of ovarian tissue carries the risk of cancer cells being present in the tissue). This should be clearly documented and communicated to the patient.

Potentially or proven infectious (i.e. HIV, HBV, HCV positive) materials collected from autologous donors should be handled in such a way that no cross-contamination with tissues and cells from other donors can occur in the tissue establishment. To ensure this, a vigorous screening process is required for all autologous donors to check for the presence of any infection. Written SOPs should be present for these situations (see Chapters 6 and 7).

4.3.2. Allogeneic donors

The guidelines for excluding or including donors presenting certain risks vary between countries and regions and are determined by local disease prevalence and risk assessments. Therefore, this list of risk criteria should be regularly reviewed and modified according to local circumstances, as epidemiological changes and future developments in diagnostics occur.

In spite of these limitations, donors should be considered as high-risk if one or more of the following conditions are present.

4.3.2.1. Unknown cause of death

If the cause of death (COD) is not known, the donation cannot be permitted, because death may have been due to a disease that could be transmitted to recipients of tissues and cells. The only exception would be in those cases where an autopsy is performed and can clarify the COD after tissue procurement.

4.3.2.2. Infectious diseases

4.3.2.2.1. 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) should be excluded. Donors with bacterial septicaemia may be evaluated and consid-

ered for (avascular) cornea donation, but only if the corneas are stored by organ culture (see Chapter 16).

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.

4.3.2.2.2. Chronically persistent infection

Consider the history of bacterial and protozoic diseases that can lead to chronically persistent infections, including tuberculosis, brucellosis, leprosy, melioidosis, Q fever, chlamydiosis, salmonellosis and tularaemia. Specific attention should be paid to tick/arthropod-borne diseases such as borreliosis, bartonellosis, rickettsiosis, trypanosomiasis, leishmaniasis, babesiosis and ehrlichiosis. The risk of transmitting these infectious agents with specific tissues must be assessed, and negative effects for the recipient(s) excluded.

4.3.2.2.3. Proven viral infection

Persons who have been diagnosed with (or have tested positive for) HIV, HCV or HBV are excluded from donation.

4.3.2.2.4. Recent history of vaccination with a live attenuated virus/bacterium [4]

Vaccinations with live vaccines 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 (not injectable, inactivated influenza), varicella-zoster, rotavirus, measles, mumps, rubella, oral polio (not injectable, inactivated), oral cholera (not injectable, inactivated *Vibrio cholerae*) and yellow fever. Vaccinia for smallpox should be deferred for 8 weeks;
- b. Bacterial: bacillus Calmette-Guérin (BCG), oral *Salmonella typhi* (not injectable, inactivated).

4.3.2.3. High-risk factors

4.3.2.3.1. Behavioural risk factors for HIV, HBV, HCV and syphilis

One major concern is the risk of unintended transmission of HIV, HCV or HBV infection; the incidence and prevalence of HIV and HCV infection

varies depending on different risk factors [5, 6, 7, 8] and the causes of *de novo* infection vary between European regions [9].

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 following behaviours:

- i. People who have injected drugs by intravenous, intramuscular or subcutaneous route for non-medical reasons in the preceding 12 months (intranasal drug use should be interpreted as similar to the subcutaneous route);
- ii. Tattoos, ear piercings, body piercings and/or acupuncture are very popular in some European countries; usually they are applied by sterile methods but in case of doubt the associated risk should be considered similar to that of non-medical injections;
- iii. Persons who have been newly diagnosed with, or have been treated for, genital ulcerative disease (e.g. syphilis, gonorrhea, *chlamydia* or genital ulcers) in the preceding 12 months;
- iv. Men who have had sex with men (MSM) in the preceding 12 months;
- v. Persons who have had sex in exchange for money or drugs in the preceding 12 months;
- vi. Persons whose sexual behaviour, including frequent changes of sexual partner, puts them at risk of acquiring severe infectious diseases.

4.3.2.3.2. Personal risks, exposure events [5]

Exposure events that increase the risk of acquiring a communicable disease can occur at any time during life. They include accidents, certain medical therapies, occupation and travel to, or residence in, an area endemic for certain diseases. Here are examples of other risk factors.

- a. Persons from a high-risk region for endemic disease, e.g. HIV-1 group O, human T-cell lymphotropic virus (HTLV-I). The Caribbean is, for example, high-risk for HTLV-I [10];
- b. Exposure to someone else's blood when that person was known to be infected with HIV, HBV or HCV in the preceding 12 months;
- c. Sharing a residence in the past 12 months with someone who has HBV or clinically-active HCV;
- d. Persons chronically transfused with blood or blood products, who should be carefully evaluated case by case for the risk of disease transmission;
- e. Persons with haemophilia or related clotting disorders who received human-derived clot-

ting factor concentrates before 1987, in a period when more advanced methods for manufacturing those products were not widely used;

- f. Patients with chronic haemodialysis;
- g. People who have been in a lockup, jail, prison or juvenile correctional facility for more than 72 consecutive hours in the preceding 12 months;
- h. Children aged under 18 months born to mothers with infection by HIV, HBV, HCV or HTLV, or who are 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 analytical tests;
- i. A history of travel to, origin in or relatives in malaria-endemic areas;
- j. A bite from an animal suspected of having rabies in the preceding 12 months;
- k. Occupational or other exposure to a toxic substance in amounts sufficient to affect tissues and cells and affect the outcome of human application (e.g. ethylene glycol);
- l. 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), Q fever, antibiotic-resistant diseases, prion disease and HIV-1 group O, rabies, Ebola virus and Zika virus [11]. In Europe, regular monitoring of the Rapid Communication Reports originating from the Eurosurveillance website 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 [12].

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. Regional risks can vary. For infections in which the agent has been fully cleared from the donor's blood on recovery, the donor should be deferred from donation until they are no longer infectious (usually 2 weeks from cessation of symptoms). In cases of known contact with an infectious agent, the donor should be deferred for approximately twice the length of the incubation period [13].

Table 4.1. Grading of selected central nervous system tumours (WHO 2016 classification)

Diffuse astrocytic and oligodendroglial tumours	I	II	III	IV
Diffuse astrocytoma, IDH-mutant		•		
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	I	II	III	IV
Pilocytic astrocytoma	•			
Subependymal giant cell astrocytoma	•			
Pleomorphic xanthoastrocytoma		•		
Anaplastic pleomorphic xanthoastrocytoma			•	
Ependymal tumours	I	II	III	IV
Subependymoma	•			
Myxopapillary ependymoma	•			
Ependymoma		•		
Ependymoma, RELA fusion-positive		•	•	
Anaplastic ependymoma			•	
Other gliomas	I	II	III	IV
Angiocentric glioma	•			
Chordoid glioma of third ventricle		•		
Choroid plexus tumours	I	II	III	IV
Choroid plexus papilloma	•			
Atypical choroid plexus papilloma		•		
Choroid plexus carcinoma			•	
Tumours of the pineal region	I	II	III	IV
Pineocytoma	•			
Pineal parenchymal tumour of intermediate differentiation		•	•	
Pineoblastoma				•
Papillary tumour of the pineal region		•	•	
Meningiomas	I	II	III	IV
Meningioma	•			
Atypical meningioma		•		
Anaplastic (malignant) meningioma			•	
Embryonal tumours	I	II	III	IV
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	I	II	III	IV
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	I	II	III	IV
Schwannoma	•			
Neurofibroma	•			
Perineurioma	•			
Malignant peripheral nerve sheath tumour (MPNST)		•	•	•
Mesenchymal, non-meningothelial tumours	I	II	III	IV
Solitary fibrous tumour/haemangiopericytoma	•	•	•	
Haemangioblastoma	•			
Tumours of the sellar region	I	II	III	IV
Craniopharyngioma	•			
Granular cell tumour	•			
Pituicytoma	•			
Spindle cell oncocyoma	•			

Source: adapted from: Louis DN, Ohgaki H, Wiestler OD et al. (2016) World Health Organization histological classification of tumours of the central nervous system. International Agency for Research on Cancer, France [18].

The European Centre for Disease Prevention and Control (ECDC) publishes review-based and rapid risk assessments of communicable diseases transmissible through substances of human origin (blood, tissues and cells, organs), for example, Rapid risk assessment: Zika [14].

The risk of transmission varies according to the infectious agent.

4.3.2.3.3. Risk of transmission of prion diseases

Transmissible spongiform encephalopathies (TSE), which include Creutzfeldt–Jakob disease

(CJD), are rare neurological degenerative diseases that are progressive and inevitably fatal. They are associated with transformation of the normal form of prion protein (PrP^C) into an abnormally-folded form (PrP^{Sc}). There are four clinical forms: sporadic (sCJD), which is the most common; variant (vCJD); genetic (gCJD); and iatrogenic (iCJD). Gerstmann-Sträussler-Scheinker (GSS) and fatal familial insomnia (FFI) are also forms of inherited prion disease although distinct from CJD [15]. 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 ECDC recommendations – see www.cdc.gov/prions/ – is suggested and the risk of TSE transmission should be considered in the following cases:

- a. persons diagnosed with any form of CJD, GSS or FFI;
- b. any suspicion of prion-associated disease, such as rapid progressive dementia;
- c. a diagnosis of dementia without a confirmed primary cause (unless prion-associated disease has been ruled out by microscopic examination). If dementia has a primary cause (e.g. dementia of vascular origin), donation can be accepted;
- d. degenerative or demyelinating disease or a disorder of unknown aetiology involving the central nervous system;
- e. persons who lived in the UK between January 1980 and December 1996 for longer than 6 months;
- f. persons treated with pituitary gland hormones or growth hormone of human origin;
- g. recipients of cornea, sclera and *dura mater*, as well as persons who have undergone undocumented neurosurgery in which the *dura mater* may have been used;
- h. persons informed that they are a public health risk for CJD (eg > 80 blood donor exposures) and therefore told not to donate blood.

4.3.2.4. Malignancies

4.3.2.4.1. Haematological malignancies

Myeloid neoplasias and leukaemias are malignant diseases caused by dysregulated multipotent haematopoietic stem cells and should be considered as absolute contraindications to donation. Other myeloproliferative diseases may also affect the stem cells; thus these donors require special attention, and donation of living cells is not recommended. Other

malignancies are not a generic contraindication and are discussed below.

The major subtypes of myeloid neoplasm and acute leukaemia, according to the updated World Health Organization (WHO) classification, are listed in Table 4.2 [1].

An investigation must be performed to rule out any haematological alterations that would be suggestive of any of the malignancies listed in Table 4.2. 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 [17], such as

- a. Altered haemoglobin (men > 18.5 g/dL; women > 16.5 g/dL) and/or haematocrit (men > 55.5 %; women > 49.5 %) should be carefully assessed, to discard a possible disease such as polycythemia vera.
- b. Platelet count:
 - < 50 × 10⁹/L is highly indicative of a haematological disorder,
 - 50-100 × 10⁹/L should be carefully assessed to discard a possible haematological problem,
 - > 450 × 10⁹/L should be carefully assessed to discard a possible disease such as essential thrombocytosis;
- c. Altered white blood cells (> 50 × 10⁹/L) should be carefully assessed to discard a possible disease such as chronic myeloid leukaemia.

4.3.2.4.2. Non-haematological malignancies

A history of malignancy should be considered an absolute exclusion criterion except for:

- a. primary basal cell carcinoma;
- b. *in situ* carcinoma of the cervix;
- c. some appropriately evaluated grade-I and -II primary tumours of the central nervous system (CNS) according to the WHO classification [18] (see Table 4.1 where the grade of each tumour is marked • in the corresponding column). Malignancy gradation in the CNS should be properly evaluated, including a complete histological exam and not just a simple biopsy, taking into account possible heterogeneity of the mass.

In addition, donors with malignant diseases can be evaluated and considered for cornea donation (see Chapter 16), with the exception of 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.

Table 4.2. WHO classification of myeloid neoplasms and acute leukaemia

Myeloproliferative neoplasms (MPN)	
Chronic myeloid leukaemia (CML), <i>BCR-ABL1</i> ⁺	• AML with mutated <i>NPM1</i>
Chronic neutrophilic leukaemia (CNL)	• AML with biallelic mutations of <i>CEBPA</i>
Polycythemia vera (PV)	• Provisional entity: AML with mutated <i>RUNX1</i>
Primary myelofibrosis (PMF)	AML with myelodysplasia-related changes
• PMF, prefibrotic/early stage	Therapy-related myeloid neoplasms
• PMF, overt fibrotic stage	AML, NOS
Essential thrombocythemia (ET)	• AML with minimal differentiation
Chronic eosinophilic leukaemia, not otherwise specified (NOS)	• AML without maturation
MPN, unclassifiable	• AML with maturation
Mastocytosis	• Acute myelomonocytic leukaemia
Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of <i>PDGFRA</i>, <i>PDGFRB</i>, or <i>FGFR1</i>, or with <i>PCM1-JAK2</i>	• Acute monoblastic/monocytic leukaemia
Myeloid/lymphoid neoplasms with <i>PDGFRA</i> rearrangement	• Pure erythroid leukaemia
Myeloid/lymphoid neoplasms with <i>PDGFRB</i> rearrangement	• Acute megakaryoblastic leukaemia
Myeloid/lymphoid neoplasms with <i>FGFR1</i> rearrangement	• Acute basophilic leukaemia
Provisional entity: Myeloid/lymphoid neoplasms with <i>PCM1-JAK2</i>	• Acute panmyelosis with myelofibrosis
Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)	Myeloid sarcoma
Chronic myelomonocytic leukaemia (CMML)	Myeloid proliferations related to Down syndrome
Atypical chronic myeloid leukaemia (aCML), <i>BCR-ABL1</i> ⁺	• Transient abnormal myelopoiesis (TAM)
Juvenile myelomonocytic leukaemia (JMML)	• Myeloid leukaemia associated with Down syndrome
MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)	Blastic plasmacytoid dendritic cell neoplasm
MDS/MPN, unclassifiable	Acute leukaemias of ambiguous lineage
Myelodysplastic syndromes (MDS)	Acute undifferentiated leukaemia
MDS with single lineage dysplasia	Mixed phenotype acute leukaemia (MPAL) with t(9;22) (q34.1;q11.2); <i>BCR-ABL1</i>
MDS with ring sideroblasts (MDS-RS)	MPAL with t(v;11q23.3); <i>KMT2A</i> rearranged
• MDS-RS and single lineage dysplasia	MPAL, B/myeloid, NOS
• MDS-RS and multilineage dysplasia	MPAL, T/myeloid, NOS
MDS with multilineage dysplasia	B-lymphoblastic leukaemia/lymphoma
MDS with excess blasts	B-lymphoblastic leukaemia/lymphoma, NOS
MDS with isolated del(5q)	B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
MDS, unclassifiable	B-lymphoblastic leukaemia/lymphoma with t(9;22) (q34.1;q11.2); <i>BCR-ABL1</i>
Provisional entity: Refractory cytopenia of childhood	B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); <i>KMT2A</i> rearranged
Myeloid neoplasms with germ line predisposition	B-lymphoblastic leukaemia/lymphoma with t(12;21) (p13.2;q22.1); <i>ETV6-RUNX1</i>
Acute myeloid leukaemia (AML) and related neoplasms	B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
AML with recurrent genetic abnormalities	B-lymphoblastic leukaemia/lymphoma with hypodiploidy
• AML with t(8;21)(q22;q22.1); <i>RUNX1-RUNX1T1</i>	B-lymphoblastic leukaemia/lymphoma with t(5;14) (q31.1;q32.3) <i>IL3-IGH</i>
• AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i>	B-lymphoblastic leukaemia/lymphoma with t(1;19) (q23;p13.3); <i>TCF3-PBX1</i>
• APL with <i>PML-RARA</i>	Provisional entity: B-lymphoblastic leukaemia/lymphoma, <i>BCR-ABL1</i> -like
• AML with t(9;11)(p21.3;q23.3); <i>MLLT3-KMT2A</i>	Provisional entity: B-lymphoblastic leukaemia/lymphoma with <i>iAMP21</i>
• AML with t(6;9)(p23;q34.1); <i>DEK-NUP214</i>	T-lymphoblastic leukaemia/lymphoma
• AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); <i>GATA2, MECOM</i>	Provisional entity: Early T-cell precursor lymphoblastic leukaemia
• AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); <i>RBM15-MKL1</i>	Provisional entity: Natural killer (NK) cell lymphoblastic leukaemia/lymphoma
• Provisional entity: AML with <i>BCR-ABL1</i>	

Source: 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 [16].

Any vascularised ocular tissues, such as sclera, limbal tissue or cells derived from limbal tissue, are not covered by this exclusion and should not be used for transplantation.

Conditions such as cirrhosis or serious gastrointestinal disorders should be carefully evaluated as they increase the risk of malignancies. It is also important to acknowledge that certain malignancies, particularly from older donors, may not be detected during procurement without a thorough autopsy inspection (e.g., prostate malignancy in donors >70 years).

4.3.2.5. *Intoxication*

If ingestion of or exposure to a toxic substance (e.g. cyanide, lead, mercury, gold, arsenic, pesticides) caused death, the quality of some types of tissues and cells may be affected and, as a result, cause harm to recipients if those tissues or cells are used for human application.

4.3.2.6. *Deferred for blood donation for unknown reason*

If it is known that the potential donor was excluded or deferred from donating blood by a blood collection establishment, and the specific reason for deferral is not known, the donor is excluded from donation.

4.3.3. **Relative contraindications for allogeneic donors**

Below are listed the potential risks that have to be analysed on an individual basis, considering the potential harm and benefit. If a risk is justified, it should be communicated to the recipient.

- 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 progenitors);
 - ii. breastfeeding;
 - iii. health risks for donor themselves (e.g. specific procedure or superovulation).
- b. Transplantation with organs
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 level of immunosuppression in combination with the possibility of tracing the medical details of the organ donor.

c. **Impact of immunosuppressive agents in the donor**

Treatment with immunosuppressive agents can weaken the immune system and thus influence the reliability of serological tests (Chapter 5). Evaluating the effect of the immunosuppressive agents on the haemogram (erythrocytes, leucocytes and thrombocytes) can be indicative for immunosuppression. 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 tissue-specific chapters).

d. **History of genetic disease**

A family history of genetic disease is a risk factor that should be assessed; where the occurrence of genetic disease in the family history cannot be traced/assured, this increases the risk of transmission of genetic disorders, especially in ART (see Chapter 24), and should be regarded as an exclusion criterion (see Chapter 21).

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 evaluated on an individual basis. If the reason is not known, the donor is considered ineligible for tissue donation (see section 4.3.2.6).

f. **Xenotransplantation [19, 20, 21, 22, 23]**

The reference to 'transplantation with xenografts' is clearly stated as an exclusion criterion for donors at 1.1.13 of Annex I in Directive 2006/17/EC. However, the absence of a formalised definition for its interpretation has previously led to some ambiguity in its application. Similar terms – such as 'xenotransplantation product', 'xenogeneic cell-based medicinal product' and others – have been used within different healthcare sectors, and a uniform consensus on terminology is paramount. In recent years different Scientific and Technical Committees have established and adopted the fundamental opinion that the term 'xenotransplantation' is applicable to any procedure 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 [23].

The scientific community continues to apply the principles of this approach, and xenotransplantation products include those which utilise living non-human animal cell, tissues or organs used for transplantation. With similar equivalence, the US Guidance for Industry has adopted the scientific opinion and further states that any biological products, drug or medical devices sourced from non-living cells, tissues or organs from non-human animals are not considered xenotransplantation products (e.g. porcine insulin and biological heart valves). The same opinion is the basis of permanent deferral (i.e. the exclusion criteria) for the assessment of potential blood donors.

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. The risk management strategy for the control of infectious agents is primarily focused on the application of selective sourcing, effective collection and handling, and measures applied for elimination/inactivation or removal of agents. Where relevant the medical device and medicinal sectors are regulated by these standards to achieve high standards of quality and safety, and the products are thus viewed as risk-mitigated.

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. The potential loss of this donor pool by these excluded donations is often predicted to be insignificant in relation to the transplantation needs of local clinics.

4.3.4. 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.

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Related documents:

Appendix 12. Sample donor assessment form;

Appendix 13. Sample donor physical assessment form;

Appendix 14. Practical guidance for the evaluation of pigmented skin lesions and differential diagnosis of melanoma.

Chapter 5. Donor testing

5.1. Introduction

Use of tissues and cells for human application can result in unintentional transmission of disease. However, such events can be prevented 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. Controls include:

- a.* ensuring that the screening programme includes relevant infectious diseases and their appropriate individual screening target(s);
- b.* selecting a suitable testing laboratory;
- 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 appropriately validated 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 vital 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.

5.2. General concepts

Tissue establishments must ensure that all donations of human tissues and cells are subjected to those biological tests mandated by national and any other applicable legislation.*

SOPs should be in place that define the criteria for acceptance or rejection of tissues and cells based on those test results. The Responsible Person who will interpret test results should be knowledgeable about infectious-disease test kits, and decisions must meet the expectations in regulations or, if regulations are not prescriptive, follow professional standards of practice [1].

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 used for testing of donor samples has been accredited, designated, licensed and/or authorised by the appropriate authority to carry out such testing.

* In EU member states, Annex II of Directive 2006/17/EC, amended by Directive 2012/39/EU, stipulates mandatory laboratory tests and general testing requirements for living and deceased donors of tissues and cells.

5.3. 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, storing or testing donor samples must be aware of these requirements to ensure optimal assay performance. If inadequate or otherwise compromised samples are provided and tested, the results may not be valid, which increases the risk of donor-related transmission of infectious diseases.

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. In cases where haemodilution is known to have occurred, ideally pre-transfusion/infusion samples should be obtained for testing purposes. See section 5.3.2.

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 pink to red. This darker colour can promote a higher density reading by the optical component of test equipment, leading to a 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 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 viral agents can be impaired if the donor has received immunosuppressive treatment prior to sample collection. In such situations, adding molecular screening tests (i.e. NAT) can be valuable because detection of viral nucleic acid in blood samples is generally not affected by immunosuppressive therapy [1, 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 a Re-

sponsible Person (RP) before release of tissues or cells for clinical application.

5.3.1. 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 must be carried out on plasma or serum of the donor according to the specification laid out by the manufacturer of the test kit. Testing must not be performed on other fluids or secretions, such as aqueous or vitreous humour, unless the assays selected 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 antibodies may not yet have been produced against an infection, thereby increasing the risk of hidden infections in child donors. See section 5.5.1.

Donor sample collection and manufacturer's test instructions must be followed with regard to:

- the type of sample collection tube (no anti-coagulant or a specific anti-coagulant) required for the test being carried out;
- sample storage and transport conditions post-collection, which can include centrifugation and/or separation within time limits or specimen refrigeration/freezing; and
- 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 14). The date and time when the sample was drawn must be accurately documented. It is recommended that at least two donor identifiers, such as the donor's full name, date of birth and/or medical record number, be used. In the case of a sample from a deceased donor, 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). It is good practice for the identity of all donor samples to be confirmed by a second person, if possible, from the procurement team, and this confirmation process should be documented [3]. If any donor

blood samples were drawn before death, they can be validated for use, 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 or a different time.

For obvious safety reasons, the collection of donor blood for infectious-disease testing must always occur as close as possible to the donation event. Personnel who collect, or otherwise obtain, donor blood samples to be used for this critical testing must consider factors that could influence sample degradation and cause false-negative or false-positive test results, e.g. time of sample collection, temporary storage conditions.

An adequate volume of whole blood must be collected, 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 evaluated before blood collection. 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). If the volume needed is not clear to personnel collecting blood samples from a donor, filling three or four 6 mL blood tubes to their limit should be sufficient. 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. Under normal circumstances, two or three full 6 mL blood tubes should be sufficient for all types of testing. To avoid unintended consequences, personnel who collect donor blood samples should be familiar with the requirements of the testing laboratory, and written procedures should provide specific direction.

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 must be followed and can differ among tests [3]. In all cases, validated transport containers and validated shipping procedures must 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 must 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.

5.3.1.1. *Deceased donor*

In the case of a deceased donor, blood samples must have been obtained just before death 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].

Some studies have demonstrated the potential for blood sample collection to occur more than 24 hours after death; however, validation of each infectious-disease test kit using such specimens is necessary to support an extension [6, 7, 8, 9, 10]. Acceptance of such practice is controlled by national regulation.

5.3.1.2. *Living donor*

In the case of living donors, blood sampling should 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 without there being a point-of-return when irreversible measures for preconditioning of the recipient had 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 5.5.2, and also Chapters 17, 19, 21, 24 and 29.

5.3.2. 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). 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.

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 15 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]:

- estimate TPV of donor (weight in kg $\times$ 40 mL/kg; or, weight in kg $\div$ 0.025);
- estimate TBV of donor (weight in kg $\times$ 70 mL/kg; or, weight in kg $\div$ 0.015);
- calculate total blood (mL) received in the last 48 h (A);
- calculate colloids (mL) received in last 48 h (B);
- calculate crystalloids (mL) received in the last 1 h (C);
- add B + C and compare to TPV (fluid volumes are compared);
- add A + B + C and compare to TBV (mass/fluid volumes are compared);
- 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, additional testing using molecular tests (i.e. NAT) for the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) will help reduce risk and should also be performed.

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.

A further, although uncommon, occurrence resulting from transfusion of human plasma in the *ante mortem* care of the donor is the possibility of passive transfer of antibody from a blood donor to the tissue donor. However, in most situations, this is limited to antibody to *cytomegalovirus* (CMV) and, given the normal screening panel, such passive transfer can generally be easily identified.

5.4. 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 must 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 for a donor of haematopoietic stem cells, the laboratory used should be accredited and participate in an appropriate external quality assessment programme [5]. See section 5.5.1. and Chapter 21.

Tissue establishments can undertake these testing protocols themselves or have a written agreement with any laboratory that carries out donor infectious-disease testing on their behalf [11]. Tissue establishments should evaluate and select a testing laboratory on the basis of its ability to generate reliable and appropriate results, and keep relevant records. In addition they must 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 that the laboratory is competent to perform this work and is using appropriate assays and procedures (ideally, with kit designed for donor screening rather than for confirming a diagnosis). There must be evidence that good laboratory practice is being followed and that personnel are appropriately trained and experienced in relevant testing procedures. 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 quality system.

In addition, test records at the laboratory must be retained for 10 years and contain the date of receipt of the blood sample at the testing facility, a record of each test kit used to test donor blood samples (i.e. manufacturer, lot number, expiry), and the results of donor testing, including repeat testing (if applicable). See Chapters 2 and 14.

5.5. Tests to be carried out

The donor screening assays selected must be validated and used in accordance with current scientific knowledge. All assays used for donor testing within the EU should be Conformité Européenne (CE)-marked (see Appendix 16). 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 use other available peer-reviewed data [12, 13].

As a minimum requirement, the biological tests listed below must be carried out on the serum or plasma of the donor according to the manufacturer's instructions for each test kit [3]:

- a. HIV 1 and 2: anti-HIV-1 and anti-HIV-2 (antibodies to HIV-1 and HIV-2), or antibodies to HIV-1 and HIV-2 plus HIV-1 antigen in a combination assay;
- b. HBV* – HBsAg (surface antigen) and anti-HBc (total antibodies to hepatitis B core antigen) (total, i.e. IgG and IgM);
- c. HCV† – anti-HCV (antibody to HCV);
- d. Syphilis‡ – a treponemal-specific test or a non-specific treponemal test can be used.

Testing for HTLV-I antibodies must be undertaken for donors living in or originating from high-prevalence areas, or with sexual partners originating from those areas, or if the donor's parents originate from those areas [14, 15].

The minimum requirement for donor testing for viral infectious agents is antibody detection for HIV 1/2, HBV, HCV and human T-cell lymphotropic virus (HTLV-1; when indicated), plus detection of antigen for HBV.

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. In addition, special screening considerations are ap-

* If anti-HBc total (i.e. IgG and IgM) is positive (repeat reactive) and HBsAg is negative, further testing is necessary to determine hepatitis status in the donor. Such determination could include anti-HBs, anti-HBe, anti-HBc IgM, and HBV DNA detection (see section 5.5.1). In these circumstances, risk assessment must be carried out and documented by the person responsible in the tissue establishment, who will determine donor eligibility and tissue release for clinical use. This strategy also applies when tissue or cell distribution occurs from procurement directly to the clinical team responsible for human application or for direct use.

† Although anti-HCV assays are suitable for screening purposes, if an HCV combination assay (HCV Antigen + anti-HCV) is available it would provide sensitive detection.

‡ A validated screening algorithm must be applied to exclude active infection with *Treponema pallidum*. A non-reactive test for syphilis (specific or non-specific) can allow tissues and cells to be released. When a non-specific test is undertaken, a reactive result will not prevent procurement or release if a treponemal-specific (confirmatory) test is non-reactive. A donor whose specimen is reactive for a treponemal-specific test requires thorough risk assessment by a Responsible Person to determine if the donation should be released for clinical use.

plicable to other paediatric donors and additional testing for communicable diseases may be indicated. See section 5.5.1.

5.5.1. Additional tests

Because NAT assays are more sensitive, and deceased donors cannot be retested after 6 months, serious consideration should be given to also carrying out NAT tests for HIV, HBV and HCV. Considerations that support the use of NAT assays for each deceased donor include:

- the medical and behavioural history obtained from a proxy for a deceased donor can be less reliable than collecting this information from a living donor; and
- if the donation includes multiple tissue types that 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.

To optimise detection when using a molecular (e.g. NAT) assay, the donor blood sample should be tested individually; samples tested should not be pooled.

In regard to additional testing for donors originating from (or whose sexual partners or parents originate from) high-prevalence areas for specific diseases, one should refer to international scientific evidence, such as that provided by the European Centre for Disease Prevention and Control [14, 15]. Additional testing that may be considered (depending on the donor's history and/or the characteristics of the tissues or cells donated) includes:

- ABO (ABO) group;
- RhD (D antigen);
- human leucocyte antigen (HLA);
- diagnostic tests for malaria;
- West Nile virus RNA (i.e. NAT);
- test(s) for Zika virus;
- antibodies to *cytomegalovirus*;
- antibodies to *Toxoplasma*;
- antibodies to Epstein–Barr virus (EBV);
- antibody to *Trypanosoma cruzi* (the causative agent for Chagas' disease).

Results of blood cultures can be very useful tools to aid in the determination of bacteraemia in a donor of tissues and/or cells (see Chapter 9).

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 and HTLV. Other diseases that

can be transmitted vertically from mother to foetus may also be relevant, such as Zika virus, malaria or Chagas disease. Additional testing of the birth mother to establish risk may be indicated.

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

Test kit assays for infectious diseases 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, but there are additional considerations because the donor is available for more testing.

For example, 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.

Repeat sampling and serology testing is required after 180 days, unless any of the following specific exemption criteria are met. If samples from a living donor undergo serology testing and are also tested by molecular tests (i.e. NAT) for HIV, HBV and HCV, re-testing after a time interval is not required. Because molecular testing can increase sensitivity in the detection of recently acquired infections, molecular testing of all donors using this technology is highly recommended as standard practice. Other circumstances where re-testing a living donor is not required include: 1) if the tissue/cells have been processed using an inactivation step that has been validated for the virus(es) concerned; and, 2) if the tissue/cells will not be stored longer than 180 days prior to use.

For testing individuals involved in assisted reproductive technology (ART), see Chapter 24.

5.5.3. 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 positive 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 Chapters 13 and 14).

SOPs based on risk analyses must 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).

5.6. 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.

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 mix-ups are avoided and prevent misinformation. Laboratories and tissue establishments must 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 public health.

Reporting procedures should ensure that accurate, rapid and verifiable results are provided. In addition, there must be a system in place to ensure prompt alerts using an immediate notification system when a positive infectious-disease test result occurs. Other precautionary measures in reporting may include [16] the following:

- 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;

- 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;
- using clearly interpretable, computerised graphic symbols to highlight positive results;
- including the titre of antibodies and/or the related positivity threshold next to the viral negative/positive result;
- use of formal laboratory reporting structures and accreditation or certification pathways to improve quality standards;
- use of widely recognised international units of measurement.

5.7. Archived samples

If any donor sample remains after all required testing has been completed, it is desirable to freeze and store aliquots of serum and/or plasma under the appropriate conditions and for an appropriate period of time. In some countries, archiving donor samples may be required. Archive 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. 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 2).

5.8. References

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Related documents:

Appendix 15. Sample haemodilution algorithm;

Appendix 16. Validation of screening for infectious disease assays for use with blood from deceased donors.

Chapter 6. Procurement

6.1. 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 undertaking the process. This quality system must guarantee adequate training of all personnel involved, as well as written standard operating procedures (SOP) that require documentation for all stages of the process. Procurement professionals should take measures to ensure appropriate safety and quality parameters are in place.

Procurement of human tissues or cells can take place only after donor consent/authorisation requirements have been satisfied, as described in Chapter 3. Tissues and cells must also be identified, packaged and labelled correctly (see Chapter 13), and then transported to the tissue establishment or clinical team for direct use, in accordance with established requirements.

Chapter 2 sets out the general quality management expectations 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.

The definition of requirements related to procurement activities requires the acknowledgement that each of the various types of donation (e.g. deceased donation, either single tissue or multi-tissue, and living donation) represents a complex of processes and different risk factors that must be consid-

ered in order to assure the quality of the tissues and cells procured as well as the safety of (living) donors and recipients.

The criteria, including the location and standard of the premises, that apply in procuring tissues or cells from living donors are equivalent to those for the treatment of patients. However, in deceased donation there are additional considerations to take into account; as specified in Chapter 3, procurement from a deceased donor may take place not only in a hospital but in a mortuary or forensic department, and in those cases it is important to define conditions and requirements to guarantee the quality and safety of the procured tissues and cells.

Deceased donation can also occur after organ donation, and in those cases sterility 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.

This chapter provides guidance for tissue procurement in general, but with a specific focus on deceased donors and multi-tissue procurement.

6.2. Personnel

Procurement activities must be undertaken by personnel with appropriate qualifications, training, expertise and experience. This includes successful completion of a comprehensive technical and/or clinical training programme, including the broader ethical, legal and regulatory context of 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 living or deceased donors.

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

To promote compliance with donor-selection criteria and procurement procedures, the tissue establishment 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 employed by the tissue establishment responsible for these steps but expectations and responsibilities pertaining to procurement must appear in their job description (see Chapter 2).

6.3. Facilities, equipment and materials

6.3.1. Facilities

Procurement activities must be authorised by the appropriate and competent health authority. Each procurement event must take place in an appropriate facility and follow the required clinical/technical procedures (see section 6.4). The procedure must take into account the risk of microbial or other contamination of procured tissues and cells, and steps should be taken to minimise the risk. For reasons of privacy and control of contamination, access to the area where procurement takes place must be restricted during the actual procurement of tissues or cells. In addition, the donation of tissues or cells by living donors must take place in an environment that ensures their health, safety and privacy. A risk assessment to determine the suitability of the procurement site, depending on the types of tissues or cells to be procured, must be carried out.

Table 6.1. Factors and criteria to be considered in risk assessment of the procurement procedure

Factor	Risk				
	Low				High
Duration of exposure of procured tissues/cells during procurement	no exposure (closed system)				≥ 3 h
No. of personnel present while tissues/cells are exposed to the environment	1-2 persons				≥ 6 persons
Reduction of bio-burden during or after procurement	closed system	validated anti-biotic/substances treatment	only substances intended to reduce microbiological contamination (e.g. glycerol)	only washing intended to reduce microbiological contamination	no reduction
Reduction of bioburden during processing	validated sterilisation	substantial microbial reduction	limited microbial reduction (e.g. antibiotics)	only washing intended to reduce microbiological contamination	no reduction
Risk that contaminants will not be detected in the tissue due to the limitations of the sampling method	tissues preserved in culture medium (contamination is visible or revealed during microbiological testing of the medium)	culture of transport media and/or washing solution	a biopsy of tissue tested from each individual tissue	swabbing	no detection method
Route of application	superficial coverage (e.g. corneas, skin, amniotic membrane) or application in intra-uterine cavity	durable implant in a poorly vascularised site	small durable implant in a well vascularised site	large durable implant in a well vascularised site	direct application into the blood stream (infusion)

It is highly recommended that the facility where procurement takes place is:

- a. of adequate size in the floor space, work-tops and benches that will be used;
- b. appropriately located to ensure cleanliness and privacy;
- c. furnished with sufficient and suitable lighting;
- d. in a good state of repair;
- e. free of pests; and
- f. provides a sufficiently clean or cleanable environment that will not increase the risk of contamination of the cells or tissues during their procurement.

Before procurement, steps to minimise the potential for contamination must include cleaning of all work surfaces with an appropriate and effective disinfectant. The procurement area must also be cleaned appropriately after the procurement, including proper and safe disposal of single-use instruments, consumables and any other waste, including clinical waste that poses a biohazard. Any re-usable instruments will need to be cleaned and sterilised. If a tissue establishment (or third party carrying out the retrieval) uses the general services of the host facility to clean the procurement area and/or sterilise any re-useable instruments, the tissue establishment must have a written agreement with the host facility and the procedures used must be inspected and validated.

6.3.1.1. Defining the requirements of a procurement area

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

- operating theatre or equivalent;
- dedicated procurement area with routine air-quality monitoring and controlled cleaning (e.g. tissue establishment procurement room);
- dedicated clean area (controlled cleaning);
- non-dedicated area, with local cleaning of the procurement space.

A risk assessment based on the factors detailed in Table 6.1 will help define an appropriate procurement area, including air quality, depending on the level of risk and any subsequent steps taken during processing.

Taking into account the criteria defined in Table 6.1, some conclusions could be reached and exemplified as follows:

- Musculoskeletal tissues that are to be processed aseptically and supplied for application without a robust decontamination or terminal sterilisation will be exposed to the environment during procurement and processing for extended periods, cannot be sampled easily (therefore, detection of contaminants might be missed) and they will be implanted directly into a vascularised bed in the recipient. For these reasons the conclusion of the risk assessment is likely to be that they should be procured in an operating theatre environment or equivalent.
- The ocular surface is constantly exposed to the environment, not just during procurement. After death, owing to lack of tears and blinking, the ocular surface will inevitably be contaminated with environmental bacteria and fungi. For these reasons and following an appropriate risk assessment, it is not usually considered necessary to procure eyes or corneas by *in situ* excision in a location with a controlled, defined air quality. However, steps must be taken to reduce the bioburden on the ocular surface before procurement, especially for corneas procured by *in situ* excision, and a local sterile field must be created around the eye. If the whole eye is procured, further steps must be taken in the eye bank to reduce bioburden before excision of the corneoscleral disc. Furthermore, for corneas stored by organ culture, microbiological testing of the organ culture medium during corneal storage is essential to further mitigate the risk of microbiological contamination.
- Peripheral blood stem cells are collected and processed in closed systems. Therefore, particular environmental requirements may be necessary and they may be collected in a blood donor clinic according to the results of the appropriate risk assessment.
- Bone-marrow procurement is done in an open system by aspiration and without any decontamination procedure during processing. In addition, the final recipient is highly immunosuppressed and the application is done directly into the blood stream. Therefore, the risk assessment should specify the need to perform procurement in an operating theatre.
- Female gametes are collected in a closed system but processed within a location with Grade A air-quality environment using antibiotics complements as well. However, since sedatives are given intravenously during the opera-

tional intervention, the risk assessment should specify the need to perform procurement in an operating theatre. Additional specific situations in ART are described in Chapter 24.

6.3.2. Equipment and materials

Materials (i.e., consumables and reagents) and equipment (i.e., surgical instruments, packaging and containers) used during procurement must be managed in accordance with standards and specifications and with due regard for relevant national and international regulations, standards and guidelines for the intended use of the donated tissues and cells (see Chapter 2). Validated sterile instruments, CE (Conformité Européenne)-marked devices (where available) and sterile single-use materials (e.g. drapes, gloves, fluids) must be used for tissue and cell procurement. Instruments or devices must be of good quality, validated or certified specifically (e.g. surgical grade) for procurement, and must be maintained in good working order. This must include visual inspection and scheduled calibration of devices, where appropriate, against relevant defined standards at specified intervals. Routine maintenance inspections (validation procedures), at least annually, of equipment used for procurement are encouraged and a re-validation 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-useable instruments are used, a validated cleaning, disinfection, packaging and sterilisation process for removal of infectious agents must be used and each event documented. A system must be in place that allows traceability and tracking of critical equipment and materials to each tissue or cell procurement event and to the donor.

In EU member states, critical reagents, materials reagents and materials must meet documented requirements and specifications, and when applicable, the requirements of Directive 93/42/EEC concerning medical devices and Directive 98/79/EC on *in vitro* diagnostic medical devices.

Personnel conducting procurement activities must be provided with protective clothing appropriate for the type of procurement. Usually, this will extend to being scrubbed as for surgery and 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.

6.3.2.1. 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. Labelling must be appropriate to ensure the identification and traceability of tissues and cells. Labelling must be resistant to storage conditions to avoid the loss of identification of tissues and cells.

Guidance on coding, packaging and labelling is provided in Chapter 13.

6.4. 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 tissue establishment or end-user needs. These SOPs must outline the correct steps to be taken for each stage of procurement. 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 sections 6.2 and 6.3.2). Review at least annually (or as required) of procurement SOPs by a Responsible Person must be undertaken and updates may be necessary owing to clinical, scientific or technical progress. Procedures must be authorised and appropriate for the type of donor and the type of tissue or cells procured and must be standardised.

The SOPs must be readily accessible so procurement personnel can follow 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 3);
- b. assignment and appropriate use of a unique identifier/code (see Chapter 13);
- c. knowledge of selection (risk) criteria required for donor assessment, including physical examination of the donor (see Chapter 4);
- d. knowledge of the types of blood and other samples required for mandatory laboratory tests to ensure that they are of appropriate quality (see Chapter 5);
- e. steps that minimise the risk of microbiological contamination during procurement (see this chapter, as well as Chapters 2, 9 and 16-29);

- f. procurement steps that protect the properties of the tissue and cells required for clinical use (see this chapter and Chapters 16-29);
- g. for deceased donation, how to reconstruct the donor's body so it is as similar as possible to its original anatomical appearance;
- h. considerations for packaging, labelling and transportation of procured tissues or cells to the tissue establishment or, in the case of direct distribution, to the clinical team responsible for their human application or direct use (see this chapter and Chapters 13 and 14);
- i. considerations for collecting, packaging, labelling and transporting samples of donor blood or other samples to the laboratory for testing (see this chapter, and Chapters 5 and 13);
- j. procedures that protect the health and safety of the living donor (see Chapters 17 and 23-29).

In addition, the tissue establishment is expected to have procedures in place to notify, without delay, other tissue establishments or the relevant Health Authority of all available information concerning (see Chapter 15):

- a. knowledge of deviations from approved procedures that occurred or that are suspected to have occurred; and/or
- b. any serious adverse reaction in a living donor that may influence the quality and safety of the tissues or cells procured.

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.

Additional procedures and policies that minimise the risk of microbiological contamination during procurement must be considered, including those listed below (see also Chapters 16-29):

- a. the maximum number of personnel permitted to be present during procurement must be defined and respected;
- b. preparation of the donor's skin 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 skin disinfection should account for the elimination of bacterial spores as well as vegetative micro-organisms and therefore include suitable disinfectants, their concentrations and durations of exposure;
- d. before use, materials and equipment must be visually inspected by procurement personnel

to ensure they meet specifications (e.g. sterile, seals not broken, equipment functioning as expected);

- 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 (tissue-specific chapters), but generally include:

- a. *Post mortem* procurement time limits. It is recommended that tissue should be procured within 24 h after death if the body has been cooled or refrigerated in the first 6 h after death, or within 12 h of death if the body has not been refrigerated.

Refrigeration of the body aims to reduce microbiological growth. Alternative time limits for procurement should be validated by quality assessments and tests for microbiological contamination.

It may be possible to extend procurement times up to 48 h after death if processing has been validated to guarantee quality and microbiological safety: in these cases the blood samples for serological testing should still be taken within 24 h after death to minimise the risk of haemolysis (See Chapter 5 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 consists 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;
- 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 18);
- b. Review of donor documentation including medical history, laboratory tests (if completed), lawful consent/authorisation;
- c. Physical examination of the donor (Appendix 13);
- 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, eyes/corneas, cardiovascular and musculoskeletal. Justifications for this recommended procurement flow include:

- Skin is the first procured tissue because the donor is placed in 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.
- Eyes are recommended to be procured after skin to avoid eye bleeding from the sockets if the donor has to be placed in a prone position following enucleation of the eyes.
- 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 must have been sutured to maintain as far as possible

the sterility 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 the procurement teams involved know that tissues will be procured after organs, first to prepare the body before starting surgery, and second to guarantee sterile condition during the whole procedure and 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).

6.4.1. Processing at the procurement stage

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 tissue establishment (see Chapters 2, 9 and 16-29). Therefore, simultaneous undertaking of processing steps during the procurement phase, or in the procurement area, is not recommended. However, if processing, including shaping, cleaning, sizing and final packaging (for direct distribution) at the procurement site is unavoidable, its duration and extent should be limited to the minimum necessary, and a Grade A air-quality environment (surrounded by, at least, air quality Grade D) for the processing steps is desirable (e.g., a laminar flow cabinet located in the operating room). Records supporting the validation of the processing site must be available for inspection. If this level of control is not possible, an in-process (active) environmental-monitoring method should be used; preferably, active air monitoring using a viable particle counter and culturing method or, as a minimum control, microbiological settle plates can be used. Sample cultures of the tissues or cells procured should also be taken (see Chapter 9) and an appropriately validated culture method must be used (see Chapter 2). Ultimately, the

procurement environment, if it is also used as a processing environment, must be specified and must achieve the quality and safety required for the:

- a. types of tissues and cells procured;
- b. types of processing steps and tissues or cells that will be used (e.g. none; exposure only to antibiotics; a validated inactivation method; or, a validated sterilisation method);
- c. types of clinical application (as well as consideration of the immune status of the recipient, if applicable).

Selection of the use of suboptimal conditions must be supported by written justification and be authorised by the relevant Health Authority.

6.4.2. Temporary storage and transportation to the tissue establishment

Once the tissue is procured and until it arrives at the tissue establishment, critical variables related to maintaining the quality of the tissues or cells (e.g., temperature, sterile packaging) must be controlled (see Chapter 13). Records to demonstrate compliance with specified storage conditions must be completed and maintained.

6.5. Documentation

Procurement is a critical activity. Therefore tissue establishments must have procedures in place that address the retention of procurement records, which 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 in compliance 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 be capable of linking the records to the particular donor of the procured tissues and cells (see Chapter 14). 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 13). This coding must be in place to ensure an effective and ac-

curate 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 (i.e. first 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.

In summary, before the procurement of tissues and cells may proceed, an authorised person (e.g., the team leader in a procurement team) must confirm and record the following as part of the procurement record:

- a. donor identification;
- b. that consent for the procurement has been obtained in accordance with local laws;
- c. how and by whom the donor has been reliably identified.

To ensure that all steps are traceable and verifiable, the tissue establishment (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. Care should be taken to maintain donor confidentiality if the procurement report is forwarded to the recipient's clinical team. This procurement report, depending on the type of donor, could contain the following:

- a. donor identification data (first 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 stem-cell (HPC) donor);
- b. unique coding number. This will be the donation identification sequence of the Single European Code for EU countries, a code generated by a health authority or through use of an internationally recognised coding system such as ISBT 128 or Eurocode (Chapter 13);
- c. the environmental conditions of the procurement facility (location or description of the physical area where procurement took place) (see Appendix 17);
- d. a list of observations during the physical examination of the donor's body (but, for a living donor, only when such an examination is justified) (see Appendix 13);
- e. a description and identification of procured tissues and cells, including samples for testing of infectious diseases;

<i>f.</i> the identification of the person who has overall responsibility for the procurement session (including his/her signature); <i>g.</i> date, time (where relevant, start and end times) and location of procurement; <i>h.</i> type, volume, manufacturer and the lot/batch/serial number of reagents, additives, and the tissue and cell transport solution(s) used; <i>i.</i> name and address of the tissue establishment; <i>j.</i> name and destination of the tissues and cells.	<i>c.</i> 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); <i>d.</i> if possible, whether procurement took place before or after autopsy and whether or not an autopsy is planned; <i>e.</i> when applicable, a description of other tissues and cells from the same donor sent to different tissue establishments, including their identification; <i>f.</i> if applicable, information regarding reconstruction of the donor's body.

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;

If procurement from a living donor involves a directed donation, the recipient's identification must be documented to avoid confusion.

Related documents:

Appendix 17. Sample form to assess the suitability of the working environment;

Appendix 18. Sample donor identification form.

Chapter 7. Processing and storage

7.1. Introduction

‘Processing’ means all operations involved in the preparation, manipulation, preservation, packaging and inactivation of micro-organisms in tissues or cells intended for human application. Storage occurs 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 during storage and that cross-contamination or loss of traceability is avoided.

The opportunity to process tissues and cells brings great advantages. The aims of processing include:

- a. facilitating and optimising clinical use by dividing a donation into multiple, ready-to-use units of tissues or cells;
- b. preservation of the required properties of the biological material, making extended storage for future use possible;
- c. reducing the risk of disease transmission or adverse reactions by removing those elements that are not necessary for the success of the transplant and the inactivation of microbes or even sterilisation in circumstances where cell viability is not required.

Processing includes a range of activities such as (but not limited to) cutting, grinding, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, separation, concentration or purification of cells, freeze drying, freezing and cryopreservation.

Although it brings great benefits, processing can also introduce risks. The potential risks include:

- microbial contamination from the environment, the operator or cross-contamination from other tissues or cells,
- errors in identification or labelling, or
- a detrimental impact on key characteristics of the tissues or cells which renders them clinically ineffective.

For these reasons, all the necessary steps must be carried out within a comprehensive quality management system, must be documented in standard operating procedures (SOPs) and must be thoroughly validated to demonstrate that the quality and efficacy of the final product have not been compromised and that contamination or cross-contamination has not been introduced during processing.

Procurement is defined as the technique for obtaining different tissues directly from the donor, e.g. procurement of corneas by *in situ* excision or procurement of menisci by arthrotomy. However, if the eye or whole knee was removed from the donor, and corneas and menisci were then excised at the procurement site, this activity would be classified as processing. Processing in the procurement facility, either during or after procurement, is not recommended because it is important to prevent microbial contamination, or cross-contamination of procured tissues. This chapter provides generic guidance on the processing and storage of tissues and cells carried out by tissue establishments (tissue establishments). Further,

more specific, guidance is provided in Part B of this Guide.

7.2. Acceptance criteria: receipt at the tissue establishment

Each tissue establishment must have a documented policy and specifications against which each consignment of tissues and cells (including blood samples from donors) is verified. These specifications must include the technical requirements and other criteria considered by the tissue establishment to be essential for the maintenance of acceptable quality. When the procured tissues or cells arrive at the tissue establishment, 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) should be cross-checked with the contents of the package.

The packaging, the tissues and cells received and any accompanying samples should all be examined to ensure that they have not been damaged or tampered with during transit.

The following should be checked and recorded:

- 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;
- c. transport conditions (unless a validated transport method has been used) and storage temperature and time in transit;
- d. 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. status of the tissues or cells (e.g. quarantine);
- i. associated samples (including blood).

The tissue establishment must ensure that the tissues and cells received are quarantined and stored in a defined, separated and adequate location and 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 tissue establishment 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 section 6.5);
- 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, 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 tissue establishment 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 tissue establishment 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 tissue establishment. Such transport should be direct, without intermediate stops where possible, to ensure the safety and maintenance of the temperature conditions of the tissues and cells and prevent unauthorised access.

Quality-control checks of procurement and transportation methods should be reviewed regularly by tissue establishments to ensure that the integrity of tissues or cells and the storage temperatures are maintained during procurement and transit.

7.3. Coding

Tissue establishments must ensure that human tissues and cells are correctly identified at all times. Upon receipt of the tissues and cells, the tissue establishment should 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 and storage.

Tissues and cells should be labelled at all stages of processing and storage (see Chapter 13 for further guidance on labelling and Chapter 14 for further guidance on traceability). The label must include at least the following information:

- a. unique identification;
- b. identification of the tissue establishment;
- c. type and characteristic of the product;
- d. batch number (if applicable);
- e. recipient name (if applicable).

The coded data must be entered in a register maintained for the purpose.

7.4. Processing and storage

7.4.1. General

Tissues and cells should be appropriately processed and preserved for clinical use. Tissue establishments must include in their SOP all processes that affect quality and safety.

Tissue establishments must ensure that the equipment being used, the working environment, process design, and validation 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 tissue establishment personnel.

If a tissue establishment entrusts one of the stages of processing to a third party, a written agreement is needed between the tissue establishment and the third party. The tissue establishment 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 cardiac arrest) until processing and storage must be defined. Procurement, processing and storage times must be documented in the records for tissues and cells.

The reagents used in preservation and 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 avoided if possible or at least be justified by the institution for information of the possibility

of medicinal allergies (such as to antibiotics) of the recipient. Whenever possible, reagents used for procurement, processing and preservation should be approved for human use and should be CE (Conformité Européenne)-marked. Reagents that are not of appropriate grade must undergo validation for the 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 or supported by extensive medical literature. 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. description of the 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. qualitative and quantitative requirements, with acceptance limits;
- d. storage conditions and precautions;
- e. the maximum period of storage before re-examination.

7.4.2. Processing methods

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 (see the general text on validation in Chapter 2 and section 7.10 below).

The processing procedures must undergo regular critical evaluation to ensure that they continue to achieve the intended results.

A written change-control procedure must be followed before any significant change is implemented in processing. A change-control procedure ensures 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 SOP;
- d. need to re-validate the process;
- e. effects on quality-control (QC) analyses;
- f. need to inform regulatory authorities;
- g. need to train personnel;
- h. effect on risk analyses.

Pooling of different tissues and cells from two or more donors during processing is not recommended. The only exception is where it has been demonstrated to be the only way of providing sufficient clinically effective tissues or cells. If performed, traceability must be fully ensured.

7.4.2.1. Processing procedures and equipment

The main processing procedures include, but are not limited to:

- a. cleansing,
- b. cutting,
- c. separation,
- d. shaping,
- e. washing,
- f. grinding,
- g. centrifugation,
- h. soaking in antibiotic or antimicrobial solutions,
- i. cell separation, concentration or purification,
- j. filtering,
- k. lyophilisation,
- l. freezing,
- m. cryopreservation (which includes vitrification),
- n. thawing/warming,
- o. glycerolisation,
- p. demineralisation,
- q. decellularisation,
- r. sterilisation and inactivation.

Cleansing of procured material from surrounding tissues and blood is an initial processing step. Usually scalpels and scissors are helpful for that procedure.

Cutting is the one of the main procedures performed in the tissue bank. It allows initial cleansing of procured tissues for future shaping and formation of grafts. For this procedure various types of cutting devices should be used, depending on type of tissue. For bone cutting different types of saw are used – oscillating saw, bandsaw or rotary saw – whereas for soft tissues (e.g. skin, tendon) scissors or scalpels (including those with single-use blades) are used.

Separation is used to remove the particular type of tissue to be processed from another type, e.g. to divide dermis and epidermis; or during processing of amniotic membrane, which should be separated from a chorion that is lying under and adhering to it. This procedure, separation, also describes the collection of particular types of cell, e.g. CD34⁺ cells from peripheral blood.

Shaping allows formation of a final graft of particular dimensions, as during cutting; similar instruments are used.

Washing is performed as an initial step in processing, e.g. removal of blood from tissues or during formation of grafts to remove cells, e.g. bone marrow or fat from bone grafts; also to remove chemical compounds used during processing, e.g. methanol/chloroform solution used for defatting. This procedure also decreases the potential bacterial contamination of tissues. Several types of washing solution may be used, e.g. distilled water, 0.9 % NaCl, balanced salt solution, phosphate-buffered saline, chlorhexidine, povidone-iodine, alcohols.

Grinding by different types of mill, e.g. liquid nitrogen bone mill, is used to pulverise bone tissue into smaller pieces, used as filling material during bone-reconstruction procedures.

Centrifugation allows concentration and separation of cells from suspension or different fractions of suspensions.

Soaking 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 inactivate potentially present infectious contaminants, e.g. 1M NaOH for inactivation of prions [1].

Cell separation, concentration or purification is used as an initial step for HPC processing or for *in vitro* cell cultures.

Filtering procedure is used for collecting bone marrow into a collection bag, as well as for preparation procedures of various solutions using 0.22 µm antibacterial filters.

Lyophilisation or freeze drying is a dehydration of tissues by freezing and then reducing the surrounding pressure to allow the frozen water in tissue to sublime directly from the solid phase to the gas phase. Lyophilised tissue should contain no more than 5 % of residual moisture. Lyophilisation stops tissue autolysis and allows storage at room temperature.

Freezing (see §7.7.1) is used for pre-processing storage of procured tissues and in-processing storage

between different processing steps of non-viable tissues.

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 such as dimethyl sulfoxide or glycerol. Controlling the cooling rate minimises the risk of cellular damage from intracellular freezing and the cryoprotectants protect cells against slow-cooling (solution effects) injury. The type and concentration of cryoprotectant and the cooling and warming rates need to be optimised for each tissue and cell type. Vitrification is ice-free cryopreservation. The crystallisation of ice is avoided by an extreme elevation of viscosity during cooling achieved by a combination of high cryoprotectant concentrations and rapid cooling.

Glycerolisation is a procedure for soaking tissues, mainly skin, in a glycerol solution where the concentration has been increased from 50 up to 85 %, to dehydrate the skin by osmosis and diffusion out of the cells and extracellular matrix (ECM).

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 performed in 0.5 M or 0.6 M HCl solution.

Decellularisation is a process of removal of cells from the extracellular matrix (ECM) of a tissue or organ. The choice of decellularisation agents depends on tissue type, and its thickness and density. The complexity and length of the decellularisation protocol (the process requires the combination of several techniques) depends on the extent of the structural and biological properties which should be conserved after processing (e.g. macrostructure, ultrastructure, matrix and basement membrane proteins, growth factors). Decellularisation can be classified according to type of compounds and techniques used [2]:

- chemical,
 - acid and bases, e.g. paracetic acid, calcium hydroxide, sodium sulfide, sodium hydroxide,
 - hypotonic and hypertonic solutions,
 - detergents – ionic, non-ionic and zwitterionic, e.g. Triton X-100, sodium dodecyl sulfate (SDS), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS),
 - alcohol, e.g. glycerol, methanol,
 - other solvents, e.g. acetone, tributyl phosphate (TBP),
- biologic,
- enzymes (nuclease, trypsin, collagenase, lipase, dispace, thermolysin and α -galactosidase),

- non-enzymatic agents, e.g. chelating agents, ethylenediamine tetra-acetic acid (EDTA), ethylene glycol tetra-acetic acid (EGTA), toxins, latrunculin, serine protease inhibitors, phenyl-methylsulfonyl fluoride (PMSF), aprotonin, leupeptin,
- physical,
 - temperature (freeze–thaw),
 - force and pressure,
 - NTIRE – non-thermal irreversible electroporation [2].

Sterilisation refers to any process that eliminates or inactivates transmissible infectious agents (pathogens) containing nucleic acids, e.g. vegetative and spore forms of bacteria and fungi, parasites, viruses. The probability of viable micro-organisms being present on or inside a product unit after sterilisation is described by the sterility assurance level or SAL. SAL 10^{-6} would assure that less than one out of a million contaminants would survive on or inside the product following sterilisation. Depending on the risk posed by the use of various specimens, different values of SAL (10^{-3} , 10^{-6}) may be recommended. For tissue allografts, a value of SAL $\leq 10^{-6}$ is recommended.

There are several methods of sterilisation, and only some of them can be recommended for sterilisation of tissue grafts. Those include the following.

- chemical:
 - alcohol
 - paracetic acid
 - supercritical CO₂
- physical:
 - heat (pasteurising)
 - ionising radiation

Inactivation methods applied for tissue and cell banking include soaking in antibiotic or antimicrobial solutions, filtration and glycerolisation (see above).

Equipment and instruments should be of quality appropriate to their intended functions. Requirements for equipment are described in more detail in Chapter 2.

7.4.3. Requirements of processing facilities

Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be designed, validated and monitored to ensure that the air quality is appropriate for the process being carried out (see Chapter 8).

7.4.4. 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 in order 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 (see Chapter 8).

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. In some cases, e.g. for ocular tissue, single-use surgical instruments for procurement and processing are available and recommended. Furthermore, tissues or cells that must be processed further (e.g. lyophilisation, sterilisation) should be treated as a single donation. Each tissue or cell product should have a batch number that is also recorded in the processing records.

In case of pooling during the process, a risk evaluation should be in place to preclude cross-contamination. Pooled tissues or cells should be treated as a single batch while ensuring that the original donations are fully traceable.

7.5. Packaging and labelling

Packaging and labelling are described in more detail in Chapter 13.

7.6. Quality control

7.6.1. General

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 at least the test method, the sample size and the accepted criteria. The minimum requirements for evaluation of each type of tissue and cell are described in tissue- and

cell-specific chapters (see Chapters 16-29). The results of all tests or procedures should become part of the permanent processing record.

If in-process controls are undertaken in the processing area, they should be carried out so that there is no risk to the processing steps being followed.

7.6.2. Microbiological control

In many cases, it is not possible to exclude contaminated material during processing because the tissue originates from naturally contaminated parts of the body, and disinfection is not 100% effective. 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. Additionally, validated methods of decontamination or inactivation during processing of tissues and cells, or sterilisation of non-viable tissues, have an effect on the microbiological status of the tissues and cells.

Microbiological controls should be done on starting or incoming tissues or cells. Chapter 9 describes microbiological assessment of the starting or incoming tissues and cells, in-process controls and the final product. In-process testing should be undertaken at relevant processing steps; for instance, after a stage of decontamination or inactivation, or after washing or a change in storage solution. Whenever possible, a tissue or cell sample of the final product should be tested. In addition (or, in exceptional cases such as an exclusive sample), spent storage or culturing medium or final washing solutions can be used for testing with an evaluated and validated method. Every effort should be made to take representative samples. It is widely recognised that swabbing is not an effective method of tissue sampling and that small tissue samples are frequently not representative. Chapter 9 also describes methods of microbiological controls. Sampling and testing methods must be validated to show the representativeness of the sample and the suitability of the selected methods.

Various procedures exist for securing microbiological control, such as decontamination by antibiotics, or physicochemical methods. Some standard sterilisation procedures, such as heat sterilisation or sterile filtration, are not applicable to preparations of tissues and cells. 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 should be shown for relevant micro-organisms in the tissue or cell preparation itself and not only in an aqueous solution. Some micro-organisms may

survive the antibiotic treatment, but are not detected by microbiological testing due to sampling error, and can start to grow and reproduce when conditions change. The terminal sterilisation designation applies when the product, in its final package, is submitted to a chosen sterilisation procedure resulting in a sterility assurance level (SAL) $\leq 10^{-6}$ (see above, 7.4.2.1).

Non-conforming products must be identified and separated from conforming products. The fate of non-conforming products will be decided by the RP in charge of the tissue establishment.

7.7. Storage

7.7.1. Methods of storage

Following processing, tissues and cells should be stored according to currently accepted good practice, based on the best available scientific evidence and according to good manufacturing practice (GMP), as appropriate, for tissues and cells. All procedures associated with storage of tissues and cells must be documented in SOPs.

Currently used methods of tissue and cell storage include:

- storage in organ culture media above room temperature (e.g. 28-37 °C for corneas);
- hypothermic storage (refrigeration) between 2-8 °C;
- storage at room temperature (for freeze-dried products);
- Ultralow temperature storage (for slow freezing and ultra-rapid/vitrification procedures).

Freezing can disrupt tissues and cells. Hence, the method of freezing used must take into account the eventual use of tissues and cells. Since cell viability must be maintained, penetrating cryoprotectants – such as dimethyl sulfoxide (DMSO), 1,2-propanediol (PROH) or glycerol – and non-permeable agents such as sucrose must be added before tissues or cells are frozen. In such cases, the rate of cooling must be controlled to prevent formation of ice crystals within cells, which can result in damage and loss of tissue integrity. Once cryoprotectants are added, cells may be placed in a freezing device (i.e. a controlled-rate freezer) that gradually reduces the temperature of the mixture in accordance with a given protocol. Where possible, the rate of cooling should take into account the volume of the cells being cooled. Post-thaw viability of cells should be assessed to determine the effectiveness of the cryopreservation and storage method used.

In the case of multi-layered tissues, diffusion of the cryoprotectant into the tissue and the effectiveness of control of freezing will depend on whether the tissue consists primarily of the extracellular matrix or dense cellular layers. Loss of tissue integrity can occur in certain regions (e.g. inner layers) if the cryoprotectant is distributed unevenly. Hence, the size and type of tissue must be taken into consideration when validating the method used for cryopreservation.

The rate of cooling for each tissue and cell preparation should be monitored and recorded to ensure that any deviations from the validated protocol are noted. If post-thaw viability and structural integrity is not compromised, tissues may be cryopreserved by placing them in a freezer without the addition of cryoprotectants.

7.7.2. Storage temperature

Refrigeration devices/incubators containing tissues and cells should be suitable for the intended use and procedures for monitoring such devices should be appropriate so that tissues and cells are maintained at the required storage temperature. Regular monitoring and recording of temperature, together with suitable alarm systems, should be employed on all incubators, storage refrigerators, freezers and liquid nitrogen tanks (see Chapter 2).

Temperature ranges for storage of tissues and cells are shown in Table 7.1 [3].

Table 7.1. Temperature range for storage of tissues and cells

Storage condition	Temperature limits (°C)
Cryopreservation	< – 140
Deep frozen ^a	– 80 to – 60
Frozen ^b	< – 15
Refrigerated ^b	2 to 8
Cold or cooled ^b	8 to 15
Room temperature ^b	15 to 25
Organ culture	28 to 37

a. Based on general practice.

b. Based on the *European Pharmacopoeia*.

7.7.3. Avoiding contamination and cross-contamination

Every effort should be made to avoid cross-contamination of material stored in vessels containing liquid nitrogen. Tissues and cells immersed in liquid nitrogen should be double-wrapped during storage (this may not apply for reproductive cells; see Chapter 24). This is highly important for storage with

liquid nitrogen owing to the accumulation of microbial contaminants in liquid nitrogen storage vessels. The seals and the material employed must be validated 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.

7.7.4. Quarantine

All human tissues and cells that are stored before determination of their suitability must be kept under quarantine. Quarantined tissues and cells should 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 products.

7.8. 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 storage condition. The selected maximum storage period can be based on national legislation. If it is not based on national legislation, it should be based where possible on data from published studies, stability testing by the establishment, or retrospective evaluation of the clinical results for tissues and cells supplied by the establishment.

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. tissue availability;
- d. package integrity over time;
- e. expiry of storage solutions;
- f. stability at the storage temperature;
- g. overall risk assessment of quality assurance: donor evaluation, donor testing (kits), regulations.

When relevant for the type of tissue or cell, the time of procurement should also be indicated.

In certain specific cases, it could be possible to prolong the expiry date, especially in the case of cryo-preserved HPC (i.e. cord blood), reproductive tissues and cells for partner donation or in cases of fertility preservation (see Chapters 24 and 25).

7.9. Risk assessment

A documented risk assessment approved by the Responsible Person (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 2. Remote alarms and auto-dialler systems must be checked regularly.

7.10. Processing and storage validation

If processing is carried out according to GMP, the processing validation must 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 signed declaration of validation acceptance or rejection by the quality manager (QM) or the RP.

Validation studies should be carried out by applying 'worst case' scenarios. The equipment used for validation studies should be fully qualified, and measuring devices should be calibrated to traceable standards. Validation experiments should be repeated at least in triplicate, though this will depend on the degree of variability in the data, to ensure reliably repeatable results. For an example of a validation study, see Appendix 4 (Example of cleanroom qualification), Appendix 5 (Example of incubator qualification), Appendix 6 (Example of a process validation) and Appendix 7 (Examples of method validation (oocyte vitrification) in assisted reproductive technology).

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 tissue establishment 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 provided to demonstrate that the process is equivalent to that applied in the scientific literature.

Where specific steps have been 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. However, they should all be fully validated. Decontamination methods, such as antibiotic soaking, should be validated to demonstrate effectiveness against a range of contaminants similar to those routinely 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, tissue establishments should monitor tissue and cell quality to ensure a state of quality control is maintained throughout the processing part of the product lifecycle. This will provide assurance of the continued capability of the process and its quality controls to produce finished tissues and cells that meet the desired quality and to identify changes that may improve product quality or performance. Relevant process trends (e.g. quality of incoming materials or components, in-process and finished product results, cases of non-conform-

ance and defect reporting) should be collected and assessed to verify the validity of the original process validation or to identify required changes to the associated controls. Documentation and tracking of patient outcomes constitutes a critical element of ongoing process verification. For new or significantly changed processes, a project of close clinical outcome monitoring should be agreed with clinical users.

Regarding storage validation, homogeneity and reproducibility are fundamental requirements of tissues and cells storage-banking. No matter which type of storage strategy used, it will be fundamental to demonstrate either that the number of units stored in individual ampoules or other devices is sufficient to generate a cell culture having similar physiologic features to the original cells or that the culture will be good enough to achieve a successful transplantation procedure. Stored material should be audited to ensure that both maintenance and documentation are updated and that any changes have been accordingly recorded.

7.11. Release

7.11.1. Release procedure

Release is the act of certifying compliance of a specific tissue or batch of tissues with the requirements and specifications. Before any tissue is released, all relevant records (including donor records, processing and storage records, and post-processing quality-control test results) must have been reviewed, approved and documented as acceptable by the RP according to the relevant local SOP and national regulations. There must be an SOP that details the circumstances, responsibilities and procedures for the release of tissues and cells.

At the time of release, donor records and tissue- or cell-processing records should be reviewed to ensure that the material is suitable for transplantation and implantation. The review should include:

- a. approval of donor eligibility by the RP or designated person;
- b. review and approval of the processing and storage record (including environmental monitoring records);
- c. final inspection of the label and container to ensure accuracy and integrity;
- d. results of screening tests on incoming material and in-process testing;
- e. specifications for final product release based on testing results used to determine final release (e.g. microbiology test results; if necessary and

justified, release of the final product can be undertaken on a 'negative-to-date' basis).

The items indicated in the processing and storage record should contain at least:

- a. the procurement file and/or release statement of the person responsible for procurement;
- b. type(s) of tissues and cells processed and/or stored (number of units per device or ampoule);
- c. quantitative and qualitative description of the tissues and cells processed, preserved and/or stored;
- d. date and time of each stage of processing and storage, identification of persons responsible for each step and the identifying reagents and materials used (batch number and expiry date);
- e. status of tissues and cells at all stages of processing and storage (i.e. quarantine, release for therapeutic use, release for manufacture of medicinal products, *in vitro* research, etc.);
- f. use of antibiotics, antibiotic composition and incubation period (if applicable);
- g. type and amount of reagents used;
- h. procedures and records concerning the processing of tissues and cells (if applicable);
- i. processing data (preparation, culture technique, incubation, treatment chemicals);
- j. data on techniques of decontamination, sterilisation or viral inactivation;
- k. results of specific quality testing, depending on tissue and cell type (e.g. human leucocyte antigen (HLA), histology, radiology results, tissue or cell viability, number of CD34 cells);
- l. procedures and records concerning the preservation of tissues and cells (e.g. cryopreservation, trace of the cooling curve, glycerolisation, lyophilisation), if applicable;
- m. date and time of storage;
- n. method of storage;
- o. storage temperature;
- p. expiry date;
- q. identification of tissues and cells (i.e. donor identification code plus product code).

Access to registers and data must be restricted to authorised persons. These records must be kept for a minimum of 30 years after clinical use or discard of tissues and cells.

The person responsible for the release of tissues or cells should sign a statement that specifies fulfilment of all ethical, legal requirements and quality release criteria, thereby releasing the tissues and cells for storage in an inventory of tissues and cells that

are available for transplantation, human application or research/educational use.

Released tissues should be physically separated and visibly different (by labelling and/or packaging whenever possible, or by any other means, e.g. computerised systems) from quarantined tissues. The tissue establishment must provide clinical users with instructions for using the tissue/cells. Clinical users must be reminded that they must report any adverse events or reactions to the tissue establishment.

7.11.2. Exceptional release

In exceptional circumstances, a tissue establishment may agree with an organisation responsible for human application of tissues and cells (ORHA) that tissues or cells that do not meet the normal criteria for release are released and used in a specific patient on the basis of a risk–benefit analysis taking into consideration the alternative options for the patient and the consequences of not providing the tissues. For more information see Chapter 11, section 11.5.

7.11.3. Disposal of human tissues and cells

There must be a documented policy for discarding tissues and cells that are unsuitable for clinical use. Records should include details of date, methods of disposal and reasons for discarding the material. The material should be appropriately handled and disposed of in a manner compliant with local control of infection guidelines. Human tissues, cells and other hazardous waste items should be disposed of in such a manner as to minimise the hazards to the tissue establishment'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 for the human body. For HPC products it is necessary to document that the conditions for disposal defined prior to collection 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.

7.12. References

1. Taylor DM. Inactivation of prions by physical and chemical means. *J Hosp Infect* 1999;43(Suppl):S69-S76.
2. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials* 2011;32(12):3233-43.

3. European Directorate for the Quality of Medicines & HealthCare. European Pharmacopoeia, General notices, general chapter 1. *Ph. Eur.* 8th edition. Strasbourg, France: Council of Europe; 2013.

Chapter 8. Premises

8.1. Introduction

In general, tissue establishments must have suitable facilities to carry out the activities for which accreditation/designation/authorisation or licensing is sought. These activities include processing of tissues and cells while exposed to the environment, which must take place in an environment with specified air quality and cleanliness in order to minimise the risk of contamination, including cross-contamination between donations. This chapter provides generic guidance on the facilities used for processing of tissues and cells. This chapter also gives guidance on validation and monitoring of clean rooms, and measures to prevent cross-contamination (gowning, personnel and material flows, cleaning). 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 categories of materials and products – tissues/cells in quarantine, released for processing, rejected, returned, recalled or for investigative use – with clearly segregated areas

provided for each category (and physically separate areas or storage devices, or secured segregation within the device, must be allocated in both quarantine and released storage locations for holding certain tissue and cells collected in compliance with special criteria);

- d. clean and dry, and maintained within acceptable temperature limits. Where special storage conditions are required (e.g. temperature, humidity) these should be maintained, checked and monitored. The necessary air conditioning capacity for the room must be calculated based on the actual heat load of the equipment and the environmental factors.

In addition, printed packaging and labelling materials may be considered critical and special attention should be paid to their safe and secure storage.

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 include at least:

- a. good ventilation;
- b. oxygen-level monitoring;
- c. a floor resistant to liquid nitrogen;
- 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 LN₂ supply and for prompt removal and transfer of products 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 temperature and oxygen level in the room and the level of liquid nitrogen in the tanks, is essential. Personnel must be trained to react based on different alarms. Personnel need to be trained to use personal protective equipment.

8.3. Requirements of processing facilities

Facilities for aseptic and clean, non-sterile processing must be dedicated to this activity, and must be designed, validated and monitored to ensure that air quality is appropriate for the process being carried out. An international standard, such as the EU Guidelines to good manufacturing practices for medicinal products for human and veterinary use (known as GMP) [1] and/or ISO 14644-1 Cleanrooms and associated controlled environments [2], should be followed regularly to achieve the appropriate air quality.

Processing of tissues and cells should be carried out in clean areas. In these areas, 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.

- Grade A (normally provided by a laminar airflow hood). Laminar airflow systems should provide a homogeneous air speed in the range 0.36-0.54 m/s (guidance value) at the working position in open, cleanroom applications (GMP, Annex 1). Maintenance of laminar flow should be demonstrated and validated. Uni-

directional air flow and lower velocities may be used in closed isolators and glove boxes.

- Grade B (the background environment for the Grade A zone if tissues or cells are processed according to GMP). The risk-assessment tool for defining the air quality can be used to select the background (Table 8.2).
- Grades C and D (clean areas for carrying out less critical stages in accordance with a documented risk assessment of the processing activities) or as background for an isolator.

Comparison of different cleanroom standards is shown in Table 8.1 [1, 2].

Whichever classification is applied, facilities should have:

- a. floors, walls and ceilings of a non-porous material with smooth surfaces to minimise the shedding or accumulation of particles (viable and non-viable) that are easily disinfected;
- b. temperature control and (based on risk assessment) humidity control;
- c. a filtered air supply that maintains positive pressure and an airflow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively; a combination of negative and positive pressure can also be used to achieve specific biosafety requirements;
- d. a documented system for monitoring temperature, air-supply conditions, pressure differentials, particle numbers and bacterial colony-forming units (environmental monitoring, see below at 8.5.4);
- e. a documented system for cleaning and disinfecting rooms 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;
- i. access limited to authorised personnel.

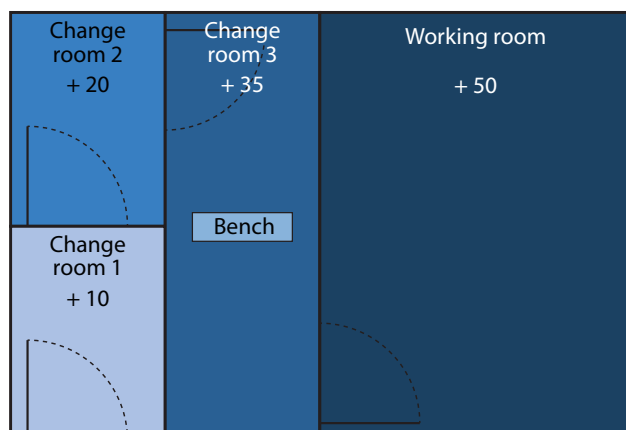
Table 8.1. Air cleanliness classifications in Europe

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	29	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

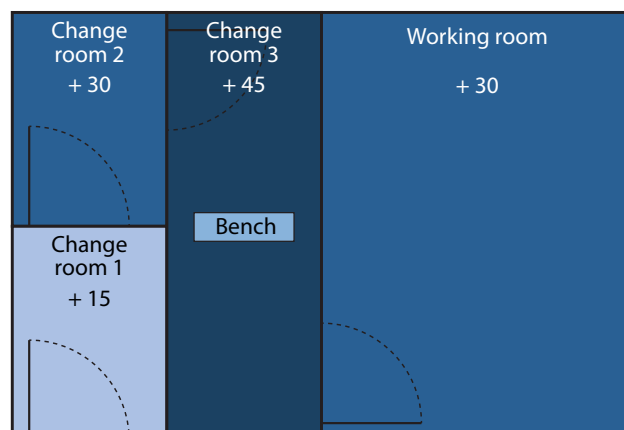
Source: [1, 2].

Figure 8.1. Schematic plans indicating air pressure in typical GMP facilities

Typical GMP facility design



Gene therapy GMP facility design



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 and processing method). Parameter settings should not interfere with the defined cleanliness standard. For temperature and relative humidity, the generally accepted guidance values are $18 \pm 2^\circ\text{C}$ and 30 %-65 % (ISO 14644-4) respectively [3]. Once the environmental temperature and relative humidity requirement have been set to guarantee the safety and quality of the product, staff comfort and energy consumption can also be taken into account.

To minimise the risk of cross-contamination, a positive pressure relative to surrounding areas of a lower grade should be created. The pressure differential between adjacent zones of different grades should be 10-15 Pa (GMP, Annex 1) with the maximal air pressure in the working room [1]. This forms a 'pressure cascade', which ensures that the air flows only outwards and limits the entry of contamination into the clean rooms (Figure 8.1).

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). The required precautionary activities or need for a special contained laboratory (processing room having reduced air pressure relative to the adjacent rooms) should be determined by documented risk analyses. Risk analyses should consider risks relating to cross-contamination of other tissues and cells processed at the tissue establishment. In addition, risk analyses should consider personnel safety according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [4].

However, when working with viruses (see Chapter 26) the cleanroom installation must also

protect the environment, and therefore the working room should have reduced air pressure relative to adjacent rooms (biosafety level 2 and biosafety level 3 contained laboratories). A possible solution is to increase the air pressure in the change rooms to result in the working room having reduced air pressure with respect to the last change room (Figure 8.1). Another specific safety consideration when working with viruses for genetic cell modifications is the protection of the worker. For this reason the use of biosafety cabinets is mandatory. In many cases Class II biosafety cabinets are sufficient but, depending on the virus present, an isolator cabinet is recommended.

Residue manipulation is another issue when working with virus. To protect the environment all material must be autoclaved before leaving P2/P3 (biosafety level 2/3) contained laboratories. The use of autoclaves inside these laboratories, or in between contained laboratories and the next room, is common. However, validating autoclaves in GMP facilities is complicated by the use of water. One possible solution is to place all residues in hermetic containers and autoclave them in another room of the facility. If this is the case, these procedures should be validated.

Figure 8.1 shows schematic plans indicating the air pressure in different rooms of a GMP facility for conventional or gene therapy use. Conventional GMP facilities are designed to protect the sample from any contamination and therefore there is an air pressure increase in subsequent rooms of 10-15 Pa, with the maximum air pressure in the working room. However, gene therapy GMP facilities must be designed to protect both the samples 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.

Table 8.2. Risks of environmental contamination that should be considered when determining air-quality specifications of processing facilities (EuroGTP guidance)

Risk	Explanation
Tissue or cell contamination during open <i>versus</i> closed processing	Processes that are mostly 'closed' need a less stringent specification than those that involve hours of open processing.
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 environmental contaminants.
Suboptimal detection of contaminants due to the sampling method	If the only options for final microbiological sampling are swabbing or testing of unrepresentative samples, the risk that environmental contaminants will be undetected is higher than in processes where 5-10 % destructive testing of final products can be performed [6].
Transfer of contaminants at transplantation	Tissues that are minimally processed, cellularised, or 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 <i>versus</i> temporary) and site of transplantation both affect the risk of transfer of contaminants.

The utilisation of isolator technology to minimise human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured products. 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 validation. Validation 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).

8.4. Selecting the appropriate air quality for processing

According to GMP, aseptic processing must be done in Grade A with Grade B background environments. For tissue 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 environment may be acceptable if one of the following applies:

- a validated microbial inactivation or validated terminal sterilisation process is applied; or,
- 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,
- 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 trans-

mitting bacterial or fungal infection to the recipient than with transplantation of tissues and cells; or,

- 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].

Many national requirements are more stringent, requiring Grade A with a surrounding environment B or C for certain processes.

The specification of the air quality of the processing facilities 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 should be taken into consideration when determining the air-quality specifications, especially, when less stringent conditions are applied, as shown in Table 8.2. 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, more stringent air-quality specification should be adopted. See also Chapter 7 and the tissue-specific recommendations provided in Part B of this Guide.

8.5. Qualification and monitoring

Clean rooms and laminar flow hoods must be classified, re-qualified and monitored in accordance with EN ISO14644 [2, 3, 7, 8] and EU GMP Annex 1 [1].

The maximum concentration of airborne particles for each grade is given in Table 8.1.

8.5.1. Classification of clean rooms and laminar flow hoods

For classification of a clean area, the required tests and acceptance criteria should be defined in the approved qualification (validation) protocol.

For particle count, the minimum number of measurement points (number of sampling locations = NL) of the facility is calculated as the square root of its area in m² rounded up to a whole number (NL = $\sqrt{A}$). Measuring points should be distributed evenly and at the same height.

For classification purposes, portable particle counters with a short charge tube should be used to avoid loss of particles.

Qualification in operation may be carried out during routine or simulated operations.

For classification purposes in Grade A zones and Grade B at rest, a minimum sample volume of 1 m³ should be obtained at each measurement site in accordance with Annex 1 of the EU GMP. In other cases, the minimum air sample volume per measurement point 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).

Samples with too-high particle counts should not be automatically rejected. A single measurement can be rejected due to equipment malfunction, failure of the procedure or because of exceptionally clean air, for the following reasons: the calculations will be repeated for the other points, at least three measurements will be taken into account in the calculations, no more than one measurement value will be omitted during calculations, and the cause of incorrect results will be documented and approved.

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 qualification protocol.

8.5.2. Monitoring particle concentration

For monitoring purposes, parameters for control of clean areas should be supported by data obtained during qualification studies.

Clean rooms and laminar flow hoods should be routinely monitored while in operation. Measuring points should be determined on the basis of a formal risk-analysis study and the results obtained during qualification of cleanrooms.

Adequate warning and alarm limits should be set on the basis of the results of the monitoring of

particles. If the limits are exceeded, processing procedures should be modified and appropriate corrective actions taken.

Monitoring systems for airborne particles may include: independent particle counters, a sequential sampling system, or a combination of both.

The system selected must be adapted to the sampling rate of the appropriate particle size. If using sequential systems, particle losses because of the length of the tubes and kinks in the tubing should be considered.

Selection of the 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 clean rooms 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.

Monitoring of particles $\geq 5.0 \mu\text{m}$ in Grade A and B zones is particularly important as a diagnostic tool for early detection of failure. Occasional counting of single particles can occur as a result of external interference. However, 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 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.

Warning and alarm limits should be determined by the nature of processing. For example, bone cutting may generate numerous particles, and corneal lamellar cutting may generate numerous aerosols, but the recommended at-rest state after a recovery time period should be attained;

Temperature, relative humidity and differential pressure of clean areas should be monitored every day.

8.5.3. Qualification

Qualification (validation) of cleanrooms and laminar hoods is required to support and verify the operating parameters and limits for the critical parameters. The specified acceptance criteria set for your facilities should be verified, and therefore testing of certain parameters and specifications should be performed. Table 8.3 specifies optional test methods characterising the performance of cleanrooms, clean zones and laminar airflow hoods. The choice of tests should be based on factors such as the design of the installation, operational states and the required level of certification. The selected test can be repeated on a regular basis as a part of routine facility monitoring programme based on a formal risk analysis.

All these tests should be undertaken by qualified professionals at least in an at-rest situation in accordance with EN ISO14644-3 [8]. Biohazard laminar airflow hoods should also be certified to national or international performance standards at the time of installation and recertified annually.

8.5.4. Microbiological monitoring

Microbiological monitoring of clean rooms and laminar flow hoods is mandatory and should be done in accordance with:

- a. EN ISO14698 *Cleanrooms and associated controlled environments – Biocontamination control* [9]; and
- b. EU GMP Annex 1.

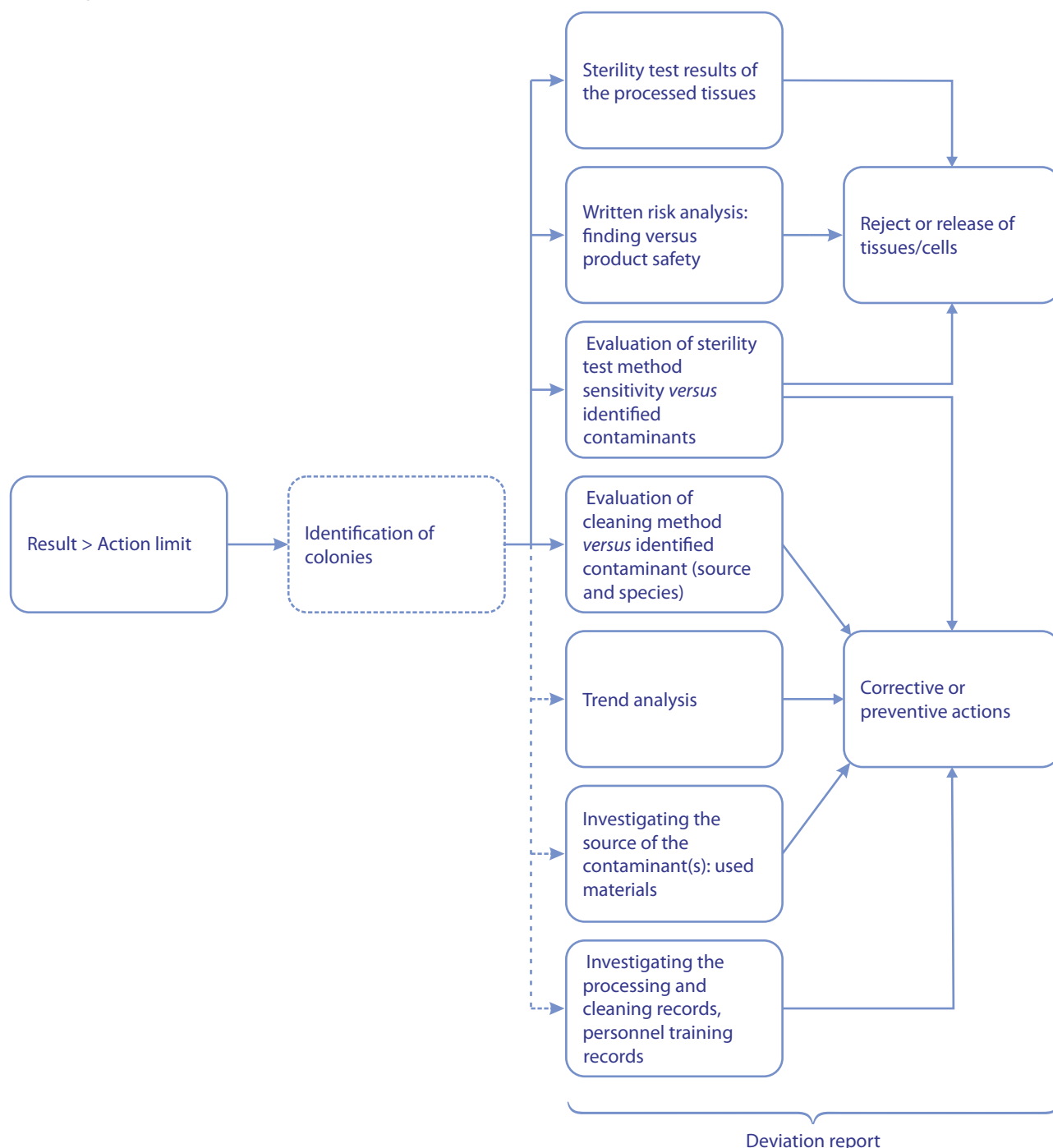
Microbiological monitoring is required during processing and during processing validations, and to demonstrate whether cleaning and sanitation methods are adequate (routine cleaning and cleaning after maintenance breaks). Different sampling methods, reagents and incubation methods are described in Chapter 9.

Microbiological monitoring done during the processing of tissues and cells (with settle plates, volumetric air sampling, contact plates, swabs) should provide information about the quality of the environment during processing and allow tracking of trends in the clean room. Surfaces (with contact plates and swabs) and personnel (with glove prints or fingerprints, i.e. FIPS) should be monitored after critical operations. The frequency of sampling should take into account the processes and activities of the staff. At least for aseptic processing (in Grade A/B clean rooms), monitoring must be frequent during the entire processing period. Sampling methods used in operation should not interfere with zone protection. Results of monitoring should be considered when making the decision whether tissues or cells can be released.

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	6 months if classification is ≤ ISO 5 12 months if classification is > ISO 5
Airflow test	Average airflow velocity and air changes per hour	12 months
Air pressure difference	Differential pressure between different areas	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 the resistance across the filter changes abnormally
Temperature and relative humidity		12 months
Recovery test	The time required for a clean room to recover after a particle-generation event – normally tested for clean rooms 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

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 to 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.

A sampling plan may be developed to evaluate the impact of the personnel on the environment, the effect of the environment on the product and the effectiveness of cleaning and disinfecting procedures.

To define and control microbiological hazards it is necessary to identify the potential risks relating to each processing step and of the tissues or cells themselves, as well as the probability of these risks and mitigation actions to minimise the risks.

Tissue establishments must have a monitoring programme that specifies:

- acceptance limits of microbial contamination (action level, alert level);
- sampling plan and frequency;
- sampling methods and equipment (see Chapter 9);
- sampling culture media and incubation of samples (see Chapter 9);
- analyses and evaluation of results (including trend analyses);
- handling of out-of-specification results.

Table 8.4. Recommended limits for microbial monitoring (EU GMP Annex 1)

Grade	Recommended limits for microbial contamination ^a			
	Air sample (cfu/m ³)	Settle plates, diam. 90 mm (cfu/4 hours) ^b	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

a. These are average values.

b. Individual settle plates may be exposed for less than 4 hours, in which case the limits should be appropriately reduced.

Recommended limits for microbiological monitoring of clean areas during operation are shown in Table 8.4. Alert and action levels for microbial contamination should be determined and 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 routinely and, if necessary, updated if there are changes to processes. The alert level emphasises an acceptable number of 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, detected colonies must be identified to the genus and species, and for other cleanliness grades according to the microbial-monitoring programme of the tissue establishment. Figure 8.2 describes topics and actions to be considered if microbiological-monitoring results exceed the action limit. Any presence of fungi or yeasts must be considered to denote non-conformity and should be identified.

After such results are obtained, tissue establishments should evaluate if the finding will affect the risk that tissues or cells could have been contaminated during processing. Tissue establishments should also evaluate if corrective or preventive actions should be initiated. All investigations that are carried out should be reported in a deviation report.

Table 8.5. 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	Appropriate clean 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
	Shoes	Appropriate clean shoes or overshoes
	Gloves	Sterile, non-powdered rubber or plastic gloves
Grade A/B In general, the protective clothing material should shed no fibres, and clothing should retain the particles shed by the body	Facemask	Sterile, single-use. Eye protection/coverage is dependent upon the process
	Cap	Headgear should totally cover hair, beards and moustaches; it should be tucked into the neck of the suit
	Suit	Sterile coverall
	Shoes	Sterilised or disinfected footwear, boot-like structure to enable the trouser-legs to be tucked inside the footwear
	Gloves	Sterile, non-powdered rubber or plastic gloves

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:

- minimise the risk of contamination of tissues and cells;
- reduce the environmental bioburden;
- 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 clean rooms. 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 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 must follow a written procedure designed to minimise contamination of clean area clothing or transfer of contaminants to the clean rooms. Wrist-watches, 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 working area. Minimum requirements modified from EU GMP Annex 1 are listed in Table 8.5. Clothing should be pocket-less, made of lint-free material, with tightly 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.

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:

- fingers of gloves;
- facemask;
- sleeve (forearm) of a suit;
- front of the suit at chest height;
- 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. All cleaning procedures should be documented. Cleaning should be done by personnel trained for the procedure, cleanroom environment, workflows and gowning. Using a sporicide is highly important, but agents that have sporicidal activity tend to be too harsh in everyday use for equipment surfaces. For this reason it is recommended that a sporicide is used in rotation with another effective disinfectant that is more suitable for regular use. Cleaning products are made up of broad-spectrum disinfectants containing quarternary ammonium compounds, stabilised chlorine dioxide, hydrogen peroxide and sodium hypochlorite.

Certain cleaning products might be detrimental for certain tissues and cells and should therefore on the one hand 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 [10]. Disinfectants and detergents used in Grade A and B areas should be sterile before use. Microbiological monitoring of the clean room 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 (usually 1:100 dilution) and the surface allowed to dry.

Inactivation of prions should be considered if risk of prion contamination has occurred, e.g. 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 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 [11].

Storage facilities should be cleaned according to a schedule. Also, handling and disposal of wastes should include appropriate collection, storage and transportation procedures.

8.8. References

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Chapter 9. Principles of microbiological control

9.1. Introduction

This chapter addresses the control and examination of microbiological, endotoxin and mycoplasma contamination of human tissues and cells, which is critical for ensuring the quality and safety of human tissues and cells used for human application. This chapter also defines the approaches to, and requirements for, effective and meaningful microbiological testing of preparations of tissues and cells and the environments in which they are processed. Guidance on microbiological monitoring for particular tissue and cell processes is provided in relevant tissue- and cell-specific chapters. However, this chapter defines the general principles that should be adopted in developing a comprehensive strategy for microbiological testing. The testing methods to be applied in the laboratory are described and, most importantly, the principles of validation of those methods are defined.

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 to the advice of a suitably qualified expert microbiologist.

9.2. Microbiological examination of donors

Microbiological blood cultures are a useful tool for the diagnosis of bacteraemia and other infections in deceased donors of tissues and cells. The blood samples must be of sufficient quantity, collected

properly and accompanied by relevant clinical information. Compliance with aseptic techniques has to be assured in order to evaluate the origin of a detected contamination properly.

For blood cultures, a clear protocol that addresses skin disinfection, the amount of blood obtained and the number of blood cultures should be followed. 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 a bacteraemia in the donor. It is recommended to take at least 2-4 blood cultures (each aerobic and anaerobic), ideally at different time points and from different vessels. The blood cultures have to be incubated for ≥ 5 days.

- Blood samples for culture can be obtained before or after 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. Blood cultures are valuable to evaluate quality and safety of specific tissues and cells especially if the tissues and cells are not terminally sterilised. The evaluation depends on numerous factors and should be based on knowledge of the particular settings, e.g. time points of blood withdrawal [1-7].

- In the situation where blood cultures are obtained sometime after cardio-circulatory arrest (in which organs and tissues may be at a higher risk of endogenous microbiological contamination) the information provided by blood cultures may be questionable because the course of *post mortem* distribution of micro-organisms is (to a large extent) unknown, and the results can be influenced by agonal spread and *post mortem* bacterial translocation. The conditions under which the deceased donors are stored may be highly variable. On the other hand, information about *post mortem* bacteraemia and fungaemia at the time of procurement may also be useful to assess the quality and suitability of tissues and cells.

The main objectives of *post mortem* cultures in the context of cell and tissue donation are three:

- i. to record infections that could be either clinically unsuspected or clinically suspected but not proven *ante mortem*,
- ii. to evaluate the efficacy of antimicrobial treatment in the case of procurement of tissues, and
- iii. to check contamination from the procurement team and environment as well as cross-contamination between tissues.

The theories supportive of the ambiguity of *post mortem* culture results are agonal spread and *post mortem* bacterial translocation. However, while the first is less common than assumed and prevention is difficult, it appears that the last is completely overcome if the body is refrigerated soon after death [1, 2]. 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 should produce false-positive cultures if the body is appropriately cooled, if the tissue procurement is performed within 24 hours after death (see Chapters 16 to 23) and if the samples for microbiological culture are collected early during tissue procurement, with minimum possible manipulation of the deceased donor before manipulation of the gastrointestinal tract [1, 2]. However, a positive culture, in the particular context of tissue and 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 because they may affect the suitability of particular tissue and cell preparations and of other tissues that eventually could have been affected by

cross-contamination, or may imply that particular attention should be applied to decontamination and/or sterilisation methods.

9.3. General considerations for microbiological control of human tissues and cells

The approaches outlined in this section cover the microbiological control of procurement, processing, storage and release of tissues and cells. The approaches cover the minimum standards to control the microbiological safety of preparations of human tissues and cells such as heart valves, blood vessels, bone preparations, skin, amniotic membranes, corneal tissues or products of haematopoietic progenitor cells (HPC). 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 scenario, to false-negative results.

9.3.1. Microbiological concepts for the detection of bacteria and fungi

9.3.1.1. Microbiological examination of procured tissues and cells

9.3.1.1.1. Sampling

In principle, microbiological examination must be carried out on a sample of the procured tissues and cells. In addition, where applicable, a sample of the storage, transport or rinsing solution should be tested. In cases where the nature of the procured tissues and cells does not allow sampling of the starting material, an alternative sampling approach may be undertaken (e.g. surrogate testing on liquids and/or cells last in contact with the starting material).

9.3.1.1.2. Testing

Considering the nature of the procured tissues and cells and any subsequent processing steps, the microbiological testing approach should follow the procedures outlined in Chapters 2.6.1, 2.6.27, 2.6.12 or 2.6.13 of the *European Pharmacopoeia* (Ph. Eur.). For each procedure, aerobic, anaerobic and fungal testing under appropriate incubation conditions must be conducted. Testing should be extended to specific micro-organisms known to represent potential contaminants where transmission may become relevant for infection. These micro-organisms may not be detectable with common culturing media; therefore,

additional tests for specific infectious agents, such as mycoplasma or mycobacteria, should be undertaken. If applicable, an exclusion list for non-acceptable micro-organisms should be compiled.

9.3.1.2. *In-process testing*

A risk assessment for microbiological contamination should be carried out, considering the nature of the tissues and cells, the origin, procurement, critical steps during processing and their intended application. Critical steps during processing are steps in which exposure to micro-organisms and contaminations of the tissues and cells may occur (e.g. during manual processing of tissue preparations). In particular, the risk of microbiological contamination should be assessed when using band saws or bone mills, and in cutting tissues to size.

In-process testing should be performed at relevant steps of the production process. Generic criteria for undertaking in-process testing are:

- a. testing for any type of contamination before a decontamination procedure (e.g. antibiotic soaking) that has not been validated to remove all contaminants; in this case, a list of contaminants (usually pathogens and micro-organisms) that are not guaranteed to be removed by the decontamination procedure should be defined and used for method validation;
- b. after a stage of decontamination or inactivation, before final storage. Ideally a disinfectant or antibiotic-free period should precede sampling;
- c. when applicable at relevant washing or changing of the storage medium, particularly when decontamination processes cannot be applied.

For in-process testing, storage or the transport solution, used tissue or cell culture medium or other suitable samples should be tested on a regular basis according to the quality management system.

9.3.1.3. *Microbiological examination of the final product or preparation*

9.3.1.3.1. Sampling

After completion of all processing steps, representative samples of every final product – if possible – and tissue or cell culture media, storage or preservative solutions – if applicable – must be tested.

In exceptional cases, if sampling of the final product is not feasible, used tissue or cell culture-medium storage, rinsing or washing solution can be

used instead of microbiological examination of the final product.

If an appropriately validated terminal sterilisation process is applied, testing of samples within a batch has to be undertaken to monitor the effectiveness of the terminal sterilisation process.

9.3.1.3.2. Testing

Microbiological control of the final product should be done in accordance with *Ph. Eur.* 2.6.1. However, automated culture systems can be used for cell suspensions and other liquids (*Ph. Eur.* 2.6.27). Many types of bacteria and fungi found in environmental and clinical settings are detectable by such test methods. If there is a need to extend the testing to micro-organisms with specific culture conditions, suitable culturing methods should be applied.

If release of the product is necessary before the end of the officially verified/required incubation period, '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 are used for product release. Final testing is still ongoing after the product is released and will be completed in line with the above-mentioned requirements. If micro-organisms are detected after product release, predefined measures such as identification and antibiotic sensitivity of the species must be carried out and information must be provided to clinicians caring for the patient. As an alternative, rapid microbiological methods should be considered, especially for preparations of tissues and cells with a short shelf-life. Independent of the applied method, their suitability must be shown with respect to specificity, sensitivity and robustness. Deviations from these standards should be justified.

In general, suitable microbiological tests must be applied for each batch of a final preparation of tissues or cells after final packaging. If this is not applicable, sampling must be completed immediately before final packaging or as late as possible during the manufacturing process.

9.3.2. Testing for mycoplasma

Depending on the type of preparation and manufacturing 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). To identify and assess the contamination risk of specific tissues, a sufficient number of samples from different tissue batches should be examined. Possible sources for mycoplasma 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 manufacturing process.

Mycoplasma can penetrate sterilising-grade filter membranes with a nominal pore size of $\leq 0.2 \mu\text{m}$. 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 immunocompromised patient. However, 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.

Testing should be conducted at manufacturing steps at which mycoplasma contaminations would most likely be detected, such as after pooling or collection but before washing steps. Mycoplasmas are cell-associated micro-organisms that may even locate within the cell, so testing should always include the cellular matrix, if possible.

9.3.3. Testing for bacterial endotoxins

The need for routine testing for endotoxins is dependent upon the intended application of the tissues and cells, and the estimated impact of endotoxins on the recipient. If deemed necessary, it should be carried out according to *Ph. Eur.* 2.6.14. In any procedure in which animal products are used (e.g. collagenase), endotoxin testing should be done. Endotoxins in pancreatic islets will negatively affect insulin production and the outcome of transplantation.

The presence of endotoxins in products can result in responses ranging from fever to irreversible and fatal septic shock. Endotoxins are major components of the outer membranes of most Gram-negative bacteria. If bacteria are growing actively they shed small amounts of endotoxins (and large amounts if they die).

Endotoxins have important roles in cell cultures because they can alter the evolution of cell cultures and thereby impair the safety and efficacy of the product. The potential sources of endotoxins in cell cultures are:

- a. glassware and plastic ware used in the laboratory;
- b. washing solutions or water used to prepare media and solutions;
- c. media and sera used during cell culture;
- d. any components and additives.

Hence, it is recommended to use raw materials certified to be free of endotoxins by their manufacturers.

Each laboratory that works with cell cultures should have a specific risk assessment and risk analysis that should include when and how to carry out an endotoxin test, together with all microbiological controls that are considered necessary. It is recommended to carry out an endotoxin test in the final product before release to the patient.

Endotoxins can be detected using the specific limulus amoebocyte lysate (LAL) bacterial endotoxin test (BET). Because of the great variability in the responses of cells to endotoxins, it is not possible to state the critical level at which endotoxins begin to interfere with the function and growth of cells. The endotoxin limit that can be accepted in cell products is based on the route of administration (intravenous or intrathecal), the threshold pyrogenic dose and the volume of the injected product. For certain cellular products that must be administered immediately and that cannot be cryopreserved without damaging the viability and quality of cells, the availability of a rapid testing method for endotoxin testing is fundamental. The BET quantifies only the amount of endotoxin, not the biological impact. Cell-based assays such as the monocyte activation test (*Ph. Eur.* 2.6.30) might be more suitable for estimating the biological effects [8-10].

9.3.4. Explanatory notes – microbial controls required for specific processing methods

9.3.4.1. Processing using closed systems

For specific types of product such as HPC, and similar preparations in which a closed system is used for processing and where no further cell-cultivation steps are conducted, repeated testing steps are not suitable and do not yield more information on the microbial status of the product. In such cases, a reduced testing strategy that relies on single testing of samples taken at an appropriate time point may be applicable. For microbial testing of HPC preparations, methods need to be validated before use, i.e. matrix validation [11].

9.3.4.2. Processing with terminal sterilisation

For preparations that undergo a validated sterilisation process, the requirements of *Ph. Eur.* 5.1.1

should be considered where methods of sterilisation are described (steam, dry heat, ionising radiation, gas). In particular, it must 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.

Procedures and precautions employed for sterilisation are such as to give a sterility assurance level (SAL) of $\leq 10^{-6}$.

If the release of products sterilised in their final container is intended to rely on process data only and not on final product testing for sterility ('parametric release'), then validated procedures for all critical production steps and a fully validated sterilisation method must be applied. This approach includes validation of procurement of tissues or cells, transportation, washing, antibiotic treatment and other processing steps, packaging and storage. Authorisation by the health authority is needed for such an approach.

9.3.4.3. *Processing that includes decontamination of tissues and/or cells*

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. Because of virulence mechanisms such as facultative intracellular parasitism, biofilm formation and persistence in a resting metabolic state without growth (which is reversible upon withdrawal of antimicrobial agents or stress conditions), microbial cells can escape these treatments. Knowledge about the acceptable quantitative and qualitative microbial load of the starting material – the bioburden [12] – before antimicrobial treatment is necessary.

Efficacy studies focusing on the usually expected initial state of tissues or cells as well as the type and concentration of antimicrobial agents should be carried out. Based on the outcome of those studies, only temporary treatment and exclusion of specific contaminants in the incoming material should be carried out. Further processing after the decontamination step should be conducted without antimicrobial agents. Methods for final product testing must be evaluated carefully with respect to possible

inhibition of microbial growth due to decontaminating agents or their residues.

9.3.4.4. *Open processing without terminal sterilisation*

Most tissues and cells, including preparations which have been decontaminated, are processed using open processing methods. Open processing is any processing step in which tissues and cells are exposed to the environment at any stage between procurement and packaging. This section relates to open processing methods, where the preparation is not subjected to terminal sterilisation. In general, any risk of contamination from the environment or consumables used during open processing must be reduced. The requirements for microbiological sampling and testing are expected to be most stringent in these situations, and aseptic conditions must be maintained during procurement, transportation and the whole manufacturing process.

If open processing takes place without terminal sterilisation, the sampling and microbiological assessment should include the starting material, the transport solution and any solutions used to wash tissues and cells. *Ph. Eur.* 2.6.1 provides a means of verifying that the tissues and cells are sterile. Alternatively, depending on the nature of the tissue or cell-based preparation, the approaches in *Ph. Eur.* 2.6.27 may be applied. If a preparation is not required to be sterile or cannot be rendered sterile, *Ph. Eur.* 2.6.12 and 2.6.13 describe tests that allow quantitative enumeration of micro-organisms. Such testing may require use of validated methods employing special media and/or conditions to enable growth of such micro-organisms and their detection. In addition, the final product should be tested to ensure quality and safety for clinical use.

9.4. Microbiological testing

For each procedure, aerobic and anaerobic testing must 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 those of environmental or clinical origin. If indicated, control tests must be carried out for specific micro-organisms that may not be detectable with the culturing media recommended in the relevant chapters of *Ph. Eur.* (e.g. *Mycobacterium* spp., fastidious micro-organisms).

Table 9.1. Incubation conditions for sterility testing

	Culture medium	Incubation temperature °C	Incubation period
Aerobic	Soya-bean casein digest medium (TSB)	20-25	14 days
Anaerobic*	Fluid thioglycollate medium	30-35	14 days
Fungi	Soya-bean casein digest medium (TSB)	20-25	14 days

* Fluid thioglycollate medium will also detect aerobic bacteria.

Table 9.2. Alternative incubation conditions^a for sterility testing

	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) ^b	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 °C (automated system)

a. Incubation period is > 7 days with an automated growth-based method and may be extended up to 14 days. Incubation period is 14 days with a manual method.

b. 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.

9.4.1. Sterility testing of solutions or tissue samples to *Ph. Eur.* 2.6.1

Conditions for sterility testing are detailed in Table 9.1.

Precautions should be taken against microbial contamination during a test (*Ph. Eur.* 2.6.1). At the least, sub-cultivation should be carried out in a class-A laminar airflow cabinet properly disinfected before the test and no other activity should be conducted at the same time.

The preferred microbiological control procedure is dependent on the sample material.

9.4.1.1. Membrane filtration method

This method uses membrane filters having a nominal pore size $\leq 0.45 \mu\text{m}$ whose effectiveness to retain micro-organisms has been established.

9.4.1.2. 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 must be carried out (see *Ph. Eur.* 2.6.1).

Ph. Eur. does not include specific microbiological growth media for the detection of fungi because fungi are detected along with aerobic bac-

teria in soya-bean casein digest medium. However, other media and /or incubation temperatures may be used, provided that they pass the growth-promotion and validation tests (according to *Ph. Eur.* General Notices on alternative methods). Alternative incubation conditions are shown in Table 9.2.

9.4.2. Microbiological testing using automated culture systems (*Ph. Eur.* 2.6.27)

9.4.2.1. Incubation conditions

Use of an automated culture system may be advantageous, especially for liquid sample material which already results in turbidity of the culture media immediately after inoculation. Unlike *Ph. Eur.* 2.6.1, automated culture systems are mostly preset to an incubation temperature of 35-37 °C, which is based, for example, on the testing of blood and blood products.

Incubation in automated culture systems should, ideally, be carried out over 14 days, especially if risk assessment identifies potentially slow-growing micro-organisms; otherwise 7 days may be sufficient. The incubation time can be adapted to specific requirements arising from the characteristics of the preparation. Incubation times should be validated.

The time and temperature of incubation may 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 9.2 are recommended as alternatives on the basis of risk assess-

ment, taking into account the expected microbial flora and environmental conditions.

Preparations of tissues and cells with a short shelf-life may be released based on an intermediate readout of the test before the incubation period is completed. In the case of a positive readout during the incubation period after release of the preparation, identification of the microbial species and a resistogram must be carried out and the information forwarded to the physician.

9.4.2.2. Sample volume

For automated culture systems, sample volumes recommended up to 10 mL can be inoculated per culture bottle. Very small sample amounts of < 1 mL may bear the risk of an increased sampling error, leading to false-negative results, if only a low microbial count is present in the product. Certain conditions, such as the usual small initial microbial count and early sampling during the production process or delayed microbial growth in the product due to inhibiting substances or unfavourable temperature are reasons for this. Therefore, a large amount of sample should be envisaged for inoculation (if applicable and appropriately validated).

9.4.2.3. Samples without antimicrobial additives

Microbial growth media without any adsorbents, such as resin or activated carbon, should be used. If sensitive organisms are not identified as a possible contaminant in the risk assessment, it is not necessary to add a fastidious organism supplement (FOS).

9.4.2.4. Samples with antimicrobial additives

Microbial growth bottles with resin or activated charcoal should be used (if membrane filtration cannot be carried out due to the nature of the sample).

The type, amount and mixture of antimicrobial agents used in manufacturing processes (and therefore present in samples for microbiological testing in automated culturing systems) is highly variable. Culture bottles containing adsorbing substances are established for the testing of patients' blood with therapeutic doses of a limited number of antibiotics or antimycotics. Therefore, such samples must be validated very thoroughly for residual antimicrobial activity to prove the suitability of the chosen method.

9.4.2.5. 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 period of 12 h is exceeded, the results of the automated culture system must be verified by subculture. In doing so, at least one control smear must be made per negative culture bottle and cultivated under identical conditions.

9.5. Notes on the validation of microbiological methods for the testing of preparations of tissues and cells

9.5.1. Growth-promotion test

Each batch of the microbiological culture medium to be used must be tested for its growth-promoting capacities – a 'growth-promotion test' in accordance with *Ph. Eur.* 2.6.1 and 2.6.27. In general, it is recommended to include possible relevant microbial contaminants from the respective tissue preparation or cell preparation or the environment – for instance, *Propionibacterium acnes* and *Micrococcus* spp. – in the assays because of their specific growth properties.

If these certificates are not available, the procurement facility or tissue establishment must demonstrate appropriate growth-promoting capacity.

Growth-promotion testing should be done for the plates and media used in microbiological monitoring. There should be a formal programme that determines the properties of media for a defined list of organisms. Growth-promotion testing must show that the media are suitable to consistently recover environmental contaminants (if they are present). The standardised list should comprise organisms based on the literature and/or environmental isolates, and should include a reasonable range of 'representative' micro-organisms that could be encountered in manufacturing environments (e.g. Gram-positive rods, Gram-positive cocci, filamentous moulds or yeasts, Gram-negative rods). The list should contain a minimum of five unique microbial strains [13].

9.5.2. Method validation

The method must be validated in the presence of the intended sample material (e.g. transport medium, final product). The basis for method validation is the 'method suitability test' laid down in *Ph. Eur.* 2.6.1 as well as the 'Method validation' laid down in *Ph. Eur.* 2.6.27.

The same conditions must be chosen as for routine testing (e.g. culture conditions, sample type, sample amount). The method suitability test must be

undertaken using the bacterial and fungal species indicated in *Ph. Eur.* 2.6.1. *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* – as well as the fungi *Candida albicans* and *Aspergillus brasiliensis* – should be incubated under aerobic conditions; *Clostridium sporogenes* should be incubated under anaerobic conditions.

It is recommended to complement the microbial spectrum by tissue-specific and/or contaminating micro-organisms such as *Propionibacterium acnes* and *Micrococcus* spp. which are typical skin contaminants.

For instance, *Propionibacterium 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. *Propionibacterium acnes* grows under anaerobic or microaerophilic conditions as a ‘slow-grower’ and is associated with particularly long detection time, so it may be included in method validation. If any other micro-organisms are considered to be relevant during processing and if present in the environment, they should also be included in validation studies.

The sensitivity of the chosen method should be shown by inoculating 10-100 colony-forming units (CFU) of the selected micro-organisms in the presence of the product. The microbial count of the dilution used for inoculation must be verified using a suitable method for each assay (e.g. plating on solid media). If a method cannot be used to detect microbial counts of 100 CFU, the limit of detection must be evaluated by experimental studies. Applicability of this method must be assessed in connection with its impact to ensure microbial safety of a product.

Each micro-organism species should be tested. For evaluation of the robustness of the method, it is recommended that testing of the same organisms is repeated at different time points (independent experiments) and that assays are repeated in the same way with defined deliberate variations (different staff, batches of consumables, and days).

For comparison, a positive control (without product) must be included in the test for each test strain. For negative control, a suitable amount of sample of the product to be tested must be incubated in the aerobic and anaerobic culture medium, at least in duplicate without inoculating micro-organisms. Positive and negative controls must also be prepared in the event of repeat tests.

Inoculated media must be incubated under the conditions applied in routine testing (temperature, duration) and checked for growth at regular intervals.

Test assays and controls must be evaluated in predetermined intervals during and at the end of the

incubation period. Samples for subculture must be taken from positive detected tests as quickly as possible. In the case of microbial growth, the micro-organisms must be identified.

If inhibition of microbial growth by the sample material is identified at validation, the method must 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 antimicrobials.

9.5.3. Documentation and interpretation of results

All materials used and working steps undertaken must be documented.

Interpretation of results should include at least the following factors:

- assessment of the growth of micro-organisms in the presence of the product to be tested and in controls;
- specification of the microbial count for evaluation of the method;
- period of time until a positive result has been detected for test assays and positive controls;
- proof of identity of inoculated micro-organisms.

For negative controls or test assays without detection of micro-organisms, the total incubation period and results of the subculture (including the methods used) must be specified.

9.6. Interpretation of results and actions to be taken

In general, source material that demonstrates contamination must be rejected unless the preparation undergoes decontamination and/or terminal sterilisation, and the detected quantity and quality of micro-organisms 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 antimicrobial treatment is initiated. Such source materials should be evaluated on the basis of qualitative (exclusion list for objectionable micro-organisms) and quantitative (microbial count, bioburden [12]) microbiological control tests, and specifications

should be given. 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 – e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE) and extended-spectrum beta lactamases (ESBL) – as well as possible toxin-producing micro-organisms, such as *Pseudomonas aeruginosa*, *Streptococcus* group A, *Staphylococcus aureus*, *Clostridium* and *Bacillus* as well as yeasts and filamentous fungi need to be evaluated carefully, and if appropriate, the tissues and cells should be rejected.

In the case of locally acquired contamination or a local infection, the microbiological result applies only to the tissue where the contamination was detected and to tissues that could have been cross-con-

taminated. If bacteraemia, septicaemia (anamnesic or blood culture) or any other distribution of the objectionable micro-organisms (at procurement, storage, transport, manufacturing) cannot be excluded, other tissues should be rejected.

For contaminated autologous preparations or preparations received from a specific heterologous donor, whereby a repeated procurement cannot be conducted or involves a high degree of risk, risk assessment based on the urgency of the application, judgment of infectious risk and treatment options must be conducted. In application of such preparations, measures must include full identification of the contaminating micro-organism and their resistograms, as well as adequate prophylaxis of the donor/recipient if the product must be used.

Table 9.3. 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 must be taken into account in the design of sampling strategies. Sample sizes $\geq 1 \text{ m}^3$ 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 the only 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 or strips 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 $\approx 25 \text{ cm}^2$, if possible. Tested surfaces must 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 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 $\approx 5\text{-}10 \text{ s}$ taking care not to damage the agar surface.

Table 9.4. 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			
<i>Alternative incubation conditions</i>			
Aerobic*	Trypticase soy agar irradiated	30-35 °C	2-3 days
Fungi	Sabouraud agar	20-25 °C	5-7 days

* When applicable, consider also anaerobic testing in the same culture conditions as aerobic testing but in anaerobic atmosphere.

9.7. General considerations for microbiological monitoring of the environment and reagents

Guidance for planning and carrying out microbiological monitoring of cleanrooms, laminar flow hoods and other equipment is described in Chapter 7.

Microbial samples can be taken using four sampling methods: volumetric air sampling, settle plates, contact plates and glove prints – or finger-prints (FIPS). A nonselective 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 must be well considered. The 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 validated for the growth of diverse bacteria and fungi and it must be possible to demonstrate that the residues generated by the sanitation programme do not interfere with micro-organism recovery. Table 9.3 summarises the characteristics of these sampling methods.

9.7.1. Incubation of samples

Environmental-monitoring samples should be incubated at a minimum of two different temperatures to detect bacteria and fungi. 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 (>90 %) of micro-organisms of interest can be demonstrated consistently [13].

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 must be adapted accordingly.

9.7.2. Data analyses

Reading of plates and media should be done according to a defined, standardised procedure. Identification of colonies should be undertaken according to the environmental monitoring programme of the tissue establishment. According to EU GMP, detected colonies in Grade A areas must be identified to the genus or species; in Grade B areas, detected colonies should be identified.

9.8. References

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Chapter 10. Distribution and import/export

10.1. Introduction

This chapter describes the requirements for distribution of tissues and cells and defines recommended controls for their import and export. The term ‘distribution’ should be understood to mean transport and delivery of tissues or cells intended for human application. 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 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 tissue establishment:

- to a clinical facility within the same country, where they will be applied (i.e. distribution);
- to another tissue establishment within the same country for local distribution.

Cross-border movement of tissues and cells includes transfers:

- to a tissue establishment outside the country (i.e. export);
- from another country to a clinical facility or tissue establishment in the country (i.e. import).

For a movement 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. 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’). See section 10.5.5 for further information.

10.2. Release

Prior to distribution, a comprehensive record review should ensure that all elements of collection, processing and storage have met the established quality criteria, including identity of the product. In case of incomplete eligibility of the donor the product must be released only for documented urgent medical need (see §11.5 on release in Chapter 11). An alternative plan of transport or shipping should be available in case of emergency situations, to prevent possible clinical complications to the recipient. The courier should be able to contact the receiving facility on a 24-hour basis in case of delay during transit.

10.3. Transport

The choice of mode of transport should take into account any general regulations governing transportation of biological substances.

Critical transport conditions, such as temperature and time limit, must be defined to ensure maintenance of the required properties of tissues or cells [1]. Unfrozen products are usually transported refrigerated (2-8 °C) or at room temperature. Frozen products are transported at –80 °C (dry ice), –20 °C (ice packs) or at < –140 °C (Liquid Nitrogen Vapour phase). For unfrozen products, such as bone marrow, there are conflicting recommendations for storage and transportation – e.g. 4 °C *versus* room temperature [2-4] – so the transplant centre is normally requested to define the transport conditions they wish to be applied. 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, should be determined by validation and documented. For instance, if liquid nitrogen is used to maintain very low temperatures, the dry-shipper shall contain sufficient absorbed liquid nitrogen to maintain the storage chamber temperature < –140 °C for a defined period of time, at least 48 hours beyond the expected time of arrival at the receiving facility. 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 should be secured and labelled appropriately (see Chapter 13).

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 tissue establishment 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 2) and must cover a requirement that any related serious adverse events should be identified and reported to the Health Authorities (see Chapter 15).

10.4. Allocation

The allocation of tissues and cells should be guided by clinical criteria and ethical norms. The allocation rules should be equitable, externally justified and transparent.

The procedures for distribution of tissues and cells by authorised tissue establishments 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.

10.4.1. Visual examination

Packaged tissues or cells should be examined visually for appropriate labels, expiry date, container integrity and security and any evidence of contamination prior to being dispatched (see Chapter 13).

10.4.2. 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.

10.4.3. Documentation

The place, date and time of pick-up and delivery (including time zone where relevant) and identity of the person receiving the tissues and cells should all be recorded and maintained in the tissue establishment from which the tissues or cells are distributed.

Any transportation must be accompanied by specific documentation attached to the package (see Chapters 6, 11 and 13).

10.4.4. Recall and return procedures

An effective recall procedure must be in place in every tissue establishment, 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 15.

10.5. Import and export

10.5.1. Underlying principles

Import and export between countries should be done only through legally authorised tissue establishments that can guarantee they have sufficient competence to evaluate safety and quality and also 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 of procured human material intended for processing, storage or banking in a tissue establishment or cell establishment in their country.

As a general rule, if clinicians or healthcare facilities identify a need to import tissues or cells, they should organise this through a written agreement with a licensed tissue establishment 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.

10.5.2. Import

Tissue establishments 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 tissue establishment 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 should be equivalent to those in place within the importing country. Imports should be accepted only from countries that have es-

tablished procedures to authenticate the legitimacy of exporters and the provenance of the donated material they supply. Exporters 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 tissue establishments for ensuring the equivalent safety and quality requirements, 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 §10.5.5 below for EU requirements for import.

10.5.2.1. Routine importation

The importing tissue establishment should assess and document that the exporting tissue establishment complies with the quality and safety recommendations in this Guide. This 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 tissue establishments 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 tissue establishment in the importing country should form part of the contract and should specify the methods to be followed to ensure main-

tenance 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 tissue establishment so that complete traceability is ensured.

The agreement should specify how tissues and cells will be identified. Unique identifying codes should allow traceability and a formal and unambiguous identification of all tissues and cells (see Chapter 13).

Agreements between importing tissue establishments 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 tissue establishment.

10.5.2.2. 'One-off' importation

There may be cases where exceptional or one-off importation is necessary for a single patient. In these cases, the importing tissue establishment should ensure that there exists a documented evaluation of the safety and quality of the tissues or cells being imported. The importing tissue establishment should keep the documentation obtained from the exporting tissue establishment 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 Health Authorities. The Health Authority should take all the necessary measures to ensure that imported tissues and cells respect the national quality and safety standards.

10.5.3. 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 13. 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.

However, it should be noted that a study published in 2002 concluded that even 10 passages

through the hand-luggage control system resulted harmless for HPC and lymphocytes in terms of viability and potency. Interestingly, the radiation dosage of passage through the hand-luggage control system is of $1.5 + 0.6 \mu\text{Sv}$ compared to a radiation dose of $60 \mu\text{Sv}$ received by the HPC during a 10 h flight [5]. 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 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.

10.5.4. Acceptance at the tissue establishment

Each importing establishment should 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 noncompliance should be reported to the exporter. Consignments should be examined for any evidence of tampering or damage during transport.

Tissues and cells should be stored in quarantine in an appropriate secure location under defined conditions until such time as they, along with their associated documentation, have been verified as conforming to requirements. The acceptance or rejection of received tissues and cells should be undertaken and documented in accordance with the guidance shown in Chapter 11.

10.5.5. 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 (EU) 2015/566 stipulates that tissues and cells must be imported into the EU by 'importing tissue establishments'* authorised for such imports by competent authorities. It also lays down the obligations of the

* An importing tissue establishment 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'.

importing tissue establishments and the competent authorities of EU member states who need to 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 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 tissue establishments are laid down, specifying the information and documentation that needs to be provided or made available to Health Authorities in EU member states when tissue establishments apply for import authorisations. Such information and documentation relates to the importing tissue establishment 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 tissue establishments 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 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 tissue establishment applicants when applying to be accredited, designated, authorised or licensed for the purpose of import activities, the content of the authorisation certificate for importing tissue establishment and the information to be provided regarding the third country supplier.

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,* whereas tissues and cells imported under direct authorisation of the competent authority of

EU member states (i.e. in emergency[†] situations or for immediate transplantation) are not affected by the new procedures.

In the EU, distribution and shipment of all cells classified as advanced therapy medicinal products (ATMP) 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).

10.5.6. 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.

Tissue establishments should 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.

10.6. 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. 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

* 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 shall 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 shall not be considered to be "one-off imports".'

† 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'.

important to ensure that there is good co-operation between organisations that allocate internationally. Registries should be in place for all imported and exported tissues and cells to ensure transparency in the process.

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Chapter 11. Organisations responsible for human application

11.1. Introduction

Tissues or cells that have been released, ready for human application, should not be distributed (see Chapter 10) without an order from a physician or other authorised health professional. Once tissues and cells arrive at a clinical application unit – hospital, clinic, doctor’s surgery, dental surgery (office) or assisted reproductive technology (ART) centre – for application in a patient, the responsibility for maintaining the quality-assurance chain is transferred to that organisation. This includes the need to store and handle tissues and cells correctly, inform patients of the risks involved in the use of the material, maintain traceability, and detect report and investigate adverse outcomes. The SOHO V&S (Vigilance and Surveillance of Substances of Human Origin) project, funded by the European Union (EU), considered these issues in a working group and developed a guidance document for clinical users on the basis of personal experiences and good practices that have been described in the American handbook for practitioners [1]. Much of the text in this chapter has been adapted from that guidance document [2].

11.2. Appropriate use

The risk associated with the human application of tissues and cells of an untoward and/or unintended response in the recipient (including transmission of a communicable disease) that is fatal, life-threatening, disabling or incapacitating – or which results in, or prolongs, hospitalisation or

morbidity – is generally regarded as being very low, particularly if the donated tissues and cells have been highly processed, blood and other cells have been removed or it has been terminally sterilised. However, the application of human substances always carries some risk. Adverse outcomes are rare, but pathogens such as viruses and bacteria have been transmitted by a wide range of tissue and cells types. GvHD (graft *versus* host disease) also represents a very common risk in haematopoietic stem cells (HPC) recipients. These events are due to failures in monitoring the quality and safety of tissues provided for clinical use [3].

Clinicians must give careful consideration to the risks and benefits of treating a patient with substances of human origin, such as tissues or cells, and consider the availability of alternatives before making a decision. Once the physician has decided that the application of tissues or cells is the most appropriate treatment, all health professionals involved in treating the patient should be conscious of their exceptional nature. Tissues and cells are donated altruistically for the benefit of patients in need and are often in short supply. Hence, only the required amount should be ordered and wastage should be avoided.

11.3. Choosing a supplier of tissues or cells

An organisation responsible for human application (ORHA) is a healthcare establishment or a unit of a hospital or another body that carries out

human application of human tissues and cells, such as those mentioned above. In most cases, procured tissues and cells require additional procedures such as processing or storage before their release for transplantation and therefore they are provided to ORHA by tissue establishments. In cases of direct distribution, procurement organisations – especially centres that procure haematopoietic progenitor cells (HPC) or lymphocytes for allogeneic (related and unrelated) use, which are known as ‘HPC collection centres’ – send the donation directly to the ORHA for immediate transplantation without any intermediate steps such as processing or storage.

Before requesting tissues or cells from a particular tissue establishment or procurement organisation (e.g. HPC collection centre), the ORHA should confirm that the supplying tissue establishment/procurement organisation is working to appropriate standards of safety and quality. Due to the obligation to maintain donor–recipient anonymity, in the case of procurement of HPC or lymphocytes for unrelated allogeneic use, there is no direct interaction between the tissue establishment/procurement organisation and the ORHA (HPC transplant centre). The HPC Donor Registry is responsible for ascertaining whether the collection centre has fulfilled appropriate safety and quality standards as well as for ensuring traceability of the cellular products.

If the tissue establishment is within the EU, it should be able to provide an accreditation, designation, authorisation or licensing certificate from the appropriate health authority (competent authority for tissues and cells). This certificate should specify the types of tissues or cells and the general activities for which the establishment is authorised. The establishment should be inspected regularly by the health authority to confirm compliance with legal requirements. A similar situation occurs in the case of direct distribution, whereby a competent authority accredits, designates, authorises or licenses a procurement organisation (e.g. HPC collection centre) to send a donation directly to a transplant centre for immediate clinical use without additional processing or storage. In non-EU countries, tissue establishments (tissue banks/cell banks) – as well as procurement organisations engaged in direct distribution – should be asked to provide evidence of the quality and safety standards they follow, and of any independent quality accreditation or certification they may possess.

Choosing appropriately authorised tissue establishments or procurement organisations helps to ensure that the donors of tissues or cells have been tested and selected correctly, and that all quality system requirements are in place for distribution of

the material as well as for possible processing and storage. If the ORHA has a large volume requirement for a particular type of tissue or cell, it may consider it appropriate to conduct a quality audit of the supplier.

As mentioned above and in Chapter 14, tissues or cells must not be dispensed by tissue establishments or procurement organisations without written order signed by a physician or other authorised health professional. It is good practice for the ORHA to conclude a basic end-use agreement with the tissue establishment or procurement organisation before beginning to receive tissues or cells from them. Written agreements should be signed, dated, reviewed and renewed regularly. They must comply with relevant laws and regulations. For situations in which an ORHA and supplying tissue establishment and/or procurement organisation are within the same healthcare institution, responsibilities should be specified in the quality system documentation.

The scope of the service-level agreement should include:

- a. details for the contact person and possible forms of communication (phone, fax, e-mail);
- b. methods of ordering and delivery of tissues or cells, including liability for transport;
- c. conditions of storage and preparation of tissues or cells for use at the ORHA;
- d. procedures for disposal of tissues or cells at the ORHA;
- e. procedures for return of tissues or cells to the tissue establishment (if permitted);
- f. responsibility and procedures for maintaining traceability;
- g. procedures for reporting and investigation of adverse reactions and adverse events;
- h. procedures for the management of tissue recalls and look-back procedures.

As mentioned above, in the case of unrelated allogeneic HPC transplantation or donor lymphocyte infusion procedure, there is no direct interaction between the tissue establishment/procurement organisation and the ORHA (HPC transplant centre). In such situations a service-level agreement can be considered between the ORHA (transplant centre) and its own national HPC donor registry (see Chapter 21).

It is not recommended that ORHAs obtain tissues or cells with the participation of a broker. If such situations occur, the ORHA shall verify that the distributing tissue establishment is authorised or certified appropriately.

Tissue establishments should only distribute gametes, embryos and germinal tissue to other au-

thorised tissue establishments or ORHAs. Direct distribution to individuals should be avoided.

11.4. Receiving tissues or cells from other countries

If an ORHA wishes to order tissues or cells from another country, it is good practice to ask a reliable local tissue establishment to locate and communicate with the tissue establishment abroad. Within the EU it is a legal requirement that any imports from third countries are managed by authorised tissue establishments. The only exceptions to this rule are cases of ‘direct import distribution’. That is, imported tissues and cells may be distributed directly for immediate transplantation to the recipient as long as the supplier is provided with an accreditation, designation, authorisation or licence for this activity, or in emergency cases. In both of these cases, the health authority must authorise the import directly. An example of direct import distribution would be an import distribution of HPC donated for immediate transplantation without storage or processing.

The tissue establishments can liaise with each other to ensure that equivalent standards of safety and quality are applied. For EU member states, any tissue establishment that is authorised 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 rules (as allowed by these directives) and require formal import procedures to be followed, even if the material comes from another EU country. It is important to be aware of the national legislation in place for the importation of tissues or cells from another country.

11.5. Exceptional release

In exceptional circumstances, an ORHA may agree with a tissue establishment or procurement organisation in the case of direct distribution that tissues or cells that do not meet the normal release criteria can be released and used in a specific patient on the basis of a risk–benefit analysis, taking into consideration the alternative options for the patient and the consequences of not providing the tissues or cells. The risk assessment should be documented before acceptance of the exceptionally released material. The recipient patient’s physician should work with the Responsible Person (RP) of the tissue establishment/collecting centre in conducting the risk assessment and

risk–benefit analysis for their patient (see Chapter 7). These discussions and conclusions should be documented. The treating physician should sign his/her agreement with the exceptional departure from normal procedures where there is any risk implied for the recipient patient. The patient should also participate in (or at least be informed of) the decision process and conclusions before providing consent (see section 11.6).

11.6. Recipient consent

Although donors of tissues and cells are carefully selected and tested, an element of risk remains due to the exceptional nature of the material. Risks can be due to transmission of infection by viruses, bacteria, parasites, fungi or prions, malignancy transmission, hypersensitivity reactions (including allergy, anaphylactic reactions or anaphylaxis), unexpectedly delayed or absent engraftment, toxic effects, unexpected immunological reactions due to mismatch, and transmission of a genetic disease.

In the context of these risks (see Chapter 15), however small, patients who will receive a human tissue or cell must be made aware of the facts and give their consent to the risks involved. The Notify Library database led by the World Health Organization and hosted by the Italian National Transplant Centre (www.notifylibrary.org) is a useful tool for accessing risk information for a particular type of tissue or cell. 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 transplant;
- b. an estimate of the frequency of the adverse outcomes described;
- c. information regarding alternative therapies.

Once the appropriate information has been given, the patient (if willing) should then provide consent on a separate form, according to national requirements, that should include at least the following elements:

- a. confirmation that the risks associated with the human application have been explained and the information has been understood;
- b. acceptance of the risks in light of the potential benefits.

This consent form should be separate from the more generic consent to receive treatment or surgery.

11.7. Centralised versus decentralised management of tissues and cells

The prevalent model for the management of tissues and cells at clinical application units is that the material is delivered directly to the relevant department or operating theatre (i.e. a decentralised model). The great advantage of the decentralised model is that a specialist clinician is in control. However, maintaining traceability of tissues and cells is very difficult in such a model and compliance with the requirements for storage and handling is also problematic. Centralised models greatly improve the ability to trace tissues and cells and can significantly improve inventory control and compliance with safety and quality standards. For these reasons, a centralised model for the receipt, short-term storage and traceability of tissues and cells for human applications is strongly recommended.

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).

11.8. Incoming inspection at the organisation responsible for human application

The incoming inspection is an important step to be taken when tissues or cells are first received and before they are placed into storage or delivered to the treatment room.

ORHA personnel should verify and appropriately record that:

- a. Tissues or cells received correspond to what was ordered and to the information in the accompanying documentation, which must be complete and legible;
- b. Shipping containers and primary containers are labelled with the information required and labels are affixed and legible (see Chapter 13). Separate accompanying documents should provide information that is not included in the primary container label;
- c. The shipping container and primary container are intact;
- d. 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 frozen or 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. for refrigerated tissues or cells – wet ice and, for frozen tissues or cells, dry ice). The supplying tissue establishment should be able to provide, on request, a validation study to show that the method of transport is adequate to maintain the required temperature for a certain period of time.

The ORHA should establish a procedure for situations in which the requirements described above are not met.

Moreover, documentation of risk assessment for donors, tissue-related information and tissue-processing details shall be made available to the transplanting physician upon request, unless such information infringes the confidentiality of the donor.

11.9. Package insert/instructions and temporary storage before use

Once tissues or cells have been distributed by a tissue establishment or procurement organisation for clinical use, appropriate storage and handling becomes the responsibility of the ORHA. Instructions should be available in the package insert that accompanies the tissues or cells that describe the appropriate storage conditions and the proper handling procedures to be followed before clinical application. These instructions should be followed precisely.

Tissues and cells are stored under various temperature conditions, depending on their type, method of preservation and packaging. The range of requirements for storage temperature includes room temperature, refrigerated and frozen. Where a specific storage temperature is necessary from receipt to clinical application, the storage device (refrigerator, freezer, liquid nitrogen storage tank, incubator, etc.) 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 should be retained for a defined period of time (the American Association of Tissue Banks recommends a minimum of 10 years).

It should be ensured that during short-term storage, and before clinical application, the material is stored together with its associated documentation or the documentation should be clearly linked to the tissues or cells and easily accessible. The accompanying document should specify the presence of particular additives or reagents that may affect the recipient (e.g. antibiotics, allergens). This information should be taken into account. If there is no package insert accompanying the tissues or cells, they should not be used.

Some member states within the EU recognise short-term storage of tissues or cells as a tissue-establishment activity and do require specific authorisation to be obtained from the health authority for such activity. Therefore, it is important to be aware of the national legislation in place for the storage of tissues or cells at an ORHA.

11.10. Inspection of the container, documentation and tissues or cells

Before opening the container, or attaching an infusion device to a bag of cells, the accompanying documentation should be 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 what is shown on the label. The packaging and the tissue should be inspected for any signs of damage during transport. Where temperature during transport is critical, there should be confirmation that the required temperature was maintained during transport.

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.

11.11. Preparation of tissues or cells before use

Instructions for opening the container or package, and any required manipulation/reconstitution (e.g. thawing, washing, rehydration), as well as information concerning expiry dates after opening/manipulation and presence of potential harmful residues or reagents that may affect the recipient (e.g. antibiotics, ethylene oxide), must be provided on the label or accompanying documentation of delivered tissues or cells.

The handling instructions should be followed precisely because tissue or cell preparation is an essential part of application safety. As in the case of storage, the preparation technique depends on the type of tissues or cells, methods of their preservation and their packaging. Additionally, preparation requirements may vary among suppliers. Any departure from the instructions provided is at the discretion of the clinical user, who will take full responsibility for any adverse outcome resulting from not adhering to supplier instructions.

11.12. 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 tissue establishment for appropriate disposal. Similarly, a single unit of tissues or cells (e.g. two halves of femoral head delivered in one container) should not be used in two or more separate patients. Moreover, any additional processing of supplied tissues or cells (e.g. a division of the whole femoral head into several pieces by a physician at the ORHA) should be considered as an exceptional departure from normal requirements, and should not be undertaken without notification of the RP at the supplying tissue establishment and health authority.

Tissues or cells provided to one ORHA should not, in general, be sent to another entity for clinical use. Within the EU, this would be defined as 'distribution' and it would require specific authorisation. Tissues or cells that are received and not subsequently used in one department of a hospital may be sent to a different department or operating theatre in the same hospital but, as a minimum, the supplying tissue establishment should be informed of this. There may be nationally established rules for this scenario.

The documentation that accompanies the tissues and cells should specify whether they can be

returned to the tissue establishment if not opened or used, e.g. if the patient is not well enough for surgery or if surgery is cancelled for another reason. Most tissue establishments will not accept tissues or cells that are returned under these circumstances. Tissue establishments that do accept returned, unused and unopened tissues or cells will have to confirm that the conditions for maintenance of the required properties of tissues or cells were provided and documented continuously, and that the packaging was not tampered with.

11.13. Traceability

Coding and traceability are addressed fully in Chapters 13 and 14. In the EU, ORHAs are required to maintain traceability records from the point of receipt of the tissue until 30 years after clinical use or other final disposal. These records (mandatory in the EU) must include:

- a. identification of the supplying tissue establishment or procurement centre;
- 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.

Details of the tissues or cells applied should be in the recipient's record and in the logbook of the treatment room, including the operating theatre where they have been applied. However, these records alone are not adequate to permit rapid re-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 will allow quick and easy action in the case of a 'recall' by the tissue establishment or the health authority, or for internal follow-up/review. 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 safe storage should be clearly identified and documented.

Some supplier tissue establishments require the ORHA to return a traceability form or card providing details of the recipient for each tissue and cell supplied. A copy of the card should be retained in the recipient medical record. The details should be sufficient to unambiguously identify the recipient, i.e. at least three points of identification including a unique identifier. 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 other final disposal. Where cards or forms are returned to the supplier tissue establishment, the manner of documentation should adhere to national data protection regulations and should ensure that personal information is not visible or that the recipient's privacy is not compromised in any way.

It is highly recommended that when patients 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.

11.14. Adverse events and adverse reactions

Vigilance and surveillance (V&S) are addressed in full in Chapter 15. Effective V&S relies heavily on all health professionals involved, from procurement to application, but particularly medical staff (including surgeons) involved in tissue- and cell-procurement activities who might be aware or informed of additional safety information on donors during their follow-up.

Serious adverse reactions (SARs) can be detected during and after donation in living donors or after application in tissue or cell recipients. As adverse application outcomes might result from many diverse factors associated with the clinical procedure or the patient's underlying condition, clinicians might not consider the tissues or cells applied as a possible reason for the outcome. Tissue establishments 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 any of the stages from donation to clinical application so that similar occurrences might be prevented in the future.

For most types of well-established tissue and cell application, detailed clinical outcome reporting by the clinical user to the tissue establishment is required only in those exceptional circumstances where there is an untoward serious adverse reaction. Routine clinical follow-up and reporting of tissue and cell recipients' clinical progress is 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 not generally considered as part of vigilance. In addition,

donor follow-up on a regular basis should be performed, especially in donations after haematopoietic growth factors or other drugs (see Chapter 15).

Tissue establishments that supply tissues and cells should provide clinical user organisations with clear instructions on how to report SARs, preferably using standardised documentation. In general, any suspected SAR should be reported by the clinical users to the tissue establishment that supplied the tissues or cells immediately, before it is confirmed or investigated, to enable the tissue establishment to take appropriate precautionary actions to prevent harm to other patients and to involve the tissue establishment in the investigation process. As described further in Chapters 14 and 15, the clinician treating the recipient plays a critical part in the investigation of suspected SARs, together with the supplying tissue establishment.

11.15. Management of recalls and reviews

There are various reasons why a tissue establishment may recall tissues or cells that were distributed to an ORHA. A recall may be related to the receipt of new information regarding the donor's medical or behavioural history that implies a risk of disease transmission risk 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 tissue establishment or required by the national health authority.

When a tissue establishment 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 of tissues and cells received, with dates of use or disposal and identification

of recipients, will greatly facilitate conducting a recall. 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 scenario 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 will facilitate effective action.

A review may 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. In this case, a central logbook or database of the tissues or cells supplied will also greatly facilitate the process.

11.16. References

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Chapter 12. Computerised systems

12.1. Introduction

Computerised systems are playing an ever-increasing part in the management of business operations, including those related to healthcare. Tissue establishments 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 controlling the facility or ensuring that required environmental conditions, such as air pressure differentials or particle counts, are maintained (e.g. building management system).

Errors and malfunctions of computer systems can go unnoticed and might have serious consequences. 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 for the UK Secretary of State for Health, published 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 [1].

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].

12.2. Planning the implementation of a computerised system

Before implementation of a computerised system at a tissue establishment, it is advisable that the user has close contact with their IT department, or an IT consultant independent of any supplier of computerised systems.

The tissue establishment needs to:

- a. establish a list of minimal requirements for the computerised system including (but not limited to):
 - i. the need to manage calculations and printouts (e.g. reports and labels)
 - ii. the need for data protection (personal access to the system or parts of the system),
 - iii. the duration of and options for record storage (in general, 10 years is required for quality-system-related data and 30 years for traceability-related data in the EU),
 - iv. back-up conditions ensuring future access to stored data,
 - v. the need to connect with other computerised systems/registries (social security registries, administrative systems, financial systems),
 - vi. the need for encryption in case information is transferred over an open network,
 - vii. the need for CE labelling (in EU) if patient data, or data relevant for diagnosis or treatment of patients, are to be included in the system;

- b. Evaluate the different systems available and choose the 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;
- d. Define roles and responsibilities and their division between the user and developer/supplier/manufacturer with regard to testing, user instructions, maintenance, system improvements and access to source codes.

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 also recommended that the developer/supplier of the computerised system receives proper information about the surrounding/other 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 tissue establishment usually includes hardware, software, peripheral devices and documentation such as manuals and standard operating procedures (SOPs). To define the system, in co-operation with the vendor or developer, the user should generate a written description of the system, the functions that it is designed to carry out, and all human interactions (functional and non-functional requirements). These requirements will be the basis for subsequent testing and verification of the developed/supplied system. For further information, refer to ISO/IEC 12207, ISO/IEC/IEEE 29148, ISO/IEC 27001:2013 and ISO/IEC 27007:2011 [3, 4, 5, 6].

12.3. Verification and testing

The guidance in Chapter 2 on the verification of new equipment should be taken into account. The verification of computerised systems in a tissue establishment 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 verification;
- b. a brief description of the verification strategies for different categories of computerised systems, as well as other validation activities;
- c. an outline of the protocols and related test procedures for all verification activities of the computer system (the reporting requirements for documenting the verification exercises and related results should also be defined);
- d. the identity of key personnel and their responsibilities as part of the verification programme.

The level of verification 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 12.1 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 verification 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 (if applicable) and all database structures (e.g. file sizes, input and output formats);
- 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 back-up, maintenance and diagnostic procedures, including assignment of responsibilities;
 - ii. safety leading indicators [5, 6, 8];
 - 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 supervised changes to incorrect data;
 - vii. procedures for verification of a change;
 - viii. a training system that includes training manuals, documentation and procedures for training.

Table 12.1. An approach to verification and control of computerised systems by system category, modified from ISPE GAMP 5

Category	Description	Typical examples	Typical approach
1. Infra-structure software	- 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
2. Non-configured	Software cannot be configured to suit the specific process	- 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
3. Configured	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 lifecycle documentation retained only by supplier (e.g. design specifications) - 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
4. Custom	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 building management systems (monitoring air pressures in rooms, temperature and/or particles, temperatures of fridges, freezers and incubators) - Clinical trial monitoring	Same as for 'Configured' above, but also: More rigorous supplier assessment, with possible supplier audits

Source: International Society for Pharmaceutical Engineering. Good Automated Manufacturing Practice (GAMP) 5 [7]

Verification documents and the results of tests undertaken and approved by the supplier/vendor/developer of the system should be presented to the user. The user can then carry out tests according to a pre-defined and documented test plan [9]. 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 [10]. 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 tissue establishment.

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)

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
 - i. System components are presented with all types of expected interaction, including normal value, boundary, invalid and special case inputs. The system must produce the correct outputs, including error messages by control programs. Carrying out such testing in parallel with a reference or standard system can be useful;
 - ii. Each test case should include the input, expected output, acceptance criteria and whether the test was passed or failed. For traceability purposes 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

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. back-ups restore data in a correct way.

12.4. Change control

In case of changes in the software, the verification status needs to be re-established. If a 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. Depending on the system, it may be desirable to have a test version of the computerised system containing the same data (mirrored).

12.5. Maintenance of the system

The database should be checked periodically and systematically by qualified information technology (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. Please refer to ISO/IEC 14764 for further guidance on maintenance [10].

Security should be maintained by:

- a. an adequate change history of the system, including for software and hardware (when necessary);
- b. periodically altering electronic passwords (without re-use) and by 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 programs to detect and remove computer viruses;
- 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.

12.6. 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 tests, routine maintenance, change procedures, data-integrity checks, error investigations and operator-competence evaluations.

Appendix 19 contains an example of a checklist that can be used for internal or external audits.

12.7. 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) [7]. More specific guidance related to blood and tissues is available from the British Committee for Standards in Haematology [11].

12.8. Regulations governing verification of computerised systems in EU Good Manufacturing Practice

Regulation of computerised systems is well established in the pharmaceutical industry, with EU Good Manufacturing Practices (GMP) [12] acting as the regulatory reference in Europe. Inspectors in the EU also use the Pharmaceutical Inspection Cooperation Scheme Guidance (PIC/S) [13]. The pharmaceutical industry operates on a global scale, so many European companies maintain compliance with the US Food and Drug Administration (FDA) [14]. These regulatory documents can be useful sources of reference for tissue establishments.

If a computerised system replaces a manual operation, there should be no decrease in product quality, process control or quality assurance. 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 tissue establishments since it details the minimum requirements that should be in place [13]. Appendix 19 contains a checklist adapted from ISO/IEC 27007:2011 and the guidance document from the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

12.9. Infrastructure

Infrastructure should include consideration of design and development tools (e.g. test suites, servers, version- and configuration-control systems, modelling and architecture tools, communication tools, traceability and behavioural-modelling tools).

12.10. Failure of the system

For computerised systems that support critical processes, provision 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 annually.

12.11. 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 time and date that they were applied.

12.12. Data protection

All personal data stored in computerised systems must be stored in a secure manner, with access available only to authorised personnel. Procedures for personal data protection must comply with national legal requirements and, for EU countries, with the requirements defined in Directive 95/46/EC on the Protection of individuals with regard to the processing of personal data and on the free movement of such data.

12.13. Archiving

Critical data must be archived in a long-term stable medium and placed 'off-site' at a location remote from the hardware to ensure secure storage. 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 are installed), then the ability to retrieve archived data must be ensured and tested [12].

12.14. References

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Related document: Appendix 19. Checklist for internal (or external) revision of computerised systems.

Chapter 13. Coding, packaging and labelling

13.1. Introduction

The quality and safety of tissues and cells is dependent not only on the way they are collected or processed, but also on the way they are coded, packaged and labelled before being sent to the end-user [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. In this chapter, the coding of tissues and cells, their packaging and labelling requirements are discussed.

13.2. Coding

With an 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.

13.2.1. ISBT 128

ICCBBA (the International Council for Commonality in Blood Banking Automation) manages ISBT 128 [4, 5], which is the most widely used information standard for medical products of human origin, including tissues and cells. It is used in more than 5 000 facilities in 77 countries across six continents. ICCBBA is a not-for-profit non-governmental organisation in official relations with the World Health Organization, 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 well-defined cell and tissue product codes.

13.2.2. Eurocode

Eurocode International Blood Labelling Systems e.V. (Eurocode IBLS) [6] 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 Germany and Austria.

13.2.3. Single European Code for tissues and cells

In 2015, the European Commission adopted Directive (EU) 2015/565, amending Directive 2006/86/EC [7] as regards certain technical requirements for the coding of human tissues and cells, which establishes the EU Coding Platform and the Single European Code. The directive introduces the obligation for tissue establishments to affix a Single European Code (SEC) on tissues and cells distributed for clinical application in the EU or exported from the EU [8]. 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 is an ‘umbrella’ code that provides for standardisation across the EU, while allowing the use of ISBT 128 or Eurocode integrated within the SEC coding structure. Where ISBT 128 or Eurocode is not used, the SEC provides high-level product description codes.

13.2.3.1. Application of the Single European Code

The SEC [8-10] shall be applied to all tissues and cells distributed for human application except in the excluded circumstances described below. For the other situations where tissues and cells are released for circulation (e.g. to other tissue establishments, third parties, manufacturers of advanced therapy medicinal products), as a minimum the Donation Identification Sequence shall be contained at least in the accompanying documentation.

There are 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), 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.

Tissues/cells imported from third countries for distribution in the EU should be also 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).

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.

Application of the SEC by EU tissue establishments is mandatory, starting on 29 April 2017.

13.2.3.2. Structure of the Single European Code

The SEC is a unique identifier that consists of two elements: a donation identification sequence, essentially indicating the origin of the tissue or cells, and a product identification sequence, essentially classifying the type of tissue or cells. Further details are specified in Annex VII to the Directive (see Table 13.1).

Table 13.1. 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.

13.2.3.2.1. Donation Identification Sequence

The coding system must identify each donation event because donors can potentially donate tissues and cells on several occasions (e.g. gamete donation, HPC when alive and corneal tissue after death). Each tissue establishment authorised in an EU member state shall 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 can, based on the donation identification system in place in their country, assign a unique number for the donation, which should be 13 characters in length. The unique donation number may be created locally, by the tissue establishment or centrally (by the Health Authority), or globally as a unique number provided by an international organisation (e.g. ICCBBA or Eurocode IBLS).

Hence, taken together, these codes will ensure that each donation will have a unique Donation Identification Number that can be used to label each tissue product. In the case of pooling of tissues and cells, a new donation identification number must be allocated to the final product.

13.2.3.2.2. 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 in ISO standard format (yyyymmdd). The product code includes an identifier of the coding system used ('E' for the European Code for Tissues and Cells or 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, so a tissue and cell product 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 two or all of them in parallel.

With respect to the format, the SEC on the label attached to each product will be readable by the eye, and preceded by the abbreviation 'SEC' with the Donation Identification Sequence and Product Identification Sequence 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.

13.2.3.3. 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 that contains the EU Tissue Establishment Compendium and the EU Tissue and Cell Product Compendium.

- The EU Tissue Establishment Compendium is the register of all tissue establishments that are authorised, licensed, designated or accredited by the EU member states' 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.
- 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 and Eurocode coding systems, and has put in place bilateral agreements with their managing organisations (i.e. ICCBBA and Eurocode) to ensure that updated product codes are regularly made available and included in the EU Tissue and Cell Product Compendium.

As the result of standardisation of the Single European Code, a tissue and cell product coding system named EUTC was developed by the European Commission for operators not interested in introducing the other two coding systems. The EUTC product-coding system 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.

Each tissue and cell product will be assigned a specific code, which will identify and describe that product. It will be possible to decode the information in the SEC to obtain text that describes the tissues or cells and their origin *via* an online code translator system attached to the EU Coding Platform.

These tools will be publicly available and free of charge. Hence, the EUTC used by EU member states may be also used by other interested countries. Further information on the SEC and its application can be found on the European Commission's website [8-10].

13.3. Packaging and labelling

Packaging of tissues and cells has an important role during all banking procedures, starting from procurement through processing steps, to distribution and clinical use. Adequate packaging minimises the risk of contamination of tissues and cells, and of the persons involved in transportation, and ensures its required characteristics and biological function.

Ensuring the traceability of all tissues and cells from the donor to the recipient is a responsibility shared by procurement centres, tissue establishments and organisations responsible for human application. All these stakeholders participate and contribute to actively safeguarding, in a continuous manner, the tracking of the stages that tissues and cells go through. 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 14. 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 correlation 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 practices in packaging and labelling at all stages from donation to implantation.

13.3.1. General concepts

Packaging and labelling operations must be considered an integral part of the activities of tissue-establishment and procurement organisations. It 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, where all materials, containers, equipment and unfinished tissues and cells must be adequately identified at all times. In addition, tissues and cells obtained and/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 and/or testing and release, as well as the handling of packaging and labelling materials.

Premises for the packaging and labelling of tissues and cells, as well as the operations carried out there, must be designed to prevent any cross-contamination or mix-ups. For the same reason, simultaneous operations must be avoided (or at least adequate measures should be taken).

Primary packaging and labelling of tissues or cells must be done in an environment that must be 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 and Annex II of Directive 2006/86/EC.

13.3.2. Packaging of tissues and cells

Packaging includes all operations, including primary and secondary packaging, which procured or processed tissues and cells undergo 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 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. 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.

Packaging must ensure the integrity and maintain the sterility of the contents of the primary container. Storage containers must be appropriate for the type of tissue or cells, the temperature, the type 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.

13.3.3. 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. Material 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 material, the container must be inspected for evidence of impurities, defects, broken seals or contamination that could compromise the quality, integrity or safety of the tissue or cells.

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 section 13.2).

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 enable understanding.

For autologous or directed donations, the name and/or identifier of the intended recipient must be included in the label. Further guidance on traceability is provided in Chapter 14.

The production of labels must be controlled. When applicable, reconciliation of labels that have been edited, used and/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.

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 the Directive (EU) 2015/565, 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).

13.4. 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.

13.5. Management of packaging and labelling materials

Selected packaging material must be able to withstand the requirements of the storage temperature (ambient temperature, refrigeration, freezing, cryopreservation) and sterilisation procedure (if this is to

be applied) 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 biological 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 and secondary packaging and leaflets should be segregated and stored in access-controlled areas.

Management of packaging and labelling materials must include the following:

- 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 and/or 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 later 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.

13.6. Primary packaging and labelling for procurement operations

‘Primary packaging’ refers to the materials that are intended to 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 appropriate, and be sealable and leak-proof.

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 function of the tissues and cells.

Packaging must also prevent contamination of those responsible for packaging 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 is 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, amniotic membrane, skin or cartilage for cell cultures must be packed in sterile containers with transport medium. Whole eyes must be stored in a moist chamber. Composition of the transport medium for a particular type of tissue must maintain the biological properties of 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 unprocessed in culture medium or cryopreserved.

A unique identification code shall 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 13.2. If any of the information listed in Table 13.2 cannot be included on the primary package label, it must be provided in accompanying records inside the transport container. Table 13.2 lists information that may be provided either on the label or in accompanying documentation.

13.7. Secondary packaging and labelling for procurement operations

If secondary packaging is used after procurement, it should adhere to the same requirements as those

established for primary packaging. If labels with the required information are not attached to the primary packaging they should be attached to the secondary packaging, which should be closed and sealed.

Table 13.2. Required information on the primary container after procurement and for finished product

Primary packaging (on the label)	Procurement information	Information for finished product
unique identification number or code (preferably the unique donation identifier and unique product identifier)	✓	✓
type of tissues and cells* (e.g. name of the product)	✓	✓
identification of the collecting tissue establishment	✓	✓
identification of the processing tissue establishment		✓
identification of the tissue establishment distributing the tissues or cells*		✓
date of donation (and time, where possible)	✓	
expiry date* and time (in GMT if it will be shipped to another time zone) as applicable	✓	✓
recommended storage temperature range	✓	✓
description of any additives/attributes (as applicable)	✓	✓
volume of product and anti-coagulant (as applicable)	✓	✓
when tissues and cells are known to be positive for a relevant infectious disease marker, the package must be marked 'BIOLOGICAL HAZARD'*	✓	✓
autologous donations must be marked 'FOR AUTOLOGOUS USE ONLY', with identification of the donor/recipient*	✓	✓
in the case of directed donations, the label must identify the intended recipient*	✓	✓

*mandatory for EU tissue establishments

If any of the information listed above cannot be included on the primary package label, it must be provided in accompanying records inside the transport container.

Table 13.3. Requirements for additional information that may be present on the primary packaging label or in accompanying documentation for procured and finished product

description and (if relevant) dimensions of the tissue or cell products*
morphology and functional data where relevant*
date of distribution of the tissues and cells*
biological determinations carried out on the donor and results*
storage recommendations*
instructions for opening the container/package and any required manipulation/reconstitution*
expiry dates after opening/manipulation
a statement that the tissues or cells are suitable for transplantation following all required disease screening and testing
a statement limiting use of the material to specific health professionals
a statement that the material is intended for single use in one patient only
a statement, as applicable, that the tissues may not be sterilised or re-sterilised
a statement that it is the responsibility of the end user to maintain the tissue or cells in appropriate storage conditions prior to transplantation
instructions for reporting to the tissue establishment serious adverse reactions and/or events potentially attributable to the tissue or cells*
presence of potential harmful residues (e.g. antibiotics, ethylene oxide)*
for imported tissues or cells, the country of procurement and the exporting country (if different from the procurement country)*

*mandatory for EU tissue establishments

13.8. Outer container packaging and labelling for procurement operations

Packaged tissues and cells must be shipped in a container which is suitable for the transport of biological materials and which maintains the safety and quality of the tissues or cells contained within. Temperature conditions between recovery and processing must be appropriate to the type of tissue so as to preserve its required characteristics and biological function (i.e. temperature and duration of transport to the tissue establishment where the tissue processing will take place). The container must be closed fully 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 as described in Table 13.4.

13.9. Procurement package insert

It is recommended that the documentation sent with procured tissues or cells to a tissue establishment indicates, where applicable, that they are in a state of 'quarantine' so that it is clear that a final review regarding their release for distribution and use has not been completed. See Chapter 6 for full guidance on the requirements for procurement documentation.

13.10. Packaging and labelling during processing

Packaging and labelling of unfinished tissues and cells during intermediate phases of processing shall be applied to all packaging materials and containers to assure identification at all times.

13.11. Primary packaging and labelling for finished tissues and cells

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 13.6, with a special focus on the radiation-resistance of packaging material for tissue that will be radiation-sterilised. The expiry date will be determined 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 shall 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.

Facilities where packaging or labelling operations have taken place should be checked before starting any other operation so as to guarantee that all previous materials have been removed.

Table 13.4. Required information that must be present on the outer container

• identification of the shipping tissue establishment, including name, address and telephone number of the contact person*
• identification of the receiving tissue establishment (for tissues/cell which require further processing or storage), including the name, address, telephone number of the contact person
• identification of the organisation responsible for human application (e.g. hospital, fertility clinic), including name, address and telephone number of the contact person*
• date and time of the start of transportation, and specifications concerning conditions of transport relevant to the quality and safety of the tissues and cells
• a statement that the package contains 'HUMAN TISSUE/CELLS FOR ADMINISTRATION' and the warning 'HANDLE WITH CARE'*
• in the case of viable cellular products, use the warning 'DO NOT IRRADIATE'*
• when cells or tissue are known to be from a donor who has tested positive for a relevant infectious disease, the warning 'BIOLOGICAL HAZARD' should be used
• any other applicable hazard warnings
• if transport is 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 shipment. The lower half of the label must indicate the external transportation range of the shipment in degrees centigrade (for more details, see section 13.15)
• Shipper handling instructions on conditions of transport relevant to the quality and safety of the tissues and cells (e.g. 'do NOT DELAY', 'KEEP COOL' and 'KEEP UPRIGHT')*
• Single European Code (SEC)*

*mandatory for EU tissue establishments

Printed labels should be examined carefully to ensure that the information contained conforms to the corresponding tissue/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 shall be destroyed according to written procedures.

The information that needs to be on the primary tissue/cell package label of the finished product is detailed in Table 13.2.

If the primary container is too small to host a label with all the required information (as may be the case with e.g. gametes and embryos), the minimal information on the primary container needs to be the unique identification code. The rest of the information may be attached to the outer packaging or to an accompanying document.

The additional information that must be provided either on the label or in accompanying documentation is described in Table 13.3.

13.12. Secondary packaging and labelling for finished tissues and cells

‘Secondary packaging’ and labelling refers to materials that are not intended to come into direct contact with the tissues and cells. Special consideration must be taken 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.

13.13. Outer container packaging and labelling for finished tissues and cells

When tissues or cells are shipped for distribution purposes, every transport container must be guaranteed to maintain the conditions needed for the specific tissue or cell type. Containers should provide adequate protection against deterioration or contamination of tissues and cells that may occur during storage and transportation. Containers should be clean and sanitised before use to ensure that they are suitable for their intended use. These containers should not alter the quality, safety and efficacy of tissues and 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 the same information as specified in Table 13.4.

13.14. Package insert for finished tissues and cells

A ‘package insert’ refers to the complementary information associated with tissues and cells that cannot be placed on labels. Critical information for the clinical user must be provided.

13.15. Customs clearance

For clearance of customs, all tissues and cells supplied from abroad 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, which at present is 30029010, but there is a request that this should be altered to base code 082 with subcodes. It is important that the transport of frozen tissues – which are usually packed in dry ice or cryopreserved cells 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 IATA codes: UN1845 for dry ice or UN1977 for liquid nitrogen in a dry shipper and UN3373 for shipment of biological substances by air [11, 12]. 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 10). For tissue transport, the agreement with the shipping tissue establishment should define responsibilities for meeting the cost of transport, refrigeration and/or storage at a receiving facility for any items that may be detained pending customs enquiries.

13.16. References

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Chapter 14. Traceability

14.1. 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 has two main components: (i) it is the means to link a donor with recipients, or offspring born through Assisted Reproductive Technology (ART) and (ii) it is the means to identify and link all the steps and procedures to which tissues or cells are subjected, together with their location and the equipment and materials used, before they reach the recipient.

Traceability is 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 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 should be kept by the entities involved in the donation, procurement, processing, storage, distribution and application of tissues and cells to ensure the compliance of safety requirements, but records should never allow the disclosure of confidential information to unauthorised persons. Donor selection, procurement/collection

from the donor, processing, storage and distribution (transport) of tissues and cells involve many complex steps that impact on the quality of the tissues and cells used for clinical application.

Human error, equipment failure, use of inadequate written procedures or new risks that cannot be predicted may affect safety or quality of tissues and cells at any of these stages, 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 information or adverse incidents. For ART, traceability does not stop when the tissues and cells reach the recipient. The health of the children born as a result of ART treatment must be followed up, so that data on children's health and follow-up of pregnancies are included in the chain of traceability.

Tissue establishments play a special role in assuring traceability, collecting the data that guarantee the ability to locate and recall tissues and cells once the establishment becomes aware of information that may have implications for their quality and safety. Additionally, tissue establishments are responsible for communication with other entities such as organ

transplant units or other tissue establishments involved in the processing of additional tissues procured from shared donors. The time interval between detecting risks 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’ [1]. 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 14.2.e) allowing personnel to track and trace all steps associated with the tissues and cells long after their clinical application, making possible adequate biovigilance and follow-up procedures. Traceability underpins biovigilance (see Chapter 15). Within each tissue establishment, 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 benefit 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 of imported and/or exported units.

14.2. What is traceability?

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. 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 donation for the purposes of testing for quality and safety;
- e. the recipient of the tissues or cells;
- f. the health of the resulting children and any adverse data on pregnancies (for ART treatment).

This means traceability is a concept that must work both ways, allowing (i) tracing of procedures when recipients show any adverse reaction that could be linked with the quality of the tissues or cells distributed and (ii) tracking the fate of recipients and units associated with incidents detected after distribution.

The following are 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 ART treatment, each organisation holding tissues or cells must have records of the donor, the donation and 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. Avoiding duplication of identifiers can involve including a tissue establishment or procurement organisation identifier as an element of the identification for the donor, donation or sample. Within the European Union (EU), the Single European Code (SEC) will help to address this need (see Chapter 13). The SEC allows, within its structure, incorporation of the existing interna-

- tional coding system ISBT 128 and a code used mainly in Germany, Eurocode.
- b. Safe transfer of critical information
The 'traceability trail' depends on the accurate transcription of critical identification information. Manual transcription errors can cause breaks in the traceability trail. Use of electronic transfer of critical information (bar codes or other machine-readable codes) is recommended. If manual transcription is used, double checking of data should be implemented.
 - c. Timeliness
If a risk is identified, it must be possible to rapidly trace all implicated products or all potentially affected recipients and children conceived through ART. A delay could result in harm to patients or children conceived through ART. Systems need to be quickly accessible, with efficient links between organisations to reduce the 'traceability window period'.
 - d. Clarity of responsibilities at interfaces between organisations
To guarantee traceability, tissue establishments should distribute tissues and cells to other tissue establishments, to organisations responsible for human application (ORHA) 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 [2]. Maintaining traceability is one of the key legal and technical responsibilities of ORHA (see Chapter 11). Tissue establishments must define responsibilities and procedures prior to the distribution of tissues, cells, gametes or embryos to those organisations.
 - e. Long-term storage of secure records
For effective reviews, traceability data need to be maintained for long periods of time. 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 to ensure that records remain quickly accessible. There is a need for regular management review of data storage, with a proactive approach to prevent obsolescence. The location of traceability records may change when organisations merge or if they cease activities relating to donor selection, donor testing, procurement, processing, distribution or transplantation. In such cases, there must be an effective link between the new location of the data and the previous location.

- f. Traceability audits
Organisations must 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.

14.3. Which records must be traceable?

There must be a system of record-keeping for activities associated with tissues and cells. Records should describe procurement, donor testing, processing, storage, distribution and end use. Records should include details of equipment used, materials such as consumables that have come into contact with those tissues and cells and the identity of the members of staff who were responsible for all critical activities from procurement until implantation or disposal.

Tissue establishments must ensure that data protection and confidentiality measures are in place, in accordance with the data protection laws in the relevant country. Many organ donors are also tissue donors and cell donors, so it is important that effective links are in place between organ-procurement organisations and tissue establishments.

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 amendments to written records are signed and dated. Computer records should be maintained in a validated system (see Chapter 12) 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 validated system, such as a computer or microfilm. Records should be maintained of equipment and consumables, including the lot numbers and expiry dates of additives, cryoprotectants and packaging materials used during procurement and processing. The tissue establishment should 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 tissue establishments ensure that the relevant information retained abroad remains accessible for as long as it may be required.

The records must be kept in an appropriate archive, which is acceptable to the Health Authority of each country involved.

14.3.1. Records of identification, donor tests and clinical evaluation of the donor

Besides the information defined in Chapter 4 (on donor evaluation), tissue establishments 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 physical examination for deceased 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 cells (HPC) donors, the donor's suitability for the chosen recipient;
- 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 tissue establishments and testing laboratories, should be properly defined through a technical and legal written agreement (for details regarding technical agreements with testing laboratories, see Chapter 5, section 5.4).

14.3.2. Records of procurement of tissues and cells

Besides the information defined in Chapter 6 (on procurement), the organisation undertaking procurement should produce a procurement report and provide it to the tissue establishment.

The procurement report should contain at least:

- a. the identification of the tissue establishment receiving the tissues or cells;
- b. donor identification data (including how and by whom the donor was identified);
- 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 signature;
- 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 necessary);
- 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.

14.3.3. Records of processing of tissues and cells

Besides the information defined in Chapter 7 (on processing and storage), the organisation undertaking processing should 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;
- d. records of consumables used during processing (manufacturer, lot number, storage conditions of consumables (if appropriate), 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.

14.3.4. Records of storage and distribution of tissues and cells

Besides the information defined in Chapter 10 (on distribution and import/export), organisations undertaking storage of tissues or cells should 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 (where relevant);
- e. any incidents that occurred during storage.

In addition, when the tissues or cells are transported or distributed to hospitals or clinics for application, tissue establishments should 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.

14.3.5. Records of clinical application of tissues and cells

Besides the information defined in Chapter 11, the ORHA should keep at least the following records:

- a. identification of the supplier tissue establishment;
- 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 in the recipient;
- i. health outcomes of children born following ART.

Systems must be in place to assure the follow-up of tissue recipients and children conceived after ART treatments. Such follow-up can be achieved only if a close working relationship exists between all stakeholders: that is, the tissue establishment, ORHA, ART centre and parent(s) involved.

Some national standards require the ORHA to provide the supplying tissue establishment with details of the patient to whom the tissues or cells were clinically applied. Whether this information is sent to the tissue establishment or not, it is essential that the end user maintain these records because ultimately they are responsible for recording the fate of the tissues or cells.

14.4. References

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Chapter 15. Biovigilance

15.1. Introduction

This chapter provides general guidance on implementation of good vigilance and surveillance (V&S) practice by all those involved in the processes of transplantation and assisted reproductive technology (ART), from donation through banking to clinical use until the donated tissue or cell functions in the recipient, and to regulation of the field. Tissue- and cell-specific chapters in Part B provide additional specific guidance on vigilance in those fields, particularly Chapter 24, which details several specificities within ART vigilance.

A programme of V&S is essential for ensuring the quality and safety of tissues and cells for human application. The quality system focuses on preventing errors and maintaining a consistent standard of agreed specification for tissues and cells released for clinical application. However, occasionally, residual risks or procedural errors result in failures, disease transmissions or situations in which donors or patients are exposed to risk, even if not harmed. These occurrences can be classified into ‘adverse events’ (which are process failures that might lead to harm in a recipient or a living donor or to a loss of any irreplaceable autologous tissues or cells or any highly matched allogeneic tissues or cells) and ‘adverse reactions’ (which are adverse outcomes that have indeed occurred with harm to a donor, a recipient, or a child born through ART procedures related to *in vitro* fertilisation (IVF) with gamete or embryo donation). 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. Reporting of these incidents presents important learning opportunities that can help all procurement organisations, tissue establishments, cell therapy and ART facilities, and clinical users (and not just those involved in the incident in question) to improve their processes and to achieve higher levels of safety and quality [1, 2].

According to European Union (EU) definitions, a ‘serious adverse event’ (SAE) in the present context is 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 the patient or which might result in, or prolong, hospitalisation or morbidity. According to EU definitions, 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 morbidity.

These definitions are reflected in the World Health Organization (WHO) Notify Library for V&S of Medical Products of Human Origin (MPHO). Adverse outcomes are categorised in the library as follows:

- a. Adverse reaction
 - harm to a donor
 - harm to a recipient
 - harm to a foetus or offspring

- b. Adverse event
 - risk of harm

In summary, an adverse reaction is an incident whereby a living donor, a recipient or a foetus or child created by IVF and intra-uterine insemination (IUI) with 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. All adverse reactions and adverse events should be documented and investigated by health professionals to ensure appropriate investigation as well as corrective and preventive actions. Those that are classified as 'serious' should be notified to Health Authorities, in accordance with national or regional (e.g. EU) requirements.

Although adverse incidents may occur at all stages from procurement to distribution of tissues and cells, many of them are not severe and may be managed through the quality management system (QMS) of the tissue establishment. Conversely, serious adverse reactions and events (SARE) 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 different substances of human origin (SoHO), because they share exposure to risks from donation to transplantation (from breaches of ethical, legal and safety standards).

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, and it 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 should be reported to the tissue establishment.

If products containing tissues or cells are classified as advanced therapy medicinal products (ATMP) in the EU, the regulatory framework of pharmacovigilance must be applied. The relevant legal texts and guidelines are described on the pharmacovigilance web page of the European Commission [3]. Donation, procurement and testing of 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 ATMP are associated with adverse outcomes.

15.2. Management and quality

As for other vigilance systems, vigilance activities in the field of tissues and cells should be considered and recognised at all levels of tissue establishment that are authorised for tissue and cell activities, beginning with the strategic and senior management levels. The organisation of the vigilance system, as well as the role of the various parties involved, should be defined and broadly communicated within the tissue establishment.

Health Authorities are encouraged to draw up guidelines for vigilance systems, notification forms, surveillance methods, acceptable risk criteria and examples of SARE for tissues and cells that should be reported to them. Appropriate communication and co-ordination between procurement organisations, tissue establishments and centres carrying out clinical application are essential for an efficient vigilance system. Any 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 collect, report, investigate and communicate notifications for adverse reactions and events (ARE). Identification of a local co-ordinator, who has responsibility for V&S specified in his/her job description, is an effective measure. It is recommended that the QMS and the V&S system, both of which contribute to risk management policy, should be co-ordinated at tissue establishment level according to guidelines established by the Health Authority and under the direct responsibility of the Responsible Person (RP).

Relevant SOPs and management of collection and investigation of ARE data should be evaluated during the inspection process in the tissue establishment. Implementation of computerised and integrated systems for collection and management of ARE data is encouraged.

15.2.1. Non-serious adverse events and reactions

This chapter focuses on the procedures for detection, reporting, alert, investigation, management, evaluation and closure of adverse reactions and events (ARE) that may occur from donation until transplantation and follow-up. All ARE and non-compliances (including those with minor consequences) should be documented and reviewed regularly within the QMS of the tissue establishment. This allows trends to be monitored and actions to be taken to continuously improve quality and safety. One important role for the Health Authority is to define (and inform tissue

establishments and professionals accordingly) which adverse events and reactions should be notified to them through the vigilance system and which should be managed locally through the QMS of the tissue establishment.

15.2.2. Complaints

Complaints from any party (clinical users, donors, patients or third parties) should also be managed within the QMS. Formal acknowledgement should be sent immediately, and the complaint should be investigated promptly. Corrective actions should be communicated to the complainant (if appropriate). Each complaint should be considered for classification as an SAE or SAR and should be managed as such if it meets the criteria described in this chapter.

15.3. Adverse reactions

Adverse reactions must be detected, reported, investigated and assessed in terms of severity, imputability, probability of recurrence or frequency, and consequences. 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 to relevant stakeholders appropriately.

Several symptoms or situations suggest that an adverse reaction might have occurred in a recipient of a tissue or cell and should, therefore, be seen as 'triggers' for an adverse reaction report. Below are examples of reportable adverse reactions [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

* In certain circumstances, clinicians may knowingly transplant an infective donation (e.g. a cytomegalovirus-positive bone marrow donation). In these circumstances, patients should be informed about benefits *versus* additional risks 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.

- d. hypersensitivity reactions, including allergy, anaphylactoid reactions or anaphylaxis [Hypersensitivity];
- 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 transplantation [Volume overload];
- m. neurological reaction [Insult];
- n. severe febrile reaction [Fever];
- o. other [Other].

15.3.1. Detection of adverse reactions

Effective V&S relies heavily on all health professionals involved, from procurement to clinical application, 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 be alert to adverse outcomes and be aware when such outcomes might be associated with the tissues or cells transplanted;
- d. physicians caring for children born after non-partner ART treatment who may detect a genetic abnormality and, by reporting it, prevent further distribution of gametes/embryos from that donor;

- e. any other tissue establishment staff involved in any procurement and transplant activities;
- f. other vigilance systems (e.g. haemovigilance, material/device vigilance, pharmacovigilance), when issues of concern are detected that might affect the safety of tissues or cells for transplantation.

V&S aims at improving patient safety, so consideration should be given to the possible role of patients and patient organisations in the notification process for adverse reactions.

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 that were applied to be a possible source of the outcome. Tissue establishments 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, so that similar occurrences might be 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 tissue establishment is required only in those exceptional circumstances in which there is suspicion of an un-

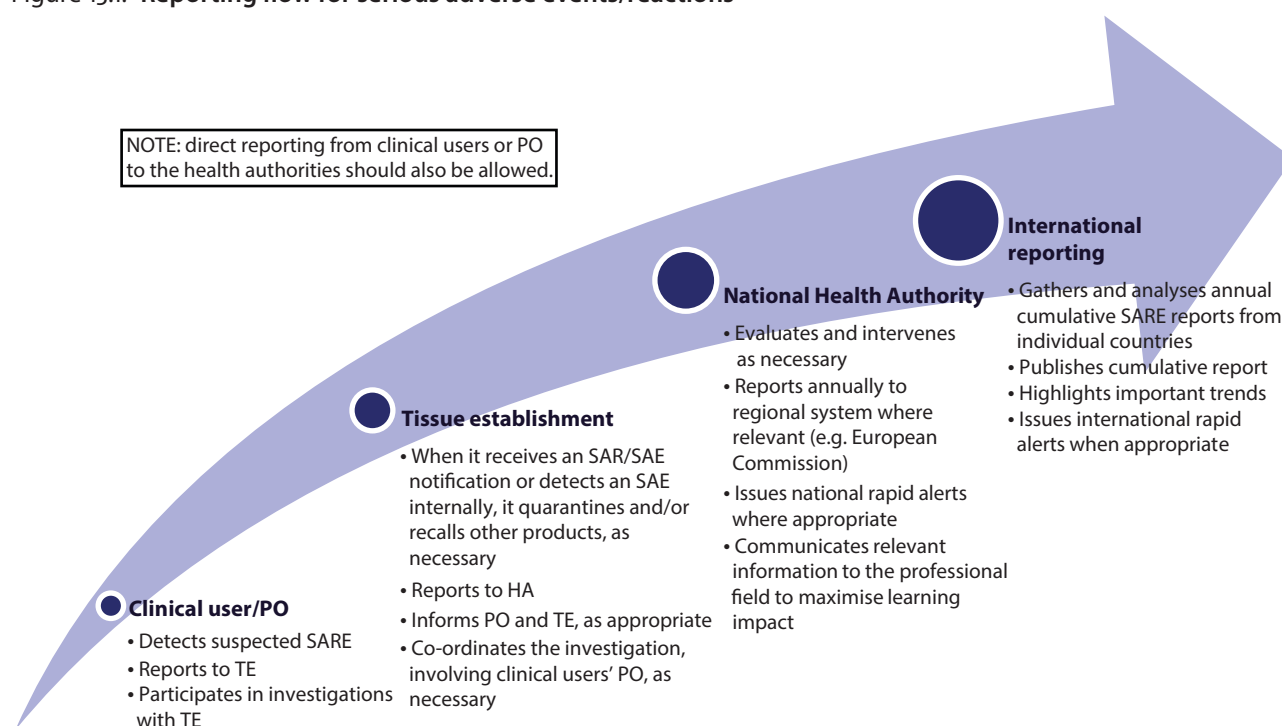
toward adverse reaction. However, reporting of the clinical progress of tissue and cell recipients to the tissue establishment might also be required for all highly-matched, life-saving transplants such as HPC infusions, or when novel tissue or cell processes have been applied or new types of tissues or cells are being transplanted. This routine clinical follow-up is not considered as part of vigilance.

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 Chapters 21 and 24).

15.3.1.1. *Surveillance for new risks*

Vigilance programmes should include an activity of scanning for new risks that have not been recognised previously. New risks may be related to donors, new techniques, new medical devices (including new ancillary products) or new reagents to which cells or tissues can be exposed during processing. 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

Figure 15.1. Reporting flow for serious adverse events/reactions



HA: Health authority; EU: European Union; PO: Procurement organisation; SAE: Serious Adverse Event; SAR: Serious Adverse Reaction; SARE: Serious Adverse Reactions and Events; TE: Tissue establishment.

Europe and publishes a weekly Eurosurveillance report that provides useful data to support the development of donor-selection policy. Moreover, the ECDC has been recently mandated to provide risk assessment on particular epidemic agents, infectious diseases or new *in vitro* diagnostic techniques in the field of tissues and cells.

15.3.1.2. *Donor follow-up for new risks to the recipient*

After donation, the living donor* should also be followed up, by documented procedures. This ensures that, if a new condition occurs that may have an impact on the recipient, proper action can be taken. This should clearly be documented. However extensive is the testing 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, preventative steps should be taken.

In cases where several organs or tissues of a deceased donor are used, and a condition is discovered with one of the organs or tissues that may have an impact on other organs or tissues as well, there should be a documented procedure to notify the other recipients' physicians.

15.3.2. **Reporting adverse reactions**

15.3.2.1. *Clinicians to tissue establishments*

Tissue establishments 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 tissue establishment that supplied the tissues or cells before investigation or confirmation. This approach allows the tissue establishment to take appropriate precautionary actions to prevent harm to other patients, and involves the tissue establishment in the investigation process. Clinical users should be encouraged to report all types of suspected adverse

reactions (serious and non-serious) to the supplying tissue establishment to allow filtering of those considered to be serious and reportable to an authority at a later stage.

15.3.2.2. *Procurement organisations to tissue establishments*

Similarly, health professionals and procurement organisations should report adverse reactions in living donors and recipients to the tissue establishment, 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.

15.3.2.3. *Reporting to regional/national programmes*

Tissue establishments must report information on SARs to Health Authorities so the benefits of consolidated data can be realised and information about the lessons detected can be shared (see Figure 15.1).

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 15.1 is the one used in the EU. It was proposed by the EU-funded project European Union Standards and Training for the Inspection of Tissue Establishments (EUSTITE) [4] for vigilance for tissues and cells and is based on the one used for haemovigilance. In the EU, all SARs that meet the descriptions of 'serious', 'life-threatening' or 'death' must be reported to the Health Authorities.

The tissue establishment is responsible for providing clinical-user entities, procurement 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 tissue establishment, with one or more SOPs that describe the process for acknowledgment of notifications, investigation, follow-up on corrective and preventive actions and reporting to the Health Authorities if criteria are met. The procedures should enable rapid action to be taken by all affected organisations to protect the safety of recipients. 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 where the donor was both an organ and tissue donor.

* This will depend on the type of donation e.g. no donor follow-up is required for cord blood donation whereas HPC donors will require a minimum of 5 years. The length of follow-up should reflect professional body guidance.

Table 15.2. Scale describing possible outcomes of an imputability investigation

	Criteria adapted from EUSTITE-SoHO V&S [10]	Criteria for infectious and malignant transmissions, adapted from the Disease Transmission Advisory Committee [9]
Not assess-able	Insufficient data for imputability assessment	Insufficient data for imputability assessment
o. 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 the following two conditions are 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

EU member states are obliged to send an annual report of the SARE they have received to the European Commission. Such international reporting allows for trend analyses on the basis of consolidated data. A published report from this programme is summarised in Appendix 21 of this Guide.

National competent authorities or regulatory agencies mandate SARE reporting for donors (as described above for the EU). However, HPC registries have implemented a global reporting system because products from HPC have specific characteristics:

- a. Grafts are always collected from living voluntary donors who do not benefit from the donation itself. Thus, every effort must be directed at minimising donor adverse reactions.
- b. Products from donors from HPC registries cross international boundaries more often than other products from tissues and cells (currently more than half of grafts donated by unrelated registry donors are used in countries different from their origin). Furthermore, most products are transported unprocessed and need to be infused < 72 h after collection, thereby cre-

ating challenges for the management of transport.

- c. SARE are rare and trends cannot be detected sufficiently at the donor centre, donor registry or at the national level.

15.3.2.5. 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 SARE. 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 depend on the type of donation, nature of the intervention and its potential impact on the individual's health [5].

Completed donor follow-up has been defined as physical, phone or laboratory contact at a given time point [6]. There should be written SOPs for follow-up of donors. For related donors, the RP 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 responsi-

bility of the relevant donor registry. The number of allogeneic HPC transplants performed globally each year continues to increase, paralleled by an increased demand for donors of therapeutic cells. Information on short- and long-term donor outcomes is of crucial importance to ensure maximal donor safety and availability. Current data, predominantly from unrelated donors, give reliable information on the frequent early events associated with donation, most of them of mild to moderate intensity. Information on the type and relative risk of serious adverse reactions is more limited. Moreover, only few data exist on long-term donor outcomes. On the basis of this need, recommendations for a minimum data set for prospective donor follow-up were developed under the auspices of and approved by the Worldwide Network for Blood and Marrow Transplantation (WBMT). Establishment of a standardised global follow-up for both, related and unrelated donors enables monitoring of the short- and long-term safety profiles of HPC donation and form a solid basis for future donor selection and counselling. Donor Outcome forms were approved and recommended by the WBMT in 2011 [7].

Reporting of donor outcome data is done through two data collection forms: a report on donation procedure and up to 30 days thereafter, and a long-term follow-up report as soon as one year has elapsed from the date of the last procedure. The forms, together with a manual, can be downloaded from the Data collection forms section of the Data management page of the European Group for Blood and Marrow transplantation (EBMT) website at www.ebmt.org/Contents/Data-Management/Pages/Data-Management.asp.

15.3.2.6. *Follow-up of donors and recipients (ART)*

Follow-up of gamete donors is not a statutory requirement in all countries. However, it is important to ensure the donor is aware of the need to inform the recruiting ART establishment of any medical information that may come to light after donation that may have health implications for any woman who receives treatment with those gametes or for any child born as a result of such treatment. The ART establishment must investigate this and ensure further screening as well as recipient/donor-conceived offspring follow-up is conducted, if applicable.

Oocyte donors who develop ovarian hyperstimulation syndrome (OHSS), post-operative complications and/or infections should also be followed up via the ART establishment's incident reporting system.

Recipients of donor gametes must be made aware that, if their offspring are diagnosed with a medical condition that could be related to the donation, they must report this to the ART establishment that carried out their treatment. The ART establishment must investigate this further.

If the gametes were provided by a donor bank, rather than procured in-house, the ART establishment must inform the donor bank. The donor bank must inform all other clinics that may have purchased the donor gametes about the problem (via the bank's recall procedures). Information should include the provisional or final diagnosis, if further genetic screening and counselling should be provided to the recipient and offspring, and if the donor should be blocked from further use.

For the above reasons it is important for donor sperm banks to provide donor gametes via licensed ART establishments rather than via an individual's home address.

15.3.3. **Investigation and assessment of adverse reactions**

Adverse reactions in recipients of tissues or cells should be investigated by a team that includes the clinician that transplanted the tissues or cells, the tissue establishment that provided them and, in more serious cases, the Health Authority in that country. Efficient co-ordination of the investigation is critical to 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. 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). Where there is suspicion of an infectious disease transmission, investigation will rely heavily on the availability of an archived sample of donor serum. It is strongly recommended that frozen serum (and/or cells or DNA) samples be maintained for every donor for vigilance investigation purposes. Consideration should also be given to keeping pre-transplant serum archives for transplant recipients to support imputability investigations. This approach is not advised for recipients of gametes or embryos.

The scale provided in Table 15.2, again developed by EUSTITE on the basis of the scale used in haemovigilance, and used in EU guidance to its member states, can be applied to describe the outcome of an imputability investigation. It is proposed that all adverse reactions be graded in terms of imputability.

Table 15.2 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 [8]. 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 able to support the process.

15.4. Adverse events

Adverse events can occur at any moment from donor selection to clinical application.

15.4.1. 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 tissue establishments and procurement organisations, those working in organisations such as testing laboratories that provide ‘third party’ services to tissue establishments, and clinical users who may also detect errors at the point of clinical use. In EU Directive 2006/86/EC, the definition of SAE includes those incidents often referred to as ‘near misses’, i.e. where an error or fault is detected and corrected without causing harm.

15.4.2. Serious adverse event reporting

Non-compliances with the quality system should be documented and investigated as part of the internal quality management system. 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. According to instructions from the European Commission to EU member states for annual vigilance reporting, deviations from SOPs in tissue establishments (or other adverse events) that have implications for the quality and safety of tissues and cells should result in SAE reporting to the Health Authority if one or more of the following criteria [11] apply (see also Figure 15.1):

- a. inappropriate tissues or 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 a mix-up of gametes or embryos;
- d. the event resulted in a loss of traceability of tissues or cells;
- e. the event resulted in loss of any irreplaceable autologous tissues or cells or any highly matched (i.e. recipient-specific) allogeneic tissues or cells;
- f. the event resulted in loss of a significant quantity of unmatched allogeneic tissues or cells.

15.4.3. Investigation and assessment of adverse events

Despite the fact that adverse events, by definition, have not (or not yet) involved harm to recipients or donors, the impact of an adverse event can be significant if considered in a broader way. The Impact Assessment tool given in Appendix 20 can also be applied to SAEs to help reach a decision on the response required.

15.5. Vigilance co-ordination

Co-ordination between various systems of vigilance (e.g. medical device vigilance, pharmacovigilance) should be in place at the local level (tissue establishment) and at the Health Authority level.

15.5.1. 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. Rapid alerts should only be issued in exceptional circumstances. The following criteria have been identified in the SOHO V&S project [12] 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 tissue establishments;
- 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 managed 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. In February 2013, this system was moved to a new secure internet platform where all rapid alerts are generated and shared.

15.6. Vigilance communication

15.6.1. 'No blame' culture

Effective communication of the results of vigilance systems is fundamental to ensuring that the benefits of these programmes are realised in practice. Regular feedback to health professionals is critical to support continued notification of ARE. All stakeholders, Health Authorities, tissue establishments 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 ART is risk-free. Programmes of training and awareness should be organised to encourage reporting. The message that reporting and disseminating V&S information can result in positive improvements for donors and patients should be promoted.

15.6.2. Vigilance experience and feedback

Health Authorities and professional societies should publish the results of their programmes without identifying individual centres, hospitals or individual people. Those tissue establishments 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 types of 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 [13, 14]. 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.

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Related documents:

Appendix 20. Serious adverse reaction or event: impact assessment tool;

Appendix 21. European report of serious adverse reactions and events associated with the clinical application of tissues and cells.

Part B. Specific requirements

Chapter 16. Ocular tissue

16.1. Introduction

Ocular tissues procured from deceased donors are used for treating loss of vision caused by 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 corneal transparency and a smooth, spherical ocular surface. The cornea is also part of the outer coat of the eye and must therefore be strong enough to withstand the intraocular pressure and help protect the delicate inner structures of the eye.

16.1.1. Corneal transplantation

A corneal transplant (keratoplasty) is an operation to remove all or part of a diseased or damaged cornea with healthy donor tissue. In Europe, the main indications for corneal transplantation include:

- Fuchs endothelial dystrophy (FED) – 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 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 [1-3].

Endothelial keratoplasty (EK) is the method of choice for endothelial dysfunction; e.g., FED and PBK. The 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 affected patients are mostly elderly and the advantages of EK over PK include much faster visual rehabilitation and, since there are no sutures required to hold the graft in place, less surgically induced astigmatism. There are currently four techniques for preparing tissue for EK:

- Descemet stripping endothelial keratoplasty (DSEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared by manual dissection.
- Descemet stripping automated endothelial keratoplasty (DSAEK) – endothelium on Descemet membrane with a thin layer of stroma, prepared using a microkeratome.

- Descemet membrane endothelial keratoplasty (DMEK) – endothelium on Descemet membrane without any stroma, prepared by manually separating Descemet membrane from the stroma.
- 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. As immunological rejection directed against the endothelium is one of the main reasons for PK failure, the advantage of DALK is that the patient’s endothelium cannot be rejected. Superficial stromal defects and scars can also be treated by anterior lamellar keratoplasty (ALK) but without the need to replace the full thickness of the stroma.

Tissue for PK and EK requires a corneoscleral disc with a viable endothelium. Tissue for DALK and ALK does not require a viable endothelium; 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. Tissue for EK may be prepared in a tissue establishment, which saves time for the surgeon and avoids the risk of damage to the tissue when prepared in the operating room.

Corneal transplant outcome – in terms of both graft survival and visual outcome – depends on the indication and reason for transplantation, the presence of pre-operative risk factors, such as vascularised cornea, glaucoma and inflammation, and postoperative complications such as allograft rejection. In the absence of other risk factors, keratoconus and FED are considered to be low-risk grafts with 5-year survivals of, respectively, 95 % and 82 % [4]. Re-grafts and PBK are more likely both to suffer allograft rejection and to fail and are therefore considered higher-risk grafts with 5-year survival of, respectively, 56 % and 54 % [4]. A major cause of graft failure is allograft rejection. Most rejection episodes can be successfully treated with topical (sometimes intraocular 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 leucocyte 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].

16.1.2. 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 epithelium (limbal stem cell deficiency) [7].

Tissues and cells 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 (see Part C, Chapters 26 and 28) [8];
- Corneal endothelial cells may be isolated from a corneoscleral disc and expanded *ex vivo* either for injection as a suspension into the anterior chamber for treatment of corneal endothelial disease (see Part C, Chapters 26 and 28) [9];
- Amnion is used for treating ocular surface conditions and as a support for limbal progenitor cells (see Chapter 17) [10];
- Autologous and allogeneic serum eye drops may be used for treating dry eye (see Part C, Chapter 29) [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:

- Introduction (Chapter 1);
- Quality management, validation and risk management (Chapter 2);
- Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- Donor evaluation (Chapter 4);
- Donor testing (Chapter 5);
- Procurement (Chapter 6);
- Processing and storage (Chapter 7);
- Premises (Chapter 8);
- Principles of microbiological control (Chapter 9);
- Distribution and import/export (Chapter 10);

- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, packaging and labelling (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

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.

16.2. Donor evaluation

16.2.1. Tissue-specific exclusion criteria for ocular tissue donation

Acceptance and exclusion criteria for cornea donation that differ from other tissues are based on the avascularity of the cornea and ocular-specific conditions that may affect the cornea.

16.2.2. 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 4).

16.2.2.1. Donor age

Provided that corneas are examined to exclude those unsuitable for transplantation based on endothelial cell density and/or stromal abnormalities (see section 16.7), the upper age limit for eye donors may be determined by the tissue establishment. 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 tissue establishment because corneas from young children lack rigidity and have a high radius of curvature. There is likely, therefore, to be little demand for corneas from such young donors for transplantation; however, corneas from these donors may be important as a source of limbal grafts or limbal progenitor cells.

16.2.2.2. Malignancies

Haematological neoplasms, retinoblastoma and malignant tumours of the anterior segment are obligatory 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 [14]; but there has been a more recent report of malignant cutaneous melanoma (MCM) metastases in peripheral, but not central, avascular cornea [15]. The Medical Advisory Board of the Eye Bank Association of America has issued an amendment to their donor selection criteria excluding donors with a history of melanoma with known metastatic disease [16]. The incidence of metastases from non-ocular tumours to the anterior segment of the eye is reportedly extremely low; however, corneas must be excluded where there is evidence of anterior segment metastases from the slitlamp examination of the eye or the corneoscleral disc [17-19].

16.2.2.3. Infections

Individuals with localised ocular infection (bacterial, viral, fungal, protozoal, parasitic) are excluded from donation of ocular tissues. This exclusion includes those with a history of past ocular *Herpes* infection.

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.

16.2.2.4. Eye diseases

The following exclude cornea donation:

- a. ocular inflammation and infection (see 16.2.1.4);
- b. autoimmune disease, e.g. sarcoidosis, rheumatoid arthritis, but only where 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 anterior dystrophies;
- b. corneal opacity, scarring, pterygium or other superficial disorders of the conjunctiva or corneal surface that involve the central area of the cornea.

16.2.2.5. Previous intraocular or anterior segment surgery

The following exclude cornea donation:

- c. previous ocular surgery that would prejudice graft outcome;
- d. receipt of a corneal, sclera or limbal allograft.

The following exclude cornea donation for PK or DALK but not necessarily for EK:

- a. refractive corneal surgical procedures including radial keratotomy, lamellar inserts and laser refractive surgery.

16.3. Procurement

16.3.1. *Post mortem* time

Ocular tissues should be procured from donors as soon as possible after cardiac arrest, preferably within 24 hours; however, Health Authorities or local practice may allow procurement up to 48 hours after cardiac arrest. For EU member states, a blood sample for the mandatory tests for transmissible disease must be obtained from the donor within 24 hours of death (see Chapter 5).

16.3.2. Procurement team

Ocular procurement personnel must be appropriately clothed and 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.

16.3.3. Procurement procedures

Since the ocular surface is exposed to the environment and, after death, there is no blinking and no tear film, the ocular surface is highly likely to be contaminated by environmental micro-organisms before procurement. Therefore, a classified area with a specified air quality is not typically required for ocular tissue procurement but other guidance given in Chapter 6 does apply.

The donor's eyelids and skin 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 may be applied to the ocular surface. A broad-spectrum antibiotic

solution may also be used. 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 families 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. There is no reported evidence of a difference in corneal quality or clinical outcome between these two procurement methods.

16.3.3.1. *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 eye should be placed, cornea uppermost, in a fixed position in a moist chamber and transported to the tissue establishment refrigerated in ice. Broad-spectrum antibiotics may be used to further minimise the risk of bacterial contamination.

16.3.3.2. *Procurement of corneoscleral discs*

After peritomy, sclerotomy is performed, maintaining wide scleral rim (*circa* 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 tissue establishment, the container should either be a corneal viewing chamber or should have a flat bottom and adequate optical properties to facilitate subsequent assessment by slit lamp and specular microscopy.

16.3.3.3. Procurement of scleral tissue

Scleral tissue is prepared in the tissue establishment from the whole eye after excision of the corneoscleral disc.

16.3.4. 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.

16.4. 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 tissue establishment should not exceed 24-48 hours.

Corneoscleral discs procured by *in situ* excision may be placed in a hypothermic storage solution or in a medium designated by the manufacturer for room temperature storage. In both cases, the manufacturer's recommendations for storage temperature should be followed or the temperature conditions during transport should be validated.

16.5. Processing preservation and storage

16.5.1. Processing facilities

The requirements of Chapter 7 on processing and storage should be applied when selecting an appropriate air-quality specification for ocular tissue processing and for environmental monitoring and quality control.

Table 16.1. Factors influencing the air quality for processing ocular tissue

Criterion	Ocular tissue-specific
Risk of contamination of tissues or cells during processing	Corneoscleral discs procured by <i>in situ</i> excision are procured and placed in a storage medium in an environment where air quality typically is not controlled. Careful cleaning of the ocular surface before excision, aseptic technique and use of antimicrobials in the storage medium help to minimise the risk of contamination. Processing whole eyes in a tissue establishment allows control of air quality (e.g., laminar flow cabinet in a room with HEPA filtered air). Cleaning of the eyes before processing is important because it has to be assumed that bacteria and fungi will be present on the ocular surface owing to lack of blinking and tear film after death of the donor. Organ-cultured corneas may be removed from their storage medium just prior to surgery to examine the endothelium by light microscopy and for further processing for EK. They are therefore re-exposed to the environment and an appropriate air quality must be applied. The EU Tissues and Cells Directive requires the equivalent of Grade A air quality with at least a Grade D background for such purposes.
Use of antimicrobials during processing	Corneoscleral discs may be stored in media containing antibiotics and antimycotics. The medium may also contain a marker (e.g., phenol red) that changes colour with a fall in pH caused by growth of micro-organisms. Turbidity of the storage medium is also an indication of contamination. Storage of corneas in organ culture not only allows the testing of samples of medium for microbial growth during storage but also ensures that any antimicrobials in the medium will be more effective, owing to the higher storage temperature than that used for hypothermic storage.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	There is typically no microbiological testing of hypothermic corneal storage media. Even if a sample of hypothermic medium is taken, the time available before transplant is limited to just a few days, which reduces the chance of detecting contaminants. Some eye banks recommend that surgeons send the remaining corneoscleral rim and storage medium for microbiological testing after preparation and transplantation of the corneal graft. For organ-cultured corneas, there is a greater chance of detecting contamination because of the extended, albeit still limited, storage period. A second sample of storage medium may be taken after transfer of an organ-cultured cornea to medium, to reverse stromal oedema and for transport to the recipient hospital, but the time before transplantation is only a few days and a negative-to-date release will apply. Therefore, there is a risk that contamination may not be detected until after transplantation.
Risk of transfer of contaminants at transplantation	Corneal tissue for the great majority of transplant procedures cannot be sterilised because living cells are required for a successful graft outcome. Post-operative endophthalmitis caused by micro-organisms transferred with the graft is therefore a risk and is a defined serious adverse reaction. It is considered to be rare. Attributing a cause is not always straightforward owing to the, albeit slight, risk of post-operative infection associated with any intraocular surgical procedure.

16.5.2. 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 tissue establishment and may not require further processing unless they are to be transferred to organ culture. When whole eyes are received by a tissue establishment, 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 [20]. Further processing of corneoscleral discs to prepare grafts for EK may be undertaken in the tissue establishment [21-22].

16.5.3. Cornea storage methods

a. Hypothermic storage at 2 to 8 °C

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.

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 [23-25]. Corneas prepared in a tissue establishment for DSAEK or for DMEK can be shipped to hospitals in hypothermic storage media.

b. Organ culture at 28 to 37 °C

A storage time of up to 4-5 weeks is typical for organ culture, although successful transplants after 7 weeks have been reported [26]. 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 except for tissue designated for superficial anterior lamellar grafting, where a viable endothelium is not a requirement.

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.

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 28 to 37 °C for up to 4-6 days, at the discretion of the RP and depending on the medium used [27-28].

Organ-cultured corneas can be prepared in the eye bank for DSAEK and DMEK with or without

pre-thinning in dextran medium [29]. The DSAEK grafts may be laid back on the anterior stroma to provide additional support during transport in dextran medium [30]. For DMEK, the graft may be supplied rolled or attached, either in the centre or at the periphery, and laid back on the stroma [22, 31]. Pre-prepared grafts for DSAEK and DMEK may be shipped to hospitals in medium at room temperature [22].

c. Storage of non-viable corneal tissue

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 or frozen [32].

16.5.4. 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 saline with antibiotics. Sclera stored in saline with antibiotics in a refrigerator should only be kept for short periods (≤ 7 days).

16.6. 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 [33-34]. 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 [35], it can be helpful for the investigation of post-operative endophthalmitis. For further information refer to Chapter 9 on the principles of microbiological control.

a. 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. 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 microbio-

logical 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 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 (< 4-6 days), 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 9. If growth is detected, the surgeon must be informed immediately to stop the tissue being transplanted. If the transplant 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. If the fellow cornea has been transplanted, the transplanting surgeon should be informed and the patient monitored.

b. 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. Testing a sample of medium is a surrogate for direct testing of tissue. The short storage period and low temperature, which would suppress microbial growth, greatly reduce the likelihood of detection of contamination in the medium.

c. Sclera

Depending on the method of storage, for example refrigerated in saline, microbiological testing should be carried out after processing. Storage in ethanol ($\geq 70\%$ v/v), glycerol ($\geq 85\%$ v/v) or gamma irradiation of the tissue may render microbiological testing unnecessary unless required by local or national guidelines.

16.7. 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, taking into account that the epithelium 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 to the optical zone 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 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 transplanted, is considered to be essential, whereas assessment at both the start and end of storage allows endothelial cell loss during storage to be determined.

There are two main methods used for endothelial evaluation by microscopy:

- vii. 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 storage medium used. It is recommended that cold-stored corneas are warmed to room temperature to enhance the quality of the endothelial image.
- viii. Transmitted light microscopy (bright field or phase contrast). To enable cell counting, brief exposure to hypotonic sucrose solution (1.8 % w/v) or 4 minutes' exposure to either balanced salt solution (BSS) or 0.9 % (w/v) NaCl is necessary 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.

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/mm²;
- ii. moderate to severe signs of polymegathism and pleomorphism;
- iii. significant (> 20 %) 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 accept-

able whereas corneas with larger areas of dead or missing cells are not.

16.8. 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, 36]. 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 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 [37, 38]. 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]. A project, part funded by the EU and led by the European Society for Cataract and Refractive Surgery, aims to establish a European Cornea and Cell Transplant Registry (ECCTR), building on the existing registries in the Netherlands, Sweden and the UK (see www.ecctr.org).

16.9. Biovigilance

Serious adverse reactions for corneal transplants include:

- a. Primary graft failure (corneal transplant never cleared);
- b. Endophthalmitis or other serious ophthalmic infection;
- c. Graft failure due to donor tissue which was out of date, scarred or had evidence of previous surgery;

- d. Malignancy possibly attributable to the transplanted tissue;
- e. Systemic infection possibly attributable to the transplanted tissue.

Serious adverse events 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 cornea supplied to surgeon.

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);
- In Record Number 20, 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;
- In Record Number 338, a case of transmission of T-cell lymphoma is described, whereby molecular analyses were used to detect the same alleles in HLA-DQ α testing of the recipient and donor of the graft;
- Record Number 720 reports 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 1663 describes 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 [14].

Further cases of adverse outcomes associated with ocular tissue can be found in the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by the substance type, adverse occurrence type and record number. A recent report, not yet in the Notify Library, concerns the identification of metastases in the peripheral, but

not central, avascular cornea from a donor with malignant cutaneous melanoma (see 16.2.2.2) [15]. 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 [16].

16.10. Developing applications for patient treatment

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 [39].

Decellularised stroma 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 [40]. Retinal pigment epithelial cells derived from human embryonic stem cells, induced pluripotent stem cells, umbilical cord, foetal brain or bone marrow are being investigated for the treatment of age-related macular degeneration (see Part C) [41].

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 (see Part C). [9].

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Chapter 17. Amniotic membrane

17.1. Introduction

Human amniotic membrane (hAM) is the innermost, semi-transparent layer of the foetal membranes, 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 fibroblasts in a collagen scaffold.

It has some unique properties. Although the exact mechanism of many hAM actions is still unclear, several beneficial effects of hAM have been suggested on the basis of its biological composition. Clinical and experimental data have shown [1-5] that hAM 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.

Collagen types I, III, IV, V and VII, laminin and fibronectin have been identified in the amniotic basement membrane and stroma. Laminin and fibronectin are especially 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, hAM 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. Moreover,

hAM produces 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) [6, 7]. These characteristics have led to the use of hAM in clinical practice in the field of ophthalmology and in the treatment of a wide variety of pathological conditions: management of burns, as a temporary or long-term wound dressing, as graft material over skin ulcers to replace mucosa, in arthroplasty and in intra-abdominal and reconstructive surgery [8-11].

In addition the special structure and biological properties of hAM make it an important potential source for scaffold material.

Stem cells derived from hAM have been described to display multilineage potential and immunomodulatory properties [12, 13]

The following generic chapters (Part A) of this Guide apply to hAM banking and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors, and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);

- i. Principles of microbiological testing (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, labelling and packaging (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

This chapter defines the additional specific requirements for hAM.

17.2. Donor recruitment and evaluation

Prior to full-term delivery, potential donors are approached to ascertain whether they would be willing to donate their placenta. A trained nurse or healthcare professional will discuss the donation process and complete the consent and medical and behavioural lifestyle assessment. General criteria for donor evaluation are described in Chapter 4. The potential donor should be evaluated before giving birth and after full consent, also with information that donation will take place only if the delivery is without any complications.

Placenta and/or procured foetal membranes should be collected only from living donors, after a full-term pregnancy.

17.2.1. Specific exclusion criteria

Diseases of the female genital tract or other diseases of the donor or unborn child that might present a risk to the recipient include:

- 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.

Individual tissue establishments may have additional exclusionary criteria.

17.3. Procurement

17.3.1. Procurement facility and procurement team

Medical staff at gynaecological clinics collect placenta and/or procured foetal membranes after caesarean section or vaginal delivery. Amniotic membrane could be contaminated by normal vaginal flora during vaginal delivery; therefore, procurement under aseptic conditions after elective caesarean section is to be preferred. If hAM is procured during vaginal delivery, different sterilisation procedures [14, 15, 16] should be applied (e.g. sterilisation by gamma irradiation). Staff undertaking procurement must be dressed appropriately for the procedure so as to minimise the risk of contamination of the procured tissue and any hazard to themselves.

17.3.2. Storage and transport after procurement

Placenta and/or procured foetal membranes should be stored at appropriate temperatures to maintain their characteristics and biological functions.

The storage and transport time of procured foetal 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 the foetal membranes are prepared < 2 h after the delivery, the placenta may be transported at room temperature [17].

The placenta and/or procured foetal membranes should be placed in a sterile receptacle containing a suitable transport medium (or decontamination solution) if transport time > 2 h [18]. The sterile packaging should then be placed inside an adequately labelled sterile container to be transported to the tissue establishment. Individual tissue establishments should validate the composition of the transport medium and determine if antibiotics are required.

The temperature during transport to the tissue establishment must be maintained. Temperature stability should be guaranteed by the container, the mode of transport used and for the time interval before processing. In cases of unexpectedly high or low environmental temperatures, 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.

Table 17.1. Factors influencing the air-quality specification for processing human amniotic membrane

Criterion	Amnion-specific
Risk of contamination of tissues or cells during processing	During processing, amniotic membranes are necessarily exposed to the processing environment for extended periods during dissection, sizing and evaluation of their characteristics.
Use of antimicrobials during processing	Soaking in antibiotics is an antimicrobial step that can be used when processing amniotic membranes. It is important to validate the antibiotic solution and to list the micro-organisms that are acceptable pre-decontamination. Since glycerolised, lyophilised and frozen amniotic membranes can be exposed to sterilisation processes, the processing environment may not be as critical as for tissue that cannot be sterilised. However, the process should be validated and maximal acceptable bioburden defined.
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of amniotic membrane for microbiological analysis following antibiotic soaking is not extensive; typically only a small amount is sampled, but the storage medium can also be sampled.
Risk of transfer of contaminants at transplantation	Although not vascularised, amniotic membrane can support microbiological contaminants and has transmitted bacteria and viruses. Amniotic membrane is mostly used in ophthalmology. Amniotic membranes are used also for other indications, such as burns, skin ulcers and arthroplasty. The use of amniotic membranes in intra-abdominal and reconstructive surgery procedures has also been described. Immuno-compromised patients, despite recent advances in therapy, are at a substantially higher risk of transmission of infection and even death from infections.

17.4. Processing and storage

17.4.1. Receipt of procured placenta at the tissue establishment

On receipt at the tissue establishment, the procured placenta should be stored at 2–8 °C (e.g. in the refrigerator). The refrigerator temperature should be monitored and continuously recorded. Processing should be carried out ≤ 48 h after procurement [18]. If the process is intended to maintain amnion cell viability, then it is recommended that the nutrient medium be changed in a controlled environment on receipt at the tissue establishment.

17.4.2. Processing facilities

In selecting an appropriate air-quality specification for hAM processing, the criteria identified in Chapters 7 and 8 should be considered. Table 17.1 outlines factors to be considered for hAM processing.

Taking the factors from Table 17.1 into consideration, it is appropriate that processing of hAM should take place in a controlled environment with defined air quality (see Chapter 7), especially for cryopreserved hAM where there is less opportunity for bacterial inactivation.

Many national requirements allow processing of tissues without subsequent microbial inactivation (including hAM) only in an air quality equivalent to Grade A as defined in European Union (EU) Good Manufacturing Practice (GMP) with a background of Grade B or C, as appropriate for the processing of

tissues at risk of contamination owing to multiple processing steps or processing at room temperature without terminal sterilisation. Within the EU, tissues exposed to the environment without subsequent microbial inactivation should be processed in environments with an air quality equivalent to Grade A, as defined in EU GMP, with a background environment at least equivalent to Grade D.

17.4.3. Processing and preservation methods

Tissue establishments may use different processing and preservation methods, according to their own standard operating procedures (SOPs) and mandatory regulations. The methods used should be up-to-date and validated.

In principle, the foetal membranes should first be rinsed several times in sterile saline until blood residues are removed completely. The amnion and chorion should then be mechanically separated according to a documented SOP. The amnion should be spread on a suitable carrier membrane (e.g. nitrocellulose), or fine mesh gauze for easier handling and if it needs to be divided into smaller pieces.

Maximum storage time will depend on the method of preservation and should be defined and validated [17, 19]. There are several methods of hAM preparation and preservation, as follows.

17.4.3.1. Cryopreservation

Before storage, amniotic membrane is usually decontaminated using antibiotic/antimycotic solution. It can then be cryopreserved in medium con-

taining glycerol or dimethyl sulfoxide. Amniotic membrane is packaged in an appropriate sealable container (bag or cryovial) and transferred to a -80°C freezer or stored in the vapour phase of a liquid nitrogen refrigerator [20, 21]. The tissue may be sterilised by irradiation.

17.4.3.2. Heat-dried amniotic membrane

The hAM is dried overnight in an oven at $40 \pm 2^{\circ}\text{C}$, then packed and sterilised by irradiation. Storage and subsequent transport should be at room temperature [18].

17.4.3.3. Air-dried amniotic membrane

After the hAM is processed in sterile conditions, it is air-dried overnight in a laminar flow hood. It can then be packed and sterilised by irradiation. Although high temperatures are not applied using this method, some properties of the amnion are lost or altered due to dehydration. Air-dried irradiated amniotic membranes should be stored and transported at room temperature [22].

17.4.3.4. Lyophilised (freeze-dried) amniotic membrane

The amniotic membrane can be cut into pieces and 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 attained. The tissue should then be packed and may be sterilised by irradiation [23]. This type of preparation induces minimal changes in the properties of the amniotic membrane and the product can be stored and transported at room temperature [24, 25].

17.4.3.5. Preservation in cold glycerol

Glycerol has long been used as a cryoprotectant. Since glycerol permeates slower 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 *circa* 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 [26, 27]. Typically, 85 % (v/v) glycerol is used to store amniotic membrane, which can then be stored at $2-8^{\circ}\text{C}$ for up to two years, although it does lose some of its biologic properties.

17.4.3.6. Antibiotic-impregnated amniotic membrane

After separation, amniotic membrane is placed overnight in an antibiotic solution composed of a range of wide-spectrum antibiotics and an anti-fungal agent and then frozen at -80°C . The resultant amniotic membrane is suitable for the management of infected wounds by providing an appropriate concentration of antibiotics to the wound surface.

Table 17.2. Microbial contaminants that should result in tissue discard if detected at any stage of processing

Bacteria
<i>Acinetobacter</i> spp.
<i>Bacillus</i> spp.
<i>Bacteroides</i> spp.
Beta-haemolytic <i>Streptococci</i>
<i>Burkholderia cepacia</i> complex
<i>Clostridium</i> spp. (notably <i>C. perfringens</i>)
<i>Corynebacterium diphtheriae</i>
<i>Enterobacteriaceae</i> (coliforms)
<i>Enterococcus</i> spp.
<i>Fusobacterium</i> spp.
<i>Klebsiella rhinoscleromatis</i>
<i>Listeria monocytogenes</i>
<i>Mycobacteria</i> spp. (for at-risk donors)
<i>Neisseria gonorrhoea</i>
<i>Nocardia</i> spp.
<i>Pseudomonas</i> spp.
<i>Porphyromonas</i> spp.
<i>Prevotella</i> spp.
<i>Salmonella</i> spp.
<i>Shigella</i> spp.
<i>Staphylococcus aureus</i>
<i>Sphingomonas maltophilia</i>
<i>Stenotrophomonas maltophilia</i>
<i>Streptococcus pyogenes</i> (Group A)
Fungi
<i>Aspergillus</i> spp.
<i>Candida</i> spp.
<i>Mucor</i> spp.
<i>Penicillium</i> spp.
Other yeasts and filamentous fungi

Note: This suggested list is not exhaustive.

17.5. Quality control

During procurement and processing of hAM, reliable macroscopic examination of the donor placenta should be undertaken to exclude visible pathological changes and ensure integrity of the tissue (to provide barrier function). Samples for detecting aerobic and anaerobic bacteria and fungal cultures should be obtained from the transport/storage medium or from the initial washings of the membrane, and from pieces of the membrane obtained both before and after antibiotic decontamination. Microbiological controls for the detection of bacteria and fungi should be carried out according to those described in Chapter 9. These approaches cover the minimum standards to control microbiological safety (see Table 17.2). 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 decontamination yield micro-organisms that are considered pathogenic and highly virulent, the tissue cannot be approved for clinical use (see Table 17.2). 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.

17.6. Distribution

For cryopreserved hAM, distribution should be in dry ice (solid carbon dioxide) or a liquid nitrogen dry shipper. Transport temperatures of cryopreserved hAM above -60°C must be avoided to ensure the stability of the product and maximum safety for the recipient. The tissue establishment must ensure that all storage processes and distribution are carried out under controlled conditions.

Air-dried and freeze-dried hAMs can be stored and distributed at room temperature, whereas those preserved in cold glycerol should be transported at $2-8^{\circ}\text{C}$.

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Chapter 18. Skin

18.1. Introduction

Donor skin is used to close full-thickness wounds. It acts as a temporary skin replacement. It is a means of reducing scarring, controlling pain, preventing infection and maintaining patient homeostasis by reducing loss of fluids, proteins and heat through the burn wound. For these reasons, it is critical (and often life-saving) in the treatment of severely burned patients. In addition, homologous skin is considered to be an excellent biological dressing for the treatment of other types of skin loss such as venous ulcers, pressure sores, decubitus ulcers, toxic epidermal necrolysis (Lyell's syndrome), surgical wounds and congenital epidermolytic skin disorders. In these cases, skin allografts promote re-epithelialisation and formation of granulation tissue (small vessels in the wound bed), shorten healing time, control pain and protect important structures (e.g. tendons, bones, cartilage, nerves).

These factors explain the constant demand for skin allografts by burn centres and reconstructive surgery units, where the capacity of these bio-products to 'take' and integrate into the wound bed is exploited.

The shortage of homologous 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 past 30 years, a huge number of biological 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.

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.* Introduction (Chapter 1);
- b.* Quality management, risk management and validation (Chapter 2);
- c.* Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d.* Donor evaluation (Chapter 4);
- e.* Donor testing (Chapter 5);
- f.* Procurement (Chapter 6);
- g.* Processing and storage (Chapter 7);
- h.* Premises (Chapter 8);
- i.* Principles of microbiological control (Chapter 9);
- j.* Distribution and import/export (Chapter 10);
- k.* Organisations responsible for human application (Chapter 11);
- l.* Computerised systems (Chapter 12);
- m.* Coding, packaging and labelling (Chapter 13);
- n.* Traceability (Chapter 14);
- o.* Biovigilance (Chapter 15).

This chapter defines the additional specific requirements for skin.

18.2. Skin-specific donor evaluation

18.2.1. Skin inspection

In addition to the standard physical examination described in Chapter 4, the donor's skin must be inspected in a particular manner before skin procurement. Skin should be checked for mechanical damage, open wounds, multiple (> 100) or dysplastic naevi, dermatitis, local infections and ectoparasites. The results must be recorded and taken into account.

18.2.2. Skin-specific exclusion criteria

Along with general exclusion criteria described in Chapter 4, there are some specific conditions that exclude skin donation. 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 transfer of diseases to the recipient and to ensure the appropriate quality of the skin for optimal functional results. The following conditions contraindicate skin donation:

- a. autoimmune dermatoses affecting skin;
- b. systemic connective tissue diseases;
- c. diseases affecting the dermis (dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria);
- d. mechanical or microbial damage to the skin;
- e. burns at the location on the body where skin is to be procured;
- f. toxicity of the skin as a result of the presence of toxic agents or poisons;
- g. presence of possible skin tumours (see Appendix 14);
- h. localised skin infections on potential donor sites.

The following relative contraindications for skin donation should be considered case-by-case:

- i. extensive lacerations or scars;
- j. skin diseases with extensive involvement, such as psoriasis, eczema or nodules;
- k. relevant skin ulcers, pressure sores, decubitus ulcers, pyoderma or mycoses;
- l. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos);
- m. diabetes mellitus with skin complications (e.g. ulcers).

The common practice is not to procure skin from donors aged <15 years but many tissue estab-

lishments do not indicate any age limits, which are basically determined by the medical director of the tissue establishment, according to characteristics and quality of tissues.

18.3. Skin procurement

It is recommended to procure the skin within 24 h after death if the body has been cooled or refrigerated within 6 h of death. It may be possible to extend procurement times up to 48 h 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 within 24 h after death (to avoid extensive haemolysis) [1]. See Chapter 5 for details on sample collection.

If the donor body was not refrigerated after death, then procurement should be completed within 12 h after death.

Skin can be obtained from deceased donors after brain death (DBD) or circulatory death (DCD). 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. Skin can also be obtained from living donors having abdominoplasty or mammoplasty procedures who consent to tissue donation. In these cases, the procurement area is prepared by depilation and disinfection and the tissue is processed to obtain full-thickness skin grafts (1-3 mm). Full-thickness grafts can also be procured by manually-operated dermatomes or scalpels.

18.3.1. Procurement team

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 multiple-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 6). Procurement teams should follow an effective and validated donor disinfection procedure, which can significantly reduce the microbial positivity rate of processed skin samples. Studies show that, whether the skin procurement is done before or after bone procurement, the contamination rate of skin is no different if the procurement process is controlled and standardised [2]; 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.

18.3.2. Skin procurement procedure

Skin is procured under aseptic conditions after adequate 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. A standardised procedure for skin disinfection should be established by the tissue establishment and allocated to all procurement sites.

The procedure should aim to reduce the bio-burden. 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.

A local sterile field using sterile drapes must be used prior to procurement to effectively prevent microbial contamination. Skin grafts are cut by electric or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs. Graft thickness is usually 200-800 µm and each graft should be cut as homogeneously as possible. Staged separate procurement of particular body areas with placement of procured material from each area into separate containers should be preferred in order to reduce eventual cross-contamination of the procured tissue. Containers and solutions for transportation of procured skin to processing tissue establishments must be sterile and certified. Pre-labelling of the containers is important to prevent mix-up of tissues and to ensure their full traceability (see Chapters 13 and 14).

18.3.3. 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. Once the tissue has been procured, appropriate garments or sealing agents (polymers) should be used to prevent leaking and oozing from sites where tissue has been obtained.

18.3.4. Temporary storage and transportation to the tissue establishment

Immediately after procurement, skin must be stored in a suitable transport medium in sterile, pre-labelled containers filled with an appropriate

transport medium. The containers shall be sealed securely, refrigerated to 2-8 °C and transported to the processing facility. Transportation at low temperatures prevents proliferation of most bacteria and fungi, and maintains viability. Antibiotics can be added to the transport medium, but at 2-8 °C even the latest broad spectrum antibiotic cocktails can fail to decontaminate skin grafts. According to Rooney *et al.* (2008) [3], approximately 22 % of skin allografts are not reliably decontaminated by antibiotic treatment. There are three likely causes as to why antibiotic cocktails are not always as efficient. First of all, bacteria can be 'hidden' in the procured skin (e.g. in the hair-follicles) where the antibiotics cannot reach them. Secondly, the optimal operating temperature of most antibiotics is much closer to 37 °C than to 2-8 °C [4]. The inclusion of an additional short antibiotic incubation step at 37 °C could be considered during processing stages. Finally, diluted antibiotic suspensions are known to lose activity relatively quickly (even when frozen). It is thus important that concentrated antibiotic suspensions are kept at low temperatures (e.g. -80 °C) and are not added to the transport media much in advance.

The container with procured skin must be cooled during transport to the tissue establishment. If skin grafts are to be glycerolised, refrigerated transportation may not be required and the recovered skin can be stored and transported at ambient temperature in 50 % glycerol solution.

The procured skin should be transferred to the processing tissue establishment as soon as possible after procurement or should be put in an initial processing fluid directly after procurement. After the skin has been received by the tissue establishment, processing of the skin should commence within 24-72 h of procurement having taken place. Before processing, the skin should be kept in a temperature-controlled refrigerator at 2-8 °C, in a physiological medium with antibiotics added for its decontamination. Before the antibiotics are added, skin samples for initial bio-burden estimation should be obtained. 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 72 h storage (e.g. adequate buffering capacity). All the manipulations where the transport containers are going to be exposed and the media changed shall be performed in a controlled air environment (clean room).

18.3.5. Procurement documentation

The organisation responsible for the skin procurement must produce a procurement report to be

provided to the tissue establishment. In addition to the generic requirements defined in Chapter 6, it must contain a description and identification of the procured skin (including samples for testing).

18.4. Skin processing

The recovered skin is processed to allow longer storage periods until transplantation in suitable patients. The methods used must be in line with current state-of-the-art procedures and validated procedures (see Chapter 7). Different tissue establishments apply specific preparation processes according to their own standard operating procedures (SOPs) and any applicable local authorisations.

18.4.1. Skin processing methods

Procured skin tissue can be processed to facilitate longer storage time and to reduce microbial contamination. Processing of procured skin generally includes soaking in antibiotic cocktails; if skin viability is to be maintained, then this is the only decontamination step that can be included and it should always be applied. The incubation temperature and the composition of antibiotic cocktails should be defined, after validation, in written procedures by each tissue establishment, assessing the initial tissue bioburden. Skin tissue can be processed according to various protocols (cryopreservation, glycerol-preservation, lyophilisation, possibly followed by gamma-irradiation). Skin grafts destined for cryopreservation should be processed immediately after receipt in order to maintain cell viability and structural integrity. Skin allografts can also be processed into de-epidermised skin and acellular dermis. All human tissues intended for human application are processed into specimens appropriate for clinical use. Processing must not change the physical properties of the tissue so as to make it unacceptable for clinical use. All processes must be validated in accordance with the guidance given in Chapter 2.

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. Procured skin allografts can be cut into specific smaller sizes according to requirements of the end user clinicians. The grafts may be provided as sheets or meshed (extended on a synthetic mesh to increase the surface area). 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 di-

mensions 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 13).

18.4.1.1. Glycerol-preserved skin allografts

Glycerol-preserved skin allografts (GPA) were developed [5] 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 low antibacterial/antiviral properties [6, 7, 8]. If there are positive microbiology results from cryopreserved skin, the skin can be processed in 85 % glycerol as a recovery procedure. The glycerol solutions used should be sterile and of high quality (e.g. see *European Pharmacopoeia* monograph 0497 – Glycerol 85 %). GPA can be stored at 2-8 °C for several years.

18.4.1.2. Unprocessed skin allografts

The use of unprocessed skin allografts is not the preferred option as it does not allow for complete donor screening and extensive microbiological testing. However, some tissue establishments use unprocessed skin allografts as it is possible to maintain structural integrity and cell viability for short periods of time (maximum 7-8 days) [4]. These allografts were initially preferred in burn centres due to their high cell viability [9].

18.4.1.3. Cryopreserved skin allografts

Cryopreservation of skin allografts aims to maintain cell viability and structural integrity. Inappropriate storage compromises the potential to restore normal metabolic activity and, thus, physiological functioning after transplantation.

Cryoprotectants such as glycerol or dimethyl sulfoxide (DMSO) are used 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. When cell viability is required for clinical use, it should be validated by qualitative (histomorphology and/or immunohistochemistry) and quantitative methods (such as tetrazolium salt MTT assay for viability). After cryopreservation, skin allografts can be stored in liquid or vapour nitrogen for several years. Storage at higher temperature (– 60 °C/– 80 °C) is a method applied for medium-term (months) preservation of viable skin allografts.

Table 18.1. Factors influencing the air-quality specification for processing of skin

Criterion	Skin-specific
Risk of contamination of tissues or cells during processing	During processing, skin is necessarily exposed to the processing environment for extended periods.
Use of antimicrobials during processing	Soaking in antibiotics is the only antimicrobial step possible for cryopreserved skin, with maintained cell viability. To minimise the risks of particulate or microbial contamination of the product or materials being handled it is indispensable to process tissues in cleanrooms (with air-quality standards as specified in Chapter 8). High concentrations of glycerol (85 %) used in the glycerolisation process have been shown to achieve long-term bacterial inhibiting effect, though it cannot be considered a sterilising agent [12, 13].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of skin for microbiological analysis following antibiotic soaking is not extensive. Sterility testing has significant statistical limitations, and can be used to detect only large-scale contamination. Final sterility testing may even be unreliable, especially if antibiotics remain on tissues [14].
Risk of transfer of contaminants at transplantation	Although not vascularised, cryopreserved skin can transmit bacterial and viral agents that cause severe life-threatening infections resulting in serious complications or even death of the patient. Although skin is placed on the external surface of the body, it is mostly used for severely burned patients whose own skin barrier is no longer functional. These patients usually develop immunosuppression by various mechanisms and, despite recent advances in therapy, have a significant risk of death from infection.

18.4.1.4. Lyophilised skin allografts

Processing of grafts by freeze-drying de-vitalises the grafts while maintaining their structure. Lyophilised skin can be stored at ambient temperature for years. A maximum limit for residual water content should be established and measured (ideally < 5 %).

18.4.1.5. De-epidermised skin and acellular dermis

De-epidermising or de-cellularising skin is a method to lower the antigenicity of the skin graft. Thicker skin obtained from deceased donors is processed aseptically to remove the epidermis and possibly the dermal cells that can accelerate tissue rejection and graft failure. Various methods for separating the epidermis from dermis are reported such as chemical (sodium chloride, phosphate buffered saline, dispase), physical (heat) or mechanical (dermatome). These methods are frequently used in association to obtain optimal de-epidermisation. Full-thickness skin obtained from living donors undergoing abdominoplasty or body-contouring procedures can be 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 [10]. The result is an intact dermal matrix that can be cryopreserved, preserved in glycerol or lyophilised.

18.4.1.6. Skin tissue decellularisation

In the last years several innovative biological products based on decellularisation of donor-derived skin tissue have been developed using biotechnological sciences.

Various methods are actually used to obtain tissue decellularisation. The best known at the time of drafting these guidelines are classified into one of two categories: simple methods (chemical, physical, enzymatic); combined methods (chemical-physical, enzymatic-physical) [11].

Chemical methods comprise hyperosmotic/hyposmotic solutions, ionic detergents or non-ionic detergents; physical techniques are based on temperature (freeze–thaw cycles), hydrostatic pressure, sonication or irradiation with ionising radiation. Combined methods are often used. The common goal of all these methods is to obtain an acellular dermal matrix characterised by an intact fibrous and collagenous architecture able to be repopulated by autologous cells of the patient after its engraftment. The absence of 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 reconstruct and regenerate damaged and/or pathological skin tissue.

The main biological characteristics of an optimal dermal matrix are biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to cytokine release), 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).

Each method used for tissue decellularisation has to be standardised and validated. Then, standardisation and validation can be repeated every two years in order to monitor the product obtained as well as to ensure maximum biological safety of decellularised tissues.

See Chapter 27 for information on the use of de-cellularised tissues as natural extracellular matrices.

Clinical indications of acellular dermal matrices are in the field of regenerative medicine and surgery. Among them are:

- Dermatology, plastic and reconstructive 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 as well as for the surgical reconstruction of the breast after mastectomy; scar tissue remodelling.
- Orthopaedics for the repair of the rotator cuff of the shoulders as well as for the treatment of skin surgical wounds.
- Maxillo-facial surgery, dentistry for the sinus lift and implant dentistry for augmentation in gum reconstructions.
- General surgery for the treatment of incisional hernia as well as post-traumatic surgical wounds.

18.4.2. Processing facilities

In selecting an appropriate air-quality specification for skin processing, the criteria identified in Chapter 8 should be considered. Table 18.1 outlines the factors to be considered for skin processing.

Taking the factors detailed in Table 18.1 into consideration, it is appropriate that processing of skin allografts takes place in a controlled and clean environment (including control of temperature, humidity, ventilation and air filtration), with validated cleaning and disinfection to ensure the best aseptic conditions that should be standardised, monitored and maintained constantly. Within the European Union (EU), tissues that are exposed to the environment without a subsequent microbial inactivation process (e.g. cryopreserved viable skin) should be processed in environments with an air quality equivalent to those of Grade A, as defined in EU Good Manufacturing Practice (GMP), with a background environment at least equivalent to EU GMP Grade D. Many national requirements for skin processing environments are

more stringent, requiring EU GMP Grade B or C as a background, which might be more appropriate for processing tissues prone to contamination due to extensive manipulation or to processing phases at ambient temperature and that are not followed by a terminal sterilisation step.

18.4.3. Sterilisation of skin allografts

When tissue viability is not required or when skin tests positive for microbiological contaminants, it can be sterilised by gamma irradiation or electron beam. Research has shown that 25-kGy irradiation of deep-frozen skin in radio-protective solutions sterilises tissue without histomorphological or physical alterations compared with normal cryopreserved skin [3].

18.5. Quality control

In addition to the standard microbiological controls described in Chapter 9, microbiological testing should be done before the start of processing and on post-processed samples of skin (without antibiotic). 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, including packaging, cryopreservation and labelling) should be sent for microbiological testing to check for aerobic and anaerobic bacteria and fungi using appropriate culture media [15]. 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 or 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 can be accepted for clinical use, without sterilisation, when bacteriological and mycological assessment (refer to Chapter 9 for acceptable microbiological examination techniques) only reveal low bioburdens of inherent inhabitants of the residential skin flora. 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 pathogens in finished product samples results in a definite rejection of the donor tissue if no validated sterilisation or decontamination method is applied (Figure 18.1).

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* and *Clostridium* spp. or any of the micro-organisms listed in Table 18.2, at any stage of the process (even if negative at the end of processing), should be discarded without corrective actions in order to remove potentially unsuitable tissue from the transplantation process. Table 18.2 is a suggested list of such micro-organisms that is non-exhaustive, can be updated and is subject to change, according to different geographical areas.

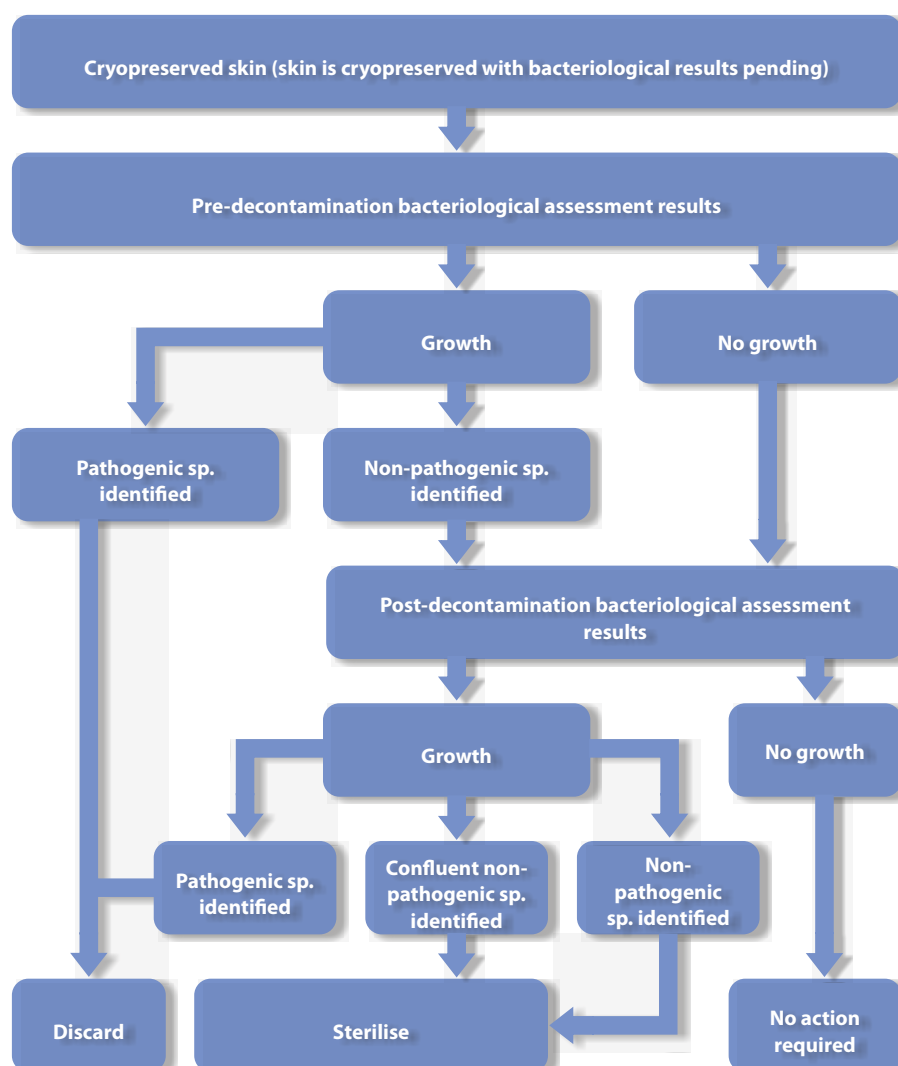
18.5.1. Skin allograft performance and quality issues

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, both qualitative (e.g. histomorphology and/or immunochemistry) and quantitative, such as tetrazolium salt assay (MTT), neutral red test (NRT) or oxygen consumption [16].

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 [16, 17, 18, 19, 20]. Comparison of unprocessed, cryopreserved (viable) and glycerolised skin allografts by the use of animals studies (immunocompetent 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 [21]. These data demonstrated that graft performance of cryopreserved skin decreased with time.

Figure 18.1. Algorithm for acceptance/rejection of skin after bacteriological assessment



Note: Pathogenic spp. during skin assessment should be understood as those defined in Table 18.2.

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 [22] 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 antimicrobial safety of the preservation method and cost should be the primary criteria for the choice of preservation method to be used for allografts.

18.6. Packaging and labelling

The grafts are packaged in foil or polythene/polyethylene sterile containers and labelled according to Chapter 13. All packages must be labelled with the name of the processing institution, a unique identifier or serial number linking the tissue to the donor, expiry date, size and type of skin graft (e.g., cryopreserved, glycerolised).

18.7. Storage

Processed skin grafts are stored in various conditions depending on processing method. Glycerolised skin is usually stored in a bio-refrigerator at 2–8 °C. Lyophilised (freeze-dried) skin can be stored at room temperature. Cryopreserved skin can be stored in liquid or in the vapour phase of liquid nitrogen in a liquid nitrogen refrigerator. Viable skin allografts can also be stored in mechanical freezers, at –45/–60/–80 °C or in ultralow freezers (–130 °C), but at higher temperatures the storage time will be shorter.

18.7.1. 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 7.

Table 18.2. Contaminants that should result in tissue discard if detected at any stage of processing

<i>Acinetobacter baumannii</i>
<i>Actinomyces</i>
<i>Bacillus anthracis</i>
<i>Bacteroides</i> spp.
<i>Burkholderia cepacia</i> complex
Carbapenem-resistant <i>Enterobacteriaceae</i>
<i>Clostridium</i> spp. (notably <i>C. perfringens</i> or <i>C. tetani</i>)
<i>Corynebacterium diphtheriae</i>
<i>Erysipelothrix rhusiopathiae</i>
<i>Fusobacterium</i>
<i>Listeria monocytogenes</i>
MRSA (methicillin-resistant <i>Staphylococcus aureus</i>)
<i>Mucor</i> spp.
<i>Mycobacterium tuberculosis</i> complex or <i>M. avium</i>
<i>Neisseria meningitidis</i> or <i>gonorrhoeae</i>
<i>Nocardia</i> spp.
<i>Pseudomonas aeruginosa</i>
<i>Salmonella typhi</i> or <i>paratyphi</i>
<i>Shigella</i> spp.
<i>Stenotrophomonas maltophilia</i>
<i>Streptobacillus moniliformis</i>
<i>Streptomyces</i> spp.
<i>Vibrio cholerae</i>
<i>Yersinia pestis</i> or <i>pseudotuberculosis</i>

Note: This suggested list is not exhaustive.

18.8. Skin allograft distribution

Skin allografts are considered life-saving therapeutic materials, so tissue establishments should have a written procedure for allocation of grafts based on clinical priority. Distribution of skin grafts for transplantation should be restricted to hospitals, tissue establishments, 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).

18.9. Biovigilance

Adverse events and reactions as well as serious adverse events and reactions shall be recorded, reported and investigated according to corresponding national regulations to Health Authorities for tissues and cells.

The Notify Library 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.

18.10. 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 bio-engineered 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 26). 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 artificial skin able to act as a physiological skin.

18.10.1. Epidermal cell suspensions

Epidermal cell suspensions (non-cultured epidermal cellular grafting) have been used in the surgical management of vitiligo since 1992 when Gauthier and Surleve-Bazeille developed a non-cultured cellular grafting technique [23]. 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 [24]. 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 suspen-

sion, a cutaneous biopsy is tripsinised 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 [25]. In consideration of the presence of melanocytes, this technique is also effective in pigmentation defects, including vitiligo and post-burn leucoderma.

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.

18.10.2. Epidermal 3D cell cultures

It was in 1975 that Rheinwald and Green [26] 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-epithelise severe burns, but also in skin pigmentation disorders (due to the presence of melanocytes) and to treat scars, vitiligo, ulcers and skin-graft donor sites.

Three-dimensional (3D) bioprinting, a flexible automated platform for the fabrication of complex living architectures, is a recent, novel approach to the design and engineering of human organs and tissues [27]. A platform consists of eight independently controlled cell-dispensing channels that can precisely place cells, extracellular matrix (ECM), scaffold materials and growth factors in any user-defined 3D pattern. After the printing process, the skin tissue is cultured in media under submerged conditions to obtain a multi-layered cell and matrix structure in which human keratinocytes are grown on collagen matrices embedded with human fibroblasts.

All cell-culture methods are relevant in the field of tissue engineering and comply, when considered for clinical applications, with the advanced therapy medicinal products (ATMP) regulations. See Part C for further information.

18.10.3. 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 banks protocols [28], a slow cooling procedure for cryopreservation is used by incubating the NAC in a cryoprotectant solution with 10 % DMSO.

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.

18.11. References

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Chapter 19. Cardiovascular tissue

19.1. Introduction

Cardiovascular 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 when the indication for their transplant procedure is not a valvular disease, saphenous veins).

The cardiovascular tissues most commonly procured are aortic valves, pulmonary valves and femoral arteries, but other cardiovascular tissues can be procured and processed, for example:

- abdominal aorta
- thoracic aorta
- saphenous vein
- patches dissected from pulmonary conduits
- pericardium.

Due to the greater resistance to infection of allografts compared with synthetic materials, the current most important clinical indication for adults is the replacement of infected valves (endocarditis) and the replacement of infected vascular prostheses. For valve replacement in newborns and young patients, human valves are usually transplanted to repair congenital malformations. They are the preferred option because they avoid the need for long-term anti-coagulant therapy (as required for the mechanical alternative) and do not tend to calcify as rapidly (as do the xenograft alternatives). Arteries are used for peripheral re-vascularisation and arterial patches are commonly used to repair congenital malformations. Iliac vessels are sometimes removed from deceased

donors along with organs for transplantation. They can be stored for up to two weeks before they are used to support organ transplantation.

The future of banking of cardiovascular tissue is to develop new procedures (e.g. decellularisation of such as valves and blood vessels) to allow recellularisation with cells from the recipient *in vitro* before implantation or *in vivo* after the tissue implantation. Tissue engineering (i.e. combining synthetic materials with decellularised human matrices) and other advanced therapy procedures represent important technical improvements for banking of cardiovascular tissue.

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. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological testing (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);

- m.* Coding, packaging and labelling (Chapter 13);
- n.* Traceability (Chapter 14);
- o.* Biovigilance (Chapter 15).

This chapter defines the additional specific requirements for cardiovascular tissue.

19.2. Donor evaluation

19.2.1. Contraindications specific to cardiovascular tissue

The following exclusion criteria are specific to donation of cardiovascular tissue:

- a.* cardiac valvulopathy of the aortic and pulmonary valves, with moderate-to-severe incompetence (the vessels can still be acceptable);
- b.* aortic dissection (detachment of the intima and adventitia);
- c.* direct (open) and massive traumas in the area of the body where the tissue is procured;
- d.* Marfanoid syndromes;
- e.* bacterial or fungal endocarditis.

Other conditions to be evaluated as part of the donor selection process are:

- f.* chronic alcoholism with myocardial dilatation;
- g.* pneumonia in previous days without evidence of effective treatment;
- h.* previous cardio-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. Common age limits are:

Arteries	male 17-45 years of age female 17-60 years of age
Aortic valves	32 weeks' gestation to 60 years of age
Pulmonary valves	32 weeks' gestation to 65 years of age

19.3. Procurement

19.3.1. Procurement team

The cardiovascular procurement teams should consist of at least two people. They should work under aseptic conditions, and be scrubbed, gowned in sterile clothing and wearing sterile gloves, face shields and protective masks.

19.3.2. Procurement procedure

For heart-valve procurement it is important, whenever possible, to procure the ascending aortic artery (including the supra-aortic trunks and the pulmonary artery with the complete bifurcation) together with the heart.

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.

Table 19.1. Factors influencing the air-quality specification for processing of cardiovascular tissue

Criterion	Cardiovascular tissue-specific
Risk of contamination of tissues or cells during processing	During processing, heart valves and vessels are exposed to the processing environment for extended periods during dissection, sizing and evaluation of their characteristics.
Use of antimicrobials during processing	Heart valves and vessels are exposed to antibiotics, and in some cases, antimycotics, with a typical decontamination period of 24 h. It is important to validate the effectiveness of the antibiotic cocktail and to list the micro-organisms that can be accepted pre-incubation as this method is not very effective compared to more robust methods that can be applied to other tissues [1].
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling of a piece of myocardium or a discarded vessel for microbiological analysis does not ensure a representative sample for analysis. Storage media or solutions used to rinse the tissue should also be sampled to make this evaluation more effective.
Risk of transfer of contaminants at transplantation	Cardiovascular tissue is vascularised and can support and transmit microbiological contaminants, bacterial and viral agents. Cardiovascular tissue is used in open surgery in well-vascularised areas and frequently to replace infected tissue (endocarditis). If it is contaminated, the risk of serious infection is considerable.

19.3.3. Tissue transportation to the tissue establishment

Common practice is to place procured tissues in a crystalloid transport solution (e.g. Ringer solution, Hanks balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin) or antibiotic cocktail, and package them in at least two sterile packaging layers after procurement.

For donors of organs, valves and vessels, it is convenient to package the heart and the vascular segments in different containers.

This package should then be placed in another container that ensures a temperature of 2-8 °C and protects the recovered tissues during transport.

19.3.4. Procurement documentation

The organisation responsible for procurement must produce a procurement report to be given to the tissue establishment. In addition to the generic requirements defined in Chapter 6, this report must contain a description and identification of the recovered material (heart, arteries, veins, valves, etc.).

19.4. Processing and storage

Procured cardiovascular tissues can be processed to facilitate longer storage periods and to reduce microbial contamination.

To ensure tissue quality, it is essential that the time between cardiac arrest and cryopreservation be as short as possible. It is recommended to procure cardiovascular tissue within 24 h after death, but only if the body has been cooled or refrigerated within 6 h of death. If the body was not refrigerated, then it is possible to procure tissue in the first 12 h after death. It may be possible to extend procurement times up to 48 h after death if processing has been validated to guarantee quality 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 5 for details on sample collection.

Time from procurement of the heart to dissection and disinfection should not exceed 24 h.

Processing of cardiovascular tissues includes dissection and evaluation of minimum functional requirements, incubation with antibiotics and, in some cases, anti-mycotics, cryopreservation and storage. The duration and temperature of antibiotic treatment and the composition of antibiotic cocktails should be defined by each tissue establishment, with prior evaluation of the initial tissue bioburden (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.

The methods used must be in accordance with current state-of-the-art and validated procedures (see Chapter 2). Different tissue establishments apply specific preparation processes according to their own standard operating procedures (SOPs) and in accordance with relevant local authorisations.

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 22 provides an example of an evaluation form.

19.4.1. Decellularisation of cardiovascular tissues

Heart valves and large vessels can be decellularised employing different methods to eliminate cellular components. Decellularisation protocols 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 include a combination of the three methods.

Quality control should guarantee maintenance of the structure and the biomechanical properties of native valves and vessels, as well as demonstrating *in vivo* function. Decellularisation protocols (especially enzymatic methods) should take into account that degraded collagen might have repercussions for *in vivo* re-endothelialisation of decellularised tissues.

See Chapter 27 for additional information on decellularisation methods.

19.4.2. Processing facilities

In selecting an appropriate air-quality specification for processing cardiovascular tissue, the criteria identified and explained in Chapter 7 should be considered alongside the factors outlined in Table 19.1.

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. Taking the factors from Table 19.1 into consideration, 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 a Grade B background environment (EU GMP) be provided.

19.5. Cryopreservation and storage in liquid nitrogen

Cardiovascular tissues can be cryopreserved by using a controlled rate freezer and 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 vessel (either a liquid nitrogen tank or mechanical freezer) and stored $< -140^{\circ}\text{C}$. Cardiovascular tissue can be stored $< -140^{\circ}\text{C}$ for ≤ 5 years. Longer storage periods should be validated.

19.6. 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.

19.7. Quality control

It is recommended that the quality-control tests on vascular grafts should consider the following minimum quality criteria:

- integrity of the vascular walls;
- minimal calcification, atheroma and fibrosis;
- anatomical suitability – non-aneurism or non-stenosis accepted.

Quality control tests for heart valves should consider the following minimum quality criteria:

- 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;

- Good morphology (no fissures, congenital defects, no/minimal calcification, etc.). Only small calcifications in the distal wall of the aorta, 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;
- 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;
- Intact structure of the tissue matrix.

Table 19.2. Contaminants that should result in tissue discard if detected at any stage of processing

<i>Aspergillus</i> spp.
<i>Candida</i> spp.
<i>Clostridium</i> spp. (notably <i>C. perfringens</i> or <i>C. tetani</i>)
<i>Enterococcus</i> spp.
<i>Flavobacterium meningosepticum</i>
<i>Klebsiella rhinoscleromatis</i>
<i>Listeria monocytogenes</i>
MRSA (methicillin-resistant <i>Staphylococcus aureus</i>)
<i>Mucor</i> spp.
<i>Mycobacterium</i> spp.
<i>Neisseria gonorrhoeae</i>
<i>Nocardia</i> spp.
<i>Penicillium</i> spp.
<i>Pseudomonas aeruginosa</i> or <i>P. pseudomallei</i>
<i>Salmonella</i> spp.
<i>Shigella</i> spp.
<i>Streptococcus pyogenes</i> (Group A)
Other yeasts and fungi

Note: This suggested list is not exhaustive.

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 9), before antibiotic and, where relevant, antimycotic incubation.

Microbiological analyses should be carried out on:

- the transport medium at the beginning of the processing procedure;
- the sub-valvular (aortic and pulmonary) myocardial tissue and vessels before antibiotic incubation;
- a final sample of each graft after antibiotic/antimycotic incubation and rinsing, and a sample of the cryoprotectant solution.

The result of the microbiological control must be negative. 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.

Table 19.2 lists some micro-organisms that, if detected in any culture of cardiovascular tissue (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 preantibiotic sample of aortic myocardium with a negative result in all other samples (e.g. transport medium, post-decontamination aortic sample, before final packaging) should result in rejection of all valves from this donor, and a risk assessment should be done for the remainder of the tissues.

It should be noted that Table 19.2 is a suggested, non-exhaustive list, and individual tissue establishments may have a different list of micro-organisms that result in tissue discard.

19.8. Cardiovascular allograft distribution

Transportation of cardiovascular tissues can be carried out using dry-shipping containers (vapour phase nitrogen $< -140^{\circ}\text{C}$). This allows re-storage of the tissues in the liquid or vapour phase of nitrogen without affecting the expiry date. If the tissue is to be re-stored at -80°C , then the expiry date must be reduced to a maximum of 6 months. 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 validated. Once cardiovascular tissues have been thawed, they cannot be re-frozen.

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 tissue establishment must ensure that all packaging

and distribution processes have been carried out under controlled conditions.

19.9. Examples of serious adverse reactions/events

The Notify Library includes some 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.

19.10. References

1. 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.

Related document:

Appendix 22. Sample form for the evaluation of heart valves.

Chapter 20. Musculoskeletal tissue

20.1. Introduction

Human bone and soft-tissue grafts are being used increasingly in surgery as valuable materials to rebuild and replace musculoskeletal structures. Bone is the most commonly banked and transplanted tissue.

Fresh autologous bone is considered to be the ‘gold standard’ bone-grafting material because it combines all the properties required in a bonegraft material: osteoinduction (bone morphogenetic proteins [BMP] and other growth factors), osteogenesis (osteoprogenitor cells) and osteoconduction (scaffold). However, use of autografts is limited by the amount that can be procured and, in most cases, allografts are used.

Allografting of bone and musculoskeletal soft tissues can in most cases allow adequate and predictable restoration 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].

Musculoskeletal tissues can be procured from donors after brain death, donors after circulatory death and living donors (e.g. in the case of a patient undergoing hip or knee prosthesis surgery), and include bones, ligaments, tendons, cartilage and other soft tissues (e.g. fascia lata). The current indications for the implantation of musculoskeletal tissues are, 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 meniscus replacement.

Allogeneic bone can be processed in different ways, depending on clinician needs and preferences. The methods include cutting or grinding into morcellised and cancellous chips, corticocancellous rings and wedges, and cortical grafts such as struts and cylinders. The preparations can be frozen or freeze-dried, and in some cases preparations are demineralised to enhance the osteoinductive properties (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, processing and preservation procedures that meet adequate quality and safety standards.

The future of musculoskeletal tissue banking is focused on the following areas:

- a. developing new preservation methods to maintain the biological properties of the grafts;
- b. developing new procedures such as decellularisation or cell therapy to improve graft incorporation in recipients;
- c. improving the safety of musculoskeletal grafts.

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. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);

- c. Recruitment of potential donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, packaging and labelling (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

This chapter defines the additional specific requirements for musculoskeletal tissue.

20.2. Donor evaluation

General exclusion criteria are described in Chapter 4. Musculoskeletal tissue-specific criteria are described below.

20.2.1. Musculoskeletal tissue: specific exclusion criteria

In addition to the general exclusion criteria described in Chapter 4, screening of donors of musculoskeletal tissue should be conducted for:

- a. diffuse connective tissue disease;
- b. metabolic bone diseases (severe osteoporosis, osteopetrosis, Paget disease, etc.);
- c. corticoid treatment (medical director should evaluate donor suitability depending on corticoid dose and treatment duration);
- d. ingestion of cyanide or heavy metals (mercury, gold, etc.);
- e. local bacterial, viral, parasitic or mycotic infection;
- f. 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, degenerative tears or lesions detected during procurement of cartilage, menisci, tendons and osteoarticular grafts.

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:

- a. for bone, the minimum age for both sexes is 6 years. No upper limit is applied unless the bone is intended to be used for structural support, in which case 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 age range is 15-65 years, although the upper limit can be extended after a biomechanical validation study.

20.3. Procurement

General principles of procurement are described in Chapter 6.

20.3.1. Procurement team in deceased donors

It is recommended that the musculoskeletal procurement team for deceased donation should be composed of at least two (but preferably three) people, limiting the maximum number of members because it can be critical to minimise the risk of contamination during procurement [4]. They should work under aseptic conditions, and be scrubbed, gowned in sterile clothing and wearing sterile gloves, face shields, glasses and protective masks.

Staff must have the experience, education and training necessary to procure tissues, including significant 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 important to define 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.

20.3.2. Procurement procedures

The methods of tissue procurement may be similar to those used by orthopaedic surgeons in the operating room or may use wider skin incisions, applying strict aseptic techniques.

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 tissues must be procured using an aseptic technique. It is recommended to establish some rules to decrease the risk of cross-contamination, i.e.:
 - 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. Surgical instruments should not be shared between sides;
 - ii. The packaging area should be independent and separate from procurement areas;
 - iii. It is recommended that a fixed procurement sequence is established, from 'cleanest' (i.e. lower limbs) to 'dirtiest' areas (i.e. abdominal cavity);
 - iv. It is recommended to change gloves and surgical blade after procuring each piece.
- d. Microbiological control: it is recommended to perform a microbiological control of each procured piece (swabbing, biopsy). These controls can be avoided only when a validated sterilisation method is further applied during processing. Sampling methods should be defined in SOPs;
- e. Tissue packaging: procured tissue must be inspected and identified appropriately before packaging and labelling to avoid mix-ups (see Chapter 13). Musculoskeletal tissues shall be packaged in a manner that minimises contamination risk, using a validated packaging system, to assure its isolation from ambient;
- f. Donor reconstruction (see 20.3.3);
- g. Procurement documentation (see 20.3.5).

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);
- Soft tissues:
 - Tendons: patellar, achilles, anterior and posterior tibialis, peroneus longus, gracilis, semitendinosus;
 - Cartilage: meniscus, acetabular labrum, costal cartilage;
 - Fascia lata.

Musculoskeletal tissues can also be procured from living donors:

- Allograft
 - Patients having a hip-replacement procedure can donate the femoral head that is being replaced by the prosthesis, and in some cases bone removed in knee replacement is also banked. This can be frozen or further processed and provided to other patients as a bone graft.
- Autograft
 - Cranial flaps removed during neurosurgical procedures where there is brain oedema. The tissue is stored and replaced in the same patient once brain swelling has diminished;
 - Cartilage can be used for producing autologous chondrocyte cultures for application in the same patient.

20.3.3. Reconstruction of the deceased donor

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 respectful reconstruction of the donor, a wooden or plastic replica bone approximating the size of the donated bone may be used to replace the procured bone. The subcutaneous tissue and skin should be sutured. The use of sutures and other materials suitable for cremation should be considered.

20.3.4. Temporary storage and transportation to the tissue establishment

Once procured, all musculoskeletal tissues should be kept at $< -20^{\circ}\text{C}$ until they are transported to the tissue establishment. If transport occurs immediately after procurement, tissues must be refrigerated.

If tissues are obtained to be preserved unprocessed (e.g. osteochondral grafts) they should be placed in a transport solution (e.g. Ringer's lactate solution, Hank's balanced salt solution) with the possible addition of nutritional/osmotic elements (e.g. albumin), antibiotic cocktail or culture medium, and packaged in at least two sterile packaging layers after procurement. This package should then be placed in another container that ensures a temperature of $2-10^{\circ}\text{C}$ and protects the procured tissues during transport.

Temporary storage must provide clearly separate and distinguishable areas for tissues and cells that remain in quarantine. To prevent mix-ups or cross-contamination, physically separate areas,

storage devices or secured segregation within a storage device/unit (i.e. refrigerator, freezer) must be allocated and prominently labelled (including at least the minimum required information – see Chapter 13). Temporary storage areas or units for tissues and cells must be monitored (and alarmed, if necessary) and checked to ensure expected environmental requirements are being met.

20.3.5. Procurement documentation

The organisation responsible for procurement must gather all relevant information associated with procurement procedures and produce a report to be given to the tissue establishment. In addition to the generic requirements defined in Chapter 6, this report must contain:

- a. Description and identification of the procured material (specifying all procured tissues);
- b. Any relevant morphological detail of procured tissues;
- c. Presence of lesions, including those produced during procurement;
- d. Non-procured standard tissues and its reason.

20.4. Processing

20.4.1. Processing methods

There are various methods of bone processing applied by individual tissue establishments. Allogeneic and autologous bone allografts from living donors can be processed in the same manner as for tissues from deceased donors.

Pooling of musculoskeletal grafts from different donors during processing is not recommended (see Chapter 7).

Processing of bone and other musculoskeletal tissue generally involves the removal of soft tissues (muscle attachments and the periosteum) and shaping using different types of saw (i.e. banding, oscillating). In addition, intraosseous blood, bone marrow and lipids can be removed using physical debridement, alcohol solutions, high-pressure water rinses, mechanical agitation, ultrasonic processes and/or supercritical carbon dioxide.

The protocols for these steps vary between tissue establishments and should be validated for the different types of tissue (chips, blocks, structural bone, tendons, menisci, etc.).

The types of graft that can be obtained include (but are not limited to):

- cancellous and corticocancellous chips, obtained from epiphyses of the long bones and vertebral bodies, and the ilium;
- cancellous blocks, obtained from epiphyses of the long bones and vertebral bodies;
- bone wedges, obtained from femur and tibia epiphysis and ilium (tricortical or bicortical);
- structural bones, long bones processed as a whole, halves, thirds or smaller grafts (i.e. femoral and tibial condyles);
- tendons with bone block, obtained from patellar or achilles tendons;
- tendons without bone block, tendons free of bone (i.e. anterior and posterior tibialis tendons);
- menisci, processed with or without bone block;
- fascia lata fragments;
- costal cartilage fragments.

Standard musculoskeletal grafts provide the osteoconductive capability (i.e. act as a scaffold being replaced by new bone from recipient). Some specific procedures can be applied to musculoskeletal grafts (e.g. demineralisation) to provide grafts with the osteoinductive capability (i.e. to create new bone by itself recruiting mesenchymal stem cells). Demineralisation comprises reducing the calcium content of bone grafts (calcium content < 5-10 %), using an acidic solution (i.e. 0.5 or 0.6 M HCl) to expose the BMP that provide the osteoinductive capability of the graft, thereby improving its incorporation in the recipient.

Living cells (i.e. chondrocytes) are recommended to be processed in a closed system to decrease the risk of cells and/or culture media contamination. Processing living cells usually involves cell cultures/*in vitro* expansion and therefore is classified as advanced therapy medicinal products (ATMP) (see Chapters 26-28).

20.4.2. Processing facilities

In selecting an appropriate air-quality specification for musculoskeletal tissue processing, the criteria identified in Chapters 7-8 should be considered. Table 20.1 outlines the factors to be considered for processing of musculoskeletal tissue.

It is important that processing of musculoskeletal allografts takes place in a bacteriological- and climate-controlled environment, including temperature and humidity control, ventilation and air filtration, with validated cleaning and disinfection. Where these grafts are not destined for terminal sterilisation, the need for an optimal processing environment is critical to the safety of the graft.

Table 20.1. Factors influencing the air-quality specification for processing of musculoskeletal tissue

Criterion	Musculoskeletal tissue-specific
Risk of contamination of tissues or cells during processing	During processing (including cutting, shaping, cleaning, grinding, etc.), musculoskeletal tissue is necessarily exposed to the processing environment for extended periods. Environmental conditions are not as critical during freeze-drying if the tissues are packaged in a validated closed system during the freeze-drying procedure.
Use of antimicrobials during processing	It is possible to soak musculoskeletal tissue in antibiotics just after procurement and before initial packaging or during processing, but these do not penetrate the tissue and can only inactivate surface contaminants. Blood and marrow removal, washing and similar preservation methods have been shown to be effective for reducing or eliminating contamination with micro-organisms. Bone can be terminally sterilised by gamma-irradiation or treated with a series of washes and chemical treatments that, together, achieve an equivalent degree of sterility (see section 20.4.3.2).
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Sampling can be done by swabbing, immersion of a tissue biopsy in culture medium after processing or by filtering and culturing washing solutions. Different sampling methods can be combined in order to detect the possibility of contamination. For bone that is processed to small pieces or ground, representative samples can be taken for culturing. For terminally sterilised bone, sampling is not an issue as the process is validated to achieve a certain sterility assurance level.
Risk of transfer of contaminants at transplantation	Bone marrow, lipids and blood components placed inside grafts act as a reservoir of micro-organisms. Decontamination methods act by removing these components from musculoskeletal tissues to decrease the risk of transmission of viral and bacterial agents. Musculoskeletal tissue is used in open and well-vascularised surgeries, sometimes linked to replacement of a prosthesis, where a significant risk of infection exists.

Within the European Union (EU), tissues that are exposed to the environment without a subsequent microbial inactivation process should be processed in environments with an air quality equivalent to Grade A as defined in EU Good Manufacturing Practice (GMP), with a background environment at least equivalent to Grade D (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.

20.4.3. Decontamination/sterilisation

20.4.3.1. Decontamination methods

The objective of a decontamination method is to produce a logarithmic reduction of the tissue bio-burden through the removal of bone marrow, lipids and blood. Different decontamination methods have been developed by tissue establishments. Decontamination methods should be validated to assure at least a $3 \times$ logarithmic reduction of bioburden preserving the biomechanical properties of the different tissues.

Most of the developed decontamination methods are based on a combination of chemical, antibiotic and mechanical steps.

a. Chemical decontamination

There are many chemicals that can be used as decontaminants or that have an inactivating effect on specific pathogens (e.g. peracetic acid,

iodophors, ethanol). The effectiveness of these agents on certain types of tissue must be validated. It is important that the chemicals used be mentioned in the documentation that accompanies the grafts, particularly if it is possible that traces of these products or their by-products remain in the tissue.

b. Antibiotic decontamination

Antibiotics may be used to decontaminate musculoskeletal tissue. The effectiveness of each 'antibiotic cocktail' should be validated and documented. The use of an antibiotic decontamination procedure might be the only method of microbial inactivation possible for grafts where cell viability is required.

c. Mechanical steps

Centrifugation and surface decontamination using ultrasonication are commonly used methods.

20.4.3.2. Sterilisation methods

Sterilisation refers to any process that eliminates/inactivates transmissible infectious agents (pathogens) containing nucleic acids (e.g. vegetative and spore forms of bacteria and fungi, parasites, viruses) [2].

Sterilisation procedures should ensure that no viable organisms are present in the sample after sterilisation. The term Sterility Assurance Level (SAL) 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, and grafts are then considered sterile.

a. Radiation sterilisation

Both gamma rays and accelerated electron beams can be used for sterilisation processes. No specific dose can be recommended as this depends on multiple factors associated with the individual process. However, it should be noted that higher doses can reduce the biomechanical strength of the bone. The irradiation process must always be documented. Gamma radiation is effective in killing bacteria, fungi, spores and, to a more variable degree, viruses. Depending on the dose, however, gamma radiation can weaken the graft. Doses of <15 kGy do not seem to adversely affect tissue strength [5]. Doses used for gamma ray sterilisation range from 17 kGy to 35 kGy and are established after calculation of the initial bioburden of the tissue. Gamma irradiation and excessive heat (> 60 °C) is known to damage bone proteins. The damaging effects of gamma irradiation are greatly increased for freeze-dried tissue. The adverse effects of irradiation can be ameliorated by reducing the temperature and inclusion of radioprotectant chemicals.

b. Pasteurisation (heat)

Unlike sterilisation, pasteurisation is not intended to kill all micro-organisms that are present in the tissue, but it aims, through heat, to reduce the number of viable pathogens that are likely to produce an infection (similar to decontamination). Validation studies should be performed to guarantee the preservation of the biomechanical properties of the tissues.

c. Sterilisation using ethylene oxide

Although an effective sterilant, ethylene oxide is no longer recommended for tissue processing due to the risks of residuals that might be mutagenic.

- c. residual moisture in lyophilised or dehydrated grafts (the maximum level to be defined according to validation studies);
- d. osteo-inductive activity (*in vivo* or *in vitro*) in demineralised bone (usually demonstrated by validation rather than testing of every batch);
- e. sterilisation indicators;
- f. no evidence of microbiological growth;
- g. number of viable cells in cell cultures (i.e. chondrocytes).

During procurement or before processing, microbiological samples should be collected to establish the initial contamination levels of tissues to assist in making a decision during quarantine regarding the release of procured material for further processing. These microbiological tests are also important as controls during the procurement procedure.

Samples for microbiological analysis should also be collected before 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 after processing must be negative. If a positive microbiology result is obtained, the tissue should be discarded or terminal sterilised. 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.

Table 20.2. Contaminants that should result in tissue discard if detected at any stage of processing or procurement

<i>Aspergillus</i> spp.
<i>Candida</i> spp.
<i>Clostridium</i> spp. (notably <i>C. perfringens</i> or <i>C. tetani</i>)
<i>Flavobacterium meningosepticum</i>
<i>Klebsiella rhinoscleromatis</i>
<i>Listeria monocytogenes</i>
MRSA (methicillin-resistant <i>Staphylococcus aureus</i>)
<i>Mucor</i> spp.
<i>Mycobacterium</i> spp.
<i>Neisseria gonorrhoeae</i>
<i>Nocardia</i> spp.
<i>Pseudomonas aeruginosa</i> or <i>P. Pseudomallei</i>
<i>Salmonella</i> spp.
<i>Shigella</i> spp.
Other yeasts and fungi

Note: This suggested list is dynamic and not exhaustive since different micro-organisms are found in each tissue establishment.

20.5. 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;

Table 20.2 lists some micro-organisms that, if detected in any culture of musculoskeletal tissues (even if detected just before processing), require the tissue to be designated as unsuitable for clinical use or to be processed. A risk assessment should be done to analyse the suitability of the rest of the musculoskeletal tissue from same donor.

See Chapter 9 for more detailed guidance on the principles of microbiological testing.

20.6. Labelling and packaging

Generic requirements are detailed in Chapter 13. Procured and processed musculoskeletal tissues are to be packaged in a way that minimises contamination risk. It is recommended that musculoskeletal tissues be at least double-packed in airtight packages or in sterile drapes as well as sterile containers. Each procured and processed tissue should be packed separately and labelled immediately.

20.7. Preservation/storage

After processing, grafts are stored at a tissue establishment during the quarantine period until the required test result from donor (blood cultures, serologies, autopsy and/or biopsy report) and tissues (microbiological test, biopsy report) are received (if required). The tissue establishment must confirm donor eligibility before releasing the graft.

Different preservation methods have been developed to maintain the biological properties of tissues for long periods of time, from processing to distribution for transplant.

20.7.1. Methods of preservation/storage

20.7.1.1. Fresh-frozen

Preservation and storage of musculoskeletal tissues (including cancellous, cortico-cancellous and cortical bone, ligaments and tendons) by deep freezing is a common method applied. 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 will diminish its immunogenicity. Freezing affects the viability of articular cartilage unless some form of cryopreservation is employed.

20.7.1.2. Cryopreservation

Cryopreservation is a process whereby tissues are preserved by cooling to temperatures $< -140^{\circ}\text{C}$. This method is suitable for the preservation of some

cell viability in cartilage. It is used for osteochondral bone grafts and for cartilage, although some centres also use it for other types of musculoskeletal tissue. Cryoprotectants – e.g. glycerol, dimethyl sulfoxide (DMSO) – are added to the medium to prevent the ice crystal formation that destroys cells.

20.7.1.3. Freeze-drying (lyophilisation)

Lyophilisation consists in decreasing the water content of the tissue to $< 5\%$ through a process of sublimation. In contrast to fresh-frozen allografts, mechanical strength in freeze-dried allografts is reduced significantly [3], so it is not recommended for structural grafts or tendons. Freeze-drying further diminishes the immunogenicity of the graft. An alternative to freeze-drying is dehydration where the water content should be $< 15\%$. Dehydration is usually performed using chemical substances.

20.7.1.4. Fresh

Preservation of unprocessed tissues at $4-10^{\circ}\text{C}$ allows maintenance of cell viability (i.e. osteochondral grafts).

Different culture mediums and storage processes have been described. The main problem of fresh preservation is to have enough time to obtain test results before releasing the graft.

The tissue establishment should validate the preservation method in order to guarantee a minimum rate (%) of cell viability.

20.7.2. Expiry date

The designated shelf-life is dependent upon the packaging system (to guarantee the integrity and sterility of the graft) and storage methods used (deep frozen, freeze-dried, fresh, etc.).

Expiry dates should be established by the tissue establishment after a validation process. Each change in the packaging should be followed by a validation study of the packaging system and the expiry date.

20.7.3. Storage temperatures

As mentioned in section 20.7.1, different preservation methods require different storage temperatures.

Type of graft	$^{\circ}\text{C}$ minimum	$^{\circ}\text{C}$ maximum
Fresh-frozen	- 20	- 30
in deep freezer	- 40	- 80
Cryopreserved	- 140	- 196
Freeze-dried*	+ 4	+ 30
Fresh	+ 4	+ 10

* At room temperature in normal conditions of humidity.

Storage time limits will be defined by expiry dates (see 20.7.2) based on the packaging and storage system and the validity of donor-selection criteria.

20.8. Distribution and transport conditions

Transportation of musculoskeletal tissues should guarantee the preservation of graft storage conditions from tissue establishment to end user.

Transportation systems will vary depending on the preservation method used:

- a. Fresh-frozen grafts can be carried using a container with dry ice. Once the graft has been thawed, it cannot be re-frozen;
- b. Cryopreserved grafts can be carried using dry-shipping containers (vapour phase nitrogen $< -140^{\circ}\text{C}$). 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. Once the graft has been thawed, it cannot be re-frozen;
- c. Freeze-dried grafts can be carried using a container just to protect the integrity of the package system.

20.9. 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 lymphotropic 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;
 - 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 disease and rheumatoid arthritis respectively; all were diagnosed during histological examination of the femoral head and resulted in discarding of allografts.

- 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;
 - 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 the donor and recipient;
 - An HIV type-1 transmission from a seronegative organ-and-tissue donor confirmed by the recipient's seroconversion 3 weeks post-transplantation (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.

For further evaluated cases of adverse outcomes associated with musculoskeletal tissue banking, search the Notify Library at www.notify-library.org. The database is publicly accessible and can be searched by substance type, by adverse occurrence type and by Record Number.

20.10. Developing applications

In the last years several innovative biological products based on decellularisation of musculoskeletal 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 decellurisation processes can be found in Chapter 27.

20.11. References

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Chapter 21. Haematopoietic progenitor cells

21.1. 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 half a century ago, the procedure rapidly established itself as a life-saving treatment for adult and paediatric patients with a variety of malignant diseases. HPC transplantation also has a role when the haematopoietic tissue is functionally damaged by congenital or acquired disorders such as severe congenital immune deficiencies, metabolic diseases or bone marrow failure. More recently, the use of autologous HPC transplantation in combination with immunosuppressive 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 cornea.

The field has developed tremendously in the past half-century in developed countries, and now many emerging countries are establishing allogeneic and autologous HPC transplantation programmes. The field has integrated medicinal and technical innovations, including the use of new immunosuppressive agents, the use of different sources of HPC, such as bone marrow, mobilised 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 unrelated, volunteer donors has dramatically increased due to the growth of donor registries in the majority of European and North American countries, which are united in the World Marrow Donor Association (WMDA).

Several other biotechnological advances, including stem cell selection, lymphocyte depletion, immune effector activation and stem cell expansion have become available. However, these advances have only entered clinical practice to a limited extent, and procurement of HPC transplantation remains relatively unchanged. Hospitals that care for recipients often obtain autologous or allogeneic HPC from hospital-based or blood establishment-based procurement and processing facilities that are located in their immediate vicinity. In more than 50 % of allogeneic HPC transplants, grafts from unrelated donors are used which very often have to be imported from other countries or continents. Each of the procurement and processing facilities works on a typically small to medium scale. Given the high rate of international exchange of donated HPC material, harmo-

nisation of the practices in this field would be of great benefit.

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. It should be noted, however, that if these cells are subjected to substantial manipulation (such as expansion), 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 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. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, labelling and packaging (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

This chapter defines the additional specific requirements for HPC transplantation.

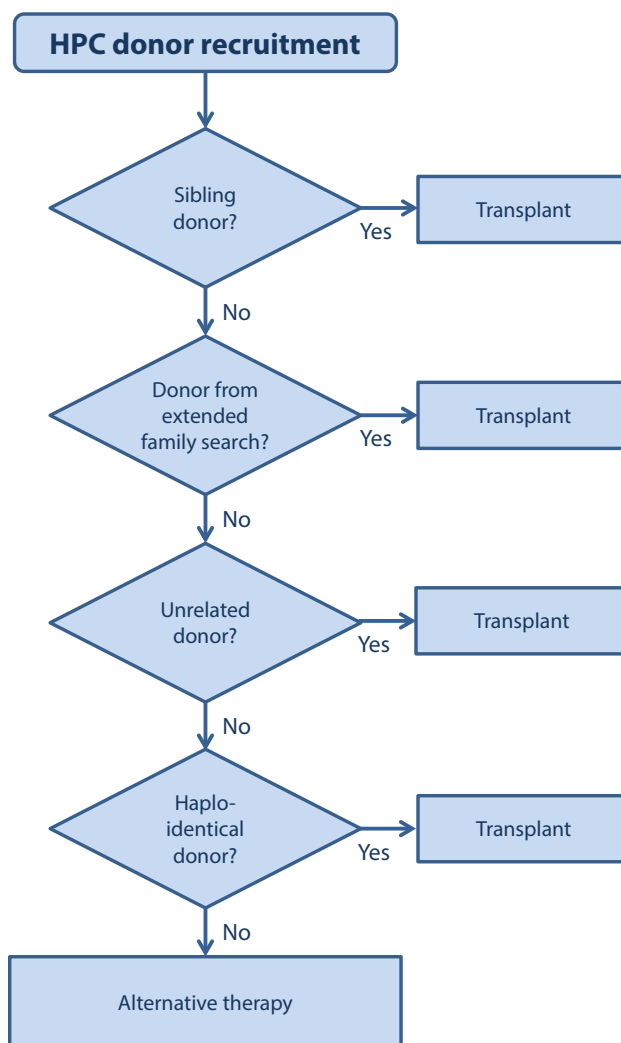
21.2. Recruitment of potential donors, identification and consent

Most of the patients who could benefit from HPC transplantation do not have a genotypically identical sibling donor. The chance of having an HLA-matched sibling donor is 25-30 % depending on the number of siblings. In some cases an extended family search can provide a HLA phenotypically

identical or HLA genotypically haplo-identical donor. This can happen in cases of consanguinity due to cultural or geographical reasons and can add an extra 10 % to 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, a search for an unrelated donor should be carried out through bone marrow registries (see Figure 21.1).

Figure 21.1. Donor recruitment algorithm



The first bone marrow donor registry was established in 1974 by the Antony Nolan Bone Marrow Trust in London. In 1988 the European Bone Marrow Transplantation group (EBMT) with The European Donor Foundation set up the Bone Marrow Donors Worldwide Organization (BMDW) based in Leiden, Netherlands (www.bmdw.org). BMDW co-ordinates the collection and listing of the HLA phenotypes and other important data of volunteer HPC donors and cord blood units. The BMDW database includes almost 29 million HPC donors and almost 700 000 cord blood units (August 2016). Since ethnic mi-

norities are under-represented in the bone marrow registries, it is very important that donor centres work with these communities to explain the need to increase the ethnic diversity of the registry and to recruit new potential donors. Once the HLA typing and personal data are entered onto the registry, further blood samples may be requested, leading to possible haematopoietic stem cell donation at any time in the future.

BMDW works closely with the World Marrow Donor Association (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). WMDA 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, WMDA offers to the donor registries an accreditation programme according to internationally accepted standards.

The main role of the registries is to facilitate interactions between transplant centres and donor centres. In most of the countries involved, the search processes – including HLA confirmatory typing requests and donor recruitment – are operated using EMDIS (European Marrow Donor Information System), an international computer network which allows fast and direct communication between registries (see Appendix 23).

Emergency rescue procedures should exist to limit consequences related to unforeseen unavailability of a donor (e.g. acute illness, accident, failed mobilisation). This could be: search for a cord blood unit, a haplo-identical donor or an autologous transplantation with previously cryopreserved autologous HPC.

21.2.1. Donor evaluation

21.2.1.1. Allogeneic donor

One of the fundamental principles of volunteer stem cell donation is the right of the potential donor to proceed to donation with a minimum of extraneous influences and pressures. Protection of the 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 [2].

For evaluation of allogeneic donors, written criteria – in the form of standard operating proce-

dures (SOPs) – should exist. Criteria must take into consideration not only the recipient's safety but also the donor's safety. 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 [3-6]. 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 transplantation 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 reach donors and recipient security, a physician involved in the HPC collection procedure must be available during the collection period, from the beginning of G-CSF (granulocyte-colony stimulating factor) injections to the post-collection period.

Criteria should include, in addition to general requirements, at least:

- a. pregnancy assessment in female donors;
- b. adequate cardiac, pulmonary, hepatic and renal function;
- c. suitability for anaesthesia (for procurement of bone marrow);
- d. assurance of adequate venous access;
- e. haematologic studies (e.g. complete blood count, chemistry, coagulation parameters);
- f. additional test if appropriate (e.g. in cases of family history or elderly donor);
- g. ABO (ABO) and Rh typing from two blood samples taken independently (for allogeneic donors);
- h. screening for red blood cell (RBC) and HLA-antibodies for allogeneic recipients;
- i. exclusion criteria (see Chapter 4);
- j. policy for making decisions in cases of 'only one' donor but who does not meet eligibility criteria (e.g. only one suitable donor with risky behaviour);
- k. a donor advocate shall be available to represent allogeneic related donors who are mentally incapacitated or not capable of full consent.

Donors with 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 [3].

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.

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 comorbidities 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. The decision process to collect from young (<18 years) and elderly donors (>60 years) must include an accurate risk assessment [3-5].

Some donors will present with comorbidities (discovered or not) during evaluation. If these comorbidities 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 made in accordance with the medical condition of the rejected donor.

If the comorbidities found during evaluation allow HPC donation, the physician in charge must manage these comorbidities during the entire donation process, including specialist consulting as needed.

There should be a written plan to care for paediatric donors, donors with comorbidities and elderly donors during recruitment [3-6].

21.2.1.2. *Autologous donors*

For autologous donors, suitability criteria for HPC donation are less strict than for allogeneic donors. For evaluation, written criteria – i.e. standard operating procedures (SOPs) – should exist. Criteria must take into consideration the recipient'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 shall be available to represent autologous donors who are mentally incapacitated or not capable of full consent at the time of HPC mobilisation and donation.

Infectious disease markers in autologous donors shall be tested as required by applicable laws and regulations (see Chapter 5). Autologous donors can donate even if the required tests are reactive or positive as long as potential cross-contamination

during HPC procurement, processing and storage can be prevented.

21.2.1.3. *Specific considerations for paediatric donors*

If minors are being considered as HPC donors, in addition to the criteria shown in Chapter 3, national regulations should 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 for age and size to the procedure.

Children should become donors only in very specific circumstances and never through public registries (see Chapter 3).

21.2.2. **Informed consent**

Informed consent is required for an allogeneic and also for an autologous donation. In cases of autologous donation, the informed consent should include terms and conditions for the HPC storage and disposal. Volunteer HPC donors joining a bone marrow registry or a cord blood bank express their commitment to donate, but they must nevertheless sign a formal consent before the HPC procurement. General considerations are shown in Chapter 3.

21.3. **Procurement**

21.3.1. **Haematopoietic progenitor cells graft sources**

HPC for transplantation are obtained from living donors only; either from the recipient patient (in the case of autologous transplantation) or from a fully or partially human leucocyte antigen (HLA)-matched allogeneic donor. Allogeneic donors can be related or unrelated to the intended recipient. In the case of an unrelated donor, procurement can be from healthy adult donors or through cord blood donations that are stored and delivered by cord blood banks. Recently, 'haplo-identical' allogeneic transplantation protocols have been used increasingly for patients lacking a fully matched donor (related or unrelated). The choice of the donor is based on the best HLA matching and other factors like age, gender, *cytomegalovirus* (CMV) status, ABO compatibility, etc.

HPC can be obtained from a variety of autologous or allogeneic sources that include bone marrow, mobilised peripheral blood and cord blood. For autologous purposes, nowadays almost exclusively peripheral blood stem cells are used as they accelerate haematopoietic recovery. In the allogeneic setting, the used graft source depends on the age 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 haplo-identical transplantation). In parallel, numerous cord blood-unrelated mismatched transplantation protocols are used widely in transplantation programmes.

Thus, 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 under intense investigation and should be evaluated in the context of biomedical research or registry studies. To date, results of prospective clinical studies comparing all sources of alternative HPC donors (especially cord blood *versus* haplo-identical) are lacking. Hence, transplantation programmes should carefully follow their own ‘local’ guidelines in defining the ‘best donor’ for each patient in each situation.

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.

21.3.2. Procurement procedures

The majority of HPC are provided using two technologies: procurement of bone marrow and apheresis. The advantages and disadvantages of these technologies are shown in Table 21.1.

A risk tool (see also Chapter 6) should be used to evaluate the contamination risk factors during procurement as shown in Table 21.2.

For HPC procurement and associated procedures – e.g. central venous (CV) 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 could serve as guidance [7].

Severe adverse reactions can occur in donors with the application of the mobilisation agents, and during and after allogeneic HPC donations. Hence, careful and documented training of clinicians 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 [2, 3, 8–11].

Table 21.1. Advantages and disadvantages of methods of haematopoietic progenitor cells procurement

Procurement method	Advantages	Disadvantages
Bone marrow collection	Donor: • Single procurement; • Use of cytokines not necessary Recipient: • Less chronic GvHD	Donor: • General or epidural anaesthesia; • Invasive procedure • Considerable risk of morbidity (associated with anaesthesia, procurement method) Recipient: • Potential tissue damage/infection at procurement site • Possible need for blood transfusion • 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/haemothorax and infection) • Potential loss of platelets • Considerable risk of morbidity (associated with mobilisation agents and extracorporeal collection systems) Recipient: • Increased risk of chronic GvHD

Source: European Group for Blood and Marrow Transplantation (EBMT) [6].

Table 21.2. Risk of contamination in haematopoietic progenitor cells procurement

HPC Sources	Air quality of procurement area	Procurement system	Microbial spectrum	Risk of microbiological contamination
HPC, marrow	classified	open	skin contaminants	low
HPC, apheresis	non classified	closed	skin contaminants (secondary contamination) venous catheter contaminants (patient)	very low
MNC, apheresis	non classified	closed	skin contaminants (secondary contamination)	very low
HPC, cord blood <i>in utero</i> / after delivery	non classified	open	vaginal, digestive tract, skin contaminants	high
HPC, cord blood (caesarean section)	classified	open	skin contaminants (secondary contamination)	low

21.3.2.1. 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, in bone marrow procurement and after-care 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 recommended for purposes 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, elaborating at least:

- preparation of media and materials used to obtain bone marrow;
- provision of containers for collected bone marrow;
- monitoring of the volume of the collected bone marrow;
- irrigation of procurement syringes;
- filtering the bone marrow;
- labelling.

Bone marrow HPC grafts contain bony spiculus, 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 collected. 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 collected. Procurement teams should not aspirate a volume more than 5 mL at each aspiration to avoid dilution of the bone marrow with blood [12]. 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 bone-marrow procurement are associated with anaesthesia, pain at aspiration sites, bruising and, rarely, local infection.

Blood cultures should be taken from bone marrow donors in presence of fever to investigate a possible microbial contamination of the procured product.

A 24-hour blood component donor-support protocol, including the provision of *cytomegalovirus* (CMV) antibody-negative (or equivalent), irradiated and leucocyte-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. Donation of autologous RBC before procurement can be considered, but should take into account potential induction of iron deficiency because the time from final selection of donor to procurement can be short. Donation of autologous blood should be over a reasonably long period before procurement but $\geq 1\text{--}2$ weeks [13]. Autologous blood must be taken in a blood-collection facility that meets applicable national/international requirements. Iron supplementation before and after bone marrow donation is recommended if possible, reducing the need for RBC transfusion.

Procurement procedures in paediatric donors shall be adjusted according to age and size [7].

21.3.2.2. *Peripheral blood*

HPC from peripheral blood should be collected in a therapeutic apheresis facility by personnel who have appropriate experience in care for haematology or oncology patients, HPC mobilisation and therapeutic apheresis. Special attention should be paid to paediatric patients and the special 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. 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) shall be monitored. 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.

21.3.2.2.1. *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 should 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, and raise the issue of inpatient *versus* outpatient administration. Donors who live far away from the transplant centre will require administration at home. However, due to a potential risk of allergic reaction, the first dose of rhG-CSF should be given under supervision of a trained healthcare professional, and the donor should be followed up for at least 30-60 minutes. The transplantation programme or the physicians in charge of mobilisation and procurement of HPC from the donor should be informed in detail of the necessary measures to be taken in case severe adverse reactions (SAR) occur, especially for

anaphylactic shock, spleen rupture, capillary leakage and acute hepatitis.

Routinely, HPC 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, administration of additional agents like HPC binding inhibitors).

21.3.2.2.2. *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. binding inhibitors 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 collected, taking into account the patients' well-being during and after procurement, as well as their needs as future recipients.

Table 21.3. **Very common adverse reactions associated with haematopoietic progenitor cells mobilisation (> 10 %)**

Agent	Adverse reaction
Rh-Granulocyte-colony stimulating factor (rhG-CSF)	Bone pain Musculoskeletal pain Thrombocytopenia Hyperleucocytosis 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: European Group for Blood and Marrow Transplantation (EBMT) [6].

In the selection process of the mobilisation agent, the World Marrow Donor Association document *Concerns about the use of biosimilar granulocyte colony-stimulating factors for the mobilisation of stem cells in normal donors: position of the World Marrow Donor Association* [14] should be consulted. The relevant mobilisation agent should be used in accordance with the latest approved Summary of Product Characteristics.

21.3.2.2.3. Apheresis procurement yield

Collected apheresis volumes vary, depending on the procurement protocol and cell separator. The target procurement 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 similar but, because of the longer time to engraftment of neutrophils and platelets associated with allogeneic transplantation, HPC 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 are used. In addition to optimising HPC procurement, apheresis should ensure that collected cells have minimal contamination with neutrophils 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 [13]. Depending on the device used for procurement of HPC, the donor may experience a marked loss of platelets, in some cases resulting in post-donation values < 100 G/l. Under such circumstances a consecutive donation has to be carefully considered. Other risks related to the procurement method are given in Table 21.2.

The targeted cell dose could be reached in one or more apheresis procedures.

Some Health Authorities do not permit the use of G-CSF in paediatric donors and so other procurement strategies may be employed, such as bone marrow donation.

21.3.2.3. Cord blood

The Council of Europe has been studying the issue of cord blood donation for several years and has always been concerned about the proliferation of cord blood banks dedicated to the procurement and storage of cord blood 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 cord blood banks and its explanatory memorandum [15], which recommends that member states allow establishment of cord blood banks only for altruistic and voluntary

donations of cord blood. The recommendation also states that the promotion of cord blood donation for autologous use and the establishment of cord blood banks for autologous use should not be supported by member states or their health services. Where autologous cord blood banks are established, the promotional material or information provided to families must be accurate, and fully informed consent to cord blood storage must be obtained. For this purpose, the Council of Europe has produced the brochure *Umbilical cord blood banking. A guide for parents*, to provide clear, accurate and balanced information about the use of cord blood in medical treatment and to guide parents through their blood-storage options [16].

Autologous (or family-use) cord blood banks 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 cord blood preservation. In any case, autologous cord blood banks must meet the same quality and safety standards as for allogeneic cord blood donation and banking.

Umbilical cord blood can be obtained at birth from fully informed women who have previously consented to allogeneic donation (including related donations) and been found to be medically eligible, following normal delivery of a healthy baby. No modification in delivery practices should be performed with a view to increasing the volume of cord blood collected. Procurement after caesarean delivery is possible, provided that the baby and mother are well. Procurement should be carried out only by trained staff.

Traceability of the collected cells from the mother and the newborn must be ensured by personnel that collect the blood. Systems to allow reporting on the health of the mother and newborn subsequent to donation of umbilical cord blood are part of the umbilical cord blood banking process.

21.3.2.4. Immunocompetent cells used after haematopoietic progenitor cell transplantation

To enhance immune responsiveness after HPC transplantation, the following cells are being used and/or evaluated in clinical trials:

- a. allo-reactive donor T-cells (donor lymphocyte infusions) for prophylaxis and treatment of relapses or mixed chimerism after HPC transplantation;
- b. T regulatory cells for the prevention and control of GvHD;

- c. natural killer (NK) cells as graft *versus* tumour (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 transplantations, or for treating GvHD;
- f. Mesenchymal stem cells to enhance engraftment in allogeneic and autologous HPC transplantations, or in treatment of GvHD;
- g. autologous chimeric antigen-receptor (CAR) T-cells.

Some of these cells are discussed in Chapter 28.

21.3.3. Temporary storage and transportation to the tissue establishment

Progressive loss of HPC occurs during non-frozen storage. Nevertheless, HPC should be 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 cells (TNC), buffering capacity of the solution, the media and anti-coagulants added, procurement volume and storage temperature. Cell viability decreases and the risk of bacterial growth increases during storage at room temperature as well as in refrigerators (Chapter 7, section 7.7.3). Therefore,

maximum storage in the non-frozen state should be ≤ 72 h. The facility should undertake a validation study of the storage and transport conditions.

21.4. 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 [7, 12]. It does not affect the main biological properties of the collected cells, which is to support the marrow re-populating ability (MRA) and the establishment of haematopoietic chimerism in a myelo-ablated or immuno-suppressed recipient in allogeneic transplant.

Generic requirements for processing facilities, together with standards, are described in Chapters 7 and 8.

The specificities of processing HPC are shown in Table 21.4 (see also Chapter 8, Table 8.2 on the risks of airborne contamination). Considering the factors detailed in Table 21.4, 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 and at least Grade D air quality as background as required by Directive 2006/86/EC. The same requirements apply for autologous or allogeneic donations.

Table 21.4. Factors influencing the air-quality specification for processing haematopoietic progenitor cells

Criterion	Haematopoietic progenitor cell-specific
Risk of contamination of tissues or cells during processing	Cryopreservation or selection of certain subpopulation of HPC is mostly closed. Processes that are closed need a less stringent specification than those that involve hours of open processing. The sterile barrier can be compromised in a moment after adding cryoprotectant, monoclonal antibodies or other solutions by sterile spikes.
Use of antimicrobials during processing	Use of antimicrobial agents during HPC processing is not applicable. Nevertheless, in some cases, even HPC contaminated by bacteria can be used (unique matching, life-saving treatment). In such cases, the recipient is protected with antimicrobial agents.
Risk that contaminants will not be detected in the final tissues or cells due to limitations of the sampling method	Obtaining adequate volumes of the sample is the main obstacle in final microbiological control, especially in CD34 selected products. Samples can be taken from the cells or residual components after processing, depending on the product volume. Procedures for microbiological detection shall be validated for inadequate quantities of sample.
Risk of transfer of contaminants at transplantation	Nature of transplant (blood cells), minimal processing and the fact that there are no applicable decontamination procedures make these cells high-risk for transfer of contaminants at transplantation. However, application of the transplant is by infusion, during which the transplant is not exposed to the environment. Hence, the risk of contamination during transplantation is minimised. A possible risk is the thawing procedure. Thawing in a water-bath bears a risk of contamination.

For all processing steps, written procedures must be established and reviewed on a regular basis with evidence of continued training of the staff. The International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, and Administration also apply to processing facilities.

21.4.1. 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.

21.4.2. Red blood cell depletion

Red cell depletion is a critical step in cases where there is major 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). Red cell depletion is almost exclusively used if bone marrow is used as the HPC graft source, whereas peripheral blood HPC are usually not red blood cell depleted. In addition the vast majority of cord blood units are red cell depleted before cryopreservation. 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 and progenitor loss associated with such procedures must be evaluated and the expected recoveries and amount of acceptable residual red blood cells must be defined.

21.4.3. 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 during the period that precedes the donation. As the titre of anti-A and anti-B antibodies that may be considered 'safe' for any particular patient is not defined, each transplant programme should establish procedures for the management of

ABO-incompatible transplants. Plasma removal is usually done by centrifugation of the collected cells. The cell loss associated with such procedures must be evaluated and expected recoveries must be defined.

21.4.4. Cryopreservation, thawing and infusion

21.4.4.1. Cryopreservation

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 sulfoxide (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 in patients with impaired renal function.

Cryopreservation is used systematically in the autologous setting and by cord blood banks that prospectively collect and store umbilical cord blood. 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 hours after procurement) without freezing. However, sometimes allogeneic HPC are being cryopreserved for logistical reasons: unavailability of the donor at the scheduled date of transplantation (procurement in advance); professional constraints; unforeseen changes in transplantation schedules; overproduction of stem cells. HPC should be cryopreserved as soon as possible. Shelf life of HPC without cryopreservation is acceptable up to 72 hours. However, cell viability decreases if cells are frozen at the end of their shelf life.

The cryopreservation method for HPC collected from peripheral blood, bone marrow or blood from the umbilical cord 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. 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. The final phase of cooling in a controlled-rate freezer is usually quicker, with the temperature drop ad-

justed 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 procedure must include evidence that the storage temperature is adequate to preserve the products.

Once frozen, HPC should be stored in vapour-phase liquid nitrogen or in liquid nitrogen at < –140 °C for all HPC. 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 from the bone marrow and peripheral blood. HPC derived from bone marrow, mobilised peripheral blood or cord blood have been transplanted successfully even 11 years after cryopreservation. A stability programme for cryopreserved products should be implemented in order to evaluate viability and potency at different storage durations.

21.4.4.2. *Thawing and infusion*

Cells can be thawed at the bedside or in a processing facility. HPC can be washed after thawing to remove most of the DMSO. For cord blood units, washing of red cell replete CB units should be performed.

The risks of washing are significant cell loss, decreased viability, and decreased chance of engraftment and microbial contamination. Hence, washing of HPC 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, although this interval may be longer if the HPC are washed. HPC should be infused using a standard transfusion set. Leucoreduction filters must not be used.

21.4.5. **Cell-selected preparations**

Specific Conformité Européenne (CE)-marked devices are available to select CD34⁺ cells from bone marrow, peripheral blood or umbilical cord 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. standard operating procedures (SOPs) – for cell-selection 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.

21.4.5.1. *T-cell depletion and depletion of allo-reactive immune effectors*

T-cell depletion is associated with positive (i.e. GvHD prevention) and negative (i.e. prolonged immunosuppression) consequences that prevents 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 eliminated, including the T-cell receptor (TCR) gamma/delta-positive T-cells not involved in GvHD and exerting anti-leukemic 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 EBV is a very encouraging strategy, especially in haplo-identical transplantation settings [17].

Other specific procedures evaluated by clinical trials include depletion of activated and allo-reactive 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 allo-reactive 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.

21.4.5.2. *Tumour cell depletion in the autologous setting*

Autologous tumour cells collected 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 immunohistochemical techniques or flow cytometry, or molecular biology techniques.

21.5. Quality control

21.5.1. **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 3 and 4 of this Guide.

21.5.2. **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 Chapter 5).

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 leucocytes in the collected cell preparations must be measured. High numbers of mature 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.

21.5.3. **Immunophenotyping and colony-forming units 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 collected 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. procurement of TNC following plasma removal, volume reduction, red blood cell depletion, etc.).

The proportion of cells of the erythroblastic lineage may be high in umbilical cord blood and should be documented. CD34⁺ cell counts are used as a marker for HPC, both in the peripheral blood of individuals undergoing mobilisation regimes and in the collected cells, whether from apheresis following mobilisation or from umbilical cord blood 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. For example, the International Society for Hemotherapy and Graft Engineering (International Society for Cellular Therapy) algorithm provides a robust and reproducible gating strategy to measure CD34⁺ cells [18, 19].

Potency assays for cell preparations used for autologous or allogeneic transplantation rely on the detection of colony-forming units (CFU) in clonogenic assays, using semi-solid media. These functional tests are hampered also 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 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. A clonogenic assay provides additional infor-

mation about the functionality of the graft; in particular, it is recommended after a long storage period. It can be used as a qualitative potency test (e.g. growth or no growth) or as a quantitative potency test. In both cases, a policy should be defined to deal with products where CD34⁺ cells clone at a low frequency. Some national regulations and guidelines define in which context CFU measurements are mandatory, as for cryopreserved cord blood units prior to release.

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).

21.5.4. 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 tissue establishment 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 products. In autologous products, infectious disease-marker (IDM) test results may be positive (this is normally not the case in allogeneic products). In allogeneic products, 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 products).

Processing and transplant facilities should agree on the cell dose (nucleated cell count, mononuclear cell count, CD34⁺ cell count and/or clonogenic assays as appropriate for the source of HPC) required to achieve reliable and sustainable engraftment. For cord blood, the number of TNC in the product is related to the clinical outcome of the recipient.

If cells are required for administration to a patient, a prescription for infusion is required. This prescription shall list the type of cell preparation that is suitable for that patient and provide specific information on dosing. If necessary, the cells may be manipulated before infusion (e.g. washing, dilution) and this should be recorded on a worksheet and on the activity report.

21.6. 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, European coding system) and must be used to promote consistency and traceability, aid international exchanges and facilitate vigilance and surveillance. See also Chapter 13.

21.7. Storage

Storage must be 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 products with incomplete or positive IDM test results are accidentally released without proper authorisation.

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.).

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 products at the defined storage temperature.

21.8. 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 cell-processing 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 shall conform to the applicable regulations and shall be secured where applicable. For cryopreserved cellular therapy products, a dry shipper should be used. During shipment of HPC products, the temperature should be monitored and records must be maintained by the shipping facility and shared with the receiving facility. See also Chapter 10.

21.9. 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* [20] 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.

Tissue establishments 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 (SARE). There is general guidance on implementation of good V&S practice, as well as definitions of SARE, in Chapter 15.

Tissue establishments should have written procedures for managing SARE. They should also provide centres carrying out clinical applications with accurate and updated information on various SARE in the area of HPC transplantation.

21.9.1. Serious adverse reactions in the recipient

21.9.1.1. 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 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.

21.9.1.1.1. 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 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 within a HPC preparation that can produce antibodies against the recipient's ABO or other RBC antigens, haemolysis can be more serious. 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.

21.9.1.1.2. 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 im-

mediately. 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.

21.9.1.1.3. Febrile non-haemolytic reactions

During HPC infusion, patients may experience febrile non-haemolytic 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 leucocytes 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.

21.9.1.1.4. Dimethyl sulfoxide toxicity

Dimethyl sulfoxide (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, dimethyl sulfoxide, 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, haemoglobinuria 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.

21.9.1.1.5. 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.

21.9.1.1.6. 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 hypervolemia due to extensive hydration before infusion, large volume of transplant, hyperosmolality of DMSO, hypothermia, lysis of graft cells or underlying cardiac conditions.

21.9.1.1.7. Allergic reactions

Allergic reactions usually manifest as urticaria and pruritis. 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/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 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.

21.9.1.1.8. 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 thrombocytopenic recipients.

21.9.1.1.9. 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.

21.9.1.1.10. 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.

21.9.1.1.11. Bacterial contamination

Bacterial contamination of an HPC product is possible. Bone marrow, which is collected into an open system, and cord blood have a higher rate of contamination than HPC collecting from peripheral blood. Contamination 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 due to the treatment, it is vital that HPC are collected 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 an interruption of sterile methods.

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.

21.9.1.1.12. Transmission of infectious and genetic diseases

HPC preparations should be tested for trans- fusion 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 neutropenia, Gaucher's disease). Autoimmune diseases transmission have been also reported (thyroiditis, type 1 diabetes, myasthenia gravis, vitiligo, etc.) [21]. 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.

21.9.1.1.13. 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. For HPC transplantation from cord blood, the time point for primary graft failure is 42 days after transplantation. Leucocyte 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 tissue establishment to enable thorough investigation of the quality and handling of grafts.

21.9.1.1.14. 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. Acute GvHD occurs < 100 days after transplantation, whereas chronic GvHD occurs > 100 days after transplantation. 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).

21.9.2. **Serious product events and adverse reactions**

In the case of serious product events and adverse reactions (SPEAR), 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, then the transplantation centre must report this matter to the tissue establishment immediately and, according to national legislation, to tissue- and cell-competent authorities.

21.9.3. **Serious adverse reactions in haematopoietic progenitor cell donors**

Deaths in unrelated HPC donors are very rare. Only one death has been reported to the World Marrow Donor Association (WMDA) since the establishment of the S(P)EAR – serious (product) events and adverse reactions – reporting system; that death was caused by haemothorax secondary to traumatic jugular central venous catheter insertion. However, a number of deaths have been reported 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 [3, 4, 20].

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. 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 [22].

21.9.3.1. *Complications in 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 hypovolemia or anaemia [8, 11, 21].

Cytopenias (anaemia, thrombocytopenia) 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.

21.9.3.2. *Complications in apheresis haematopoietic progenitor cell donors*

Complications in apheresis HPC donors are related to apheresis and administration of granulocyte-colony stimulating factor (rhG-CSF). 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.

Reports from long-term follow-up studies in unrelated and related apheresis HPC donors mobilised with rhG-CSF demonstrated 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.

21.9.3.3. *Follow-up of haematopoietic progenitor cell donors*

Chapter 15 on biovigilance also applies to HPC transplantation and must be read in conjunction with this chapter.

The donor, whether related or unrelated, should be followed up by the donation centre in the short, mid and long term according to the scheme and times suggested by scientific organisations like the Italian Bone Marrow Donor Registry (IBMDR) or EBMT. In particular, a short-term follow-up to document SAEs, and a long-term follow-up on a regular basis to document late effects of the donation or the mobilising agent, should be performed [21, 22].

21.10. Mononuclear cells

21.10.1. Introduction

Mononuclear cells (MNC) concentrates comprise leukapheresis products from unstimulated donors: a former HPC donor (i.e. donor lymphocytes infusion/DLI) or a third party donor. DLI can be administered to the selected patient after allogeneic HPC transplantation, either prophylactically to augment the anti-tumour immune response (following reduced-intensity conditioning protocols), 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 the graft *versus* tumour (GvT) effect.

For this section see also similar sections that apply to HPC:

- a. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);

- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, labelling and packaging (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

21.10.2. Processing of unstimulated leukocytes

Processing of MNC 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 can be determined by flow cytometry, and further characterisation of subpopulations of T-cells 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. DLI may be frozen in ampoules or in bags. The tissue establishment shall validate the freezing technique in order to establish the expected level of viable T-cells after thawing. Some protocols also include cell-selected preparation. See also 21.4.

21.10.3. Quality control

In addition to the microbiological testing of the cells or supernatant of the cells described in Chapter 9, the specific requirements include establishing the absolute number and the frequency of T-cells (CD3⁺ and/or subpopulations) and cellular viability. The anti-tumour effect should be evaluated at intervals as defined in the clinical protocol.

21.10.4. Biovigilance

The processing of DLI and MNC involves several steps where unexpected events that have to be documented and reported may occur (see 21.9 and Chapter 15), for instance, lower viability of frozen and thawed DLI or MNC than expected or human errors in calculating the dose of T-cells. In such cases, 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 21.9, complications due to DLI infusion such as DMSO toxicity, transmission of infectious diseases or GvHD may occur. In rare circumstances, serious product adverse events/reaction or complications in the donor are possible. Follow-up of the donor (as required after HPC donation) as stated in 21.9 and Chapter 15 is suggested.

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Related document:

Appendix 23. Donor search through registries for haematopoietic progenitor cells transplantation.

Chapter 22. Pancreatic islets

22.1. Introduction

Type-1 diabetes mellitus (T1DM) is characterised by absolute and specific destruction of insulin-producing cells that reside within clusters of cells in the pancreas known as islets. People who do not have diabetes mellitus have ≈ 1 million islets comprising 2 % of the overall pancreas. Without lifelong insulin replacement, T1DM quickly results in coma and death. Even with optimised treatment, vascular and neurological complications often develop over time. Restoring near-normal blood glucose levels can prevent these complications. This has, however, been associated with a threefold increase in severe hypoglycaemia (low blood glucose, which can result in collapse without warning, one of the greatest fears for those living with insulin injections) [1]. DM remains a leading cause of blindness, renal failure requiring dialysis or renal transplantation, and lower limb amputation.

The ultimate goal of pancreatic islet transplantation and beta-cell replacement therapy is to restore glucose-responsive insulin secretory capacity to patients with insulin-deficient DM. This includes all people with T1DM and potentially also those with insulin-deficient type-2 diabetes mellitus (T2DM). The benefits of islet transplantation (in its current form) are protection from hypoglycaemia, with improved glycaemic control overall and, occasionally, complete insulin independence. It should, therefore, be available for patients with unresolved recurrent severe hypoglycaemia despite optimised specialist management.

Hence, islet transplantation may be especially beneficial in two defined subgroups of subjects with T1DM: those with intractable hypoglycaemia and those with problematic diabetic control following renal transplantation. Also, islet autotransplantation for intractable pancreatitis or other cases requiring pancreatectomy can prevent surgically induced severe DM.

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. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate. (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, labelling and packaging (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

22.2. Donor evaluation

22.2.1. General criteria

Donor criteria for pancreatic islet transplantation are the same as those generally applied for organ transplantation [2]. 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. However, additional criteria for donation of tissues and cells (see Chapters 4 and 5 of this guide) must be applied.

22.2.2. Specific exclusion criteria for pancreatic islet transplantation

Insulin independence after transplantation requires pancreatic islets from 1 or more pancreases. Donors suffering from diabetes mellitus type 1 or 2 are excluded from donation for this clinical use.

Donor characteristics – such as type of donor (brain death *versus* circulatory death), age, body mass index, cold ischaemic time and glycated haemoglobin A_{1c} – affect the islet yield and/or function [3, 4]. The tissue establishment should establish contraindications for pancreas acceptance.

22.3. Procurement

The consistency of pancreatic islets manufacturing is highly dependent on the quality of the procured organ. Organ procurement should be conducted to ensure organ viability in transit, using similar procedures as for whole pancreas transplantation, but vascular access is not required. The tissue establishment should have agreements with procurement centres regarding organ harvesting, warm and cold ischaemia time, organ preservation methods, cold preservation fluid, and shipping conditions.

22.4. Processing and storage

Organs are transported to the designated isolation facility. Pancreases are processed by enzymatic and mechanical dissociation, and pancreatic islets are collected after density-gradient purification.

Storage of pancreatic islets in media under stringent conditions before implantation has logistical benefits (enables additional quality control tests, time to prepare patient for transplant or to ship pancreatic islets to distant transplant centre) [5]. The tissue establishment should guarantee that the composition of the storage medium does not alter

the physiological properties of insulin-producing islets.

22.5. Quality controls/release criteria

Pancreatic islet cells exhibit a wide variety of functions that should be tested during quality control procedures. The tissue establishment should define – alongside the general tissue-and-cell release criteria – additional criteria for pancreatic islet transplantation, including:

- Quantification of the pancreatic islet cell mass, or of the number of insulin-positive cells,
- Beta cell function (e.g. glucose-stimulated insulin secretion or insulin synthesis),
- Cell viability (e.g. qualitative determination by hoechst/propidium iodide or fluorescein diacetate/ethidium bromide),
- Endotoxin level [6] (see section 9.3.3 of this guide).

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 tissue establishment should define how it will deal with incomplete test results.

Following confirmation of product identity and integrity of the pancreatic islet graft, islets will be transplanted into the portal vein as an inpatient procedure. Alternative routes of administration should demonstrate adequate safety.

22.6. Packaging and distribution

Transport temperature is usually maintained at 18–25 °C.

Pancreatic islet cells are transported in liquid media, so special notice might be necessary according to airline transport regulations (see Chapters 10 and 13 of this guide).

22.7. Traceability

The attached documentation for the clinical transplantation centre should include, for example, details of the donor, organ transport/ischaemic time, 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 tissue establishment, and it should be

possible to trace also other organ recipients from the same donor, and vice versa.

22.8. Biovigilance

Any unforeseen events influencing islet isolation and storage conditions are to be considered as adverse events that should be recorded and documented. Not only viability but also failure to produce the expected level of insulin upon glucose stimulation is an example of an adverse event specific to pancreatic islets.

22.9. Developing applications for patients

In the last several years, some innovative applications have been investigated and developed, based on somatic cell and gene therapy:

- Human embryonic stem cells differentiated into pancreatic beta cell precursors [7];
- Encapsulation of insulin-producing cells;
- Hepatic insulin gene therapy (pre-clinical).

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Chapter 23. Adipose tissue

23.1. Introduction

Autologous fat transplantation in aesthetic and reconstructive plastic surgery has revolutionised surgical treatment for soft tissue augmentation in recent years. In 1893, Neuber reported the first autologous fat grafting [1]. Since the 1980s, lipofilling procedure has become one of the most popular procedures performed by plastic and aesthetic surgeons [2].

Unlike with synthetic materials, there is no risk of rejection and implementation costs are reasonable. Autologous fat transplantation could be used in both aesthetic and reconstructive plastic surgery for soft tissue augmentation. In addition, it does not induce an immune response in the recipient and, as a filler material, is abundantly available.

One of the most common indications for this therapy is reconstruction after tissue removal in patients with breast cancer. The indications for this procedure could also include lipodystrophy due to acquired immune deficiency syndrome, so positive results in infectious disease testing can be accepted, as the tissue will be for autologous use. For more than 100 years, autologous fat transplantation has been used to correct subcutaneous lipoatrophy, resulting from hemifacial atrophy, acne, trauma, lipodystrophy and scleroderma, cutaneous lupus erythematosus and defects resulting from accident, infection or surgery. Adipose tissue has been used in post-mastectomy pain syndrome; in fact, breast-conserving surgery has become a well-established alternative to mastectomy in the treatment of breast cancer, providing a less invasive treatment [3]. Fat

transplantation is efficient also for breast augmentation in patients suffering from micromastia, post-explantation deformity, tuberous breast deformity and Poland syndrome [4]. Adipose tissue has been used also for the correction of cicatricial ectropion [5] and for superior sulcus deformity [6].

Most of the clinical data obtained from adipose tissue transplantation are from patients receiving lipofilling directly after procurement in a one-step surgical procedure.

So far, the main obstacle to achieving favourable outcomes is its unpredictable long-term results due to the high rate of resorption in the grafted site, which means additional grafting and repeated procurement, leading to increasing cost and surgical risks as well as discomfort for the patient. There are several approaches to improving fat graft survival, including changes to procurement and processing techniques.

In order to avoid multiple procurements, protocols are developed to store adipose tissue in tissue establishments sufficient for several grafting events.

Autologous adipose tissue from liposuction is being used increasingly in plastic surgery for reconstructive procedures. Some of the implanted tissue is resorbed, so surgeons treating large defects frequently apply a staged approach; its absorption rate has been reported to be 30-70 % [7, 8]. This approach can be facilitated by storing all or part of the tissue collected from the initial liposuction and implanting it during subsequent interventions. 'Fat banking' eliminates the need for repeated liposuction and, thereby, reduces cost and the risk of morbidity. However, the

overall quality of the cryopreserved fat grafts is still less ideal than the fresh one.

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. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, packaging and labelling (Chapter 13);
- n. Traceability (Chapter 14);
- o. Biovigilance (Chapter 15).

23.2. Donor evaluation

The criteria for donor selection to be applied are those for autologous donation. The patient must be provided with sufficient information regarding the process (including the planned storage period and tests performed) and should sign an informed consent form.

23.2.1. Tissue-specific exclusion criteria

It should be ascertained that donors do not have any major systemic diseases or lipid disorders, and they are not underweight. If the adipose tissue is to be stored, infectious disease testing must be performed for all autologous adipose tissue patients, as described in Chapter 5. Patients known to have HIV or Hepatitis B/C can be accepted for autologous use.

23.3. Procurement

23.3.1. Procurement procedures

Risk assessment on the conditions of procurement, processing facilities and storage should be conducted, and appropriate mitigating actions should be taken to prevent cross-contamination. Particular

attention should be paid to procurement conditions, as they support the initial quality and low bioburden of the adipose tissue.

Various procurement and preparation techniques have been introduced to obtain better and more reliable survival of adipose tissue. The fat tissue is usually procured with a specific cannula with negative pressure from abdomen, thighs and hip with Coleman technique. Adipose aspirates are collected in a specific container (for example, a Luer-lock syringe).

23.3.2. Temporary storage and transportation to the tissue establishment

The adipose aspirates should be transferred immediately (at a transport temperature of 4 °C) to the processing unit.

23.4. Processing

There are several published protocols for processing adipose tissue, but there is no evidence to prefer one technique above another.

Tissue processing includes washing, centrifugation or decanting, eventually antibiotic decontamination, controlled-rate freezing ($\approx 1^\circ\text{C}/\text{min}$) with cryoprotective agents and storage $< -140^\circ\text{C}$ (in vapour phase of liquid nitrogen) to preserve maximum viability.

It should be taken into account that adipose tissue is very sensitive to external treatment (centrifuge, processing methods, temperature). In particular, Moscatello *et al.* described the requirements for cryoprotectants and controlled freezing/storage, and listed components which can affect the viability of transplanted adipose tissue [9].

- 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. Appropriate measures should be taken to minimise the risk of microbiological contamination, including possibly the addition of an 'antibiotic cocktail' to the lipoaspirate.

23.5. Quality control

To cryopreserve adipose tissue, quality control is an essential issue. Adipose tissues must be processed in sterile conditions and in an aseptic manner. All biological tests should be performed as described in Chapters 5 and 9.

Quality control must include microbiological testing of each batch. Histology and cell-viability controls are highly recommended. Microbiological analyses of procured tissue, rinsing solutions and tissue after possible decontamination should be carried out according to the *European Pharmacopeia* (*Ph. Eur.*). In cases of positive results after decontamination, the adipose tissue should be discarded.

23.6. Labelling and packaging

All bags or containers used in the collection, processing and final packaging (frozen or thawed tissue) must be labelled appropriately. For further details, see Chapter 13.

23.7. Preservation/storage

23.7.1. Methods of 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 patients), 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 mixed. Further studies need to be done in order to develop a better cryopreservation method for long-term preservation. The selection of cryoprotective agent is one of the key issues for obtaining optimal viability of adipocytes.

The most common cryoprotective agents used for adipose tissues are dimethyl sulfoxide (DMSO), trehalose and glycerol.

When autologous adipose tissue is procured and stored, positive test results will not necessarily lead to discarding the tissue. For such tissues, isolated storage possibilities should be considered in order to ensure no risk of cross-contamination or mix-ups.

23.7.2. Expiry date

A state-of-the-art research or validation study on the shelf life of preserved adipose tissue should be performed in order to determine the expiry date

according to the used processing and preservation protocol.

23.7.3. Storage temperature

Processed adipose tissue should be kept in the vapour phase of liquid nitrogen ($< -140^{\circ}\text{C}$). The thawing protocol must be gentle, when removing the cryoprotective agent as well the amount of free lipids and debris associated with loss of adipocytes.

23.8. Biovigilance

Any adverse reaction or event occurring during procurement, processing, thawing or re-injection of tissue should be notified, as described in Chapter 15.

No entries have so far been found in the Notify Library (www.notifylibrary.org) for the banking of adipose tissue.

23.9. Developing applications

Adipose tissue may also be a source of stromal vascular fraction (SVF) or stem cells (see Chapter 28). A search for clinical trials using SVF in www.clinicaltrials.gov revealed that 34 studies are currently actively recruiting for patients. Most studies are conducted to treat osteoarthritis and rheumatoid arthritis. However, the conditions potentially treated with an SVF approach include musculoskeletal, neurological, immunological, cardio-pulmonary and immunological disorders and soft tissue defects. Similarly, adipose-derived stem cells isolated from SVF and expanded *in vitro* are under investigation for a whole range of diseases [10, 11].

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Chapter 24. Assisted reproductive technology

24.1. Introduction

Assisted reproductive technology (ART) refers to medical procedures used to achieve pregnancy and live birth involving the identification, collection, processing and/or storage of at least one of the following reproductive tissues and cells: oocytes, ovarian tissue, sperm, testicular tissue and embryos. ART is carried out using freshly collected and/or cryopreserved gametes, zygotes or embryos originated from the couple being treated ('partner donation') and also from gamete donors ('non-partner donation'). These contexts are, in general, addressed separately due to the different risks involved. Ovarian stimulation or any other clinical procedure that does not involve gamete collection will not be addressed in this chapter.

ART is carried out in ART centres specialised in treating patients with fertility problems. These centres are usually a combination of a tissue establishment and an organisation responsible for human application, bringing together a clinical team and a laboratory team in a multidisciplinary unit.

ART comprises methods such as:

- a. Artificial insemination, mostly intra-uterine insemination (IUI). Sperm is provided by the partner or from a non-partner donor, processed and placed in the uterus at the estimated time of ovulation;
- b. *In vitro* fertilisation (IVF), either conventional (whereby collected and prepared sperm and oocytes are co-incubated) or intracytoplasmic sperm injection (ICSI); whereby a single sper-

matozoon is injected into a mature oocyte). IVF and ICSI involve collection and processing of gametes, fertilisation, culture and transfer of embryos into the uterus. In these insemination strategies, oocytes and/or sperm might be provided by a partner or non-partner donor;

- c. Cryopreservation and storage of gametes, embryos and/or gonadal tissue;
- d. Pre-implantation genetic testing (PGT) that uses genetic identification methods to diagnose or screen oocytes or embryos *in vitro* for genetic abnormalities in order to select embryos before transfer. These methods include pre-implantation genetic diagnosis (PGD, now named PGT-M for monogenic/single gene defects, and PGT-SR for chromosomal structural rearrangements), as well as pre-implantation genetic screening (PGS, now named PGT-A for aneuploidy screening). In some countries, not all of these types of test are allowed, or are allowed only under specific circumstances according to national legislation.

ART procedures such as cryopreservation of gametes or gonadal tissue can also be used in patients with certain diseases (e.g. cancer, some chronic diseases) for whom treatment may be potentially harmful to their fertility. In those cases, long storage of cryopreserved reproductive tissues and cells may be proposed to children, adolescents and male or female adults. This approach, called 'fertility preservation', is addressed in Chapter 25 and is also an option for fertility preservation for non-medical reasons. ART can

also be proposed to couples at risk of transmitting a serious transmissible disease – e.g. human immunodeficiency virus (HIV) or hepatitis B and C viruses (HBV and HCV) – to the partner and/or the child. These practices will be applied only after risk assessment of vertical and horizontal disease transmission and taking into account the patients' health condition. In some countries, ART can be undertaken in 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 surrogate mother carries and gives birth to a child for the intended parents.

ART is performed in most countries in Europe. Each year, the European Society of Human Reproduction and Embryology (ESHRE) publishes a report of activity in European countries, based on voluntary declarations. ESHRE data from 2012 included clinics in 34 countries and reported 640 144 treatment cycles, including 139 978 IVF, 312 600 ICSI, 139 558 transfer of frozen thawed embryo, 33 605 oocyte donation, 421 *in vitro* maturation (IVM), 8 433 pre-implantation genetic testing (PGT) and 5 549 frozen oocyte replacements [1]. In countries in which ART is well established, 1.3–6.1 % of children born per year are conceived using ART.

ART involves ethically sensitive medical activities that lead to the birth of a child. As such, the described procedures are intended to achieve efficient results in terms of delivery rates and also address the safety of patients, donors and children born after ART. For partner donation, priority is given to using the reproductive cells from the partner even if the sample exhibits poor quality (e.g. ICSI for patients with decreased sperm quality, ART between partners with a partner infected by HIV, ART between partners with a high risk of transmission of a genetic disease).

This chapter aims to provide guidelines which can help to conceive healthy singletons carried to term through ART.

In addition, the following generic chapters (Part A) of this Guide all apply to ART and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Recruitment of potential donors, identification and consent (Chapter 3, but only for non-partner donors);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);

- f. Processing and storage (Chapter 7);
- g. Distribution and import/export (Chapter 9);
- h. Organisations responsible for human application (Chapter 11);
- i. Computerised systems (Chapter 12);
- j. Packaging and labelling (Chapter 13, but only for non-partner donors);
- k. Traceability (Chapter 14);
- l. Biovigilance (Chapter 15).

This chapter defines additional specific requirements for ART.

Procedures may vary from country to country as determined by national legislation.

24.2. Quality management, risk management and validation

The implementation of a quality system in ART is mandatory and it will contribute to the success of a given ART programme.

24.2.1. Risk-assessment analysis for all laboratory activities

Risk management in ART will help in assessing and prioritising the possible existing hazards in order to monitor and control them, so the probability of an adverse event occurring will be kept to a minimum.

In order to assess the risk, the following questions need to be answered:

- a. What are the potential hazards?
- b. Who is at risk?
- c. Do control measures exist?
- d. Are there preventive measures?
- e. Who will be the Responsible Person?

24.2.2. Validation

Every piece of equipment should be validated before it enters routine clinical use, and CE-marked if possible. Such validation should include installation, operational (technical) qualification (see Appendix 7) and performance qualification by comparison with laboratory key performance indicators (KPI) for similar types of equipment already existing in the laboratory. Examples of KPIs that can be used are percentage of mature oocytes, percentage of fertilisation, percentage of good-quality embryos and pregnancy outcome, e.g. live birth rate. Based on the validation that is carried out, several specific KPIs are monitored.

Likewise, with novel and emerging technologies, it is desirable to ensure that the necessary re-

search and development is conducted before bringing new techniques into clinical practice by means of well-designed randomised control trials (RCTs) 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 24.1).

24.2.3. Materials, consumables and reagents

All consumables and media should be fit for their purpose, of embryo-culture grade in quality and preferably CE-marked.

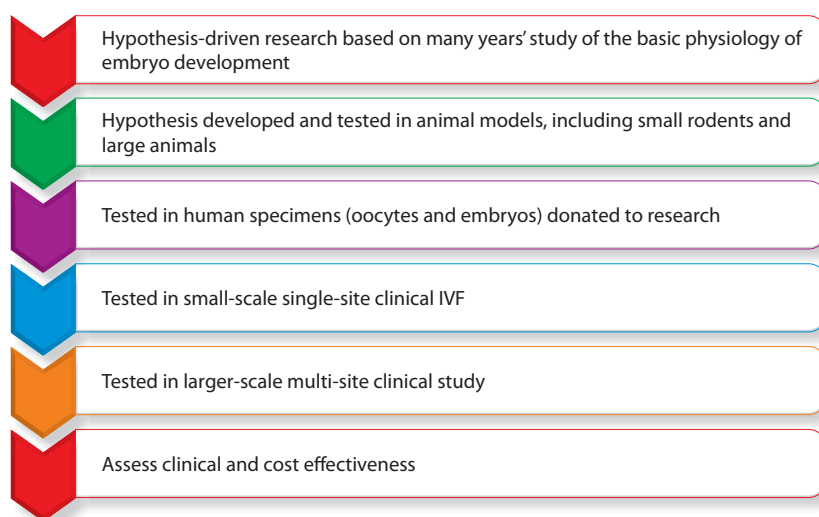
Specific culture media that fulfil the requirements of gametes and embryos are needed during all processing, fertilisation, culture, cryopreservation

and other processing activities in ART. Use of commercially produced, quality-control-tested media is recommended. If in-house prepared culture media are to be used, they must be prepared according to a validated procedure and at least tested for sterility, pH, osmolality and endotoxicity.

Use of quality-controlled media, oil and disposables is recommended. If appropriate quality-control testing for IVF purposes is not provided, this must be performed by the laboratory itself or by a designated company. 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 according to European and/or national regulations.

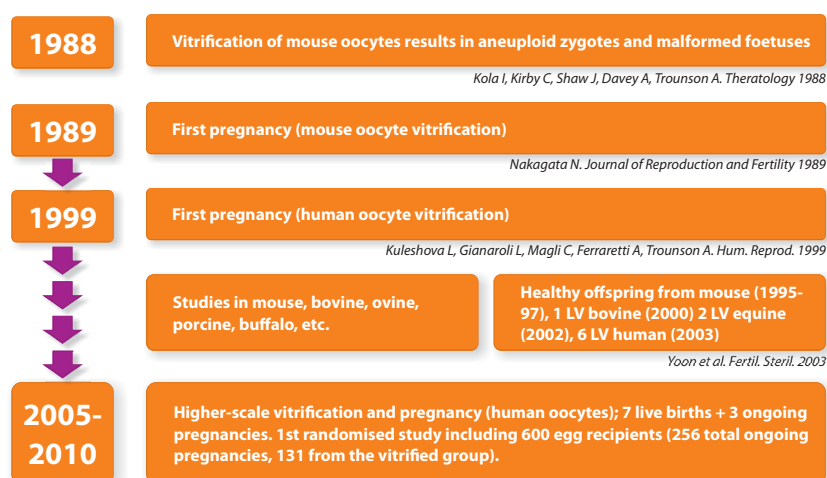
Figure 24.1. Validation of new technologies in assisted reproductive technologies

- a. Theoretical steps to be followed when introducing a new technology into clinical use in assisted reproductive technologies



Adapted from "When and how should new technology be introduced into the IVF laboratory?" *Hum Reprod* 2012.

- b. Example of a validation process in assisted reproductive technologies



Source: adapted from [2].

24.3. Recruitment of potential donors, identification and consent

24.3.1. Donor recruitment – non-partner donation

As with any tissues and cells, the donation of reproductive material should strictly follow the principles of voluntary and unpaid donation, as described in Chapter 3 (see 3.2.1.2, which specifically relates to ART).

National regulations will need to pay special attention to the existence of advertising and false or misleading promotion. In addition, the activities of tissue establishments related to donation should 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.

24.3.2. Donor consent

As mentioned above, ART can be undertaken with partner- or non-partner-donated gametes (partner donation or non-partner donation). Chapter 3 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. For partner donation also, fully informed written consent is mandatory, and this section describes additional aspects of these specific consent forms that should be addressed. In ART, consent forms may be separated for the female and male patients, although for certain treatments – and especially in partner donation – they could be combined in one document. It is important to emphasise that both partners need to sign these combined documents on partner donation. Examples of separate consent forms for ART treatment and storage are given in Appendices 9 and 10 for the female patient and in Appendix 11 for the male patient.

The couple (or individual) to be submitted to ART treatment should receive written and verbal information (during medical consultation with the physician or paramedical personnel, through information sessions, leaflets, website etc.) concerning the:

- a. possibility of adoption or foster care and alternative options for the couple to have a child;
- b. national legislation about ART and its implications for those who have access to assisted reproduction;
- c. possibility of withdrawal of consent to treatment;

- d. possibility of the physician not proceeding with the entire treatment (or some of its parts) for medical or deontological reasons;
- e. route of the ART cycle at each stage of its implementation;
- f. possible ethical issues regarding ART;
- g. possible psychological effects resulting from ART application;
- h. chances of success based on their medical history, the degree of invasiveness and the possible risks of the ART treatment (including multiple pregnancies). Specifically in IVF, 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);
- i. possible risks to the offspring resulting from ART, particularly in multiple pregnancies, and the limited follow-up data on the long-term health of those born from ART;
- j. testing for genetic and infectious disease and evaluation carried out in gamete donors in non-partner donation;
- k. possibility of cryopreservation and storage of gametes and supernumerary embryos, and the options for future use according to national legislation;
- l. total cost of the ART procedure, and existing reimbursement policies;
- m. in cases of non-partner donation and embryo donation, the implications of current national legislation about the possible anonymity of the donor and the possible right of the offspring to know their origins.

Psychological counselling should always be offered to the couple/patient.

ART treatment normally comprises a series of individual treatments, so consent forms for each ART treatment should be signed or be valid for consecutive ART treatments until the treatment is successful, until a predefined date, or until relevant circumstances change.

If the ART treatment is undertaken with cryopreserved gametes or embryos, consent for thawing should be given for each ART treatment. This policy would prevent a treatment from being initiated by either of the partners without the knowledge of the other.

In the consent form, the couple should state whether embryos or gametes should be cryopreserved or not. The couple giving consent to cryopreservation should receive information on the different options contemplated by national legislation on

the fate of cryopreserved gametes or embryos. The destiny of cryopreserved gametes or embryos could be to keep them for own reproduction, donation to other couples, donation for scientific research or destruction. The consent form could also specify the duration of storage.

There should be specific consent whenever additional methods beyond IVF/ICSI and cryopreservation are used. A very specific case in ART is the possibility, in some countries, of consent for the use of the remaining gametes or embryos after one of the partners has died ('posthumous donation'). This factor needs to be specified in the consent form.

A woman who enters IVF treatment could decide not to use all of her oocytes for her own ART treatment, but to donate some of them for reproductive purposes. This procedure is called 'oocyte sharing' and implies that this patient should also be considered as a donor in non-partner donation. In this case, the patient is considered a partner and a non-partner donor simultaneously. Screening of this patient should therefore be conducted as described in Chapters 4 and 5, and specifically for ART in 24.4.2 and 24.5.2 below.

All possible measures should be taken to avoid the possibility that women feel pressured to donate a portion of their oocytes in order to be able to receive the appropriate treatment.

24.4. Donor evaluation

24.4.1. Evaluation of partner donors

24.4.1.1. Interview

Couples who experience problems in conceiving should be evaluated together because infertility constitutes a medical problem for the couple. Both partners need to be aware of the decisions about their investigation so that they can support each other. Counselling before, during and after treatment is widely practised and is recommended since fertility problems, investigation and treatment can be a cause of psychological stress.

24.4.1.2. Taking of medical history and physical examination

Full medical history – including travel history, surgical, sexual, contraceptive, genetic, family and pregnancy history – should be taken from both partners. This information will help to determine the cause of the couple's infertility and the appropriate investigations to undertake, and will help to recommend the best course of treatment. A history of coital

frequency, dyspareunia and any sexual dysfunction should also be elicited from the couple. Both partners should also undergo a physical examination.

24.4.1.3. Screening of the female

a. Assessment of ovulation

A complete menstrual history should be taken, including age at menarche, cycle length and frequency, and the presence/severity of dysmenorrhoea. Ovulatory dysfunction can be due to hypothalamic, pituitary or ovarian dysfunction. Ovulation can be confirmed retrospectively by measurement of serum levels of progesterone in the mid-luteal phase of the cycle. Women with irregular menstrual cycles should also have serum levels of a number of hormones measured in the early follicular phase – i.e. follicle-stimulating hormone (FSH) and luteinising hormone (LH), prolactin, thyroid hormones – to assess hypothalamic pituitary function.

b. Assessment of ovarian reserve

Measurement of ovarian reserve may include biochemical tests and ultrasound imaging of the ovaries, especially antral follicle count (AFC). Biochemical tests may include basal measurements of FSH and anti-Müllerian hormone (AMH). No single test has 100 % sensitivity and specificity, so biochemical and imaging measurements are combined in an effort to improve their validity. However, there is insufficient evidence to recommend that the result of any single ovarian-reserve test should be the sole criterion for use in ART.

c. Assessment of tubal patency

Hysterosalpingography (HSG) can be performed as an outpatient procedure to assess tubal patency and uterine anatomy, and to assess the site of tubal blockage, if any. Hysterosalpingo-contrast sonography (HyCoSy) may be also used in an outpatient setting to assess tubal patency. Laparoscopy and chromopertubation test are methods used to assess and confirm the site, aetiology and degree of tubal blockage. Laparoscopy also allows the diagnosis of other conditions (e.g. endometriosis). Consideration should be given to testing of *Chlamydia trachomatis* in women with tubal damage.

d. Assessment of uterine abnormalities

Hysteroscopy may be considered if uterine abnormalities, such as submucous fibroids, polyps, adhesions or other Müllerian malformations (septae, bicornuate uterus), are

suspected on ultrasound, HSG or nuclear magnetic resonance. A concomitant laparoscopy may be indicated in certain circumstances.

e. Serology

Testing for immunity to rubella should be carried out before treatment. Vaccination should be offered to seronegative women before they commence an ART cycle.

24.4.1.4. Screening of the male

a. Semen analyses

At least one diagnostic semen analysis should be carried out before ART treatment. Procedures and reference values are described in the *WHO laboratory manual for the examination and processing of human semen* [3]. To confirm any abnormality detected, analyses of a second semen sample should be done. Results of semen analyses are used to determine the most appropriate ART method.

b. Investigations to determine cause of abnormal semen

Severe oligozoospermia (< 5 million sperm/mL) and azoospermia may be caused by testicular failure. Testicular failure may be due to trauma, infection, varicocele, gonadotropin deficiency, tumours e.g. testicular cancer, Hodgkin's lymphoma, leukaemia or associated gonadotoxic therapies, or to genetic abnormalities such as Klinefelter syndrome or Y-chromosome deletions. Men with azoospermia and severe oligozoospermia should be screened for the above-named genetic abnormalities. If a chromosomal abnormality is detected, appropriate genetic counselling should be offered. Cystic fibrosis or renal-tract abnormalities should be screened for in the presence of obstructive azoospermia. Besides genetic testing, there should be hormonal testing and a scrotal ultrasound performed in order to establish a diagnosis of testicular failure.

24.4.1.5. Inclusion/exclusion criteria for treatment

A full medical evaluation will determine if a couple is suitable for ART treatment. The risk-benefit analysis should be estimated on an individual basis.

The number of repeat cycles should be based on the individually estimated probability of a live birth.

24.4.2. 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 off-

spring (e.g. infectious disease, genetic disease) can be excluded. In addition, ensuring that the donation process does not cause harm to the health of the donor is equally important.

To donate his/her sperm/oocytes, the potential donor must be submitted to:

- a. consultation and counselling with a healthcare professional;
- b. completion of a health/medical-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. ABO (ABO) blood group and rhesus typing;
- g. genetic testing as indicated by family history and prevalence of carrier status in specific populations. Karyotype testing is strongly recommended; other extensive genetic screening for common recessive genetic mutations are now available and may be considered in order to reduce the risk of transmitting genetic disease to the child;
- h. semen analyses for sperm donors. Freeze-thaw test may also be recommended, to assess the quality of the sperm after freezing and thawing;
- i. assessment of ovulation and ovarian reserve (including endocrine work-up) in oocyte donors;
- j. informed consent before any procedure.

24.4.2.1. Exclusion criteria for oocyte donors

- a. Age < 18 years or > 36 years;
- b. Positive results in tests for genetic disease and/or infectious disease;
- c. Any risk factor to her own health;
- d. Unsuitability for donation based on interview.

24.4.2.2. Exclusion criteria for sperm donors

- a. Age < 18 years and > 45 years;
- b. Positive results in tests for genetic disease and/or infectious disease;
- c. Unsuitability for donation based on interview.

In cases in which embryos are donated, partners who have generated them should both be considered non-partner donors and comply with general criteria for non-partner donation in this section and in Chapters 4 and 5.

24.4.2.3. *Psychological examination (non-partner donation)*

In ART, psychological evaluation of non-partner donors is highly recommended and should focus on a psychological anamnesis (including but not limited to: looking at motivation, looking at pattern of personal stability, discussing the psychological ramifications of being a gamete donor, giving psychological guidance in the preparations for becoming a gamete donor) often in combination with a personality and/or psychological diagnostic test. The latter is mostly used in screening of anonymous gamete donors, and here standardised validated questionnaires are often used: the Symptom checklist (SCL-90), a multidimensional list of complaints for measuring mental and psychological symptoms; or the Utrechts Coping List (UCL), a measurement tool for coping. Others are freely available, such as the SIPP: Severity Indices for Personality Problems or the Rosenberg Self-esteem scale.

24.4.2.4. *Welfare of non-partner gamete donors*

The welfare of gamete donors is very important. For gamete donors it is important to counsel male and female donors about the psychological impact of having children if they have not yet had children themselves. Although the minimum age limit is 18 years old, it could be good clinical practice not to include very young gamete donors, but to recruit an older donor group who have proved their fertility. Although the donor can be unknown to the recipient, in some countries it is possible that the identity of the donor may be disclosed to the child when it is older. It is therefore also important, where applicable, to address the possibility of contact for future donor children for gamete donors and to make sure that agreements about future contact are clearly stated in the consent for donation.

Specific to female donors, the risk of OHSS should be minimised, as it should be in all women submitted to ART. The number of times an oocyte donor may donate should be determined by several factors: number of children and/or families achieved with this donor's gametes; medical and psychological risks to the donor; legislation in the country of donation. ART centres should be encouraged to accept female donors after they have achieved a successful pregnancy and consider the number of times a woman may donate by using appropriate registries.

24.5. Donor testing

The purpose of testing gamete donors is to prevent transmission of infectious and genetic diseases

from the donor to the recipient and offspring and to protect the staff while handling the patients and their gametes.

Testing of gamete donors will be discussed separately for each type of donation:

- a. partner donation
- b. non-partner donation.

Less strict biological testing is justifiable in the donation of reproductive cells between partners who have an intimate physical relationship (partner donation).

24.5.1. **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, tissue establishments should, based on analyses of risk, also carry out additional tests:

- d. syphilis (a treponemal-specific test or non-specific treponemal test can be used);
- e. testing for human T-lymphotropic virus (HTLV)-I 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 originate from those areas.
- f. 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* (CMV), *chlamydia* and *Trypanosoma cruzi* (infectious agent for Chagas disease).

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 partner donation. Robust procedures should be in place to prevent the risk of contamination to partner or to personnel, and 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.

If the tissue establishment 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.

24.5.2. Testing in non-partner donation

The following biological tests must be carried out for each donation:

- 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. in male donors: *Chlamydia trachomatis*. In the EU, this must be done from a urine sample by a nucleic acid test (NAT) but recent scientific data suggest ejaculate testing may be more sensitive.

In some cases, further tests may be required:

- f. testing for HTLV-I 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 originate from those areas.
- g. 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 CMV, antibody to *Trypanosoma cruzi*, Zika virus infection. Latest epidemiological updates can be found at the European Centre for Disease Prevention and Control (<http://ecdc.europa.eu/en/Pages/home.aspx>).

All serum 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 (unless NAT testing performed). Quarantine is not necessary if at each donation serology testing is combined with NAT for HIV, HBV and HCV. It is recommended that the same testing approach 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 could be considered as starting at the first day

of stimulation, and the sample for testing could be taken at that time.

24.6. Procurement/collection

24.6.1. Sperm

24.6.1.1. 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 the ejaculate can affect their survival, motility and fertilising ability, the start of diagnostic/therapeutic treatments 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 minimised. This situation calls for appropriate design and equipping of the laboratory and semen collection room. In general, patients are asked to collect a semen sample after 2-7 days of abstinence from ejaculation. 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 (preferably sperm-toxicity tested). 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. Collection should be preferably performed in a room near to the laboratory. After collection, the sample should be delivered to the laboratory as soon as possible avoiding extreme temperatures ($< 20^{\circ}\text{C}$ and $> 37^{\circ}\text{C}$). Analysis should start within one hour of collection.

Records should be kept of the type of container used, the time and place 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.

24.6.1.2. Retrograde ejaculation

In cases of retrograde ejaculation, where the sperm ends up in the urinary bladder after ejacu-

lation, sperm can be collected from the urine after voiding, where the urine pH has been increased by intake of bicarbonates.

24.6.1.3. *Collection by electro-ejaculation*

In some patients (e.g. 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. This procedure is carried out in an operating room, usually by a urologist.

24.6.1.4. *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 as described above.

24.6.1.5. *Collection of sperm from the epididymis*

Microsurgical epididymal sperm aspiration (MESA) is a surgical method for sperm collection if the vasa deferentia are blocked.

Percutaneous epididymal sperm aspiration (PESA) is a variant of this approach. It involves the use of a sterile needle to aspirate sperm from the epididymis without a surgical incision. Both approaches typically yield sufficient quantities of sperm for ICSI, but not enough for a standard IUI or IVF.

24.6.1.6. *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), and possibly by tissue removal (testis biopsy), and could be accompanied by a histopathology study. 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.

24.6.2. **Oocytes**

Oocyte collection for IVF, also known as oocyte retrieval, is the stage in IVF in which oocytes are collected from the ovaries. Before oocyte collec-

tion, the patient will be given hormonal treatment to stimulate the growth and maturation of the follicles in the ovaries (so-called 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 ovarian hyperstimulation syndrome (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.

Aspiration pressure and temperature should be controlled. The operating theatre where oocyte collection takes place should be adjacent to the IVF laboratory. If oocytes have to be transported to another centre for processing, validation of temperature in the transport unit is important.

24.6.3. **Ovarian tissue**

Collection of ovarian tissue is undertaken solely for fertility preservation purposes. This is addressed in Chapter 25.

24.7. **Processing**

Safety and quality issues covered in Chapter 7 also apply to the processing of human gametes and embryos. However, there are some specific issues that must be taken into consideration.

The following section is based largely on *Revised guidelines for good practice in IVF laboratories* by ESHRE [4]. These guidelines were drawn up by the Special Interest Group (SIG) in Embryology and published in 2016, and constitute the minimum requirements for any laboratory offering ART.

24.7.1. **Facilities for processing of gametes and embryos**

24.7.1.1. *Laboratory design*

The ART laboratory 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. Gametes and embryos are sensitive to adverse air quality, so 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 (see Figure 24.1).

Table 24.1. **Criteria to be considered in determining the air-quality specifications of assisted reproductive technologies processing facilities (EuroGTP guidance)**

Criterion	Explanation
Risk of contamination of tissues or cells during processing	Although very rare, accidental contamination during processing might occur. Working under oil in the laminar flow hood minimises this risk.
Use of antimicrobials during processing	Use of antimicrobials during processing reduces the risk of contamination. Culture media for processing of oocytes, sperm and embryos usually contain antibiotics (e.g. penicillin, streptomycin, gentamycin).
Risk that contaminants will not be detected in the final tissue or cell product due to limitations of the sampling method	Destruction testing is not possible in partner donation or in non-partner oocyte donation. In non-partner sperm donation, 5-10% of final-batch destruction testing is plausible. However, in ART, preliminary microbiological testing of donors before procurement and the methodology of processing are more important. Eventually additional testing of conditioned culture media can be of use to assess microbial contamination.
Risk of transfer of contaminants	Working under oil in the laminar flow hood minimises this risk. In ART, only cells in a minimum amount of culture media are transferred into the uterus, so the risk of contamination is very low. Furthermore, other measures such as cleaning of the cervix on transfer will help to reduce the risk of infection.

According to the European Union Tissues and Cells Directive (EUTCD), 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, it can be performed in at least a Grade D environment.

24.7.1.2. *Laboratory equipment*

All equipment must be validated as fit for its purpose, and its performance verified by calibrated instruments. All equipment should preferably be CE-marked.

The laboratory should contain all essential/critical equipment required for IVF, in numbers appropriate to the 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 the maintenance of a constant temperature during manipulation of gametes and embryos that are out of incubators must be in place (i.e. warm stages, heating blocks). Regular checks of critical parameters such as temperature and pH related to CO₂ 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 (LN₂).

24.7.2. **Handling of gametes and embryos**

As stated 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) 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 done outside the laminar hood since they need to be undertaken under an inverted microscope. Class-II hoods should be used for documented contaminated samples (e.g. HIV, HCV) because 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 pipets 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 24.13 on Biovigilance in ART).

24.7.2.1. *Oocyte processing*

Oocyte retrieval is a particularly sensitive procedure and special attention should be given to temperature and pH, as well as efficient and quick handling. An identity check before oocyte retrieval is mandatory. The time between oocyte retrieval and culture of washed oocytes should be minimal. Prolonged oocyte exposure to follicular fluid is not recommended. Appropriate equipment must be in

place to maintain oocytes close to 37 °C. Flushing medium, collection tubes and dishes for identifying oocytes should be pre-warmed. Follicular aspirates should be checked for the presence of oocytes using a stereomicroscope and heated stage, usually at 8-60 × magnification. Exposure of oocytes to light should be minimised. Timing of retrieval, number of collected oocytes and identity of the operator should be documented.

24.7.2.2. Sperm processing

Before starting a treatment cycle, at least one diagnostic semen analysis should be performed according to the protocols described in the WHO manual [3]. In addition, a test sperm preparation may be advisable in order to propose the most adequate insemination technique; an IVF/ICSI frozen back-up sample should be requested if sperm collection difficulty on the day of oocyte retrieval is anticipated.

Sperm preparation aims to:

- eliminate seminal plasma, debris and contaminants;
- concentrate progressively motile sperm;
- select against morphologically abnormal sperm.

An appropriate sperm preparation method should be chosen, according to the characteristics and origin of 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.

24.7.2.2.1. Specific treatments

Phosphodiesterase inhibitors (pentoxifylline, theophylline) or the hypo-osmotic swelling (HOS) test may be used in absence of motile sperm.

Enzymatic digestion of testicular tissue by collagenase may be applied if no sperm are observed.

24.7.3. Insemination of oocytes

Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time should be decided based on 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.

24.7.3.1. Conventional *in vitro* fertilisation

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 $0.5 \times 10^6/\text{mL}$ is used.

The final sperm suspension should be in a medium compatible with oocyte culture.

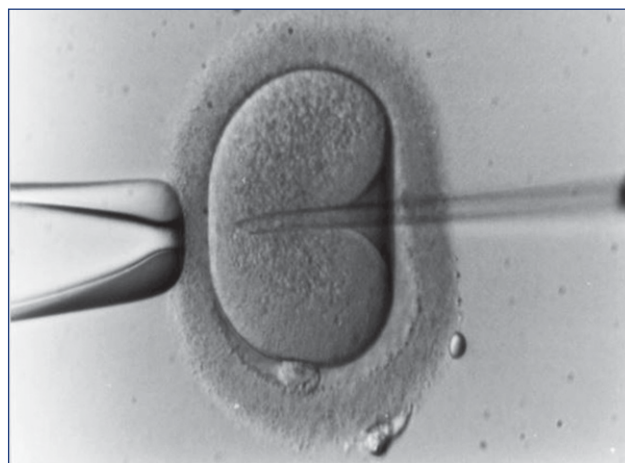
Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient.

24.7.3.2. Intracytoplasmic sperm injection procedure

24.7.3.2.1. 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.

Figure 24.2. Intracytoplasmic sperm injection



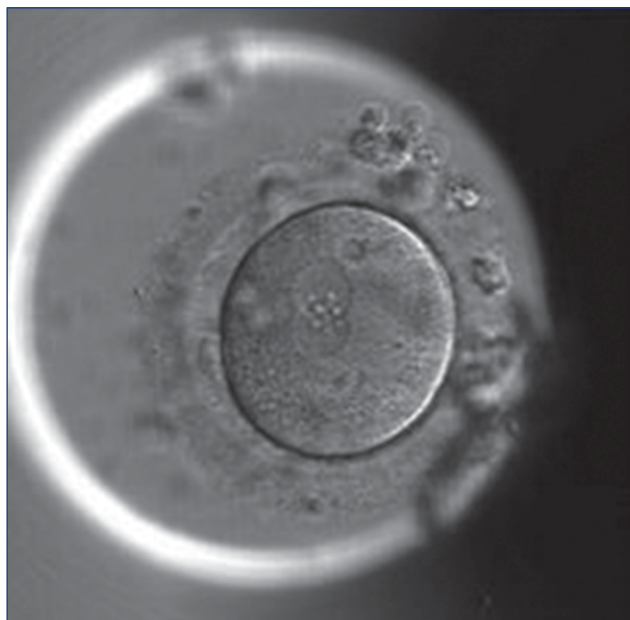
Source: Image provided by María José De los Santos Molina (Spain).

24.7.3.2.2. The injection procedure

See Figure 24.2. Records should be kept 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. The number 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) can be used to facilitate sperm

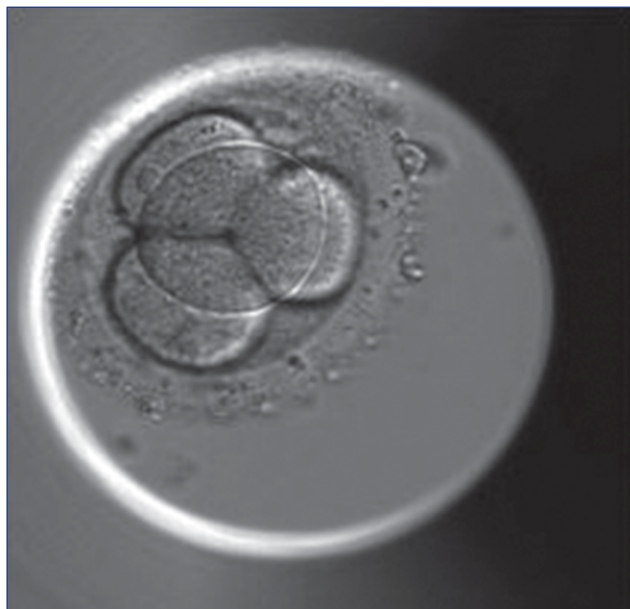
manipulation. In case of only immotile sperm cells, a non-invasive vitality test can be used to select viable sperm for injection. After injection, oocytes should be washed prior to culture.

Figure 24.3. Zygote with 2 pronuclei and 2 polar bodies



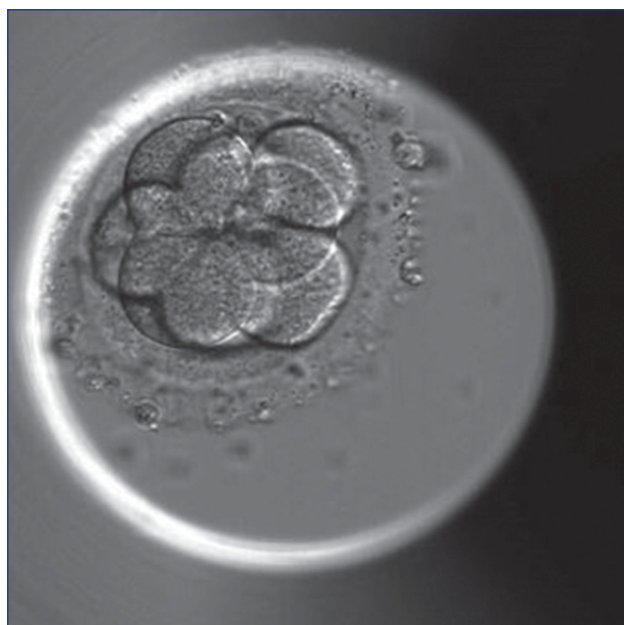
Source: Image provided by María José De los Santos Molina (Spain).

Figure 24.4. Embryo at the 4-cell stage



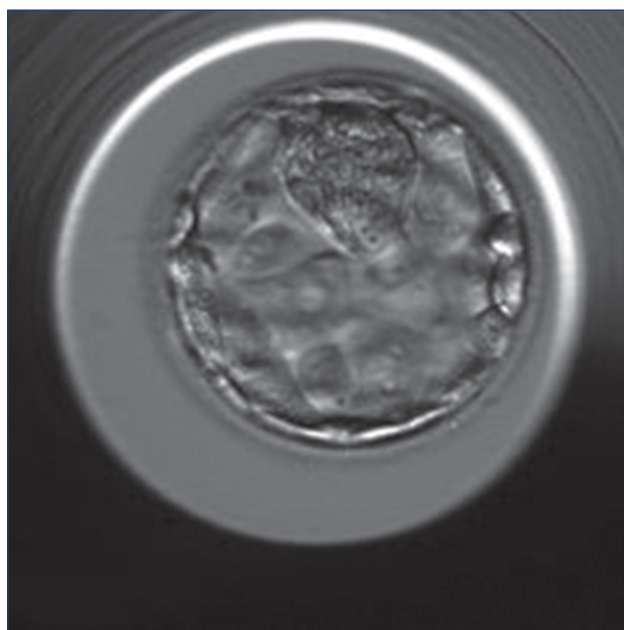
Source: Image provided by María José De los Santos Molina (Spain).

Figure 24.5. Embryo at the 8-cell stage



Source: Image provided by María José De los Santos Molina (Spain).

Figure 24.6. Embryo at the blastocyst stage

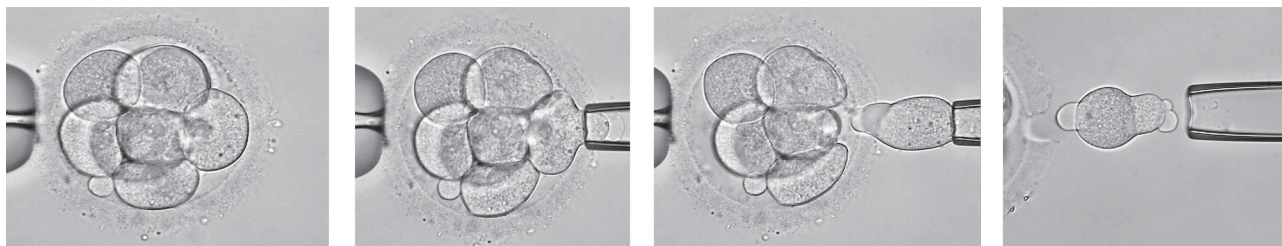


Source: Image provided by María José De los Santos Molina (Spain).

24.7.4. 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 2PN and 2 polar bodies (Figure 24.3). For conventional IVF, cumulus cells must be removed and 2PN oocytes transferred into new dishes containing pre-equilibrated culture medium.

Figure 24.7. Embryo biopsy of an 8-cell stage embryo



Source: Image provided by María José De los Santos Molina (Spain).

Fertilisation assessment should be performed under high magnification (at least 200×), using an inverted microscope equipped with Hoffman or equivalent optics, in order to verify number and morphology of pronuclei.

24.7.5. Embryo culture and transfer

In order to optimise embryo development, fluctuations of culture conditions should be minimised. Precautions must be taken to maintain adequate conditions of pH, temperature and osmolarity, 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, size and symmetry, percentage of fragmentation, granulation, vacuoles and nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade, blastocoel cavity size and morphology of the inner cell mass and 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 [5].

Embryo quality assessment records should include the operator(s), date and time of assessment, and embryo morphological characteristics. Embryo morphological characteristics and developmental stage should be noted (Figures 24.4, 24.5, 24.6).

Embryo selection for transfer is primarily based on developmental stage and morphological aspects. Other selection parameters, such as time-lapse kinetics, may be considered.

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. It is advisable not to transfer more than two embryos.

Supernumerary embryos may be cryopreserved, donated to research or discarded, according to their quality, patient wishes 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 the patient, the patient file and the culture dish(es) is mandatory immediately prior to the transfer.

24.7.6. 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 carried out by:

- a. removal of polar bodies;
- b. blastomere biopsy at day 3 (see Figure 24.7);
- c. trophectoderm biopsy at the blastocyst stage.

Cell(s) destined for genetic investigation are removed in the IVF laboratory using glass microtools on a micro-manipulation set. The embryology laboratory has the responsibility of providing unique identification between biopsied polar bodies, blastomeres or trophectoderm cells and the corresponding oocyte, embryo or blastocyst, respectively. All cells and embryos for genetic investigation must be handled individually, avoiding DNA contamination from other cells from the samples or the operator. They must be identified and labelled carefully, and tracked during the entire procedure. During these steps, double identity checks are necessary. The biopsy sample should be subjected to diagnostic procedures in a genetic laboratory.

The purpose of PGT-M (for monogenic/single gene defects) and PGT-SR (for chromosomal structural rearrangements) is to identify embryos generated *in vitro* that carry certain hereditary genetic diseases or chromosomal abnormalities and exclude them from transfer. These tests are an alternative to therapeutic abortion due to a minimised risk of transferring affected embryos.

Genetic counselling should be available to all couples known to carry a hereditary disease. The recipient should be informed that genetic testing on embryos does not substitute for prenatal analysis, such as amniocentesis, due to mosaicism of the tested embryos and the limitations of the test.

PGT-A (pre-implantation aneuploidy screening) is used to check if an embryo has the correct number of chromosomes, with no known hereditary genetic indication. PGT-A 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. Bearing in mind the conflicting 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.

In some countries, PGT may not be not allowed or allowed only in specific circumstances according to national legislation.

24.7.7. *In vitro* maturation

In vitro maturation (IVM) refers to the maturation in culture of immature oocytes in special media after recovery from follicles that may or may not have been exposed to exogenous gonadotropins before retrieval. During IVM, such oocytes progress from prophase I – i.e. from germinal vesicle (GV) – to reach metaphase II (MII) [6]. However, reaching the morphological criterion for MII (release of the first polar body) does not necessarily mean 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 should be provided to patients.

24.7.8. Processing of samples from seropositive donors

24.7.8.1. *Non-partner donation*

If initial testing for transmissible infections and/or genetic diseases proves a donor to be positive, they must be excluded from the donor programme.

24.7.8.2. *Partner donation*

In couples with one or both partners being seropositive, ART 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.

Discordant couples should be treated by ART.

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 females with seropositive partners should be offered vaccination before ART [7].

Good quality and safety laboratory practices in assisted reproductive technologies for serodiscordant couples are summarised in Figure 24.8.

24.8. Cryopreservation and storage

24.8.1. Methods for cryopreservation of human gametes and embryos

Sperm, embryos and, more recently, oocytes are being cryopreserved for future use in ART (supernumerary embryos or oocytes, 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.

Slow freezing is a method using programmed step-wise decreases in the temperature of the solution in which the specimen is cryopreserved. This 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 temperature drops (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. This is a fast method that does not require special equipment (but special consumables are required) but with the addition of specific cryoprotectants in higher concentrations (compared to those in slow freezing) for shorter exposure times.

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 (anti-freeze substances), osmotic damage and incomplete dehydration, all having an effect on the cell viability.

There are significant differences in the sensitivity of different types of male and female gametes and different-stage embryos concerning the cooling process and cryoprotectant agents used [8, 9, 10, 11, 12].

Figure 24.8. **Good quality and safety: laboratory practices in assisted reproductive technologies for serodiscordant couples**

Woman Hep B –, Man Hep B + • Vaccinate woman • Last retrieval of the day • Use Class II cabinet (sperm processing) • No PCR in semen if woman successfully vaccinated • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container	Woman Hep B +, Man Hep B + • No need of vaccination • Last retrieval of the day • Use Class II cabinet (egg retrieval and sperm processing) • No PCR in semen • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container	Woman Hep B +, Man Hep B – • Vaccinate man • Last retrieval of the day • Use Class II cabinet (egg retrieval) • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container
Woman Hep C –, Man Hep C + • Successful antiviral therapy man • Last of the day • Use of Class II cabinet (sperm processing) • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container	Woman Hep C +, Man Hep C + • Successful antiviral therapy both • Last of the day • Use of Class II cabinet (egg retrieval and sperm processing) • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container	Woman Hep C +, Man Hep C – • Successful antiviral therapy woman • Last of the day • Use of Class II cabinet (egg retrieval) • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container
Woman HIV –, Man HIV + • Effective anti-retroviral therapy • Low viral load • Use Class II cabinet (sperm processing) • PCR in washed semen, if negative: standard culture	Woman HIV +, Man HIV + • Effective antiretroviral therapy (both) • Low viral load • Last of the day • Use Class II cabinet (egg retrieval and sperm processing) • PCR in washed sperm • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container	Woman HIV +, Man HIV – • Effective antiretroviral therapy • Low viral load • Last of the day • Use Class II cabinet (egg retrieval) • Use of specific incubator • Cryostorage: Use of specific Liq N2 container or closed systems or Gas phase container

Source: Viral screening of couples undergoing partner donation in assisted reproduction with regard to EU directives 2004/23/EC, 2006/17/EC and 2006/86/EC: what is the evidence for repeated screening? *Hum Reprod* 2010, 25: 3058.

24.8.1.1. Cryopreservation of sperm

For sperm, slow freezing is still the method of choice, but rapid cooling is a possible alternative [9, 10].

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 or incubated in liquid nitrogen vapour and then plunged in the liquid phase.

Immotile and damaged sperm can 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.

24.8.1.2. 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 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 [12, 13].

24.8.1.3. Cryopreservation of embryos

Zygotes, early-cleavage embryos, morulae and blastocysts have been cryopreserved successfully and used later for 'frozen embryo transfer'. Slow-freezing and vitrification can be used [14, 15, 16], with vitrification/warming in dimethyl sulfoxide-based cryoprotectants resulting in better survival rates. Exposure time to the cryoprotectant before vitrification is crucial and must be strictly respected.

24.9. Storage

Regarding the facilities, the main aspects to be considered are location, air quality and construction materials.

From a practical point of view the storage room with the liquid nitrogen tanks should be located close to the IVF laboratory, so the cryopreserved gametes or embryos can be easily, rapidly and successfully transferred to the storage room and into the liquid nitrogen tanks.

As far as dimensions are concerned, the storage room should be designed to allocate enough tanks to store the expected number of samples. Some experts suggest calculating the space required on the basis of a 10-year linear increment in projected demand, or to have an off-site storage room in case of urgent need of extra space.

As regards temperature, even though room temperature (22–23 °C) should be adequate, setting up the room under a cold temperature might help to minimise the liquid nitrogen evaporation and water condensation that can facilitate microbial growth. Another approach can be to set up a humidity controller.

A low-levels oxygen sensor and adequate alarm system in case of liquid nitrogen leaks must be put in place for safety reasons. 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 must be in place.

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.

It is convenient to have a large-scale supply of liquid nitrogen as well as the use of temperature-isolated pipes to minimise the evaporation of liquid nitrogen during refilling manoeuvres.

24.9.1. 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 [17, 18]. However, at defined time points, contact with

patients should be made to determine the destiny of their cryopreserved material. Regular audits of all stored materials are strongly recommended.

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 section 24.3.2 on donor consent).

A periodic inventory of the contents of the cryobank is strongly recommended, including cross-referencing contents with storage records.

24.9.2. Storage temperature

Storage in liquid nitrogen or liquid nitrogen-vapours is common practice. Optimal storage temperature is based on the type of tissue, cryoprotectant and freezing method used. However, a temperature < -136 °C for gonadal tissue, embryos and gametes is appropriate, and > -130 °C is detrimental to the survival and quality of the material frozen.

24.9.3. 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. Embryos and oocytes are stored in straws, whereby one straw can hold one or more embryos or oocytes. It is, however, advisable to store only one embryo per straw to encourage single 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.

24.9.4. 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 of these environmentally induced pathogens to 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. Even though

evidence is lacking [19], 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.

24.9.5. 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 each ART centre. 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, goggles) and use special forceps for manipulation of straws.

24.10. Distribution import/export

During transport of gametes and embryos, measures need to be taken to ensure the quality and safety of reproductive tissues and cells. Before transport, some specific actions need to be taken using the appropriate documents:

- A signed transport agreement between exporting and receiving institutions;
- A protocol of acceptance to check for possible damage to container, samples and patients identification, and presence of valid documentation;
- Signed consent for sample transportation by patients or by institutions when necessary.

24.11. Packaging and labelling in assisted reproductive technologies

As addressed in Chapter 13, 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 24.9.3.

Labelling is intended to identify gametes and embryos unambiguously. Labelling and identification systems may vary between centres and countries. As mentioned in section 24.7.2, procedures must be in place that ensure correct identification of patients at all stages of handling, comprise at least two points of identification (e.g. treatment number, name, 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 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).

Cryo-devices must be clearly and permanently labelled with reference to patient details, treatment number and/or a unique identification.

Likewise, at thawing, documentation on biological material should include thawing method, date and time of thawing, identity of operator and post-thawing sample quality.

24.12. Traceability

Identification of patients and traceability of their reproductive cells are crucial aspects of ART 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, which has to clearly and easily refer to the patient's documentation. Each treatment cycle must be assigned a unique identification.

Corresponding 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 the correct identification and processing of reproductive cells must be established in the laboratory by a system of codes and checks including:

- a. Direct verification of patient identity and correspondence with their assigned unique identification is required at every critical step. Patients should be directly asked to give their own identifying information (at least full name and date of birth) before procurement or artificial 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, a double-check by a second person (witness) and/or an electronic identification system is strongly advised.
- g. Products and materials used with biological materials must be traceable. The date and time of each manipulation and 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 13, section 13.2.3).
- 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 for 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. Tissue establishments that store and distribute non-partner gametes should label containers with an appropriate unique donation identification. In the EU, the coding requirements for non-partner donation apply (see Chapter 13, sections 13.2.3.1-2).

24.13. Biovigilance in assisted reproductive technologies

Biovigilance in ART involves not only vigilance in terms of tissues and cells, but also pharmacovigilance (e.g. OHSS) and vigilance in terms of medical devices (e.g. media, incubators, aspiration needles).

In ART, the following specific characteristics of reproductive cells/embryos must be considered:

- a. oocytes and embryos are available in (very) limited numbers;
- b. reproductive cells are particularly sensitive to external factors (temperature, culture media, laboratory equipment, pollutants);
- c. any defect does not only have an impact on the recipient of the cells but could also affect the offspring;
- d. adverse outcomes are, in general, associated with a loss of gametes or embryos and subsequent loss of chance of pregnancy (unlike other tissues or cells: illness, disability).

Among other aspects, ART biovigilance should focus specifically on:

- a. sensitivity of gametes/embryos (impact of equipment, media and environment);
- b. traceability;
- c. mix-ups;
- d. complications during procurement;
- e. cross-border management of serious adverse reactions and events (SARE).

Serious adverse events (SAE) and serious adverse reactions (SAR) are defined in Directive 2004/23/EC for the EU. See also Chapter 15 of this Guide, on Biovigilance, for further explanations. Mis-identifications and mix-ups as well as total loss of reproductive cells, gonadal tissues and embryos for one cycle should be considered as SAE in the context of ART.

The definition of SAR in the context of ART should be extended to the offspring in non-partner donation for transmission of genetic diseases (see section 24.13.2-3).

24.13.1. General criteria for reporting serious adverse reactions and events in assisted reproductive technologies

In ART biovigilance, deviations from SOPs in tissue establishments or other adverse events that may influence the quality and safety of tissues and cells should result in SARE reporting to the Health

Authority if one or more of the following criteria apply:

- a. inappropriate gametes, embryos or gonadal tissues have been released for clinical use (even if they are not used);
- b. events that could have implications for other patients or donors because of shared practices, services, supplies, critical equipment or donors;
- c. a mix-up of gametes or embryos;
- d. loss of traceability of gametes or embryos;
- e. contamination or cross-contamination;
- f. accidental loss of gametes, embryos or gonadal tissues (e.g. breakdown of incubators, accidental discards, manipulation errors) resulting in a total loss of chance of pregnancy for one cycle.

Mix-ups can result from an error in the attribution of reproductive cells or embryos, which can occur at any stage of clinical or laboratory processes (collection, insemination, embryo transfer, cryo-preservation). Reasons for mix-ups (e.g. multiple processing steps, mislabelling, too many people involved, misidentification, overworked staff, absence/failure of witnessing, poor-quality systems) and the consequences of mix-ups (loss of traceability, loss of gametes or embryos, disease transmission, psychological impact, ethical/legal issues) are diverse and should be reported to the national Health Authority immediately, no matter at which stage they were detected. Causal factors should be investigated.

All patients involved should be informed as soon as possible and should be offered counselling and support.

Hospitalisation of oocyte donors should be considered as an adverse reaction (non-serious adverse reaction if it is for observation only).

In the EU, Health Authorities must be notified without delay of all SARE which may influence the quality and safety of tissues and cells, and which may be attributed to the procurement, testing, processing, storage and distribution of tissues and cells, as well as any SARE observed during or after clinical application which may be linked to the quality and safety of tissues and cells. It is highly recommended that Health Authorities should be notified of any SAR involving harm to donors.

All SARE related to stimulation and procurement (especially severe OHSS) should be reported to Health Authorities. Co-ordination between various systems of vigilance (tissues and cells, pharmacovigilance, medical-device vigilance) should be established by Health Authorities.

24.13.2. **Transmission of genetic diseases by assisted reproductive technologies with non-partner donations**

Non-partner donors may unknowingly carry genetic defects. Sperm banks, for example, could supply sperm from a donor to multiple recipients, thereby spreading genetic disease. Tissue establishments should keep in mind genetic diseases when educating non-partner donors and recipients of non-partner donations. National registries of non-partner donors are strongly recommended.

Non-partner donors should be strongly advised to inform the procurement centre/tissue establishment if they are diagnosed with a genetic abnormality. Recipients of non-partner donations should be advised to inform any doctor 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. If necessary, the distribution of gametes from that donor could be discontinued. These examples emphasise that forward and backward traceability is of the utmost importance in ART.

24.13.3. **Examples of serious adverse reactions and events in assisted reproductive technologies**

The Notify Library includes many well-documented cases of adverse occurrences in ART.

- A case of spinal muscular atrophy in a child born from an embryo created by an anonymous oocyte donor and an anonymous sperm donor. It was confirmed by molecular genetic investigations that both anonymous donors were carriers of one copy of the SMN1 deletion mutation (Record Number 92).
- A case of embryo mix-up detected after embryo transfer that resulted in a medical abortion (Record Number 978).
- A serious adverse event in which embryo-toxic oil was used during culture that led to degeneration of 11 embryos from three couples (Record Number 981).

For further evaluated cases of adverse outcomes in ART, search the Notify Library at www.notifylibrary.org. The database is publicly accessible and can be searched by substance, adverse occurrence and record number.

24.13.4. 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 SARE might occur and do not get reported to the professionals who carried out the treatment and 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 SARE to their national Health Authorities even for cross-border treatments.

24.14. Final considerations

Fair, clear and appropriate information must be provided at all stages of ART treatment. The chances of success (including the live-birth rate) should be discussed appropriately. Clinicians, embryologists, technicians, nursing staff and all professionals need to communicate frequently 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.

24.15. References

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Related documents:

Appendix 7. Examples of method validation (oocyte vitrification) in assisted reproductive technology;

Appendix 9. Sample consent form (women);

Appendix 10. Sample consent form (women);

Appendix 11. Sample consent form (men).

Chapter 25. Fertility preservation

25.1. Introduction

Fertility preservation involves actions taken 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 cytotoxic therapy treating a severe disease or for a number of possible reasons for postponing parenthood, this involves cryopreservation of gametes, gonadal tissue or embryos.

Fertility preservation techniques are usually proposed 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 individuals for non-medical reasons, such as to postpone parenting, previous to vasectomy or other reasons. Initially, fertility preservation was developed for cancer patients because treatments for cancer are always evolving and cancer survival rates are constantly increasing.

This chapter describes the indications for male and female fertility preservation and the techniques actually available for the cryopreservation of gametes and germinal tissue. The collaboration between paediatricians, oncologists and reproductive specialists is essential to ensure proper evaluation and counselling for each patient. Donor assessment and approach will depend on disease, age and treatment, and information about possible options and future use of cryopreserved gametes or germinal tissue should be discussed with potential donors, or parents (in the case of minors). It is important to realise that fertility preservation and restoration include tech-

niques that are in an experimental state, and their availability may be restricted according to national legislation.

This chapter must be read in conjunction with Chapter 24 on ART and the following chapters in Part A of this Guide:

- a.* Introduction (Chapter 1);
- b.* Quality management, risk management and validation (Chapter 2);
- c.* Recruitment of living donors, identification and referral of possible deceased donors, and consent to donate (Chapter 3, but only for non-partner donors);
- d.* Donor evaluation (Chapter 4);
- e.* Donor testing (Chapter 5);
- f.* Processing and storage (Chapter 7);
- g.* Premises (Chapter 8);
- h.* Principles of microbiological control (Chapter 9);
- i.* Distribution and import/export (Chapter 10);
- j.* Organisations responsible for human application (Chapter 11);
- k.* Computerised systems (Chapter 12);
- l.* Coding, packaging and labelling (Chapter 13, but only for non-partner donors);
- m.* Traceability (Chapter 14);
- n.* Biovigilance (Chapter 15).

25.1.1. Female fertility preservation

Female fertility preservation should be considered whenever fertility loss is predicted as a consequence of a cytotoxic treatment for a specific

disease (e.g. in cancer patients) or due to the disease itself (malignant or non-malignant, e.g. endometriosis). Non-medical fertility preservation can be also considered in young women who want to postpone maternity (age-related fertility preservation) [1], and transsexual female-to-male patients may undergo oocyte collection and storage before sex reassignment surgery.

All girls and women newly diagnosed with specific medical conditions (e.g. certain cancers or rheumatoid arthritis) the treatment of which may cause premature ovarian insufficiency (POI) should be offered fertility preservation. Fertility preservation should ideally be offered before treatment is started, but should not delay treatment.

In the case of cancer patients, all girls and women at reproductive age newly diagnosed should be referred to a fertility expert to be counselled about the risk of infertility and informed about fertility preservation. Not all cancer treatments result in a high risk of POI, but women about to receive protocols that include high doses of alkylating agents, haematopoietic progenitor cell (HPC) transplantation or abdominal or spinal radiation should be offered fertility preservation. This also applies to prepubertal girls.

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 the ovaries are exhausted of follicles, the patient will experience POI and infertility. In the case of prepubertal girls, loss of the entire stock of primordial follicles will mean 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 to reduce the quality of life significantly. 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 effect the most damage to the ovaries. Alkylating agents, such as cyclophosphamide or busulfan, are used to treat various cancer forms, including breast cancer, lymphoma and sarcoma, and are also used in the preconditioning protocol before 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, as this is the most immature and 'dormant' type of follicle [2].

Radiation therapy, whether given to the abdomen or the spine, will also affect the ovaries. Radiation therapy is very toxic to the oocytes, and doses as low as 2 Gy will destroy half of the pool of follicles. Whenever possible, the ovaries will be shielded or moved away from the field of radiation, but scatter dose is inevitable [3].

The risks of undergoing fertility preservation, including possible delay of the cancer treatment, should be weighed against the benefits of having oocytes stored for future use. Sometimes the patient's health is too impaired by the disease to justify 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 fertility preservation.

When surgery to the ovaries is planned, as in the case of endometriosis or benign ovarian cysts or borderline cysts, healthy ovarian tissue containing primordial follicles will inevitably be excised in connection with the operative procedure. These procedures may pose some threat to the reproductive potential of the patient, and in these cases fertility preservation should also be offered [4].

Certain genetic conditions – such as Turner mosaicism, galactosaemia, Fragile X mutation carrier status or blepharophimosis, ptosis or epicanthus inversus syndrome – will cause 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 fertility preservation.

Several options exist to preserve fertility in postpubertal girls and women. Oocytes, ovarian tissue and embryos can be cryopreserved depending on the characteristics of each individual case and considering the most efficient alternative for every patient. Examples of consent forms for female fertility preservation are given in Appendices 9 and 10.

Finally, transsexual men planning to undergo sex reassignment surgery may benefit from fertility preservation.

25.1.2. Male fertility preservation

Fertility preservation is indicated in all boys and men facing gonadotoxic treatment or surgical procedures affecting semen production and deposition, and all patients at risk of fertility loss should be informed about fertility preservation options. Apart

from these indications, other groups with reasons to choose fertility preservation can be men in military services and transsexual male-to-female patients wishing to store semen before undergoing sex re-assignment surgery.

Chemotherapy 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. Dividing 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 and are released from the seminiferous epithelium [5].

Significant damage is reported after treatment with alkylating agents, and different thresholds are given in the literature (e.g. for cyclophosphamide and cisplatin-based drugs). Both alkylating- and platinum-containing agents cause direct DNA and RNA damage, and so can affect even 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 [6].

25.2. Consent in fertility preservation

After recruitment and identification of the patients, informed consent for fertility preservation should be obtained by a clinician. However, since prepubertal children are also recruited for fertility preservation techniques, informed consent should in this situation be signed by the parents or legal guardians of the child. It is important that, in the case of fertility preservation for prepubertal children, care should be taken to explain the future use of banked gonadal tissue.

Individual countries may have their own legislation regarding fertility preservation, and therefore consent forms can differ. An example of a generic consent form for cryopreservation of sperm is given in Appendix 11. This form can be used as a template that can be adjusted according to national legislation or common practice of the ART centre.

25.3. Patient evaluation

Patient evaluation of postpubertal women or men undergoing fertility preservation is similar to patient evaluation for patients undergoing ART techniques (Chapter 24). The future use of the stored gonadal tissue or gametes is eventually in ART techniques with the aim of obtaining embryos in partner donation treatment.

Patient evaluation for prepubertal boys and girls needs special care in cases where gonadal tissue is removed and banked. Close collaboration between paediatric, surgical, oncologic and fertility specialists is essential concerning fertility preservation in prepubertal children. When the patients are first seen in the oncology department, the awareness of paediatric oncologists needs to be raised about the fertility preservation options. Clear instructions on whom to contact in the infertility department to discuss the matter on an individual patient basis, unrestricted access to educational patient information, and rapid and flexible access to medical consultation and surgical biopsy for tissue recovery and storage, should be provided to accommodate the short timescales available to these patients before starting their ablative treatment.

To minimise trauma to the patient, the surgical recovery of gonadal tissue should be combined with other interventions requiring anaesthesia, such as bone marrow sampling or implantation of venous ports. Close interdisciplinary co-operation between paediatric oncologists and gynaecologists, urologists or paediatric surgeons is required. In the case of prepubertal boys, measurement of testicular volume is helpful in predicting the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been induced – are comparable to those of adult patients, irrespective of the underlying disease [7, 8]. In the case of prepubertal girls, only the assessment of the ovarian reserve by Anti-Müllerian Hormone (AMH) is to be investigated. No further gynaecological investigations should be performed, since they can be perceived as intrusive and an emotional and psychological burden for these patients.

25.4. Procurement

25.4.1. Female

25.4.1.1. Ovarian tissue

Collection of ovarian tissue can be performed at any time of the menstrual cycle and can be done on short notice.

By the procurement of ovarian tissue, thousands of follicles can be preserved. The 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 semi-ovary or ovarian cortical biopsies are removed by an operative procedure under general anaesthesia and prepared for cryopreservation [9].

Ovarian tissue procurement is offered to prepubertal girls, and to postpubertal girls not ready to undergo ovarian stimulation, endovaginal 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. Despite the possibility of storing ovarian tissue at the time of sex reassignment surgery, the consent forms should highlight the present technical constraints related to its future use.

25.4.1.2. Oocytes

In order to collect oocytes, controlled ovarian stimulation is needed. This stimulation is similar to the stimulation for IVF (Chapter 24). 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 anytime in the menstrual cycle, including in the luteal phase, with apparently good results. Any premenopausal patient with a sufficient ovarian reserve can be considered for oocyte collection for fertility preservation. Postpubertal girls may sometimes be able to undergo ovarian stimulation and tolerate endovaginal ultrasound monitoring and oocyte retrieval.

Oocytes will be collected by aspiration via the transvaginal route following the same steps previously described in Chapter 24.

25.4.2. Male

25.4.2.1. Testicular tissue

Testicular tissue is mostly procured in prepubertal 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 amount of tissue procured for fertility preservation will have an effect on future testosterone production and hormone replacement therapy could possibly be needed. 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 prepubertal children can be limited, one third of the testis is generally retrieved. Immunohistochemical staining is necessary to assess the presence of SSC in the procured and stored tissue [10].

25.4.2.2. Sperm

Sperm samples are mostly obtained through masturbation.

Sperm samples can be collected in adult men, postpubertal boys and in peripubertal boys if the patient is ready to obtain a sample through masturbation [11, 12, 13, 14]. 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 postpubertal boys how to produce a sample by ejaculation, since not all patients are already sexually active.

25.5. Processing and storage

ART tissue establishments such as ART centres and banks can process and store gonadal tissue and gametes for fertility preservation. The techniques for processing and storage are described in Chapter 24.

Processing and storage of gonadal tissue require a tissue establishment with the facilities, licence and expertise to perform the procedure, and process and store the tissue. These are described in more detail below.

25.5.1. Female

25.5.1.1. Ovarian tissue

Ovarian tissue should be transported on ice in a transport medium (e.g. Leibovitz L-15), sup-

plemented with serum albumin. 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 to the required thickness of, on average, 1–2 mm. The cortex is subsequently cut into smaller fragments (5 × 5 mm). These fragments are then treated with a cryoprotectant (dimethyl, DMSO) to protect the cells from cryodamage and generally subjected to controlled slow freezing in a programmable controlled-rate freezer [15]. Vitrification of the ovarian tissue is another optional methodology.

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 enclosed oocytes (COC). These COC can be collected and subjected to *in vitro* maturation in order to obtain MII oocytes that can be collected and stored. This collection, *in vitro* maturation and storing of oocytes obtained during the processing of ovarian tissue is considered a highly innovative fertility preservation technique, since so far only one live birth has been described in Europe [16]. However, it opens the possibility of maximisation of fertility preservation in the case of ovarian tissue procurement and storage.

Transport of the collected tissue from different centres to a centralised tissue establishment is a realistic and efficient system to be considered [15].

25.5.1.2. Oocytes

Oocyte cryopreservation is the preferred option for fertility preservation in postpubertal 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 [17, 18]. The methodology is described in Chapter 24.

25.5.1.3. Embryos

Although oocyte cryopreservation is generally practised today, embryo cryopreservation can also be considered for fertility preservation in the case of couples. However, cryopreserved embryos will not be available for future use if the couple separates.

The permitted storage period of cryopreserved oocytes and embryos varies according to national legislation.

25.5.2. Male

25.5.2.1. Ejaculate

Sperm cryopreservation is performed for male fertility preservation in postpubertal 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 in predicting the chances of successful retrieval of spermatozoa and semen production in adolescents, whose semen parameters – as soon as spermatogenesis has been induced – are comparable to those of adult patients, irrespective of the underlying disease.

The methodology for sperm cryopreservation is described in Chapter 24.

25.5.2.2. Testicular tissue/epididymus

For prepubertal boys and also for azoospermic patients, surgical sperm retrieval by testicular sperm-extraction procedures should be offered. Sperm can be retrieved by microsurgical aspiration of the epididimal fluid or by testicular biopsy/testicular sperm aspiration (TESA) or testicular sperm extraction (TESE).

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. Since the reproductive potential of cryopreserved immature testicular tissue has still to be demonstrated in humans, the technique remains experimental.

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). Processing of the testicular tissue consists of cutting the tissue into small fragments, submerging the pieces in medium supplemented with a cryoprotectant (DMSO) to protect the cells from cryodamage and then subjecting them to controlled slow freezing. Vials are thereafter submerged into liquid or vapour-phase nitrogen.

25.6. Fertility restoration

25.6.1. Female fertility restoration

Once a woman has been cured of her cancer or other health condition, and her treating physician considers it safe for her to attempt a pregnancy, she can request making autologous use of her stored ovarian tissue or oocytes/embryos for ART treatment.

Transplantation 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. 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. Spontaneous pregnancies can occur after the follicles start maturing and she gets her cycle back, 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. In certain kinds of cancers, transplantation of the cryopreserved tissue is not possible due to the risk of re-introducing the original disease. This is to be considered especially in the case of leukaemia, where 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 also be advised against transplanting the tissue [19, 20, 21].

If oocytes or embryos have been cryopreserved, an embryo replacement cycle must be planned. If she is menopausal, her endometrium will be prepared in an artificial cycle.

It has to be emphasised that a significant percentage of women will experience a spontaneous return of ovarian function months after chemotherapy. For these women spontaneous pregnancies may occur and they may not need their frozen gametes or gonadal tissue [22].

Pre-menarchal 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 for the health of their bones and for 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.

25.6.2. 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.

Long-term storage of ejaculated or testicular spermatozoa does not negatively affect the quality of the frozen material, but constant storage conditions with a temperature of $\leq -140^{\circ}\text{C}$ are mandatory.

The legislation and recommendations for fertility preservation in males differ between countries. There are no strict limitations on semen quality or sperm numbers for fertility preservation strategies and there are no international guidelines for the duration of storage of spermatozoa, whether ejaculated or testicular. Good laboratory practice will include testing for infectious disease (HIV, hepatitis) and offer separate storage of infectious material. Detailed information on how to handle the samples and process sperm are given by the WHO manual [23].

When cryopreserved samples are used, intracytoplasmic sperm injection (ICSI) is recommended to improve the chances of success. Before ICSI was applied, the success rate of ART 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. In addition, since the cryopreserved material will be limited, using ICSI increases the number of ART treatments that can be performed. To date, no adverse effect of the combination of cryopreservation of semen and subsequent ART has been reported concerning the health of the offspring.

25.7. Future developments

25.7.1. Female experimental approaches

Female patients who seek fertility preservation but cannot undergo ovarian stimulation and oocyte/embryo preservation may consider using immature oocytes – either retrieved from antral follicles during the luteal phase or obtained during the ovarian cortex processing technique – to do *in vitro* maturation to produce viable embryos [24].

25.7.2. Male experimental approaches

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 potential of these cells. In principle, fertility restoration strategies include the 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. Where there is any risk of reintroduction of malignant cells 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, spermatogonial stem cells 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 [25]. While the demonstration of functional donor spermatogenesis following SSC transplantation in primates is an important milestone 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.

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 malig-

nant 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 [26]. 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. 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.

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Related documents:

- Appendix 9. Sample consent form (women);**
- Appendix 10. Sample consent form (women);**
- Appendix 11. Sample consent form (men).**

Part C. Developing applications

Chapter 26. General considerations for cell-based therapies

26.1. Introduction

The isolation and characterisation of cells from almost all body tissues presents new therapeutic opportunities. These are certainly innovative strategies, but cell therapy is not new, having its origins in blood transfusion, bone marrow and organ transplantation, tissue banking and *in vitro* fertilisation. In recent years, increasing numbers of tissue establishments have expanded their activities, providing starting materials or engaging in the preparation of more complex products based on human tissues and cells.

Some of these therapies are already well established in medical practice, such as haematopoietic progenitor cell transplantation, which has for many decades allowed treatment of a wide range of life-threatening conditions (see Chapter 21). More recently, the ability to generate new cell lineages from somatic cells (see Chapter 28), embryonic cells or induced pluripotent stem cells (iPSC) has created great possibilities for the development of cell therapy and regenerative medicine. Cells may also be genetically modified to perform a required function through *ex vivo* gene therapy or combined with extracellular matrices or scaffolds (see Chapter 27) to create tissue-engineered constructs. An overview of the different approaches to developing these therapies is presented in Figure 26.1.

Cells for human application can be obtained from and applied in the same patient (autologous use) or donated by one person for clinical application in a different person (allogeneic use).

This chapter, which serves as an introduction to Part C of this Guide, provides an overview of this developing field and addresses technical considerations for professionals in tissue establishments who may be interested in learning about or developing such activities.

It is important to note that, in different countries, these 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.

26.2. Legal framework for the development of advanced therapy medicinal products in the European Union

26.2.1. Specific regulations in the European Union

To provide a common framework for the marketing of advanced therapy medicinal products (ATMP) in the European Union (EU), Regulation (EC) No. 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal prod-

ucts (hereafter ‘the ATMP Regulation’) was adopted in 2007.

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.[‡]

Specifically, it is noted that the use of cells that have been subject to substantial manipulation, and/or the use of cells for an essential function or functions in the recipient different from their function in the donor, are regulated as medicinal products in the EU.

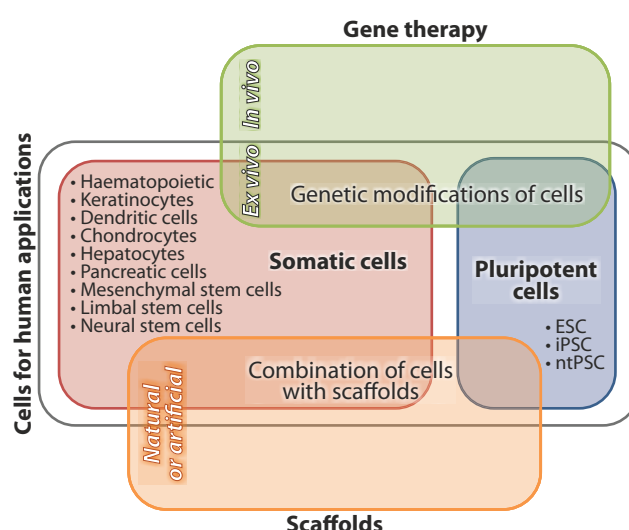
* A gene therapy medicinal product is a biological medicinal product that fulfils these two characteristics: a. it contains an active substance that contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting 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 shall 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, and b. 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 shall not be 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. 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, and 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.

The cornerstone of the ATMP Regulation is that a marketing authorisation must be obtained before the marketing of ATMP. In turn, the marketing authorisation 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 ATMP 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.

Figure 26.1. Approaches to using cells in human applications



Note: ESC, embryonic stem cells; iPSC, induced pluripotent stem cells; ntPSC, nuclear transfer pluripotent stem cells.

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 ATMP.

Where ATMP 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 ATMP 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, ATMP 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 ATMP. Furthermore, use of ATMP in an investigational setting is also subject to EU rules on clinical trials. Additional information about the EU regulatory framework for ATMP can be found at the EMA [1] and EC Internet websites [2].

It is important to highlight that, in Part C of this Guide, the term ‘cells’ or ‘cell therapy’ can be used to refer to situations that may be regulated as ATMP in the EU. 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.

26.2.2. 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 [3]. 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. 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.

26.2.3. 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 [4].

26.3. Cell types being used in developing new technologies

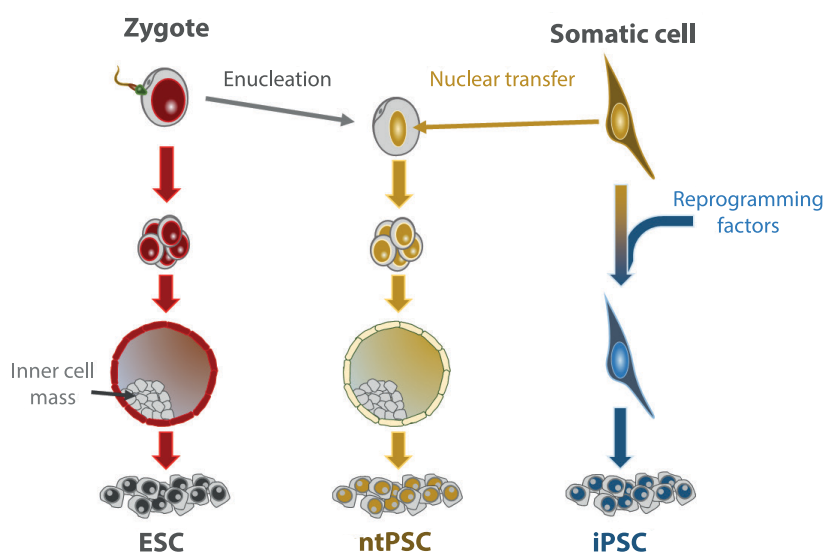
Many tissues are sources of different cell types that are currently being used or researched for the development of new therapeutic options. Due to their undifferentiated nature, stem cells can be expanded *in vitro* and differentiated into various tissue-specific cells. This unique characteristic can 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, in some countries the use of these stem cells may not be permitted, and this must be taken into consideration. Table 26.1 summarises some of the cell types currently used in developing novel cell-based therapies.

The methods most commonly used to obtain pluripotent stem cells (PSC) are shown in Figure 26.2. Multipotent and lineage progenitor cells and haematopoietic progenitor cells are discussed in further detail in Chapters 28 and 21 respectively.

Table 26.1. 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, foetal 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. For details see Chapter 28.
Lineage-committed	Progenitor cells, e.g. haematopoietic progenitor 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 <i>in vitro</i> expansion (e.g. bone marrow, peripheral blood, cord blood), or cultured <i>in vitro</i> and differentiated into more restricted cell types for clinical application.

Figure 26.2. The most common methods used to obtain pluripotent stem cells



Note: To obtain embryonic PSC (ESC), embryos are developed until the blastocyst stage, when the inner cell mass is isolated and cultured to derive the cells. Zygote can be enucleated, and a somatic cell nucleus is then transferred into it. The nuclear-transfer zygote is then developed until the blastocyst stage and the inner cell mass is isolated and cultured to obtain the nuclear-transfer PSC (ntPSC). Somatic cells can also be reprogrammed into induced PSCs (iPSC) by using a transcription factor cocktail.

26.4. Processing cells for human application

In the preparation of cells for human application, different levels of processing may be required in

order to deliver cells with the required characteristics and functionality. Some cells can be transplanted without *in vitro* expansion (e.g. bone marrow, peripheral blood, cord blood, hepatocytes, beta cells), whereas others need to be cultured *in vitro* and

differentiated into more restricted cell types and, finally, some others undergo many manipulations to render the final product suitable for clinical application. In this section some of the most commonly used processing methods are discussed.

In the EU, many of the following processing methods are considered as substantial manipulations and thus the resulting products are governed as ATMP under the medicines rules, which include specific requirements that must be respected when the activity is undertaken in the EU or if the resulting cells are intended to be used in the EU (see section 26.2 for further details).

26.4.1. Culturing cells

The origin and procurement of the starting material to isolate cells for therapeutic use is considered critical for the yield and identity/purity of the final cell population. Processing steps such as derivation and/or expansion of cells, addition of cryoprotectants and all steps involved in the culture of cells must be undertaken in an environment that is fully compliant with good manufacturing practices (GMP). The initial procurement of tissue or cells from a human should always follow the regulations and guidelines related to human tissue/cell donation, procurement and testing, and universal precautions must be applied to minimise the risks of contamination, infection and pathogen transmission [4] (see Chapters 4, 5 and 6).

Culture conditions are critical during expansion and differentiation of cells in culture. The culture conditions for a specific cell type should be defined to control the number of cellular duplications and to achieve an adequate balance between number of passages and duplications. Antibiotics are not usually included in cell-culture media because they may mask the presence of a low level of bacterial contamination and, therefore, have the potential to cause infections in some recipients. Microbiological testing is required when culturing cells, which should be done at different critical steps and before cell release (see Chapter 9).

Validation of the preparation process should be carried out with respect to maintaining genetic stability and the relevant biological properties, as well as avoiding malignant transformation.

A safety issue when culturing human cells is the use of materials of animal origin, such as media or growth factors. The use of this xeno-material should be avoided as much as possible by using human-derived factors, for example by obtaining serum from the intended recipient. If animal materials cannot be

avoided, specification and verification of the source and method of preparation of the material is required. Culture media and other reagents derived from animals must be evaluated for the risk of contamination with micro-organisms, particularly viruses and transmissible agents of human pathologies such as transmissible spongiform encephalopathies (TSE). Documentation that demonstrates the application of appropriate quality-assurance measures by suppliers of media of animal origin, including origins and veterinary certificates for the animals used in the preparation of the material (e.g. bovine serum albumin), must be obtained. Certificates must be supported by audit trails for collection, pooling, shipping and final formulation by the third-party supplier. The use of raw materials and processing materials that are supplied with a TSE certificate from the European Directorate for the Quality of Medicines & HealthCare (EDQM) minimises the risks of infection from TSE [5]. The requirements for sourcing/donation, procurement and testing are set out in Annex 2 of the GMP Guidelines and in the guideline on xenogeneic cell-based medicinal products. For further guidance on cell culture, refer to the report (by the second Task Force of the European Centre for the Validation of Alternative Methods) on Good Cell Culture Practice [6].

At the point where the cells will not be expanded any further, the downstream process begins. This includes the final collection and subsequent process steps of concentration or volume reduction of the collected cells, washing or clarification of the collected cells, formulation of the cells into an appropriate medium for preservation and then filling their final container with the formulated cells for cryopreservation and storage, or for direct delivery to patients. When a cell-based product is delivered for use, it must be shipped under appropriate conditions to the clinical site, prepared for application to the patient and then administered by a medical doctor or trained healthcare professional.

26.4.1.1. Microbiological testing when culturing cells

Culturing cells includes a broad range of procedures that differ, depending on the cell type, in many aspects such as source material, finished product, culture reagents, time in culture, expansion, differentiation, quality control analysis and storage procedures. All these differences make it difficult to establish a general rule for microbiological testing. Therefore, for each procedure, Quality Risk Management (QRM) should be applied to determine the quality-control strategy to be followed through the whole process and to identify critical

steps to reduce the possibility of contamination and cross-contamination.

As a general recommendation, both the starting material and the finished product should be tested. For the starting material, microbiological tests should be carried out on the cells (or the cell source if the cells of interest are in too low numbers) and the culture reagents to ensure the absence of bacterial, fungal or mycoplasma contaminations. It is recommended to use the methodology described in the *European Pharmacopoeia* (*Ph. Eur.*). If other methods are used, they must always be validated in advance.

When the starting material, including the cells themselves, cannot be stored in quarantine until the results of the microbiological analysis are obtained (for reasons of cell instability), the processing steps can start without the results being available. However, it would be necessary to study the potential risks of using this 'non-tested material' and document it following the principles described in the QRM protocol.

In principle, the microbiological analysis should be done either in intermediate-cell products or finished product. However, when the number of cells is scarce, it may be acceptable to perform the analysis with samples of washing media, supernatants or cell-culture media. If any microbiological contamination is detected, it is necessary to identify the strain and to investigate its source. In this case the product should not be used for clinical application unless a risk-benefit analysis indicates that it is the best option for the patient.

The finished product should be analysed for its microbiological quality before it can be released. Absence of bacteria, fungi and mycoplasma should be demonstrated. However, in specific cases when this approach is not feasible, the release of the finished product before the microbiological results are available may be justified. In this context, implementation and documentation of an adequate processing – one which provides sufficient assurance of the microbiological quality of the product when released – is essential. This will include in-process microbiological tests that have been established on the basis of a risk analysis, usually including sterility testing of the culture media and of samples from the intermediate product at critical steps. It is recommended to use tests as described in *Ph. Eur.* 2.6.1 and 2.6.27, the results of which are available in 7-14 days, depending on the method used. Alternative methods have been developed in recent years, and some of these methods have shown potential for real-time or near real-time results. Absence of mycoplasma should also be analysed at given critical steps (*Ph. Eur.* 2.6.7) and endo-

toxin determination may also be recommended (*Ph. Eur.* 2.6.14).

When cells are allogeneic it is recommended to include the analysis of adventitious viruses, both in cell source material and in the finished product, in order to avoid transmission to the patient (see *Ph. Eur.* 5.1.7 for viral safety). Adventitious viruses can be analysed by different methodologies but PCR assays are the most commonly used. In order to establish the virus that should be tested, it will also be necessary to do a risk analysis [7]. Some examples of adventitious viruses that can be included are: *adeno-associated virus* (AAV), *adenovirus*, *bunyavirus*, *cytomegalovirus* (CMV), Epstein-Barr virus (EBV), *flavivirus*, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human *Herpes virus* 1 (HHV1), HHV6, HHV7, HHV8, human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2), human *papilloma virus* (HPV), human *rotavirus* (HRV), human T-cell leukaemia virus type-1 (HTLV-1), HTLV-2, influenza, measles, Norwalk virus, *parvovirus* B19 (Parvo-B19), rubella and *enterovirus* (polio).

26.4.1.2. Considerations for quality control of other parameters for cultured cells

It is important to demonstrate that the cell-based product meets specifications before release. For this reason, quality controls should include not only cell viability and genomic studies, but also cell identity, testing of biological activity and other microbiological controls. When possible, fluorescence-activated cell sorting (FACS) to analyse the presence or absence of specific cell-surface antigens (CD) is the best method to quantify the purity of the culture. For example, haematopoietic progenitor cells should be positive for CD34 whereas mesenchymal stem cells (see Chapter 28 for nomenclature clarification) are negative for the antigen CD45 but positive for CD105, CD73 and CD90 (see Chapter 28 for specific information on cell surface antigens of several somatic stem cells). However, in many cases the short time from cell culture to release and application is challenging, and these tests must be performed during processing.

The selection of appropriate markers is fundamental in the standardisation of isolation conditions and to identify cell populations, heterogeneity and yield. However, in many cases there are no known specific surface antigens for stem cells, which makes their purification difficult.

Control of genomic stability is also critical before releasing the cells for transplant in humans. Telomerase activity, proliferative capacity and senes-

cence are also quality controls of relevance for human pluripotent stem cells.

26.4.1.3. Master cell banks and working cell banks

Some cells, including stem cells, are used to produce master cell banks, which are then banked for future use as starting materials to manufacture cell therapy products. A cell line is established from a single clone and this cell line is used to make up the master cell bank. This master cell bank must be characterised and extensively tested for contaminants such as bacteria, fungi and mycoplasmas. In addition, sterility and endotoxin testing, as well as polymerase chain reaction (PCR) testing for viruses, may be required.

Cell lines used for patient treatment are placed in cryovials, and cryoprotectants are added before the cells are frozen and stored in the vapour phase of liquid nitrogen. The use of cryoprotectants and the control of cooling and warming rates during freezing and thawing are essential to minimise cell death and to increase cell attachment after thawing. This is of special importance during cryopreservation of human pluripotent stem cells [8]. The temperature inside the liquid nitrogen tank should be continuously monitored in order to ensure that the cells are stored under stable conditions.

It is recommended that master cell banks are stored in two or more widely separated areas within a production facility as well as at a distant site in order to avoid loss of the cell line. Dual-site storage of all cells is accomplished with on-site storage and off-site storage.

Quality controls should be the same as described above for cell release and should be carried out before cryopreservation and after thawing to ensure that cell proliferation and viability, genome stability and purity of the culture have not been affected by the freezing process.

The working cell bank is a pool of expanded well-characterised cells derived from the master cell bank. The working cell bank is prepared from a single homogeneously mixed pool of cells. One or more of the working cell bank containers is used for each cell expansion. The characterisation and quality controls carried out for the working cell bank after cell thawing must be exactly the same done for the master cell bank, and the same specifications should be maintained.

26.4.2. Genetic modifications of cells

The genetic engineering of human, animal, plant and microbial cells in the laboratory became

established biotechnology practice in the latter part of the 20th century. Techniques have been developed to allow the addition, removal and editing of gene sequences within cells, with ever greater precision and the reliable expression of the products from such modified genes *in vitro* and *in vivo*. New therapeutic products, such as monoclonal antibodies, have been manufactured using such genetically modified cells grown *ex vivo* in bioreactors.

There is now much interest in using the techniques developed for *in vitro* genetic modification of cells for direct therapeutic use *in vivo* for the treatment of a variety of diseases. This area of medicine is known as gene therapy. Modern gene sequencing has allowed specific genes associated with certain diseases to be identified and new techniques allow those genes to be synthesised and modified in the laboratory. In order to have a therapeutic effect, the gene needs to gain access to the target cells and be appropriately processed within the cell. This is achieved using a gene carrier or vector.

26.4.2.1. General considerations regarding gene vectors for genetic modification of human cells

The starting point for most gene vectors is a synthetic DNA plasmid. This contains the therapeutic gene sequence flanked by suitable sequences to allow its appropriate replication and expression in the target cell and by further sequences which allow the manufacture of multiple copies of the therapeutic gene in laboratory cell culture in order to have sufficient to transfect the target cells. Plasmids are relatively small, circular forms of DNA and several techniques have been developed that enable these plasmids to gain entry to cells *in vitro*. Most are engineered to allow multiple copies to be manufactured in bacterial cell culture, then extracted and purified before being used for gene therapy.

Techniques for transfecting cells with plasmids cannot generally be used *in vivo*, although muscle cells have been induced to take up plasmids following intramuscular injection and respiratory tract epithelia using liposomal delivery, with transient expression of the therapeutic genes. They are therefore more commonly used to transfect human cells *ex vivo* as a means of genetic manipulation before the cells are used therapeutically. For example, plasmids have been used as suitable vectors for delivering the genes to *in vitro* somatic cell cultures required to derive iPSC (see section 26.3).

However, because of low transfection efficiency and generally transient nature in human cells, plasmids are not the vector of choice for *ex vivo* genetic

manipulation of human cells or *in vivo* gene therapy. Instead, they are used to manufacture synthetic viral gene vectors in laboratory cultures of human or animal cell lines. Two or more plasmids are usually used to transfect the cell line. The plasmids contain not only the therapeutic gene and required flanking sequences, but also genes coding for important viral proteins, so that multiple viral particles will be generated with the therapeutic genes appropriately packaged within each particle for delivery to the target cell. Viral vectors are based on naturally occurring virus, selected dependent on their particular characteristics such as whether they selectively transfect certain tissue types and whether they integrate their genes into the host cell chromosome, or express them extra chromosomally. Specific packaging cell lines have been produced that allow the necessary replication of the vector in culture, but do not allow viral replication genes to be packaged in the viral particles produced, rendering the vector replication non-competent. A number of viruses have been used as the basis for human gene therapy, including *retrovirus*, *adenovirus*, *lentivirus*, *Herpes simplex virus*, *vaccinia*, pox virus and *adeno-associated virus*.

Because of the potential risk of harmful infection using viral gene vectors and the unintended side-effects of genetic manipulation, tissue and cell establishments must consult appropriate experts in designing or selecting a vector for use in genetic manipulation of cells for human application. It is also essential that the vector is manufactured under highly controlled conditions, with rigorous quality control. Establishments should use experienced, specialist GMP manufacturers for this purpose.

26.4.2.2. Post-genetic modification processes

Extensive characterisation of the genetically modified cells must be performed, including establishing the number and location of integration events, sequencing of integrated sequences to establish the integrity of the molecular construct, removal of the transgene (if needed), investigation of the possibility of vector replication and viral reactivation, and confirmation of the genetic stability of the cells.

When the transgene is not intended to modify the cells or induce differentiation, a large number of experiments should be performed in order to demonstrate that modified cells have the same morphology/phenotype, genetic profile and functionality as the (pre-modification) parental cells. Unexpected changes in cell morphology, function and behaviour should be at least investigated and documented, and (depending on the magnitude of the changes) genetically modified cells must be discarded.

If the genetically modified cells are intended to have replacing activity, chromosomal integrity should be shown. Similarly, when genetic modification is performed to induce differentiation, the efficacy of such a process must be characterised and documented. When using pluripotent cells, any undifferentiated cells should be removed or killed to avoid teratoma formation after grafting. The purity criteria should be established and be within determined limits.

When genetic modification is performed for *ex vivo* production of secreted proteins of interest, pharmacokinetic studies should be designed in order to address not only expression, distribution and persistence of the transgene, but also dosage of protein release per cell and stability under *in vitro* and *in vivo* conditions. Toxicological studies should also be performed in order to avoid any unexpected effects. Similarly, when the cells that produce the gene product are encapsulated in biocompatible material, the appropriate secretion activity and potential toxicity should also be characterised and reported.

26.4.2.3. Transient expression

When transient genetic modification is intended, for example to induce cell differentiation, all genetic constructs must be removed from the final cell product. For this purpose, it is important to design not only the plasmid sequence but also the molecular strategy to verify that any traces of the plasmid used have been removed, to avoid future expression of genes or aberrant constructs.

26.4.2.4. Purity and cell selection

The purity of genetically modified cells is related to the efficacy of the transfection/transduction method used. In addition, when choosing the genetic modification method to be used, a selection marker can be introduced to increase the purity and consistency of the gene delivery method. The selection method, such as fluorescent or magnetic, should be selected depending on the intended use. Cell selection is an important step to purify genetically modified cells from those that were not successfully modified in many clinical trials. A complete description and control of the method used for the selection and/or purification is mandatory. The consistency of the method must also be demonstrated in different cell preparations.

The homogeneity and genetic stability of the modified cells should be characterised, including ascertaining that all cells in the purified population contain the intended genetic modification. The

testing methods used for this should be cell type and vector-specific as necessary.

Furthermore, any observable change in morphology, function or behaviour of the purified cell population – whether caused by the genetic modification, the process of genetic modification or the purification process – should be documented. Special attention should be paid to the proliferation and differentiation properties of the modified cells and how they compare to the original unmodified cells.

26.4.2.5. Cell banking of genetically modified cells

After genetic modification of the cells, they can be cultured, selected and/or frozen. When expression of the gene is permanent, the production of a master cell bank (see section 26.4.1.3) with modified cells is recommended. The master cell bank will give the opportunity to make the appropriate quality controls without excessive passaging of the cells.

A complete description of the post-modification steps should also be registered and appropriately controlled.

26.4.2.6. Dosage

It is critical to select the appropriate dose of the product when the cells are used to secrete a functional protein to produce a paracrine effect. The final dose will depend on several parameters, including the level of expression, the number of vector copies per cell and the number of cells grafted. Where possible, the number of grafted cells should be adjusted to administer the desired protein dose. Pre-clinical studies may help to set the correct dose, but the results obtained in animal models cannot always be extrapolated to human beings since the expression of the gene may change depending on the niche of the transplanted area. For this reason, applying a single dose in clinical trials is not feasible in most cases. Thus applying at least two doses, the minimal effective dose and the maximum tolerable, will provide important information for future clinical trials.

26.4.3. Tissue decellularisation and combination of cells with natural scaffolds

Decellularisation of donated tissues is a technique commonly performed in tissue establishments with the purpose of producing a cellular-neutralised parenchyma that may have several uses. These extracellular matrices (ECM), also known as ‘scaffolds’, may in some instances be used directly for tissue implantation (e.g. heart valves, large vessels or dermal matrices) providing structural benefits while reducing immunological rejection and the risk of con-

tamination. Alternatively, cells can be combined with these ECM and used in patients to improve or replace biological tissues.

Decellularisation and combination of ECM with cells are addressed in more detail in Chapter 27.

26.5. Safety considerations when applying cells to patients

Many of the early clinical successes using intravenous infusions of cell-based products have been seen in the treatment of systemic diseases such as graft *versus* host disease and sepsis. However, it is becoming more accepted that diseases involving peripheral tissues, such as cartilage repair, may be better treated with methods that increase the local concentration of cells. Direct injection or placement of cells into a site for tissue repair may be the preferred method of treatment, as vascular delivery suffers from a ‘pulmonary first-pass effect’, where intravenous injected cells are sequestered in the lungs.

Cells may suffer substantial functional changes resulting not only from culturing, *ex vivo* activation or genetic manipulation, but also as consequence of their human application. When cells are transplanted, the environment changes considerably and these changes can modify the morphological and functional characteristics of the cells; therefore, evaluation of tumorigenicity should also be integrated when cells are implanted into the patient. Post-grafting follow-up of each patient is critical in autologous and allogeneic applications.

Cells are not classical drugs and need specific requirements when manipulated. It is important that administering professionals have some basic knowledge about cells. Some clinical trials failed because of a lack of training of the professionals who manipulated the cells before and during transplantation. Detailed instructions should be given that include cell manipulation and tissue pre-treatment, to avoid cell death or modification of the biological properties of the cells. Key requirements include having adequate pre-clinical data, independent oversight and peer review, fair subject selection, informed consent, research subject monitoring, auditing of study conduct, and trial registration and reporting [4].

Clinical research, including trials of experimental interventions, is essential in translating cell-based treatments and requires participation of human subjects, whose rights and welfare must be protected [4]. 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 of stem cell-based interventions must follow internationally accepted principles governing the ethical design and conduct of clinical research and the protection of human subjects [9, 10]. In the EU, a specific directive regulates clinical trials [11].

Early phase trials provide the first opportunity to evaluate methods and the effects of promising stem cell-based interventions in humans. It also represents the first occasion when humans are exposed to an unproven intervention. Initial tests of a novel strategy should be tested under lower-risk conditions before escalating to higher-risk study conditions, even if the latter are more likely to confer therapeutic benefit. Clinical research should compare new stem cell-based interventions against the best therapeutic approaches that are currently available, or could reasonably be made available, to the local population. Where there are no proven effective treatments for a medical condition and stem cell-based interventions involve invasive delivery, it may be appropriate to test them against placebo or sham comparators, assuming that early experience has demonstrated the feasibility and safety of the particular intervention. We recommend the ISSCR guidelines for further details on clinical trials [4].

In the EU, use of cells falling under the definition of ATMP is governed by specific requirements and procedures, including prior authorisation by the competent authority. Evaluation of risks and benefits and the determination of the necessary data to demonstrate efficacy and safety are to be done in accordance with the EU rules on medicines if the use takes place within EU territory.

26.6. References

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Chapter 27. Decellularisation and preparation of natural scaffolds

27.1. Introduction

Tissue decellularisation is a technique that aims to remove all cells from a tissue leaving behind just the extracellular matrix (ECM). These acellular ECMs, also known as ‘scaffolds’, may be used directly for tissue implantation. The resulting scaffolds have several advantages for the effective treatment and restoration of unhealthy, missing or damaged tissue. For example, the removal of the cellular component of a tissue may significantly improve graft compatibility and enhance successful transplantation through the reduction of immunogenicity. In addition, the absence of constituent (donor) cells can help to create a microenvironment more conducive to recipient recellularisation and re-vascularisation *in vivo*.

Scaffolds can also be produced artificially, using biological or synthetic materials, which are not addressed in this chapter. However, decellularised tissues have several advantages over artificial scaffolds since they reproduce much more accurately the structure of the extracellular matrix in which cells live *in vivo*, and may even incorporate some of the chemical and mechanical signals that lead the cells to behave in a determined, ‘realistic’ way. Decellularised dermal matrix, decellularised stroma (see Chapter 16, section 16.10), large blood vessels and heart valves are examples of scaffolds currently applied for patient treatment.

The following generic chapters of this Guide all apply to the production of natural scaffolds for

human application and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Premises (Chapter 8);
- i. Principles of microbiological control (Chapter 9);
- j. Distribution and import/export (Chapter 10);
- k. Organisations responsible for human application (Chapter 11);
- l. Computerised systems (Chapter 12);
- m. Coding, packaging and labelling (Chapter 13);
- n. Traceability (Chapter 14);
- o. General considerations for cell-based therapies (Chapter 26).

27.2. Donor selection and procurement

Organs and tissues for decellularisation are mainly obtained from deceased donors. Donor evaluation and testing criteria, as described in Chapters 4 and 5, apply.

Figure 27.1. **Decellularisation methods: physical, enzymatic and chemical**

Physical • Mechanical/agitation • Freeze/thaw • Sonication	Biological (enzymatic) • Trypsin • Endonucleases • Exonucleases	Chemical • Alkaline/acid • Hypo/hypertonic solutions • EDTA/EGTA <i>Non-ionic detergents</i> • Triton-X100 <i>Ionic detergents</i> • Sodium dodecyl sulfate (SDS) • Triton-X200 <i>Zwitterionic detergents</i> • CHAPS • Sulfobetaine-10/16 • Tri(n-butyl)phosphate

Source: Extracted from [1].

27.3. Processing

27.3.1. Decellularisation

27.3.1.1. Decellularisation agents

Several procedures can be applied to decellularise human material for clinical application, but the chosen method should take into account the specific clinical use of each scaffold because the preserved properties may differ, depending on the method used.

A sterile, clinical-grade ECM should preserve the intrinsic biological properties of the ECM, while removing cells, cellular debris and alloantigens (to minimise immunogenicity), and achieving the elimination of toxins. However, it must be acknowledged that any decellularisation process carries a certain degree of ECM denaturation.

The specific decellularisation technique must be chosen to suit the final use of the ECM. Because

ECM properties vary between tissues and organs, the main properties to safeguard must be clearly identified in order to choose the correct decellularisation protocol. There are three methods used to decellularise organs or tissues: physical, biological (enzymatic) and chemical [1] (see Figure 27.1). Each of these methods has a different mode of action and effect on the ECM [2] (see Table 27.1). To ensure effectiveness, using a combination of methods is recommended.

The effectiveness of tissue and organ decellularisation depends on intrinsic tissue properties, such as the specific cell density, thickness, compaction and lipid content, as well as on the selected decellularisation agents. In addition, different decellularisation processes have different effects on the ECM properties [3] (see Table 27.2), which can be critical for the functionality of the ECM. These factors should all be taken into account when choosing the decellularisation method.

If the three-dimensional structure and its stiffness are important, collagens have to be preserved. In this case, ionic detergents would be the optimal choice, and enzymatic or alkaline-acid methods should be avoided. This consideration is valid if the preservation of any protein is important. It is worth considering that the ECM should always be able to resist the stress inherent to the transplantation procedure.

Osmotic buffers are a milder method to obtain decellularised ECM but they are slower and cannot penetrate into thicker, compact organs, such as a heart. For dense tissues or intact organs, detergents can help buffers penetrate, but they will affect the proteic ultrastructure due to disruption of protein-protein interactions. Furthermore, any residual detergents can have cytotoxic effects.

Table 27.1. **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

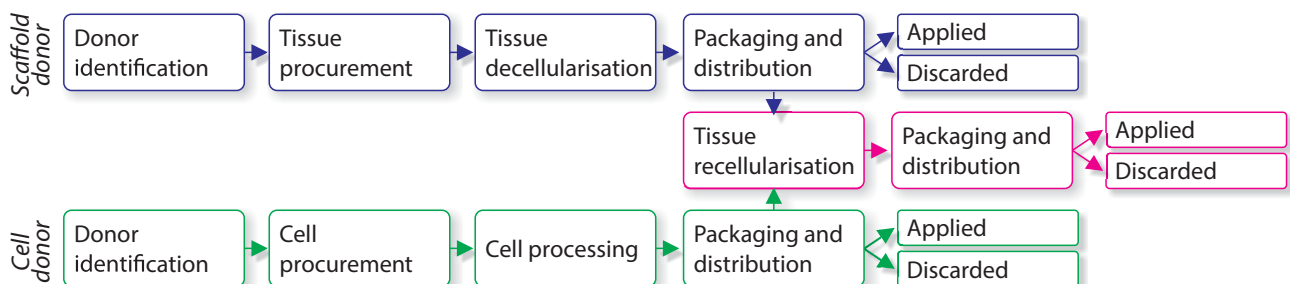
Source: Modified from [2].

Table 27.2. Modes of action and the effects of different decellularisation methods

Method	Mode of action	Effects on ECM
Physical		
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	Can lyse cells; facilitates chemical exposure and removal of cellular material	Aggressive agitation or sonication can disrupt ECM
Biological (enzymatic)		
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		
Alkaline/acid	Solubilises cytoplasmic components of cells; disrupts nucleic acids	Removes GAG
Hypo/hypertonic solutions	Cell lysis by osmotic shock	Efficient for cell lysis, but does not effectively remove cellular remnants
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 deoxycholate		More disruptive to tissue structure than SDS
Triton X-200		Achieves efficient cell removal when used with zwitterionic detergents
Zwitterionic detergents: 3-[(3-cho-lamidopropyl) 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(<i>n</i> -butyl)phosphate	Organic solvent that disrupts protein–protein interactions	Variable cell removal; loss of collagen content, although effect on mechanical properties is minimal

Source: Extracted from [3].

Figure 27.2. The different process in tissue engineering using natural scaffolds



Note: Traceability should include all these steps, from scaffold and cell donors to patient grafting after recellularisation of the scaffold.

Alcohols and other solvents can very efficiently remove lipids from tissues, but they can crosslink proteins and modify the ECM ultrastructure.

Enzymatic treatments guarantee the specific removal of certain proteins, but they may cause the unspecific digestion of desired constituents and may not be sufficient for a complete decellularisation of the whole tissue. Hence they must be used in combination with other methods.

Physical methods (such as temperature, force and pressure) can be applied but have limited efficacy and should be carefully evaluated to assess any possible damage to the ECM.

Specific combinations of mild physical, biological and chemical methods, along with the type of administration, should be tested to obtain the best results for each tissue or organ.

Reagent quality should be specified and controlled to protect the safety of the recipient and assure the consistency and effectiveness of the decellularisation process. In particular, bioburden should be minimised and controlled even if the ECM is to be terminally sterilised in accordance with principles of microbiological control (see Chapter 9). Processes for the effective removal or reduction of potentially toxic reagents used in the decellularisation process should be validated (see Chapter 2). Reagents and their suppliers should be validated to a standard equivalent to that used for processing of complete tissues (see Chapter 2).

27.3.1.2. *Sterilisation of natural scaffolds*

Sterilisation techniques may be applied to provide a higher level of safety, since scaffolds contain no living cells; however, classic sterilisation methods, such as ethylene oxide exposure, gamma irradiation and electron beam irradiation, are known to alter ECM ultrastructure and mechanical properties, which are critical for ECM functionality. Additionally, ethylene oxide treatment can cause undesirable host immune responses that impair proper function of the biological scaffold after implantation. Therefore, sterilisation by ethylene oxide is only acceptable when there is no other suitable alternative (*Ph. Eur.* 5.1.1).

Natural ECM can be sterilised by simple treatments with acids or solvents, but such methods may not provide sufficient penetration depending on the size of the organ or tissue, and may damage key ECM components. There are significant advantages to combining the decellularisation and sterilisation processes to ensure a clinically safe ECM for the recipient without affecting its ultrastructure. At the

moment, peracetic acid has been shown to be the best solution to minimise bacterial, fungal and spore contamination. Tributyl phosphate organic solvent has viricidal properties.

Supercritical carbon dioxide is under investigation as a method for the sterilisation of natural ECM. This agent reduces the bacterial and viral loads, with minor changes in mechanical properties relative to other sterilisation methods.

Absence of bacteria and other microbial contaminants, which is achieved by a correct process of sterilisation, is not the only condition required to avoid an undesired patient response caused by pathogens. Endotoxins in the ECM may induce strong biological responses in the patient, fever being the main manifestation (see Chapter 9 for detailed endotoxin testing guidance). Although these bacterial endotoxins are among the most frequent pyrogens, other micro-organisms – such as mycobacteria, fungi and viruses – also release pyrogens. Depyrogenation agents to eliminate endotoxins and intact viral and bacterial DNA that may be present should be applied when indicated. Because pyrogens are often difficult to remove from ECM, inactivation or destruction may be preferable. Acid–base hydrolysis, oxidation, heating or sodium hydroxide are frequently used to this end. However, careful evaluation to avoid affecting the mechanical or biological properties of the ECM should be made.

27.3.1.3. *Quality control of decellularised tissues*

The quality control and release criteria for tissue to be decellularised should in general be the same as for the tissue if used directly for transplantation. However, because of the effect of the reagents and processes used for preparation of decellularised tissue, some release criteria may be removed. In particular, tissue with low levels of microbial contamination may be considered as acceptable for decellularisation if the process can be validated to kill and remove such contamination. If known human pathogens are detected in the tissue samples at any stage, there should be a decision on further processing based on an approved risk-assessment algorithm, even if the tissue is sterilised throughout the process. Also, if the donor does not meet the required screening and testing criteria the tissue must not be used, even if known viricidal agents are used in the process.

The quality control of tissues after the decellularisation process should consider the following:

- effective removal of cells and cellular components,

- effective removal of microbial contamination (see Chapter 9) and any potentially toxic microbial products (e.g. endotoxins),
- effective removal of undesirable and potentially toxic reagents at the end of the decellularisation process,
- maintenance of desired ECM physical characteristics.

It may not be practical to test the decellularised product for all of the required quality controls before release for direct transplantation. Omission of some quality controls is acceptable if the processes have been fully validated to assure that the required acceptance criteria can be consistently achieved (see Chapter 2).

27.4. Packaging and distribution

Decellularised tissues for direct human application can be packaged and distributed in the same way as other tissues. Special packaging may be needed to support and protect the scaffold where the decellularisation process has weakened the structural integrity of the graft.

27.5. Traceability

Traceability is essential to ensure rapid action to prevent harm as described in Chapter 14. In the case of using decellularised tissues, traceability from donor identification and procurement of the original tissue to application or disposal of the decellularised tissue is essential.

27.6. Safety considerations for clinical application

Because of the potential harmful nature of the reagents and processes used to decellularise tissue and, where relevant, in order to determine as far as possible the safety and efficacy of the scaffolds, non-clinical safety studies as well as controlled clinical evaluations should be performed before making these products generally available for use.

The delivery of decellularised tissues may require administration through specific surgical procedures and therefore, each procedure will have special safety considerations (not detailed in this chapter).

27.7. *In vitro* recellularisation of natural scaffolds

In recent years, tissue establishments have been expanding their activities, providing starting materials or engaging in the production of recellularised scaffolds. To obtain them, cultured cells are combined with natural scaffolds to induce cell differentiation and to provide the mechanical support and required shape of a replacement tissue. The matrix material must be evaluated for compatibility with the cells to be used and should be sterile to avoid any contamination of the culture. The combined properties will depend on the intrinsic characteristics of the cell used and the scaffold used.

When decellularised tissue is recellularised, packaging and distribution must ensure appropriate preservation of the scaffold and viability and function of the cells. If the decellularisation and recellularisation processes are done in the same tissue establishment, packaging of the ECM may not be needed. However, when the two processes are carried out in different tissue establishments, packaging is required after decellularisation.

For recellularised tissues, traceability includes critical information about the cell donor (see Figure 27.2).

The Responsible Person of a tissue establishment producing recellularised tissues should carefully consider the legal requirements that apply to the activities they are undertaking. Before starting any such activities, there should be appropriate consultation with the relevant authorities to understand the regulatory environment and any licence/authorisation that may be required.

Tissue-engineered products (including the manufacturing, storage, distribution, labelling, advertising, traceability and use thereof) must comply with the relevant requirements provided under the EU medicines rules when the activity is undertaken in the EU or if the resulting products are intended to be used in the EU. For further details about EU legislation for advanced therapy medicinal products, refer to Chapter 26, section 26.2.

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Chapter 28. Developing applications for somatic cells

28.1. Introduction

Advances in medical research and the developing field for clinical applications using somatic cells for autologous or allogeneic therapies hold great promise for patients with a wide range of serious conditions. These therapies rely on a supply of cells of appropriate safety and quality. This chapter provides guidance for tissue establishments on quality and safety aspects, not only of donation, procurement and testing of the starting material for these applications but also in the further processing, storage and release of the cells. The general quality and safety demands in Chapters 1 to 15 (Part A) apply, but some specific considerations for these cells are also relevant. This chapter also aims to provide an overview of some of the cellular therapies used in the clinic but still under further development. The special considerations for donor selection, procurement, certain quality criteria, storage and administration as well as biovigilance are described in these sections, and an overview of the different steps is provided in Table 28.1.

The following chapters of this Guide all apply to these cells and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, risk management and validation (Chapter 2);
- c. Donor evaluation (Chapter 4);
- d. Donor testing (Chapter 5);
- e. Premises (Chapter 8);
- f. Principles of microbiological testing (Chapter 9);

- g. Distribution and import/export (Chapter 10);
- h. Organisations responsible for human application (Chapter 11);
- i. Computerised systems (Chapter 12);
- j. Coding, packaging and labelling (Chapter 13);
- k. Traceability (Chapter 14);
- l. General considerations for cell-based therapies (Chapter 26).

In the EU, some of the cell preparations described in this chapter fall under the definition of an advanced therapy medicinal product (ATMP). Such cell preparations are governed by specific requirements and procedures, including prior authorisation by the competent authority. When ATMP preparation takes place in the EU, or where products meeting the ATMP classification are intended to be used in the EU, their processing, quality control, storage, packaging, distribution, traceability and use must be done in accordance with medicinal product legislation, specifically Regulation (EC) No. 1394/2007. In these cases, donation, procurement and testing of such cells must comply with the requirements in Directive 2004/23/EC. For all other requirements, full GMP must be applied.

Any operator intending to process, store, distribute or use cells which might be considered to be ATMPs should seek advice from their national competent authority.

In case of doubt regarding whether a specific cell-processing activity is regulated under the ATMP regulation, a recommendation from the Committee for Advanced Therapies (CAT) can be requested. For further details about EU legislation for ATMPs (including the manufacturing, storage, distribution, labelling, advertising, traceability and use thereof), see Chapter 26.

28.2. Donor lymphocytes

28.2.1. Introduction

Donor lymphocytes infusion (DLI) is a procedure in which lymphocytes from the original allogeneic HPC donor are given to the recipient following allogeneic HPC transplantation, to augment the anti-tumour immune response. Since the donor selection, testing, procurement and human application is closely linked to the HPC, DLI are described in detail in Chapter 21. An overview is included in Table 28.1.

28.3. Keratinocytes

28.3.1. General introduction

The current gold standard of burn care [1] is early tangential excision of eschar and autologous split-thickness skin grafting to the surgical wound areas. However, once 20-30 % of the body surface area is burned, the access to healthy skin for transplantation starts to be limited. Procurement of skin transplants can be repeated from the same donor site after some weeks of healing but since every procurement includes a dermal portion eventually, due to the risk of creating a full-thickness skin wound, one can usually not procure more than 2 or 3 times. Therefore, culture of autologous keratinocytes is often the last resort for the most severely burned patients, and a useful tool in treating significant burns. The skin has many crucial functions and the main goal with the treatment is to heal the patient's skin as soon as possible and thus restore its functions to make survival from the burn possible for the patient.

Culture of human keratinocytes has been in clinical use since the beginning of the 1980s [2]. Cultured keratinocytes, i.e. epithelial cells, can be used both for autologous and allogeneic treatment of patients as described here (see also Table 28.1).

The skin comprises approximately 5 % epidermis and 95 % dermis (even though sometimes part

of the subcutaneous fatty tissue is regarded as part of the skin). The dermis is responsible for the skin's strength and pliability. Cultured skin in most applications today is composed only of epidermal cells (keratinocytes) which restores a new epithelium (epidermis) on the patient, thus closing the wound and contributing to the survival of the severely burned patient. Depending on the depth of the burn (i.e. deep dermal or full-thickness burn) the amount of remaining dermis varies greatly. As a result, the quality of the healed skin, after transplantation of cultured epithelial autografts (CEA), varies equally greatly (in structure, function and cosmetics), depending on the residual amount of dermis.

Today there are very few therapies to restore the dermis through ordinary cell culture; this is due, *inter alia*, to the morphological appearance of the dermis with a substantial extracellular matrix in a three-dimensional structure. Extensive research is needed to develop a tissue-engineered skin consisting of both dermis and epidermis that is useable for wound care. Meanwhile, when the options for skin substitutes are limited, biological acellular allogeneic dermis (prepared from donated skin) or synthetic dermal regeneration templates can be used for reconstruction of the dermis. By applying a 3D biological degradable matrix to surgical wounds, the host's cells will migrate and populate the matrix, start to develop autologous extracellular matrix while degrading the applied matrix, thereby restoring the dermal part of the skin and improving the quality of the reconstructed skin [3] (see also Chapter 18).

Cultured keratinocytes can be used in two ways, either for permanent skin cover in an autologous manner, or contributing to healing in an allogeneic situation through the natural growth-stimulating properties of the cells:

- a. Autologous keratinocytes can be cultured and guided into stratified growth, rendering keratinocyte sheets, which can be grafted in the same manner as split-thickness skin grafts [4]. A novel, alternative way is to culture keratinocytes in multiplicity and mix the cell suspension with tissue glue, to be sprayed onto the wound in a single-cell suspension [5, 6]. Epidermal cell suspensions without culturing, containing keratinocytes, melanocytes and fibroblasts, can also be applied as spray to restore epithelialisation *in vivo* (see §18.10).
- b. In the allogeneic use of cultured keratinocytes on full-thickness wounds, the cells make up a temporary skin that will eventually be sloughed and replaced by the patient's own skin (either split-thickness skin grafts or CEA). With the

use of allogeneic keratinocytes on superficial dermal wounds, the healing time can be shortened and – since the wound is superficial – the possibility of spontaneous re-epithelialisation is good.

28.3.2. Donor selection

For autologous use, 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. The sooner (after trauma) the skin biopsy is taken, the better because the patient (and tissue) will 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 burns.

For allogeneic application, donor selection must include careful examination to exclude donors with skin malignancy or other dermal conditions, in addition to the general donor evaluation and testing described in Chapters 4 and 5. The transplanted allogeneic keratinocytes will be sloughed off, and thus human leucocyte antigen (HLA) typing or ABO (ABO) blood grouping are not necessary.

28.3.3. Procurement

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 burned 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 basal culture medium with the addition of 10 % foetal bovine 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.

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 26).

28.3.4. Quality controls/release criteria

The quality controls needed, besides microbiological controls (see Chapter 9), include continuous surveillance: of keratinocyte morphology, mode of growth and expansion rate. It is important to have cellular expansion that corresponds to the patient's need for cultured keratinocytes, but it is equally important not to exceed the cells' capacity to proliferate. The possibility of proliferation within the keratinocytes is determined by different biological aspects, including the age and healthiness of the patient and the biopsy site.

28.3.5. Packaging and distribution

The packing procedure is determined by the mode of delivery, i.e. sheets or suspension. Sheets are attached to an inert synthetic carrier-membrane and placed one by one in transport liquid consisting of a basal cell-culture media (e.g. Leibovitz 15). Keratinocytes delivered for spray application are enzymatically detached from the culture vessel, washed repeatedly in basal cell-culture media and finally diluted in a minimal amount of basal cell-culture media, just enough to be in a solution. Further dilution is performed with the tissue glue to reach the appropriate cell concentration at the surgery room, just before application of the cells. The extent of dilution depends on the number of cells needed to cover the burned area of the patient.

The keratinocytes, either in sheets or in suspension, must be applied as soon as possible (or within a maximum of approximately 6 hours) after preparation.

28.3.6. Traceability

Records to secure traceability from donor to recipient, and all steps in between, are the responsibility of the tissue establishment, as described in Chapter 14.

28.3.7. Biovigilance/pharmacovigilance

As described in Chapter 15, adverse events and adverse reactions should be documented and reported. One can foresee that any event in the laboratory affecting the culture conditions, and thus reducing the amount of cells or the size of cell layer expected on the day of transplantation, should be considered as an adverse event.

28.4. Dendritic cells**28.4.1. General introduction**

Tumour vaccines based on dendritic cells (DC) are a new form of immunotherapy which is being tested in a large number of trials internationally [7]. DC have the capacity to activate tumour-specific T-cells to attack and eliminate the patient's tumour. There are several subtypes of DC vaccines, but most are derived from monocytes that are cultured in a cytokine mixture composed of GM-CSF and IL-4 and then induced into mature DC by various maturation factors. See Table 28.1 for an overview.

28.4.2. Donor selection

The majority of DC vaccines are autologous and derived from monocytes purified from the blood circulation of the patients. As described in Chapter 5, donor testing includes assays for transmissible diseases; although their presence is not an exclusion criterion, it should be documented and care should be taken to avoid cross-contamination of other cells or personnel. Medical evaluation of the patient should take into account the burden of collecting large amount of leukocytes using apheresis. Depending on the method used to obtain monocytes from the collected leukocytes, a predetermined level of circulating monocytes may be relevant.

28.4.3. Procurement

As a starting material, apheresis-derived leukocytes are normally used. Apheresis should be performed by professionals specialised in apheresis, with the precautions mentioned in this chapter for DLI collection (Chapter 21).

The blood volume processed, in order to obtain a sufficient number of monocytes for further processing, 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.

28.4.4. Quality controls/release criteria

Prior to freezing, the mature DC are tested by flow cytometry for their expression of a number of cell-surface markers which are characteristic for mature DC. These include markers such as low CD14 expression and high expression of CD80, CD83, CD86, CD1a, HLA-DR, DC-SIGN, ILT-3 and CCR-7. Secreted markers, e.g. IL-12, can be tested by ELISA assays.

28.4.5. Packaging and distribution

Matured and aliquoted DC are frozen with cryoprotectant in cryo-tubes and stored at -140°C in a freezer or in liquid nitrogen in a cell bank. Frozen cells are distributed on dry ice or in liquid nitrogen.

28.4.6. Traceability

Records for the complete process from donor selection to clinical use should be kept by the responsible tissue establishment.

28.4.7. Biovigilance/pharmacovigilance

Any adverse event during procurement of leukocytes, cell separation, culturing, cryopreservation or distribution, or any adverse reactions during administration, should be documented as described in Chapter 15. An example of such an adverse event might be lower numbers of monocytes than expected when collected by apheresis or during the cell separation procedures. The route of administration of DC may involve adverse reactions that should be recorded and if possible avoided, or at least managed according to a risk-benefit assessment.

28.5. Chondrocytes**28.5.1. General introduction**

Damaged articular cartilage has a limited capacity for self-repair. Cartilage lesions are usually associated with disability and symptoms such as pain, swelling, locking and malfunction of the joint, and if these lesions are left untreated it may lead to osteoarthritis. Autologous chondrocyte implantation (ACI) is a therapy widely used for the treatment of isolated cartilage defects. The original (first-generation) technique is based on an implantation of a suspension of *in vitro* expanded chondrocytes into the defect beneath a sealed cover of periosteum flap. Since the technique was introduced in 1987 by Brittberg *et al.* [10], over 35 000 patients have been treated worldwide.

The second-generation ACI technique includes the use of a collagen membrane instead of the periosteal flap. The use of collagen membrane simplifies the surgical procedure and reduces complications such as periosteal hypertrophy.

Further technological advances led to the development of the third-generation technique that involves both *in vitro* expanded chondrocytes and a scaffold (briefly described in Chapter 27). After the expansion in culture flasks, the cells are seeded onto a membrane or a biodegradable scaffold before implantation. The scaffold may function as a carrier for the cells or as a structure to build up the 3D environment for the cells. In most techniques only fibrin glue is used for the fixation of the graft and, since there is no need for any suturing of the periosteum/collagen membrane cover, this implantation can be done arthroscopically.

For an overview, see Table 28.1.

28.5.2. Donor selection

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 ($> 2 \text{ cm}^2$) full-thickness chondral or osteochondral defects surrounded by healthy cartilage. 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 [11].

28.5.3. Procurement

The ACI technique includes a two-stage procedure, with an initial harvest of a cartilage biopsy, which is sent for chondrocyte culture, followed by a second-stage operation that includes the cell implantation. A full-thickness cartilage biopsy (about 200-400 mg) is harvested 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 13). The biopsy should be kept cold – at about $5\text{--}15^\circ\text{C}$ – during transport to ensure the quality of the biopsy specimen. Transport should be sent directly to the tissue establishment for further processing, which should start within 48 hours. For culture conditions with autologous serum

instead of foetal bovine serum, up to 50 mL of autologous blood should accompany the biopsy.

28.5.4. Quality controls/release criteria

Living cells cannot be sterilised and therefore it is very important to ensure that all handling of the product is performed under good aseptic conditions and that all material, media and reagents used are sterile and endotoxin-free (see Chapter 9). The first sterility test for release is done a few days before the scheduled implantation, at the last media change, and the second test is made during assembly of the final product. The final result of a sterility test takes normally 10-14 days and the expiry time for the final product is normally 24-72 hours, so it is common that the cells are already released and implanted before the final results are available. However, a preliminary result of the sterility test can normally be given after 24 hours and it is upon this result that the cells can be released. An indication of the viability of cells in suspension is obtained using trypan blue. It is more difficult to test the viability of cells growing in a 3D construct. There is currently no non-destructive assay available, but other release criteria specific for chondrocytes are used.

28.5.4.1. Morphology

The morphology of the cells can be followed easily during culture using an inverted microscope. The chondrocytes should be typical of cultured chondrocytes in appearance, and the personnel who perform this subjective judgment must have good experience in this task and should have reference pictures of cultures for comparison. The cells lose the phenotype of uncultured chondrocytes during culture and become more fibroblast-like.

28.5.4.2. Population doubling

The cells should undergo only a limited and defined number of population doublings, for example 4-8 population doublings. In order to ensure a proper re-differentiation of the cells, the functional capacity of the cells after maximal expansion has to be shown, and it is important to record the population doubling during culture.

28.5.4.3. Number of cells

The number of cells must be stated on the product. This can be in form of either the actual number of cells in the vial or syringe or the number of cells per surface area that have been seeded in the scaffold. Other labelling requirements are as described in Chapter 13.

28.5.4.4. Purity

To determine the possible contaminants in the product, such as synoviocytes or other types of impurity such as bone cells, the purity of the product is assayed. A representative batch of cells can be validated for presence of mRNA markers for chondrogenic lineage, like *sox9*, and lack of (or low) mRNA expression of synoviocyte-specific genes. Since cells are dedifferentiated during culture, more specific markers of differentiation are not tested.

28.5.4.5. Chondrogenic potential

Attempts to predict the chondrogenic potential of the cells and thus the chondrogenic repair capacity have been made [12], but currently no clinical potency marker exists. Thus, functional properties of the cells and an appropriate surrogate marker are still to be defined and research is needed.

28.5.5. Packaging and distribution

The first-generation product (cells in a suspension) is aseptically filled in syringes or vials. The third-generation product is aseptically packed in a sterile two-container system. The advantages of having primary packaging that is also sterile on the outside is that it can be taken directly to the operation table and be handled by sterile personnel. The product should then be placed in an outer secondary packaging that ensures the sterility and the temperature and should be approved for transport of biological substances. Implantation is normally done within 48 hours, depending on the expiry time for the product. For the accompanying information sent to organisations responsible for human applications (ORHA), see Chapter 11.

28.5.6. Traceability

Records with all information, from procurement to implantation, must be kept. Reference samples may also be archived.

28.5.7. Biovigilance/pharmacovigilance

Several adverse events that affect the growth and differentiation of chondrocytes may occur during the relatively long *in vitro* expansion period. In addition, if for example the degree of contaminating cell types unexpectedly exceeds the release criteria, this should be recorded and managed as described in Chapter 15.

28.6. Hepatocytes

28.6.1. General introduction

Hepatocyte transplantation is an experimental treatment for patients with metabolic liver diseases or acute liver failure, or as a temporary support for patients with chronic liver failure while waiting for an organ transplant. 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. As the liver has a high redundancy in function, selective replacement of a fraction of the liver cell mass is clearly therapeutic and replacement of the whole liver by liver transplantation may not be required. It is assumed that replacement of 10-20 % of the liver with healthy donor hepatocytes can correct a wide range of inherited metabolic liver diseases [13]. In patients with acute and chronic liver failure, hepatocyte transplantation can give 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 surgically invasive approach resulting in lower morbidity and mortality; one donor liver provides enough hepatocytes (without the need for cell expansion) for several recipients, possibly improving the ratio of donor organs to patients on the waiting list; viable and functional hepatocytes can be isolated from marginal donor livers usually not accepted for whole organ transplantation; and, in contrast to whole organs, cells can be cryopreserved and stored until needed. See Table 28.1 for an overview.

The transplantation procedure is in most cases an infusion into the portal vein. The spleen and the peritoneal cavity have also been used successfully.

The generic chapters of this guide apply to hepatocyte isolation and transplantation, and must be read in conjunction with this chapter. This chapter defines the additional specific requirements for liver tissue and hepatocyte isolation and transplantation.

28.6.2. Donor selection

Liver tissue for hepatocyte isolation can be procured from donors after brain death (DBD) and donors after circulatory death (DCD). Liver tissue can also be procured from living donors in a so-called domino procedure where a patient is undergoing a liver transplantation when the indication for the transplant is not considered to be a contraindication for the hepatocyte recipient [14]. In theory a healthy

living donor could also donate a part of their liver. However, this procedure has not been 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.

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 cause disease, e.g. hyperoxalosis, familial amyloidotic polyneuropathy;
- b. alterations to the liver vessels that could complicate perfusion and isolation of hepatocytes;
- c. donor characteristics that might affect hepatocyte quality, such as age of donor, size of liver tissue, degree of steatosis and length of both warm and cold ischaemia [15-16].

28.6.3. Procurement

Liver tissue is usually procured from deceased donors by medical staff at a surgical department or transplant unit. Liver tissue can also be obtained from a living donor, e.g. a patient undergoing liver transplantation when the indication for the transplant procedure is not considered to be a contraindication for the hepatocyte recipient. Staff performing the procurement must be dressed appropriately for the procedure so as to minimise both the risk of contamination of the tissue to be removed and any hazard to themselves.

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.

28.6.4. Quality controls/release criteria

Hepatocytes exhibit a wide variety of functions that can be individually tested. In general, quality testing should be 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.

Cryopreserved hepatocytes [17] have the advantage that more extensive quality testing can be

performed, which is not possible for unprocessed hepatocytes due to time constraints. However, current cryopreservation protocols induce severe hepatocyte damage, which decreases both viability and function.

Cell number and an indication of viability by the trypan blue exclusion test should be evaluated for all hepatocyte isolation, and these are the most important release criteria. In general, viability above 50 % is a minimum release criterion for clinical transplantation. The trypan blue exclusion test detects only cell-membrane damage; it cannot detect apoptotic cells or determine metabolic or physiological function.

The following quality tests could also be considered as possible release criteria:

- a. Plating efficiency, ability to adhere to extracellular matrix;
- b. Cytochrome P450 activities;
- c. Urea cycle activity, metabolism of ammonia into urea;
- d. 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. 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.

28.6.5. 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 [18].

28.6.6. Traceability

Records covering the complete procedure – from donor selection to recipient transplantation – should be kept at the tissue establishment. 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.

28.6.7. 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. 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 (see also Chapter 15 for management of adverse reactions).

28.7. Mesenchymal stem cells

28.7.1. General introduction

Mesenchymal stem cells (MSC), also referred to as mesenchymal stromal cells [19], are multipotent stem cells that can differentiate into a variety of cell types, including osteoblasts (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells). This phenomenon has been documented in specific cells and tissues in living animals and their counterparts growing in tissue culture.

While the terms MSC and bone-marrow stromal cells (BMSC) have been used interchangeably, neither term is sufficiently descriptive, as discussed below.

Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into haematopoietic and connective tissue, whereas MSC do not differentiate into haematopoietic cells.

Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSC, the term fails to convey the (relatively recently discovered) roles of MSC in the repair of tissue.

The term MSC, used by many labs today, can encompass multipotent cells derived from other non-bone-marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle or the dental pulp of deciduous baby teeth; see also Table 28.1.

The International Society for Cellular Therapy (ISCT) encourages the scientific community in all written and oral communications to adopt this uniform nomenclature (MSC) when cells meet specified stem cell criteria. The ISCT defines the specific MSC criteria thus:

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC.

First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSC must differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*. [19]

These criteria will probably change as new knowledge unfolds. MSC are characterised morphologically by a small cell body with a few cell processes that are long and thin. The cell body contains a large, round nucleus with a prominent nucleolus, which is surrounded by finely dispersed chromatin particles, giving the nucleus a clear appearance. The cells, which are long and thin, are widely dispersed; and the adjacent extracellular matrix is populated by a few reticular fibrils but is devoid of the other types of collagen fibrils.

The immunoregulatory and regenerative properties of MSC make them an attractive tool for the development of treatments of autoimmunity, inflammation and tissue repair [20, 21]. MSC do not induce alloreactivity but generate a local immunosuppressive microenvironment by secreting cytokines. However, MSC interfere with dendritic cell activation, and they suppress lymphocyte activation and T-cell function *in vitro*. They have been shown to reverse inflammation in several experimental animal models, and clinical studies indicate that MSC are immunosuppressive also in humans as they reverse steroid-refractory GvHD and other inflammatory conditions [22, 23]. MSC are a heterogeneous population of cells, with functions depending on both source and *in vitro* culturing conditions. MSC are also investigated for tissue-engineering purposes mainly for osteo-articular diseases: bone and cartilage regeneration. The mechanisms behind their tissue-regenerating ability and their immunomodulating capacity, and the extent to which the two processes interact, require further elucidation. In view of the increasing interest in using MSC for human application, the safety and quality aspects to bear in mind are mentioned in this section.

28.7.2. Donor selection

Under resting conditions, MSC express HLA class I but not class II alloantigens. When cultured *in vitro* with allogeneic lymphocytes, MSC do not 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. However, MSC that are to be used for their regenerative capacity should preferably be autologous.

Donors should be evaluated for their own safety, and for the safety of the recipient, according to the criteria described in Chapter 4.

28.7.3. Procurement

MSC can be isolated from haematopoietic tissues, such as bone marrow, peripheral blood and umbilical cord blood, but also from parenchymal non-haematopoietic tissues, such as muscle, fat or liver. The youngest, most primitive MSC can be obtained from the umbilical cord tissue, namely Wharton's jelly. Umbilical cord-derived MSC have more primitive properties than other, adult MSC obtained later in life, which might make them a useful source of MSC for clinical applications.

The two main sources of MSC presently used for human application are bone marrow- and adipose-derived. The latter is one of the richest sources of MSC: there are about 500 times more stem cells in 1 gram of adipose tissue than in 1 gram of aspirated bone marrow.

28.7.4. Quality controls/release criteria

It is challenging to identify markers that may be predictive for the potency of a specific product with a specific indication. However, surrogate markers will be needed to address functional properties and consistency of the cells. Further release criteria, apart from sterility (absence of bacteria, mycoplasma and fungi; see Chapter 9), might be the haematopoietic contaminating cells to be excluded from the culture. Often, phenotypic release criteria include cultures predominately expressing CD73, CD90 and CD105, but the relevance of these remains to be clarified.

It is therefore necessary to define and validate the release criteria depending on the culture conditions used and the clinical protocol.

28.7.5. Packaging and distribution

When distributed to the clinic for administration to the patient, the cells should be either transferred to vials with the pre-decided dose for administration or distributed directly in a pre-labelled syringe, depending on whether the MSC are processed in the close vicinity of the clinic.

Frozen vials of MSC are often thawed at the bedside by diluting with isotonic saline solution, at

least 4× volumes (to avoid toxicity by the cryoprotectant to the MSC), and administered immediately to the patient. A small portion of the thawed, diluted MSC can be used to verify cell numbers and viability.

28.7.6. Traceability

Records to ensure traceability from the donation to the recipient should be kept with the tissue establishment.

28.7.7. Biovigilance/pharmacovigilance

As indicated above, the MSC consist of a heterogeneous population; their phenotype and function are dependent on source and culture condition. Accordingly, any deviation from the expected endpoints (according to the predefined criteria) should be considered an adverse event that should be recorded.

28.8. Limbal stem cells (ocular surface)

28.8.1. General introduction

In the normal human eye, the epithelial cells of the cornea and conjunctiva are responsible for the continuing regeneration and homeostasis of the ocular surface. Cells with progenitor characteristics have been identified in the corneoscleral limbus (the transitional zone between the cornea and sclera) and these have the capability to continually repair and replace the epithelial surface of the cornea [24]. These cells are known as limbal stem cells (LSCs). Limbal stem cell deficiency, a term which covers 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 [25]. These anomalies can be painful and are very difficult to treat because of the significant impairment of the patient's vision which, in most cases, progresses to legal blindness. LSCs have the characteristics of undifferentiated multipotent stem cells and are capable of undergoing proliferation and differentiation into the corneal epithelium. They may also be genetically modulated for therapeutic purposes [26].

Currently, approaches to the treatment of many ocular surface diseases focus mainly on three strategies: transplantation of portions of keratolimbus (Kenyon technique), either from the healthy fellow

eye or from a cadaveric donor; *ex vivo* LSC expansion from a limbal explant; *ex vivo* expansion of isolated LSCs and *in vitro* culture. Achieving effective control of the underlying inflammatory process and preventing tissue rejection is the key objective [25, 26]. In addition to LSCs, corneal endothelial cells [27] derived from the corneoscleral disc are being used for treatment of corneal endothelial disease; see Chapter 16 and Table 28.1. Recently, retinal pigment epithelial cells [28], induced to differentiate from different sources of pluripotent stem cells, have been investigated for the treatment of age-related macular degeneration; see Table 28.1 for an overview.

28.8.2. Donor and tissue selection

Donors should be tested for infectious diseases as described in Chapter 5, although in the case of autologous transplantation a positive test result may not be an exclusion criterion.

The treatment of unilateral LSC deficiency involves *ex vivo* expansion of a tissue explant or isolated LSCs from the unaffected limbal region of a patient's healthy eye. The autologous cell population is isolated and the final aim is to expand the limbal epithelial cells for transplantation 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 transplantation of the cells (see Chapter 27). 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 transplantable tissue include the culture of epithelial cells lining the autologous oral mucosa. Allogeneic sources of transplantable 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 [29, 30].

28.8.3. Procurement

Extraction as well as *ex vivo* expansion/*in vitro* cultures must be carried out in strict laboratory conditions.

The explants (autologous or allogeneic) are obtained by a biopsy of healthy ocular tissue from the limbal region and maintained in sterile culture medium until processed. The most widely applied technique, with minimal manipulation of tissue, is the explant culture system, in which the specimen

obtained by biopsy is directly placed in the centre of the amniotic membrane.

Epithelial cells (LSCs) grow out from the biopsied tissue and proliferate to form a multi-layered epithelial construct.

An alternative approach, with additional manipulation of the tissue, is to obtain single epithelial cell suspensions by enzymatic isolation (a one- or two-step enzymatic method) from limbal specimens.

28.8.4. Quality controls and release criteria

Microbiological tests that simulate normal processing conditions by replacing the cells with culture media are essential. Three simulations of all the critical stages of the process should be performed before the implementation process [31].

Conventional microbiological (bacteria and fungi) assays should be performed at baseline, during the process and in the final product before clinical use. Specific tests for the presence of *Mycoplasma* spp. are conducted after handling the final product (see Chapters 9 and 26). Morphological control of culture cells and bioengineered products can be achieved with the use of a phase-contrast microscope. Basic cell-culture techniques can be applied, such as cell viability tests with vital dyes and techniques for cell counting using an automated cytometer or cell-counting chambers.

In the explant culture system the final product requires less handling, and adequate microbiological control of the final product (LSCs on amniotic membrane) is needed to obtain approval for clinical use. Further release criteria are to be established during the pharmaceutical and clinical development of the cell-based product. In the enzymatic approach, in addition to the controls mentioned, the cell product may be tested for the presence of holoclones (clonogenic assay), high expression of the transcription factor deltaNp63alpha or ABCB5 and negative or very low expression of markers both for differentiated epithelial cells CK3 and CK12 and, where used as a feeder layer for LSC expansion, markers or PCR-based assays for the presence of 3T3 murine fibroblasts [29, 30].

28.8.5. Packaging and distribution

The purpose of packaging and labelling operations is to protect the product and to provide identification and information for the user. Packaging and labelling are key elements in the Quality Management System to ensure that appropriate standards are maintained during a product's life cycle [31].

The final cell product should be conditioned in a suitable sterile container immersed in unprocessed complete cell-culture medium and maintained at an appropriate and controlled temperature until arrival at the transplant centre (see also Chapter 13). A maximum period should be set for delivery and it is recommended that the product is implanted on the same day as its release for clinical use, unless longer storage is adequately justified. As described in Chapter 11, the essential information about usage of the cells should be included in accompanying documentation to the ORHA.

28.8.6. Traceability

Special consideration should be given to the reagents that come into contact with tissues and cells, and which may be left as residues when cells are applied in the recipient. The following links in the process are particularly important: between the donor and donation at the tissue centre; between donation and the final cell product at the manufacturing site; and between the final product and the recipient at the transplant centre. All records and information should be kept and stored at the tissue establishment(s) or according to national legislation.

28.8.7. Biovigilance/pharmacovigilance

As described in Chapter 15, deviations from SOPs, from donation to clinical application, should be recorded and documented, as well as adverse reactions after application.

28.9. Antigen-specific T-cells

28.9.1. General introduction

Technology is available for the capture and isolation of cells based on the affinity of cell-surface receptors for specific proteins or peptides immobilised on a suitable insoluble matrix. This technology can be used to isolate donor T-Cells from peripheral circulation with specific affinity for pathogenic or other antigens that can then be transplanted to elicit a beneficial immune response in the recipient (adoptive immunotherapy). For example, viral infection in immunocompromised patients after haematopoietic stem cell transplantation is a frequent cause of morbidity and mortality. It has been possible to reconstitute the antiviral immunity of the recipient against specific viruses – e.g. *cytomegalovirus* (CMV), Epstein–Barr virus (EBV) and *adenovirus* – through isolation and adoptive transfer of donor-derived

virus-specific T-cells. Also, pre-established virus-specific T-lymphocytes from allogeneic HLA-typed third-party donors may be used after HLA-mapping and selection of a suitable HLA-match [32]. If such banked virus-specific T-cells are to be used, a risk assessment based on the degree of HLA-mismatch and the risk of graft *versus* host disease (GvHD) or graft rejection must be considered by a qualified specialist in allogeneic stem cell transplantation.

T-Cells can also be modified, using gene-transfer technology (see Chapter 26), to express antibody-like receptors to selected antigens. These are synthetic proteins normally consisting of single-chain variable fragments (scFv) of an antigen-specific antibody fused with other proteins to ensure that it is displayed on the surface of the T-Cell with appropriate transmembrane activity and effector properties in response to the desired target. Because these synthetic receptors consist of a fusion of different proteins, they are known as chimeric receptors, and T-cells modified in this way are called chimeric antigen receptor-T (CAR-T) cells [33]. Because of the need for appropriate expression and processing of the chimeric proteins in the host cells, integrating retroviral or lentiviral gene vectors (see Chapter 26) are commonly used for gene transfer to create CAR-T cells. This approach is used for adoptive immunotherapy where sufficient naturally occurring antigen-specific T-cells cannot be isolated from a donor, most notably in the case of tumour cell antigens. Clinical trials with such engineered CAR-T-cells represent a promising development of specific anti-tumour responses; for a brief overview, see Table 28.1 [33, 34].

28.9.2. Donor selection

Donors should be tested for transmissible diseases in accordance with Chapter 5; in addition, the presence of circulating antibodies against the specific target antigen should be determined before assays for specific T-cells are initiated.

The patient's own cells are normally used for CAR-T-cell therapy. As well as the transmissible disease testing just mentioned, consideration should be given to the possible presence of wild-type virus of the same type as the basis of the gene vector employed and to the likelihood of the formation of replication-competent virus.

28.9.3. Procurement

A sample of heparinised venous blood (30–50 mL) from the stem cell donor is usually enough to select the virus-specific T-lymphocytes

from the mononuclear fraction of the blood. Otherwise, apheresis procedures following the specifications described above can be used (see Chapter 21).

For CAR-T-cells, a sample of heparinised venous may be enough, since the CAR-T-cells are then expanded *in vitro* and *in vivo*. However, the mononuclear cell fraction isolated through an apheresis process is normally used as starting material to ensure a higher dose of T-Cells for cell culture.

28.9.4. Quality control

The specific requirements for release include assays to determine their virus-specificity according to pre-established criteria for IFN- γ production that can be quantified using ELISpot assay or flow cytometry. Depending on the defined and validated protocol, the functional assay can be complemented with phenotypic release criteria using flow cytometry. Expected viability after thawing should also be established if the specific T-cells are stored frozen for repeated *in vivo* transfer. Where T-cells expanded in cell culture are used for cancer treatment, dosing may have to be synchronised with chemotherapy and the cells may need to be available freshly prepared. This may require a planned two-phase release (see Chapter 26).

28.9.5. Storage and distribution

Cultured and released cells can be cryopreserved $< -140^{\circ}\text{C}$ in liquid or vapour phase of nitrogen, or deep freezer with liquid nitrogen back-up.

Precautions should be taken to prevent cross-contamination of infectious agents if stored in the liquid phase of nitrogen, including the use of double containers.

28.9.6. Traceability

Records, with all information from procurement to *in vivo* administration, should be kept by the tissue establishment.

28.9.7. Biovigilance/pharmacovigilance

Whenever adverse events occur during the processing, or adverse reactions during application, of the T-cells this should be documented and reported (see Chapter 15). As an example, T-cells that do not fulfil criteria for specificity as measured by *in vitro* methods can still be used as they may fulfil the biological effect measured *in vivo*.

28.10. Natural killer cells

28.10.1. Introduction

Natural killer (NK) cells were described, first in mice, and later in humans as non-B, non-T lymphoid cells with a non-major histocompatibility complex (MHC)-restricted cytotoxic activity against transformed or virally infected cells. The ‘missing-self’ theory by Kärre *et al.* [35] and the identification of killer Ig-like receptors (KIR) acting as inhibitory or activating signals have contributed to the understanding and better design of clinical trials. NK cells are bone-marrow-derived from CD34⁺ progenitors, and migrate upon differentiation to lymphoid organs and peripheral blood. Their development and homeostasis are dependent on IL-15, and they express the adhesion molecule CD56 but lack the T-cell receptor and CD3. NK cells can be divided into at least two subpopulations according to their surface density of CD56 expression:

- CD56^{bright} in a resting stage are considered to be regulatory NK cells that produce high levels of cytokines and are more proliferative, but poor mediators of NK cell cytotoxicity.
- CD56^{dim} in a resting stage are potent cytotoxic cells mediating natural killer cytotoxicity as well as antibody-dependent cytotoxicity through CD16 (Fc γ RIII).

NK cells can be activated by several cytokines and they produce a wide variety of cytokines and chemokines: granulocyte-colony stimulating factor (G-CSF), tumour necrosis factor (TNF)- α and TNF- β , IFN- γ , tumour growth factor (TGF)- β , macrophage inflammatory protein 1-beta, and regulated on activation, normal T-cell expressed and secreted (RANTES). It is still not clear whether the *in vivo* effect of NK cells is a result of direct killing or indirectly through cytokine production, engaging other parts of the immune system. NK cell-based immunotherapies against malignancies involve using either the autologous NK cells *in vivo*, by cytokine stimulation, or by adoptive transfer of autologous or allogeneic NK cells. There are many different protocols and clinical studies using NK cells against malignancies, as reviewed by Cheng *et al.* [36], but not covered in this chapter.

28.10.2. Donor selection

Depending on the clinical protocol, autologous NK cells can be used, either by activation of the pre-

sumed NK cells *in vivo*, or by *ex vivo* selection and/or activation.

Increasing knowledge of MHC recognition (classical or non-classical) and interaction, and of the haplotypes of KIR, suggests that donor selection for protocols with adoptive transfer of allogeneic NK cells could include MHC typing for HLA-C, HLA-E and possibly also HLA-F and HLA-G as well as KIR. The aim would be to select a donor whose ligands for the inhibitory signals were absent in the recipient. For adoptive transfer of *ex vivo*-activated NK cells with additional *in vivo* activation, special care should be taken to reduce the possible toxic effects of cytokines, depending on the underlying disease and the dose of the cytokine(s).

28.10.3. Procurement

NK cells can be procured from peripheral blood, by apheresis carried out by experienced personnel, as described in Chapter 21, section 21.3.2.2.

Smaller amounts of NK cells to be expanded *in vitro* are obtained from 30-50 mL anti-coagulated venous blood. *In vitro* expanded NK cells can also be obtained from CD34⁺ stem cells from cord blood after *in vitro* differentiation and maturation with defined cytokines.

28.10.4. Quality controls/release criteria

In addition to microbiological testing as described in Chapter 9, release criteria should include the defined level of phenotypic NK cells as established by flow cytometry, and possibly also a functional assay (cytokine production or cytotoxic assay).

28.10.5. Packaging and distribution

The processed and released NK cells are aspirated into a syringe for direct administration to the patient, with labelling containing the recipient identification and, if applicable, the dose of NK cells. Attached documentation should contain additional information to prevent errors if other patients or clinical trials are ongoing in the clinic (see Chapters 11 and 13).

28.10.6. Traceability

Records, with all information from procurement to *in vivo* administration, should be kept by the tissue establishment.

28.10.7. Biovigilance/pharmacovigilance

Adverse events during the procurement or processing that should be documented could involve less NK cell recovery than expected after a cell-separation step or a lack of functional activity measured *in vitro* as a quality control. Adverse reactions that should be documented could involve unexpected side-effects related to the administration of NK cells or to the additional activating cytokines, all of which should be documented.

28.11. Stromal vascular fraction from adipose tissue

28.11.1. General introduction

Adipose tissue is a source of stromal cells similar to those identified in bone marrow. The so-called stromal vascular fraction (SVF) isolated from fat is 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 (ASC) (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 [37-39].

28.11.2. Donor selection

Commonly, SVF cells are used autologously. Donor testing includes assays for transmissible diseases as described in Chapter 5; although their presence is not an exclusion criterion, it should be documented and special actions should be taken to avoid cross-contamination to other cells and to ensure the safety of personnel.

28.11.3. Procurement

The SVF can be isolated from either resected adipose tissue or aspirated using tumescent liposuction. Although a common procedure is still lacking, in general minced adipose tissue is digested by enzymes including collagenase, dispase, trypsin or the like [39]. However, mechanical procedures have also been reported [40]. After neutralising the enzymes, the released elements defined as SVF are separated from mature adipocytes by differential centrifugation.

28.11.4. Quality controls/release criteria

Currently, as there is no single marker to identify SVF cell subpopulations, the use of a combination of fluorochrome-labelled antibodies to surface antigens is suggested. The following markers or marker combinations should be considered for identifying the stromal cells within the SVF: CD45⁺/CD235a⁺/CD31⁺/CD34⁺, CD13⁺, CD73⁺, CD90⁺, CD105⁺. Alternative positive stromal cell markers, including CD10, CD29 and CD49, can be applied. Viability is recommended to be > 70 % to allow for good cell expansion. A fibroblastoid colony-formation unit assay and testing of the differentiation potential of the ASC within the SVF might enhance information on the quality of the cellular product [29].

However, it is necessary to define and validate release criteria that are specific to the chosen clinical setting.

28.11.5. Traceability

Records to ensure traceability, from the donation to the recipient, should be kept with the tissue establishment.

28.11.6. Biovigilance/pharmacovigilance

Although recent literature supports a paracrine role for SVF cells in regenerative settings, these same secreted cytokines may have adverse effects in the presence of tumour cells, e.g. recruiting the homing and promoting the proliferation of cancer cells following transplantation [39]. Hence, the safety of SVF treatment has to be evaluated carefully.

Table 28.1. Overview of developing cellular therapies

	Starting material	Processing steps	Parameters for specificity (quality control)	Storage	Transport/distribution/administration
Donor lymphocytes (see §28.2 and Chapter 21)	Apheresis product	Obtaining lymphocytes (T-cells): Fraction from HPC-collection or Unstimulated apheresis with settings for mononuclear collection	Phenotype (CD3⁺, CD4⁺, CD8)	≤ -140 °C	Administer immediately or Frozen vials thawed at bedside
	Allogeneic origin				
Keratinocytes (see §28.3)	Biopsy	Isolation of keratinocytes: 1. Mechanical or enzymatic in combination with mechanical	- Morphology - Expansion rate	Ideally, use fresh. If necessary, store at ≤ -140 °C. Short period (1-2 days) of culture is needed after thawing, before distribution	Keratinocytes in sheets attached to synthetic carrier membrane and kept in culture media or Keratinocytes in suspension as spray – administration: immediate
	Autologous or allogeneic origin	2. Culture with or without serum and feeder layer			
Dendritic cells (see §28.4)	Leukocytes collected by apheresis	Isolation of monocytes: 1. Physical isolation (counterflow centrifuge elutriation) or Affinity column separation based on surface markers (CD14) 2. Culture with growth factors for differentiation into dendritic cells. 3. Maturation step with required specificity.	- Phenotype (CD80⁺, CD83, CD86⁺, CD1a⁺, HLA-DR⁺, CD14⁺) - IL-12 production	≤ -140 °C	Transport at low temperature Administration: immediate after thawing at bedside
	Autologous origin				

Table 28.1. Overview of developing cellular therapies (continued)

	Starting material	Processing steps	Parameters for specificity (quality control)	Storage	Transport/distribution/administration
Chondrocytes (see §28.5)	Cartilage biopsy Autologous origin	Isolation of chondrocytes: 1. Mechanical and enzymatic 2. Culture for 2-3 weeks as adherent cell layers (expansion) and/or Culture for 4-5 weeks expansion and attachment to scaffold for 3D support	- Morphology - Limited population doubling (max. 8 x) - Purity of the population (mRNA)	Not to be stored	Chondrocytes are suspended into syringes/ampoules for administration within 48 h
Hepatocytes (see §28.6)	Liver Allogeneic origin	Isolation of hepatocytes: 1. Mechanical (2-8 °C) 2. Collagen perfusion through hepatic vessels in three steps: i. Calcium chelator ii. Removal of the chelator iii. Collagenase (37 °C) 3. Filtration and low-speed centrifugation	- Enzyme production, depends on disease of intended recipient - Cytochrome P450 activities - Viability	$\leq -140^{\circ}\text{C}$	Distribution at 2-8 °C Administer as soon as possible
Mesenchymal stem cells (see §28.7)	Bone marrow, peripheral blood, umbilical cord blood, adipose tissue, muscle, liver and umbilical cord tissue (Wharton's jelly) Allogeneic or autologous origin (depending on source)	Generation of functional mesenchymal stem cells: <i>In vitro</i> culture with growth factors. Culture conditions depend on source of cells.	Phenotype: Depends on the protocol for <i>in vitro</i> culture, but usually CD73 ⁺ , CD90 ⁺ and CD105 ⁺	$\leq -140^{\circ}\text{C}$	Preparation of non-cryopreserved cells in syringe or Frozen vials thawed at bedside
Limbal cells (see §28.8)	Biopsy from the limbal region of the eye Autologous or allogeneic	Generation of limbal cells: Biopsy is cultured on a feeder layer (irradiated) or Directly attached to amniotic membrane	- Morphology - Clonogenic assay (holoclones) - Transcription factors - Phenotypes for epithelial cells (CK3⁺, CK12⁺) The parameters assessed will depend on culture system.	No storage	2-8 °C during transport to the clinic. Administration the same day as released
Retinal pigment epithelial cells	Embryonic stem cells, pluripotent stem cells, cord blood, foetal brain or bone marrow	Generation of retinal pigment epithelial cells: <i>In vitro</i> culture with growth factors	- Immunohistochemistry - Phagocytosis - Melanin content - Up regulation of retinal pigment epithelial cells-specific genes	$\leq -140^{\circ}\text{C}$	Thawed and re-suspended cells distributed to operating room at 2-8 °C

Table 28.1. Overview of developing cellular therapies (*continued*)

	Starting material	Processing steps	Parameters for specificity (quality control)	Storage	Transport/distribution/administration
Corneal endothelial cells (see §28.8)	Corneoscleral disc Allogeneic origin	Isolation of corneal endothelial cells: 1. Mechanical, released during medium storage 2. Expanded <i>in vitro</i>	- Morphology - Expression of ZO-1 and Na⁺/K⁺-ATPase	No storage	Distributed to operating room at 2-8 °C
Antigen-specific T-cells (see §28.9)	Blood from heparinised sample or apheresis product Allogeneic origin	Selection of virus-specific T-lymphocytes: 1. Obtain mononuclear fraction 2. T-cell selection based on i. phenotype with peptide-HLA-multimers or ii. Interferon gamma (IFN γ) production after stimulation with specific peptides and antigen-presenting cells 3. Expansion including re-stimulation	Specificity for the virus measured by - IFNγ production (ELISA or flow cytometry assay) - Phenotype	$\leq -140\text{ }^{\circ}\text{C}$	Administered fresh or Frozen vials thawed at bedside
CAR-T cells (see §28.9)	Blood from heparinised sample or apheresis product Autologous origin	Preparation of CAR-T cells: 1. T-cell selection 2. Insertion of the modified gene for the chimeric receptor 3. <i>In vitro</i> expansion	Specificity for the antigen measured by - Expression and functionality of insert by cytokine production - Phenotype	$\leq -140\text{ }^{\circ}\text{C}$	Administered immediately or Frozen vials thawed at bedside
Natural killer cells (see §28.10)	Blood from heparinised sample, apheresis product or cord blood Autologous or allogeneic origin	Selection of functional NK cells: 1. Obtain mononuclear fraction 2. Positive and/or cell selection using phenotypes: i. T-cell depletion (CD3) and/or ii. NK cell enrichment (CD56) 3. (Optional) activation and expansion <i>in vitro</i>	- Phenotype - Function measured by cytokine production and cytotoxicity	$\leq -140\text{ }^{\circ}\text{C}$	Administered immediately or Frozen vials thawed at bedside
Stromal vascular fraction (see §28.11)	Adipose tissue Autologous or allogeneic origin	Isolation of stromal vascular fraction: Enzymatic and/or mechanical. No culture	Phenotype (heterogeneous populations)	$\leq -140\text{ }^{\circ}\text{C}$	Administered immediately or Frozen vials thawed at bedside

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Chapter 29. Other substances of human origin for clinical application

29.1. Introduction

This chapter introduces several other substances obtained from humans for autologous or allogeneic use. In general, their regulatory classification is challenging because they do not fit clearly into one group of therapies or another, whether the criterion applied is anatomical origin, method of application, mode of action or complexity of processing.

Hence, a wide variety of approaches to regulation exist in Europe [1], and in some cases these substances are banked outside any regulatory framework.

Given the significant commercial interest in developing these services 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.

Regardless of their regulatory status, ensuring safety and quality requires a similar approach to that applied to the tissues and cells discussed in the other chapters of this Guide. Thus, the guidance on selection and testing of donors, quality management and traceability described in the generic section (Part A) of this Guide can be applied usefully to the banking of these substances to provide an appropriate framework for safe and effective services to patients.

29.2. Breast milk

29.2.1. Introduction

Human milk contains essential nutrients and bioactive components that promote the growth and development of the newborn. Milk not only covers the nutritional needs but also facilitates the process of maturation of various organs such as gut or brain. It is highly nutritious and contains a complex combination of immunological and anti-infective constituents that promote health, protect against infection and support a baby's immune system. For these reasons, a mother's own milk is universally accepted as the optimal nutritional source for neonates and infants.

Unfortunately, not all infants can receive milk from their mothers. Where this is the case, official bodies such as the World Health Organization (WHO), the American Academy of Pediatrics (AAP) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), as well as many scientific associations, consider donated human milk, obtained and processed in human milk banks (HMB), to be the best alternative, especially in preterm infants [2-4]. Donor human milk should not be substituted for a mother's own milk if available. Given the significant impact that human milk banks can have on infant health outcomes, the WHO has asked member countries 'to promote the safe use of donor milk through human milk banks for vulnerable infants'.

Milk banks are medical institutions that collect, process, store and distribute human milk prescribed for babies that are medically fragile, preterm babies, those with low birth weight, babies from mothers with delayed lactation, infants recovering from serious gut complications and surgery, and newborns affected by feeding intolerance, malabsorption syndromes or infectious diseases. The first milk bank was established in Vienna in 1909. Since then, milk banks have been established in many countries: currently 207 human milk banks exist in Europe [5], more than 300 in South America (217 in Brazil) and 19 in North America. The numbers and activities of milk banks are growing, driven by studies indicating that premature infants show better development if they are fed human milk rather than artificial formula milk. With donated human milk, there is a lower rate of infection, better long-term psychomotor development, a lower rate of necrotising enterocolitis and a better tolerance to the introduction of enteral feeding.

In addition, it has been shown that where new milk banks are established there is an increase in the rates of breastfeeding on discharge from hospital of these babies. It is now widely accepted that milk banks and the availability of donor breast milk encourage and support breastfeeding.

The risks associated with feeding with human donor milk are similar to those in the food industry (i.e. contamination or cross-contamination with bacteria or fungi, with subsequent recipient infection) but are also equivalent to those associated with transfusion and transplantation (i.e. transmission of viruses and other infective agents).

The following generic chapters (Part A) of this Guide all apply to milk banking and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Processing and storage (Chapter 7);
- g. Principles of microbiological control (Chapter 9);
- h. Organisations responsible for human application (Chapter 11);
- i. Computerised systems (Chapter 12);
- j. Coding, packaging and labelling (Chapter 13);
- k. Traceability (Chapter 14);
- l. Biovigilance (Chapter 15).

*In the European Union (EU), human breast milk may fall within different national legal frameworks for which appropriate quality and safety requirements need to be applied (e.g. food, tissues and cells).**

29.2.2. Donor recruitment

The donation of human breast milk must be voluntary and unpaid.

Promoting milk donation is carried out through a variety of different channels: written material (e.g., in prenatal clinics, paediatric surgeries, pharmacies, shops for maternity products), media, social networks, associations for breastfeeding programmes or educational guidance, and direct contact with pregnant women by their doctors and midwives.

A woman must be of legal age and lawfully competent to take this decision: she must be nursing her own baby, who must be adequately fed, before giving milk to the Milk Bank. It is acceptable to have donor mothers who are mourning their dead baby. Because milk 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 a milk donor.

29.2.3. Donor evaluation

Milk bank processing cannot guarantee complete elimination of toxic substances and potential infectious elements that may be contained in the milk. For this reason human milk, 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 4, the following conditions contraindicate the donation of milk [6, 7, 8, 9]:

- a. Donor's behavioural risks

* 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 breast 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 [1].

- i. Smoking tobacco 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 of substances containing xanthine (coffee, tea, cola or cacao) equivalent to ≥ 300 mg per day. Occasional consumption may be accepted if milk collection is avoided for at least 48 hours.
 - v. Vegetarians or vegans who do not supplement their diet with vitamin B12 adequately during pregnancy or lactation. Donors may be accepted if an adequate level of vitamin B12 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, treatments with acupuncture needles that are not properly sterilised or disposable, endoscopic examinations or treatments made with flexible instruments (e.g. colonoscopy or bronchoscopy) and transplantation of organs, tissues or cells should exclude donation for 4 months [6].
- 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., chicken pox, mumps, measles) should be excluded for a period equivalent to the incubation period or, if not known, for 4 weeks. Those that suffered the disease in the past, but are immunised, can donate.
 - iii. Women with close contact (sexual contact or living in the same house) with patients with viral hepatitis should be excluded temporarily for 4 months, depending on the virus causing hepatitis [6].
 - iv. Women with mastitis or fungal infections of the nipple or areola should be excluded.
 - 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, including haematological malignancies, must be excluded since viruses have been shown to play a role in the development of some types of tumour. However, women with the following can be accepted as donors:
 - Cervical *in situ* carcinoma and localised skin tumours (basocellular carcinoma and squamous carcinoma) if they are removed and the donor has recovered.
 - Some childhood solid tumours such as neuroblastoma, Wilms tumour and retinoblastoma. These are considered cured if the diagnosis was made before the donor was 5 years old and there has been no recurrence.

29.2.4. Milk donor testing

It is recommended to determine the absence of viral nucleic acid (HBV, HCV and HIV) in pasteurised milk.

In addition to the tests outlined in Chapter 5, testing for HTLV I/II and *Trypanosoma cruzi* should be performed for donors living in or originating from high-prevalence areas or with sexual partners originating from those areas, or if the donor's parents originate from those areas.

It is not necessary to repeat donor testing during the period of milk donation if there is no change to the donor's risk status.

Seropositivity for *cytomegalovirus* (CMV) is not considered a contraindication as long as the milk is pasteurised.

29.2.5. Procurement

The milk can be expressed by hand or with a breast pump. Before each collection it is essential to wash the hands thoroughly, and clean and disinfect all the components of breast pumps [8].

Most recommended containers are rigid plastic, suitable for food use and made from a variety of materials such as carbonate, polyethylene or polypropylene. It is recommended that containers should be sterile and single-use. Re-using containers requires cleaning and disinfection. Containers sterilised with ethylene oxide, such as those used to collect urine samples, must not be used. Glass containers may be

used but are not recommended because of the risk of breakage and even the possibility of micro particles of glass being present in the milk [9].

Some milk banks use plastic bags of polyethylene as an alternative to rigid containers; but these bags can easily rupture with the risk of contamination. Use of a double bag is therefore recommended if bags are used.

If the milk is to be frozen, the container should not be filled completely. The containers must be labelled with a donor code, donor's given name and family name, and the date of collection.

29.2.6. Temporary milk storage and transportation to the milk bank

Milk collected at home must be kept at room temperature for the shortest possible time. After collection, the container must be sealed and cooled in order to avoid bacterial growth and degradation of the milk [10].

It can be stored at 4 °C for up to 24 hours and then frozen at ≤ -20 °C. Some HMB accept the pooling of different collections of milk but the new milk must first be refrigerated before being added to previously collected milk [11, 12].

If a woman has accumulated milk before being accepted as a donor, medical and behavioural history must be evaluated retrospectively for suitability, and the milk can be accepted only if it has been appropriately preserved and identified.

Milk must be frozen within 24 hours of its collection, and it is mandatory for it to be transported frozen to the milk bank. Dry ice may be used as the refrigerant during transport. It is recommended that the milk bank is responsible for the transportation of milk. If third parties are used, there must be a formal agreement in place, with the milk bank covering transport conditions to ensure the safety and quality of the milk.

The transport process must be validated or temperatures monitored during transport to ensure the milk is kept under appropriate conditions.

Evidence of the integrity of the containers on arrival at the milk bank must be documented.

29.2.7. Processing

It is common practice in HMBs to increase safety and reduce the risk of contamination by pasteurising the milk. However, in some countries (e.g., Norway) raw untreated milk from CMV-negative donors is widely used.

Recent guidelines for pasteurisation recommend a temperature of 62.5 °C for 30 minutes, the so-called Holder pasteurisation [13]. It is recommended that the heated milk should be cooled to 25 °C within 10 minutes, although a final temperature of 10 °C or lower is preferable [9].

While it has been shown that pasteurisation does not affect the macronutrient composition (protein, carbohydrates and lipids, including polyunsaturated fatty acids) of milk, new treatments are under development to provide the same level of safety without affecting the other biological components of human milk [13, 14, 15].

The pasteurisation process begins with the thawing of milk either slowly, overnight in a refrigerator, or quickly by immersion in a water bath with stirring at a controlled temperature, not higher than 37 °C [6].

Milk must be processed under hygienic conditions. Individuals handling open containers of milk must wear a hair covering, gloves and a clean gown, apron or lab coat to prevent contamination of the milk. Although it may not be considered strictly necessary, an increasing number of HMB manipulate the milk in a laminar flow cabinet.

After thawing, some HMB combine or pool milk from multiple donors. This practice of pooling may increase uniformity in the product and provide more consistent nutrient content; however, if there is contamination of pooled milk, it may be difficult to trace the source of the contamination. If milk pooling is practised, the HMB must decide whether pooling will be allowed between different donors, the maximum number of donors whose milk may be pooled, and when pooling will take place (before or after pasteurisation).

Before pasteurisation, a sample of milk from each batch should be taken for microbiological testing. This sample may also be used for a macronutrient analysis (see 29.2.8 Quality control).

The pasteurisation process can be carried out in a shaker water bath, or equipment specifically designed for pasteurising milk may be used. The equipment used for pasteurisation must be calibrated at least every 12 months [6].

During pasteurisation, bottle caps must remain above water level to prevent contamination, unless caps and equipment designed for submersion are used. A control bottle containing the same amount of milk or water as the fullest container of milk in the batch shall 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 must be monitored and recorded.

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.

29.2.8. Quality control

The first control to be done with donated milk, before processing, is the evaluation of its organoleptic characteristics. The donated milk must be discarded if the appearance, colour or smell are inappropriate or if foreign bodies/impurities are detected.

29.2.8.1. Microbiological evaluation

The microbiological evaluation of donated human milk includes the testing of samples taken both before (10 µl sample volume/agar plate) and after pasteurisation (200 µl sample volume/agar plate). Current French legislation and Australian and Spanish guidelines recommend that every batch of milk should undergo bacteriologic testing before and after pasteurisation. However, British guidelines developed by the National Institute for Health and Care Excellence (NICE) [6] recommend a different testing schedule. Although testing every batch before pasteurisation is recommended, the guidance proposes that it is not necessary to test every batch after pasteurisation; rather, a batch of milk should be tested either at least once a month or after every 10 cycles of pasteurisation. If any new processes, equipment or staff are introduced, or there are concerns about any part of the process, additional batch testing should be undertaken. Italian guidelines [7] propose testing samples before pasteurisation only for the first donation or when appropriate hygiene standards of the donation cannot be assured. Periodic random sampling is also recommended. The Italian guidelines [7] adopt the same schedule as the NICE guidelines for the post-pasteurisation samples [9].

A similar level of discrepancy is observed in the criteria of acceptability of the milk when bacteriologic analysis is performed, as is shown in Table 29.1.

The criteria for discarding pasteurised milk vary from no growth acceptable to 10^5 CFU/mL.

29.2.9. Labelling and packaging

In addition to the information about labelling set out in Chapter 13, labels for packaging pasteurised milk may contain information about the nutritional value, such as the concentrations 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, although both smaller containers (10 mL) and larger (250 mL) are also useful.

29.2.10. Preservation/storage

The most common method of milk preservation is freezing $\leq 20^\circ\text{C}$. Milk that has not been frozen is acceptable for use (or for freezing) only if kept for less than 24 hours from collection.

Despite its advantages, very few milk banks use lyophilisation as a method of preservation. However, the Milk Bank CHU de Bordeaux in Marmande uses only lyophilisation and processes more than 12 000 litres of milk per year [16].

29.2.10.1. Expiry date

There is no unanimous agreement about the expiry date for milk. In most European countries, it is accepted that milk should not be kept more than 3 months at -20°C before pasteurisation whereas the USA and Canada accept storage for up to 12 months. After processing, milk may be stored for between 3 and 12 months, depending on the country [9].

In the case of milk stored at -80°C , no more than 12 months' storage both before and after pasteurisation is the recommended maximum [8].

Table 29.1. Criteria for the discard of milk before pasteurisation

NICE Guidelines	Total bacteria $> 10^5$ CFU/mL	<i>Enterobacteriaceae</i> $> 10^4$ CFU/mL	<i>Staphylococcus aureus</i> $> 10^4$ CFU/mL
Italian Guidelines	Total bacteria $> 10^5$ CFU/mL	<i>Enterobacteriaceae</i> $> 10^4$ CFU/mL	<i>Staphylococcus aureus</i> $> 10^4$ CFU/mL
French legislation	Aerobic flora $\geq 10^5$ CFU/mL	<i>Staphylococcus</i> coagulase-positive $\geq 10^4$ CFU/mL	
Australian Guidelines	Confluent bacterial growth $> 10^5$ CFU/mL	Any <i>enterobacteriaceae</i> , <i>enterococci</i> or potential pathogens capable of producing heat-stable enterotoxins	

29.2.10.2. Storage temperature

Freezing milk at $-20^{\circ}\text{C}/-30^{\circ}\text{C}$ before and after processing slows down but does not stop lipolysis, which affects milk quality. But, if milk is preserved at -80°C , lipolysis is stopped and milk properties can be maintained for a longer period of time [12, 17].

29.2.11. Distribution and transport conditions

Distribution of milk to hospitals must be so conducted as to ensure product traceability between donor and recipient, as described in Chapter 14 of this guide. The milk bank must keep records of the documentation of each donor, the processing pool, storage and final destination (distribution, disposal, expiry date), and the hospital must document how the milk is used.

During transport, milk must remain frozen 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 transport procedure must be validated, or else 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.

29.2.12. Biovigilance

As described in Chapter 15, deviations from the SOP, from donation to application, should be recorded and documented, as well as adverse reactions after application [18].

In addition to milk banks, hospitals must 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.

Milk should not be thawed in a microwave oven, since that significantly reduces the amount of vitamin C, the total IgA content and lysozyme activity [19].

Milk thawed in the refrigerator can be kept at 4°C for up to 72 hours if the container has not been opened. Once opened, the package should be consumed within 24 hours. Furthermore, thawed milk must not be re-frozen.

29.2.13. Developing applications

Several techniques have been investigated to eliminate pathogens in milk without affecting its biological properties. These include:

- high-temperature short time (HTST) or ultra-high short time (UHST),
- ultra-pasteurisation or ultra-high temperature (UHT).

There are also methods for reducing micro-organisms in food that do not use heat. While not technically pasteurisation, they achieve the same effect and are known as cold pasteurisation. These include:

- high-pressure processing (HPP) or pascalisation,
- ultraviolet (UV) irradiation,
- ultrasonication,
- high intensity pulsed electric field (PEF).

Even if such techniques are shown to be effective and preserve important bioactive components of human milk better than Holder pasteurisation, they may be difficult to translate into practice, given the lack of appropriately scaled equipment for use in milk banks.

29.3. Faecal microbiota

29.3.1. Introduction

Faecal microbiota transplantation (FMT) was first reported in 1958 by Eiseman *et al.* [20]. Since then, promising reports of FMT have suggested a clinical cure rate of $\approx 90\%$ for recurrent *Clostridium difficile* infection (CDI) refractory to antibiotic therapy [21]. The transplant can restore the diversity of gut microflora, which may confer protection against toxigenic *Clostridium difficile* [22, 23].

In a systematic review and meta-analysis conducted recently, 11 studies concluded that FMT is a promising intervention for serious recurrent *Clostridium difficile* infection after conventional treatments have been exhausted [24], and a small randomised controlled trial published in 2013 confirmed this finding [25]. A systematic review published in 2015 showed that FMT was successful in 85 % of recurrent and 55 % of refractory *Clostridium difficile* infection [26]. The effectiveness of FMT for treatment of ulcerative colitis has also been reported [27].

The European Society of Clinical Microbiology and Infectious Diseases strongly recommend FMT in combination with oral antibiotic treatment for the

treatment of multiple recurrent CDI unresponsive to repeated antibiotic treatments [28].

The following generic chapters (Part A) of this Guide all apply to FMT and must be read in conjunction with this chapter:

- a. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Principles of microbiological control (Chapter 9);
- i. Distribution and import/export (Chapter 10);
- j. Organisations responsible for human application (Chapter 11);
- k. Computerised systems (Chapter 12);
- l. Coding, packaging and labelling (Chapter 13);
- m. Traceability (Chapter 14);
- n. Biovigilance (Chapter 15).

*In the EU, faecal microbiota may fall within different national legal frameworks, for which appropriate quality and safety requirements need to be applied (e.g., medicinal products, tissues and cells).**

29.3.2. Donor evaluation

Traditionally, donors have been close relatives or spouses. Preference should be given to close relatives who do not live at the same address [29]. However, there would be considerable benefits in having unrelated pre-screened donors to allow more rapid transplantation if it is needed. There may be benefits in using donors from different households, where the gut microbiome would be different and would provide enhanced diversity in the patient.

* 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 faecal microbiota 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 [1].

Along with the usual donor-exclusion criteria described in Chapter 4 of this Guide, some specific exclusion criteria have also been proposed:

- a. history of inflammatory bowel disease, irritable bowel syndrome [30], idiopathic chronic constipation or chronic diarrhoea;
- b. history of gastrointestinal malignancy or known polyposis;
- c. antibiotic treatment within the preceding 3 months [30];
- d. use of major immunosuppressive medications (e.g., calcineurin inhibitors, exogenous glucocorticoids, biological agents);
- e. systemic anti-neoplastic agents;
- f. recent ingestion of a potential allergen (e.g., nuts) where the recipient has a known allergy to this agent.

Relative exclusion criteria that might be appropriate to consider [30] are:

- a. history of major gastrointestinal surgery (e.g., gastric bypass);
- b. metabolic syndrome;
- c. systematic autoimmunity (e.g., multiple sclerosis, connective tissue disease);
- d. atopic disease (e.g., asthma, eczema, eosinophilic disorders of the gastrointestinal tract);
- e. chronic pain syndromes (e.g. chronic fatigue syndrome, fibromyalgia).

Donors should be selected and tested carefully for all relevant transmissible diseases as defined in Chapter 5 of this guide. In the case of fresh FMT, blood samples must be taken for testing within the 7 days prior to donation.

Donors should also be screened for intestinal pathogens and infectious disease agents that could be harmful to the recipient [29], including:

- a. *Clostridium difficile* toxins by polymerase chain reaction or evaluation for toxins A and B by enzyme immunoassay (EIA);
- b. *Salmonella*;
- c. *Shigella*;
- d. multi-resistant bacteria to antibiotics;
- e. *Campylobacter*;
- f. *Helicobacter pylori* (in the case of nasogastric administration of the transplant);
- g. exclusion of all pathogen parasites;
- h. faecal *Giardia* antigen;
- i. faecal *Cryptosporidium* antigen.

Using a risk-based approach, donors could be screened for other intestinal pathogens and infec-

tious disease agents that could be harmful to the recipient [29, 30]:

- a. *Adenovirus*;
- b. *Astrovirus*;
- c. *Calicivirus* (*norovirus*, *sapovirus*);
- d. *Picornavirus* (*enterovirus*, virus Aichi);
- e. *Rotavirus*;
- f. Hepatitis A and E virus;
- g. *Vibrio*;
- h. *Listeria*;
- i. ova;
- j. protozoa;
- k. babesiosis;
- l. malaria;
- m. Chagas disease.

The use of fresh faecal microbiota for FMT does not allow proper screening of the faecal material for intestinal pathogens and infectious disease agents at the time of donation. In the case of fresh FMT, faecal material must be taken for testing within 5 days prior to donation [29]. Frozen faecal microbiota has been shown to be equally effective in treating CDI [31].

29.3.3. Procurement

There is no specific preparation of the donor. The donor can be advised to avoid food that can be allergic for the recipient, such as peanuts, other nuts and shellfish within the 5 days prior to donation. Consider the use of a gentle osmotic laxative the night before donation [29, 30].

29.3.4. Processing

The stool should be processed preferably within 6 hours after donation [29, 30], following the safety and quality issues covered in Chapter 7. Donor faeces are obtained and diluted with water, preservative-free normal saline 0.9 % or milk, homogenised and filtered to remove large particles.

29.3.5. Quality control

From a scientific perspective, quality control is complicated by the material's complexity and inconsistency across samples. The microbial and metabolic contents of human stool are known to vary enormously across individuals and over time within individuals. Unless the active components are identified, purified and tested, it will not be possible to guarantee that the product is consistent across batches.

29.3.6. Preservation/storage

Highly filtered faecal microbiota mixed with a cryoprotectant could be stored at -80°C until required for use [32].

29.3.7. Distribution and transport conditions

Recipients may receive a bowel lavage before transplantation to reduce the *Clostridium difficile* load in the intestines.

The ideal volume for instillation has not been established. However, smaller volumes (e.g., 25-50 mL) could be used for delivery via a nasoduodenal tube or nasogastric intubation; larger volumes (e.g., 250-500 mL) could be used for instillation via colonoscopy [29, 30].

29.3.8. Developing applications

FMT can also be used to treat various diseases, including Parkinson, multiple sclerosis, fibromyalgia and chronic fatigue syndrome, among others [33].

The NICE has also published guidance on interventional procedures on FMT [34].

29.4. Serum eye drops

29.4.1. Introduction

Serum eye drops is a serum component for use by patients suffering from severe ocular disease, and specifically where the patient has not responded to conventional treatments. Serum eye drops can be prepared for autologous use from the donor's serum or they can be allogeneic. The serum is separated from a unit of whole blood collected from a patient, or an allogeneic donor, which can be provided undiluted or diluted in saline and dispensed in small aliquots into dropper bottles 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 is not only lacrimal substitute, but also contains growth factors and other biochemical components mimicking natural tears more closely. This is the reason why serum eye drops have become a popular second-line therapy in dry eye treatment [35-37].

The following generic chapters (Part A) of this Guide all apply to serum eye drops and must be read in conjunction with this section:

- a. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);

- c. Donor evaluation (Chapter 4, in the case of allogeneic donors);
 - d. Donor testing (Chapter 5);
 - e. Processing and storage (Chapter 7);
 - f. Premises (Chapter 8);
 - g. Principles of microbiological control (Chapter 9);
 - h. Distribution and import/export (Chapter 10);
 - i. Computerised systems (Chapter 12);
 - j. Coding, packaging and labelling (Chapter 13);
 - k. Traceability (Chapter 14);
 - l. Biovigilance (Chapter 15).
- a. elevated bilirubin or protein (regular whole blood donors do not regularly undergo these tests but, if the patient is visibly jaundiced, he/she will be excluded on medical grounds, or if the serum shows evidence of elevated bilirubin or protein, the serum should be discarded);
 - b. active viral or fungal infection;
 - c. certain medications that may injure the cornea.

This chapter defines the additional specific requirements for serum eye drops.

Within the 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 as regards certain technical requirements for blood and blood components. Serum eye drops per se may fall within different national legal frameworks in the EU for which the appropriate quality and safety requirements need to be applied (blood, tissues and cells, medicinal products).

29.4.2. Donor evaluation

In the case of autologous donation, special attention should be paid to avoiding the development of anaemia where there is repeated collection of blood to prepare serum eye drops.

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.

In allogeneic donation, allogeneic serum eye drops can be prepared in advance and be ready for use in emergency cases. The ABO (ABO) blood group for allogeneic eye drops is not clinically important, so it is recommended that donations are collected from blood groups that have the least impact on blood supply. It is also recommended to collect from male donors as this routinely provides a larger yield.

The following conditions contraindicate for eye-drops donation, in addition to those specified in Chapter 4 of this Guide:

29.4.3. Processing and storage

There must be written protocols for all procedures related to blood collection 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 should be collected in a sterile container/blood bag, without anti-coagulant. The collected blood volume depends on local procedure but cannot be more than for regular blood donors.

The unit of blood should be refrigerated within 10 hours of collection. It should then be stored at 4 °C for a minimum of 48 hours to allow the blood to clot before the serum can be separated. Processing must then take place within a minimum time period of 48 hours and a recommended maximum of 72 hours following venepuncture.

Eye-drop preparation should be carried out using aseptic technique. It is strongly recommended to use a closed system for aliquoting. Microbiological control for each batch is mandatory (see Chapter 9). The volume of one aliquot shall be adjusted for daily dose to minimise microbiological contamination during application and storage at patient's home. All bags that are used in the collection, processing and/or aliquots of final packaging must be properly labelled (see Chapter 13).

Eye drops must be stored frozen and transported in an appropriate container, to maintain the required temperature. The routine shelf-life of frozen eye drops is 3-6 months; however, an extended shelf life up to one year can be applied if stability and sterility studies are performed.

29.4.4. Clinical application

The patient must be given appropriate information about the blood collection and testing as well as eye-drops preparation. Patient should get written instructions for storage and handling of the eye drops at home.

It is strongly recommended that the ophthalmologist monitors the patient's progress in a system-

atic way because research continues on the benefits of using serum eye drops.

Any adverse reaction or events that occur during production or usage of eye drops should be notified.

29.5. Platelet-rich plasma and platelet-rich fibrin

29.5.1. Introduction

Platelet-derived products are used as source of growth factors and cytokines. They are normally used as autologous products and can be prepared *ex tempore* during the same surgical procedure or in advance. When they are prepared in advance, this should be done by a blood or tissue establishment.

Platelet-rich plasma (PRP) is blood plasma that has been enriched in platelets. As a concentrated source of autologous platelets, PRP contains several different growth factors and other cytokines, in concentrations 5 to 10 times higher than usual, which can be used to stimulate healing of soft tissue by injecting this concentrated plasma in the tissue where healing or effect is desired. There are primarily 3 isomers of platelet-derived growth factor (PDGF), namely $\alpha\alpha$, $\beta\beta$ and $\alpha\beta$, 2 transforming growth factors, TGF- β 1 and TGF- β 2, endothelial growth factor (EGF) and vascular endothelial growth factor (VEGF). PRP also contains proteins responsible for cell adhesion: fibrin, fibronectin and vitronectin [38]. The content of bioactive molecules depends on the production protocol [39].

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, plastic surgery and dentistry, including oral and maxillofacial surgery. As of 2016, 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.

Platelet-rich fibrin (PRF) is a second-generation PRP where autologous platelets and leucocytes 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 [40]. PRF has a dense fibrin network with leukocytes, cytokines and structural glycoproteins, as well as growth factors (e.g., TGF β 1, 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 that has faster regeneration potency than connective tissues [41].

PRF is used to accelerate the healing of soft and hard tissue and as a scaffold for endodontics.

The following generic chapters (Part A) of this Guide must be read in conjunction with this section:

- a. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Donor evaluation (Chapter 4);
- d. Donor testing (Chapter 5);
- e. Processing and storage (Chapter 7);
- f. Premises (Chapter 8);
- g. Principles of microbiological control (Chapter 9);
- h. Distribution and import/export (Chapter 10);
- i. Organisations responsible for human application (Chapter 11);
- j. Coding, packaging and labelling (Chapter 13);
- k. Traceability (Chapter 14);
- l. Biovigilance (Chapter 15).

In the EU, PRP and PRF may fall within different national legal frameworks, for which appropriate quality and safety requirements need to be applied (e.g., medicinal products, blood, tissues and cells).

29.5.2. Procurement and processing

29.5.2.1. Procurement and processing in platelet-rich plasma

To prepare PRP, blood must first be drawn from a patient at the time of treatment. The platelets are separated from other blood cells and their concentration is increased during centrifugation. A 30 mL venous blood draw will yield 3-5 mL of PRP, depending on the baseline platelet count of an individual, the device used and the technique employed. The blood draw occurs with the addition of an anti-coagulant, such as citrate dextrose A, to prevent platelet activation prior to its use.

An initial centrifugation to separate red blood cells is followed by a second centrifugation to concentrate platelets, which are suspended in 3-5 mL of the final plasma volume. After the first spin step, the whole blood separates 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.

For the production of pure PRP, the upper layer and superficial buffy coat are transferred to an empty sterile tube. The second spin should be just adequate to aid in formation of soft pellets (platelet-erythrocyte) at the bottom of the tube. The upper portion of the volume that is composed mostly of platelet-poor plasma is removed. Pellets are homogenised in the lower third part (5 mL of plasma) to create the PRP.

There are many PRP systems commercially available, which facilitate the preparation of ready-to-apply platelet-rich suspensions in a reproducible manner. All operate on a small volume of drawn blood (20-60 mL) and on the principle of centrifugation.

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 leucocytes are obtained. Differences in the concentrations in platelets and white blood cells influence the diversity of growth factors concentration.

29.5.2.2. *Procurement and processing in platelet-rich fibrin*

For the preparation of PRF a sample of blood is collected from the patient in tubes without anti-coagulant 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 (≤ 2 minutes) 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 prepared using 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 [42].

29.6. Autotransplantation of teeth

Autotransplantation refers to the repositioning of an autogenous erupted, semi-erupted or unerupted tooth from one site into another in the same individual.

As with any other kind of tissue transplantation, it is recommended that the following generic chapters (Part A) of this Guide that may apply and must be read and taken into account, in conjunction with this section:

- a. Introduction (Chapter 1);
- b. Quality management, validation and risk management (Chapter 2);
- c. Recruitment of living donors, identification and referral of possible deceased donors and consent to donate (Chapter 3);
- d. Donor evaluation (Chapter 4);
- e. Donor testing (Chapter 5);
- f. Procurement (Chapter 6);
- g. Processing and storage (Chapter 7);
- h. Principles of microbiological control (Chapter 9);
- i. Distribution and import/export (Chapter 10);
- j. Organisations responsible for human application (Chapter 11);
- k. Computerised systems (Chapter 12);
- l. Coding, packaging and labelling (Chapter 13);
- m. Traceability (Chapter 14);
- n. Biovigilance (Chapter 15).

Although in the era of dental implants, transplantation of teeth is not a common procedure, autotransplantation of immature teeth can have a success rate of 70 to 98 % [43, 44], if the tooth is atraumatically transplanted from the donor site to a suitable recipient site and the extra-oral time is kept to a minimum. When the tooth cannot be transplanted immediately, teeth may be preserved and stored in a tissue establishment.

To prevent ice injury, there are two possible preservation methods: vitrification or a slow rate/control cooling. In both methods cryoprotectants are used (see Chapter 7). It is important to remove dental pulp at the time of extraction before banking procedure.

Both mature and immature teeth should be apico-ectomised. This allows re-vascularisation and ingrowth of new pulpal tissue after transplantation.

29.7. References

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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.

- *Aide-mémoire* on Access to safe and effective human cells and tissues for transplantation, World Health Organization, available at www.who.int/entity/transplantation/AM-HCTTSer-vices.pdf, accessed 4 November 2016.
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Appendix 2. Acronyms and other abbreviations

3D	three-dimensional	CAT	Committee for Advanced Therapies
AATB	American Association of Tissue Banks	CBC	complete blood count
AAV	<i>adeno</i> -associated virus	CD	cluster of differentiation
Ab	antibodies	CDC	Centers for Disease Control and Prevention
ACI	autologous chondrocyte implantation	CD-P-TO	European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe
AFC	antral follicle count	CE (marked)	Conformité Européenne
AIDS	acquired immunodeficiency syndrome	CEA	cultured epithelial autografts
ALK	anterior lamellar keratoplasty	CFU	colony-forming units
AMH	Anti-Müllerian hormone	CFU-GM	colony-forming units-granulocyte/monocyte
Anti-CMV	antibody to <i>cytomegalovirus</i>	CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
Anti-EBV	antibody to Epstein–Barr virus	CJD	Creutzfeldt–Jakob disease
Anti-HBc	antibody to hepatitis B core antigen	CLET	cultivated limbal epithelial transplantation
Anti-HCV	antibody to hepatitis C virus	CMV	<i>cytomegalovirus</i>
Anti-HIV-1	antibody to HIV-1	CNS	central nervous system
Anti-HIV-2	antibody to HIV-2	CNT	Centro Nazionale Trapianti (Italy)
ARE	adverse reactions and events	COC	cumulus enclosed oocyte
ART	assisted reproductive technology	COD	cause of death
ARTHIQS	Assisted Reproductive Technologies and Haematopoietic stem cells for transplantation Improvements for Quality and Safety throughout Europe [joint action]	COHS	controlled ovarian hyperstimulation
ATMP	advanced therapy medicinal products	COMET	cultivated oral mucosal epithelial transplantation
aW	available water	DALK	deep anterior lamellar keratoplasty
BET	bacterial endotoxin test	DBD	deceased by brain death
BFU-E	burst forming units erythroblast	DBM	demineralised bone matrix
BM	bone marrow	DBO	Department of Biological Standardisation, OMCL Network & HealthCare (at the European Directorate for the Quality and Medicines & HealthCare)
BMDW	Bone Marrow Donors Worldwide Organization	DC	dendritic cells
BMP	bone morphogenetic proteins	DCD	deceased by circulatory death
BMSC	bone marrow stromal cells		
BSS	balanced salt solution		
CAR	chimeric antigen receptor		

DH-BIO	Committee on Bioethics of the Council of Europe	FDA	Food and Drug Administration (USA)
DLI	donor lymphocyte infusions	FED	Fuchs endothelial dystrophy
DM	Diabetes mellitus	FIPS	fingerprints
DMEK	descemet membrane endothelial keratoplasty	FMEA	failure mode and effects analysis
DMSO	dimethyl sulfoxide	FMECA	failure mode, effects and criticality analysis
DQ	design qualification	FMT	faecal microbiota transplantation
DSAEK	descemet stripping automated endothelial keratoplasty	FNHTR	febrile non-haemolytic reactions
DVT	deep-vein thrombosis	FOS	fastidious organism supplement
EATB	European Association of Tissue Banks	FSH	follicle-stimulating hormone
EBMT	European Society for Blood and Marrow Transplantation	GAG	glycosaminoglycans
EBV	Epstein-Barr virus	G-CSF	granulocyte-colony stimulating factor
EC	European Commission	GEMM	granulocyte-erythrocyte-macrophage-megakaryocyte
ECCTR	European Cornea and Cell Transplant Registry	GF	growth factors
ECDC	European Centre for Disease Prevention and Control	GM	granulocytes and macrophages
ECM	extracellular matrix	GM-CSF	granulocyte macrophage-colony stimulating factor
ECVAM	European Centre for the Validation of Alternative Methods	GMP	Good manufacturing practice [EU document]
EDQM	European Directorate for the Quality of Medicines & HealthCare	GPA	glycerol-preserved allografts
EDTA	ethylenediamine tetra-acetic acid	GTP	good tissue practice
EEBA	European Eye Bank Association	GV	germinal vesicle
EGF	endothelial growth factor	GvHD	graft-versus-host disease
EGTA	ethylene glycol tetra-acetic acid	GVT	graft-versus-tumour
EIA	enzyme immunoassay	HACCP	hazard analysis and critical control points
EK	endothelial keratoplasty	hAM	human amniotic membrane
EMA	European Medicines Agency	HAV	hepatitis A virus
EMDIS	European Marrow Donor Information System	HBc	hepatitis B core antigen
EQSTB	European Union project 'European Quality System for Tissue Banking'	HBsAg	hepatitis B surface antigen
ESBL	extended-spectrum beta lactamases	HBV	hepatitis B virus
ESC	embryonic stem cell	HCV	hepatitis C virus
ESHRE	European Society for Human Reproduction and Embryology	HEPA	high-efficiency particulate air
EU	European Union	HES	hydroxyethyl starch
EURO CET	European Registry for Organs, Tissues and Cells	HHV	human <i>Herpes</i> virus
Eurocode IBLS	Eurocode International Blood Labelling Systems	HIV	human immunodeficiency virus
EuroGTP	Euro Good Tissue Practices [EU project]	HLA	human leucocyte antigen
EuroGTP-II	Good [Tissue] Practices for demonstrating safety and quality through recipient follow-up [EU project]	hMG	human menopausal gonadotrophin
EUSTITE	European Standards and Training in the Inspection of Tissue Establishments [EU project]	HPC	haematopoietic progenitor cells
EUTC	European Code for Tissues and Cells	HPV	human <i>papilloma</i> virus
FACS	fluorescence-activated cell sorting	HRV	human <i>rotavirus</i>
FACT	Foundation for the Accreditation of Cellular Therapy	HSC	haematopoietic stem cells
		HSG	hysterosalpingography
		HSV	<i>Herpes simplex</i> 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	OHSS	ovarian hyperstimulation syndrome
ICSI	intracytoplasmic sperm injection	ONT	Organización Nacional de Trasplantes (Spain)
ICU	intensive care unit	OQ	operational qualification
IDM	infectious disease marker	ORHA	organisation responsible for human application
IEC	Independent Ethics Committee	Parvo-B19	Parvovirus B19
IFN	interferon	PBK	pseudophakic bullous keratopathy
Ig	immunoglobulin	PBSC	peripheral blood stem cells
IL	interleukin	PCR	polymerase chain reaction
iPSC	induced pluripotent stem cell	PESA	percutaneous epididymal sperm aspiration
IQ	installation qualification	PGD	pre-implantation genetic diagnosis, see PGT
ISCT	International Society for Cellular Therapy	PGS	pre-implantation genetic screening, see PGT
ISO	International Organization for Standardization	PGT	pre-implantation genetic testing (formerly known as PGD and PGS, see above)
ISPE	International Society for Pharmaceutical Engineering	PGT-A	pre-implantation genetic screening for aneuploidy screening
ISN	International Society for Nephrology	PGT-M	pre-implantation genetic diagnosis for monogenic/single gene defects
ISSCR	International Society for Stem Cell Research	PGT-SR	pre-implantation genetic diagnosis for chromosomal structural rearrangements
IT	information technology	<i>Ph. Eur.</i>	<i>European Pharmacopoeia</i> , 9 th edn, Strasbourg: Council of Europe 2016
IUI	intra-uterine insemination	PIC/S	Pharmaceutical Inspectorate Co-operation Scheme
IVF	<i>in vitro</i> fertilisation	PK	penetrating keratoplasty
IVM	<i>in vitro</i> maturation	PN	ProNucleus
JACIE	Joint Accreditation Committee–ISCT & EBMT	PO	procurement organisation
KIR	killer immunoglobulin-like receptors	POI	premature ovarian insufficiency
KLAL	keratolimbic allograft	POSEIDON	Promoting Optimisation, Safety, Experience sharing and quality Implementation for Donation organisation and networking in unrelated haematopoietic stem cell transplantation in Europe [EU project]
KPI	key performance indicator	PQ	performance qualification
LAL	limulus amoebocyte lysate	PRF	platelet-rich fibrin
LH	luteinising hormone	PRIVILEGED	Privacy in Law, Ethics and Genetic Data [EU project]
LSC	limbal stem cells	PROH	1,3-propanedial
MCM	metastatic cutaneous melanoma	PRP	platelet-rich plasma
MESA	microsurgical epididymal sperm aspiration	PVP	PolyVinylPyrrolidone
MII	metaphase II	QC	quality control
MNC	mononuclear cells	QM	quality manager
MPHO	medical products of human origin	QMS	quality management system
MRA	marrow re-populating ability	QRM	quality risk management
MRSA	methicillin-resistant <i>Staphylococcus aureus</i>	RANTES	regulated on activation, normal T-cell expressed and secreted
MSC	mesenchymal stromal (stem) cells	RATC	rapid alerts for tissues and cells
MTT	tetrazolium salt assay	RBC	red blood cell
NAC	nipple–areola complex	RCT	randomised control trial
NAT	Nucleic acid amplification technique/nucleic acid test	RhD	Rhesus D antigen
NEC	necrotising enterocolitis		
NICE	National Institute of Health and Clinical Excellence		
NK	natural killer		
NRT	neutral red test		
NtPSC	nuclear-transfer pluripotent stem cells		
OA	osteoarthritis		
OECD	Organisation for Economic Co-operation and Development		

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
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
TBV	total blood volume
TCR	T-cell receptor

TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TGF	tumour growth factor/transforming growth factor
TNC	total nucleated cells
TNF	tumour necrosis factor
TPV	total plasma volume
TRALI	transfusion-related acute lung injury
TSE	transmissible spongiform encephalopathy
TTS	The Transplantation Society
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.	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.
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).	Artificial insemination	See Intrauterine insemination.
Adverse event	Any untoward occurrence associated with the procurement, testing, processing, storage or distribution of tissues and cells. (See also: serious adverse event.)	Aseptic techniques	Procedures designed to prevent contamination from micro-organisms and spread of infection.
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.)	Assisted reproductive technology	All treatments or procedures that include the <i>in vitro</i> handling of human oocytes, spermatozoa or embryos for establishing a pregnancy. This includes, but is not limited to, intra-uterine insemination, <i>in vitro</i> fertilisation, intracytoplasmic sperm injection, embryo transfer, gamete, germinal tissue and embryo cryopreservation, oocyte and embryo donation and gestational surrogacy.
Allogeneic	Refers to tissues and cells removed from one person and applied to another of the same species.	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.
Allograft	Tissues or cells transplanted between two genetically different individuals of the same species. The term is synonymous with 'homograft'.	Autologous	Refers to tissues or cells removed from and applied in the same individual.
Ambient temperature	The temperature of the surrounding environment. In temperature-controlled facilities, ambient temperature equals the room temperature and is usually 17-21 °C for thermal comfort. Referred to as 'room temperature' in this Guide.	Azoospermia	Absence of spermatozoa in the ejaculate.
Amniotic membrane	The innermost layer of the placental membrane; it surrounds the foetus during pregnancy.	Bacteraemia	The presence of viable bacteria in the circulating blood.
Antibiogram	See: resistogram.		

Banking	Processing, preservation, storage and distribution of tissues and cells for human application or other purposes, including research and training.	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.
Barcode	An optical machine-readable representation of data relating to the object to which it is attached.	Colonisation	The natural, biological presence or spread of micro-organisms.
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.	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.
Biobank	A collection of biological material and the associated data and information stored for research purposes. Also known as a bio-repository.	Competent authority	See: Health Authority.
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.	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.
Blastocyst	An embryo, 5 or 6 days after fertilisation, with an inner cell mass, outer layer of trophoblast and a fluid-filled blastocoele cavity.	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.
Blastomere	Undifferentiated cells formed after cleavage of the fertilised oocyte (zygote).	Contained laboratory/area	According to EU GMP, an area constructed and operated in such a manner (and equipped with appropriate air handling and filtration) so as to prevent contamination of the external environment by biological agents from within the area.
Blood groups	ABO or ABo. Both forms are widely used, but this Guide uses O. The O is from German <i>ohne</i> ('without') and means the same as o ('zero'): these are red blood cells without A or B antigens on the cell surface.	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.
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.	Controlled ovarian stimulation	Pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes.
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).	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.
Cell	The smallest transplantable and functional unit of life.	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.
Cell culture	Growth of cells in a nutrient medium <i>in vitro</i> .	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.
Clean area/environment	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.	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 of viable tissues and cells (including gametes and embryos) to preserve viability, either by freezing or vitrification, or alternatively (to extend their viable life) by low-temperature storage.	Donor after circulatory death	A donor who is declared dead based on circulatory criteria. Also known as deceased non-heart-beating donor.
Cryoprotectant	A chemical compound that is able to protect cells and tissues against freezing injury. Also used as a compatible solute tolerated in high concentrations by cells and tissues for cryopreservation by vitrification.	Donor evaluation	The procedure for determining the suitability of an individual, living or deceased, as a donor of cells or tissues.
Cumulus cell	See: oocyte cumulus complex.	Donor selection	See: donor evaluation.
Deceased donor	A person declared to be dead according to established medical criteria and from whom cells, tissues or organs have been recovered for the purpose of human application. See also Donor after brain death; Donor after circulatory death.	Double embryo transfer	Transfer of two embryos, selected from a larger cohort of available embryos.
Decontamination	The process of removing or neutralising contaminants.	Embryo	Product of the division of the zygote to the end of the embryonic stage, 8 weeks after fertilisation. In assisted reproductive technologies, the term is commonly used for the pre-implantation stages.
De-epidermisation	Process by which epidermis is removed from skin.	Embryo biopsy	The removal of cells (blastomeres or trophectoderm cells) from the embryo for the purpose of genetic analysis.
Delivery rate	Number of deliveries expressed per 100 initiated cycles, aspiration cycles or embryo-transfer cycles. When delivery rates are given, the denominator (initiated, aspirated or embryo-transfer cycles) must be specified. It includes deliveries that resulted in the birth of one or more live and/or stillborn babies. Delivery of a singleton, twin or other multiple pregnancy is registered as one delivery.	Embryo donation	Transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.
Denudation	The removal or stripping of the cumulus cells from the oocyte.	Embryo transfer	Procedure in which one or more embryos are placed in the uterus or Fallopian tube.
Design qualification	The first step in the qualification of new equipment or facilities.	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.
Deviation	Departure from an approved instruction/protocol or established standard.	End user	A healthcare practitioner who undertakes human application procedures.
Direct use	Any procedure in which tissues and cells are donated and used without banking/storage.	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.
Discontinuous gradient centrifugation	Sperm-preparation technique based on sedimentation of sperm at different rates depending on motility.	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.
Disinfection	A process that reduces the number of viable micro-organisms, but does not necessarily destroy all microbial forms, such as spores and viruses.	Expert	Individual with the appropriate qualifications and experience to provide technical advice to a health authority inspector.
Disposal (of tissues/cells)	The act or means of discarding tissues and/or cells.	Expiry date	The date after which tissues or cells are no longer suitable for use. Also known as 'expiration date'.
Distribution	Transportation and delivery of cells or tissues intended for human application.	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).
Donor	An individual, living or deceased, who is a source of tissues or cells for human application and for other purposes including research.	Facility	A physical building or part of a building.
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.		

Fallopian tube	A long duct in the female abdomen that transports the oocytes that have been released from the ovary to the uterus.	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 referred to as 'haematopoietic stem cells'.
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.	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'.
Fertilisation	Penetration of the oocyte by the spermatozoon and combination of their genetic material, resulting in the formation of a zygote.	Haemolysis	Damage to red cells resulting in the release of haemoglobin into serum/plasma.
Fertility preservation	Cryopreservation of reproductive tissues or cells before treatments that may be potentially deleterious for reproductive cells.	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.
Final product	Any tissue or cell preparation intended to be transplanted or administered after the final release step.	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.
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-human application interventions.	Human application	Use of tissues or cells on or in a human recipient.
Freezing	As used in this guide, it means storage of tissues at high sub-zero temperatures without cryoprotectant.	Human error	A mistake made by a person rather than being caused by a poorly designed process or malfunctioning of a machine such as a computer.
Freeze drying	See: lyophilisation.	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 a human recipient.
Fungaemia	The presence of fungi in the circulating blood.	Identification of tissues and cells	The labelling of tissues and cells to uniquely designate their origin, use or destination. See also: labelling.
Gamete intrafallopian transfer	ART procedure in which both gametes (oocytes and spermatozoa) are transferred to the Fallopian tubes.	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.
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.	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.
Good manufacturing practice	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.		

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.	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.
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.	Laparoscopy	A surgical procedure in which a small incision is made through which a viewing tube (laparoscope) is inserted.
<i>In vitro</i> fertilisation	Assisted reproductive technology procedure that involves extracorporeal fertilisation.	Limbal stem cells	The population of stem cells residing in the basal epithelium of the limbus, giving rise to the corneal epithelium.
<i>In vitro</i> maturation	Refers to the maturation of immature oocytes after recovery from follicles that may or may not have been exposed to exogenous gonadotrophins before retrieval. Also, the <i>in vitro</i> process of maturation from immature dendritic cells (DC) to mature DC.	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.
Incident	A generic term for an adverse reaction or adverse event.	Limbus	The area bridging the junction between the cornea and sclera
Incident reporting (adverse event reporting, serious/critical incident reporting)	A system in a healthcare organisation for collecting, reporting and documenting adverse occurrences that impact on patients and are inconsistent with planned care (e.g. medication errors, equipment failures, violations).	Live birth rate	Delivery of one or more infants with any signs of life per initiated ART cycle.
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.	Living donor	A living person from whom cells or tissues have been removed for the purpose of human application.
Inner cell mass	A group of cells in the blastocyst that give rise to the embryonic structures and the foetus, the yolk sac, the allantois and the amnion.	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.
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.	Malignancy	Presence of cancerous cells or tumours with a tendency to metastasise, potentially resulting in death.
Inspection	On-site assessment of compliance with local/national regulations on tissues and cells carried out by officials of the relevant health authority.	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.
Installation qualification	The second step in the qualification of new equipment or facilities.	Medicinal product	Any substance or combination of substances presented as having properties for treating or preventing disease in human beings, or any substance or combination of substances 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.
Intra-cytoplasmic sperm injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.	Meiotic spindle	Spindle apparatus composed of microtubules that support and segregate chromosomes during meiotic division.
Intrauterine insemination	Procedure in which processed sperm cells are transferred transcervically into the uterine cavity.	Metaphase II oocyte	Mature oocyte at the metaphase of the second meiotic division.
Keratoplasty	Corneal transplantation.		
Key performance indicator	A quantifiable measure or a set of quantifiable measures used to trace performance over time.		

Micromanipulation	Technology that allows micro-operative procedures to be done on the spermatozoon, oocyte, zygote or pre-implantation embryo.	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.
Microsurgical epididymal sperm aspiration	Aspiration of epididymal spermatozoa as an open operation under an operating microscope.	Organ culture	Culture of the whole or parts of an organ in medium in vitro to preserve cell-cell and cell-matrix interactions and maintain structure and function.
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.	Organisations 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.
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.	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.
Next of kin	A person's closest living blood relative or relatives.	Ovulation induction	Pharmacological treatment of women with anovulation or oligo-ovulation with the intention of inducing normal ovulatory cycles.
Non-compliance	Failure to comply with accepted standards, requirements, rules or laws.	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.
Non-partner donation	Donation of reproductive cells between a man and a woman who do not have an intimate physical relationship; also called 'third party donation'.	Packaging	Packaging, including primary and secondary packaging, aims to protect tissues and cells and to present them to the operator (starting or in-process packaging) or to the clinical user (final packaging) in a suitable manner. See also: primary packaging; secondary packaging.
Oligozoospermia	Total concentration of spermatozoa $< 15 \times 10^6/\text{mL}$.	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.
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 shall not normally occur more than once for any given recipient. Imports from the same third-country supplier taking place on a regular or repeated basis shall not be considered to be 'one-off imports' (Directive 2015/566/EC).	Partner donation	Donation of reproductive cells between a man and a woman who declare that they have an intimate physical relationship.
Oocyte cumulus complex	Oocyte surrounded by the granulosa cells and corona radiata.	Percutaneous epididymal sperm aspiration	Sperm aspiration by percutaneous puncture of the epididymis by a fine-needle technique.
Oocyte sharing	Refers to a female partner who enters an ART treatment and decides to donate a specified number of her retrieved oocytes.	Performance qualification	The fourth step in the qualification of new equipment or facilities.
Operational qualification	Third step in the qualification of new equipment or facilities.	Pericardium	A double-walled sac that contains the heart and the roots of the great vessels.
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.	Placenta	An organ that connects the developing foetus to the uterine wall to allow nutrient uptake, waste elimination and gas exchange via the mother's blood supply.
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.		

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 or mixing in a single container, of 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 diagnosis	Analyses of polar bodies, blastomeres or trophoctoderm from oocytes, zygotes or embryos for the detection of specific genetic, structural and/or chromosomal alterations.
Pre-implantation genetic screening	Analyses of polar bodies, blastomeres or trophoctoderm from oocytes, zygotes or embryos for the detection of aneuploidy, mutation and/or DNA rearrangement.
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.
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.
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.
Pyrogenic	Producing or produced by heat or fever.

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 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 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 or retrieval	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.	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.
Reproductive cells	Oocytes and spermatozoa intended to be used for the purpose of assisted reproduction.	Sporicidal	Refers to a substance, agent or product used for killing bacterial spores.
Resistogram	The result of a test for the sensitivity of an isolated bacterial strain to different antibiotics. Also known as an antibiogram.	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.
Return	Sending back recalled tissues or cells to the tissue establishment that supplied them for human application.	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.
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.	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, and grafts are then considered sterile.
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.	Storage	Maintenance of a product under appropriate controlled conditions until distribution.
Sclera	Fibrous white outer coat of the eye.	Storage temperature	Temperature at which tissues and cells must be stored to maintain their required properties.
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.	Supernumerary embryos	Excess embryos after embryo transfer.
Septicaemia	A systemic disease caused by the spread of pathogenic micro-organisms or their toxins via the circulating blood.	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.
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 life-threatening, disabling or incapacitating conditions for a patient or which might result in, or prolong, hospitalisation or morbidity (Directive 2004/23/EC).	Swim up	A preparation technique based on the ability of spermatozoa to swim in the culture medium.
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).	Tendon	A tough band of fibrous connective tissue that usually connects muscle to bone and which can withstand tension.
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.	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	Extraction of testicular spermatozoa by biopsying the testes through single or multiple small incisions.	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.
Third countries	Term used within the EU to refer to countries that are not members of the EU.	Unique identification code	A code that unambiguously identifies a particular donor and donation (e.g., a unique donation + tissue product code). See: coding.
Third party	Any organisation that provides a service to a procurement organisation or tissue establishment on the basis of a contract or written agreement.	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.
Tissue	An aggregate of cells joined together by, for example, connective structures and performing a particular function.	Vasa deferentia	Tubes that transport sperm from the epididymis to the ejaculatory ducts.
Tissue bank	See: tissue establishment.	Verification	Preferred term for the validation or qualification of IT systems/software.
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.	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.
Toxicity	Degree to which a substance can damage an organism.	Viraemia	The presence of viruses in the blood.
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 and the tissue establishment or the processing facility that receives, processes or stores the tissue and cells, and the ability to identify 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.	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. Devitrification is the formation of ice during warming of a vitrified solution.
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.	Withdrawal	Process instigated by a tissue establishment to recall tissues or cells that have been distributed.
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.	Xenograft	Graft of tissue taken from a donor of one species and grafted into a recipient of another species.
Transport	The act of transferring a tissue or cellular product between distributing or receiving facilities under the control of trained personnel.	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 <i>ex vivo</i> contact with live non-human animal cells, tissues or organs.
		Zygote	A diploid cell resulting from the fertilisation of an oocyte by a spermatozoon, which subsequently divides to form an embryo.

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 – SCl 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 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 and 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 DOP.	External contractor to perform DOP testing of facilities in accordance with ISO 14644-3. Aerosol concentrations must be $\leq 0.01\%$ of the upstream concentration.				
2.	Air exchange rate testing. Tested in accordance to 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 SOP978.	Full set of plates must be exposed whilst 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 whilst 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/working has 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 clean room as per SOP 4007 for a minimum of an 8-week period.	Clean room 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 clean room 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.

Clean room 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 clean-room 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 a 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 is 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. assessment of its viability or histological structure).

It was determined that the maximum amount of skin that would be transported would be 6 000 cm², 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.

* Reproduced with permission from: Winters M, Lomas R. The principles of process validation and equipment qualification. In: Fehily D, Brubaker S, Kearney J, Wolfinger L, editors. *Tissue and cells processing: an essential guide*. London, UK: Wiley-Blackwell; 2012.

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 as closely as practically possible real-life conditions).

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 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 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 operatives need to do is confirm that they have complied with the relevant standard operating procedures.

Appendix 7. Examples of method validation (oocyte vitrification) in assisted reproductive technology

If and when a validation is performed, a randomised clinical study with primary and secondary end points should be defined and, in the case of a multi-centre study, agreed upon between the participating ART centres (see Figure 1).

However, for a single ART centre it may be difficult to perform a randomised study, considering the

number of patients that needs to be included to override biological variations. As an alternative, the new method of randomised controlled trials (RCT) can be used to validate the standard method in parallel with the new method following the layout shown in Figure 2.

Figure 1. Validation of a new oocyte vitrification method in a multicentre study

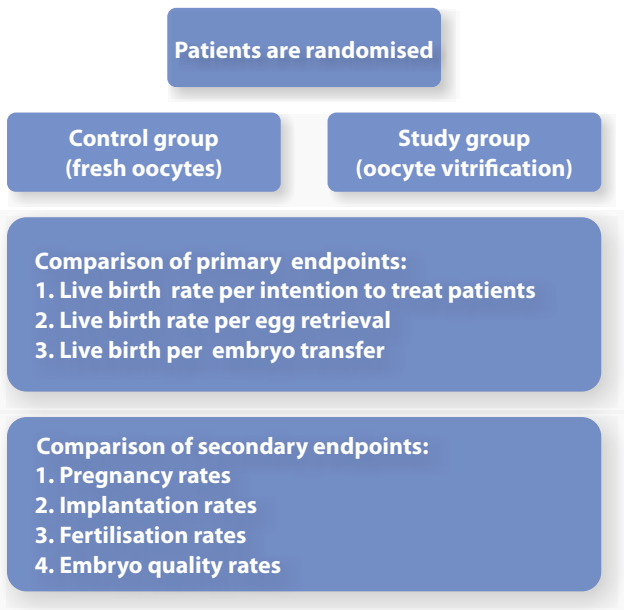
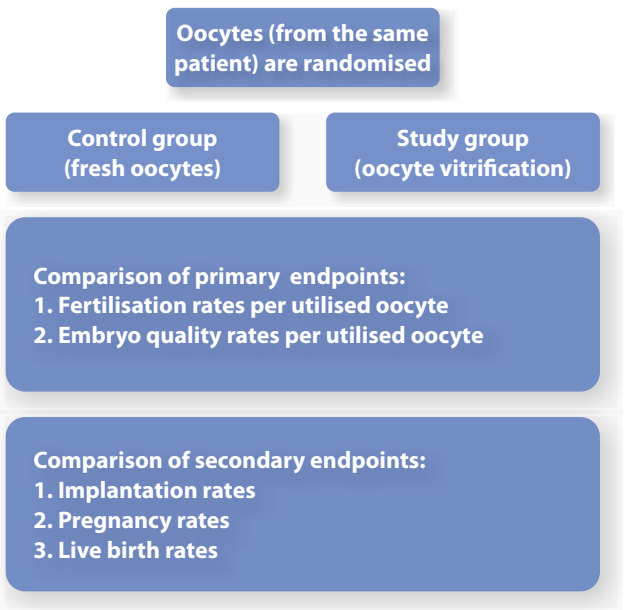


Figure 2. Validation of a new oocyte vitrification method in a single centre



Appendix 8. **Sample consent form**

FORM FRM4281/3.2

Effective: 12/05/14



Consent in accordance with the Human Tissue Act (2004)

Blood and Transplant

Unique Tissue Number

K								

ODT Donor number

Consent - Solid Organ and Tissue Donation

Directions for Completion:

1. This form must be completed in accordance with the Human Tissue Act 2004.
2. This six page form should be completed by the Specialist Nurse-Organ Donation (SN-OD)/ Nurse Practitioner – Tissue Services in **black or dark blue ink**, and signed and dated as appropriate.
3. Where an option box requires completion, it **must** be initialled by the SN-OD/Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator.
4. The original should be retained by the SN-OD/ Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator for the donor file.
5. A copy should be made for the patient's medical records.
6. Another copy should be offered to the patient's family.
7. A copy should be sent to relevant tissue banks.

NOTE: The term 'patient' is used throughout the form to refer to the potential donor.

FORM FRM4281/3.2

Effective: 12/05/14

**Consent in accordance with the Human Tissue Act (2004)****Blood and Transplant**Unique Tissue Number ODT Donor number **PATIENT DETAILS****Section 1**

Name		NHS number	
Address		Hospital number	
		Date of birth	
		Age	years months
		(If under 3 years record years and months)	
Postcode			

CONSENT FOR ORGANS AND TISSUE**Section 2**Complete **Box A** if the patient is/has giving/given first person consent**OR**Complete **Box B** if consent is given by the person ranking highest in the qualifying relationship**Box A**

Name of patient

gave/gives* first person consent for the donation of the following organs/tissue for transplantation via the Organ Donor Register/donor card/expressed wish/will*
(*Delete as appropriate)

Box B

I, the of

(Name) (Relationship to the patient) (Patient's name)

give my consent for the donation of organs and tissue as detailed below.

NOTE:

Proceed to Section 3 for Telephone Consent for Organ and/or Tissue Donation
 Proceed to Section 4 for Organ and Tissue Donation
 Proceed to Section 5 for Tissue Only Donation

OBTAINING CONSENT VIA TELEPHONE**Section 3**

To obtain consent via telephone is in accordance with the Human Tissue Act 2004 and the Human Tissue Authority Codes of Practice 2009 – it is not a legal requirement for relatives to sign a consent form. However, the interviewer **must** ask the following and **initial** the appropriate boxes:

Do you agree to the conversation about donation between (name of HCP) of NHS Blood and Transplant and you being voice recorded? The recording will be stored as proof of the information that I give to you and of the consent and information that you give to me.

Yes	No
☐	☐

For the purpose of the recording can you tell me again your full name and relationship to (name of the patient)

☐	☐

May we use the recording and case details for training purposes?

☐	☐

NOTE:

Proceed to Section 4 for Organ and Tissue Donation
 Proceed to Section 5 for Tissue Only Donation

FORM FRM4281/3.2

Effective: 12/05/14

**Consent in accordance with the Human Tissue Act (2004)****Blood and Transplant**Unique Tissue Number ODT Donor number **CONSENT FOR ORGAN DONATION****Section 4****Organ Group****All Abdominal
Organs**

Yes

No

☐☐**Organ**

Yes

No

Coroner Objection

Outside Criteria

Kidneys

☐☐☐☐

Liver

☐☐☐☐

Liver hepatocytes

☐☐☐☐

Pancreas

☐☐☐☐

Pancreas Islet cells

☐☐☐☐

Bowel

☐☐☐☐Other (consider
multivisceral
donation where
appropriate)

If YES

Please specify

**All Cardiothoracic
Organs**

Yes

No

☐☐

Heart

☐☐☐☐

Lungs

☐☐☐☐

Blood Vessels

☐**CONSENT FOR TISSUE DONATION****Section 5****All Tissue**

Yes

No

Coroner
Objection☐☐☐**Tissue**

Yes

No

Coroner
ObjectionOutside
Criteria

Eyes

☐☐☐☐Whole heart for
heart valve donation☐☐☐☐

Skin

☐☐☐☐

Bone

☐☐☐☐

Tendons

☐☐☐☐

Meniscus

☐☐☐☐

Spleen

☐☐☐☐

(tissue only donation)

Specific Tissue

Yes

No

Coroner
ObjectionOutside
Criteria

Corneas

☐☐☐☐

Sclera

☐☐☐☐Whole heart for
heart valve donation☐☐☐☐

Pericardium

☐☐☐☐

Femur(s)

☐☐☐☐

Iliac crest(s)

☐☐☐☐

Achilles tendons

☐☐☐☐Semi tendinosus
tendons☐☐☐☐

Patella tendons

☐☐☐☐Anterior tibialis
tendons☐☐☐☐

Meniscus

☐☐☐☐

Blood Vessels

☐☐☐☐

Please specify

Other

☐☐☐☐

Please specify

FORM FRM4281/3.2

Effective: 12/05/14

**Consent in accordance with the Human Tissue Act (2004)****Blood and Transplant**Unique Tissue Number K ODT Donor number **REQUIRED INFORMATION TO SUPPORT ORGAN AND TISSUE DONATION****Section 6****IF APPLICABLE (Organ and Tissue Donation)**

Do you consent to the patient being transferred from their place of death to the dedicated donation facility for the donation procedure to be undertaken?

Yes

No

☐
☐
I have been informed of and am aware of the following:

That blood and tissue samples will be obtained from the patient (and the patient's mother where the patient is under 18 months old **and/or** the patient has been breast fed in the last 12 months) for testing, including tissue typing, HIV, Hepatitis, HTLV and Syphilis. These samples may be subsequently stored for future testing as necessary. In the event of any screening results that may have implications for the family, relevant individuals may be contacted if their health could be affected.

That blood and tissue samples e.g lymph node and spleen that have been obtained for screening will be subsequently biopsied, analysed and stored for future testing as necessary.

Blood vessels will be retrieved and stored to support surgical procedures and if not used within 14 days will be disposed of in accordance with the hospital/tissue establishment policy. The tissue donated for transplantation will be stored for extended periods in tissue establishments whilst it is prepared for transplantation.

The patient's medical records will be accessed by relevant healthcare professionals to obtain a past medical/behavioural history. This information may be passed on a need-to-know basis to other healthcare professionals in support of the transplantation process. This information may also be retained by the Organ Donation Teams/Tissue Establishments.

Organs and / or tissue which you have agreed to donate may be used in research prior to transplantation to improve transplant outcomes for the recipient. The organs and / or tissue will still be transplanted. Do you consent to this?

Yes

No

☐
☐
CONSENT FOR SCHEDULED PURPOSES**Section 7**

Organs and/or tissue can also be used for the scheduled purposes listed below.

Scheduled Purposes Include:

Research
Education or Training related to Human Health
Clinical audit
Quality Assurance
Performance Assessment

Organs and/or tissue may at times be found to be unsuitable for transplantation once removed.

Do you consent to organs and/or tissue being used for a scheduled purpose listed above if they are removed for transplant and subsequently found unsuitable?

Yes

No

☐
☐

Other organs and/or tissue unsuitable for transplantation may also be donated to aid future health care.

Do you consent to organs and/or tissue being removed solely for a scheduled purpose as listed above (please detail in notes on page 6)?

Yes

No

☐
☐
NOTE:

Organs and/or tissue will be used and stored for a scheduled purpose in accordance with The Human Tissue Act (2004)

Organs and/or tissue that are not used for a scheduled purpose, or following the completion of a scheduled purpose, will be disposed of as per local establishment policy in accordance with the Human Tissue Act (2004).

FORM FRM4281/3.2

Effective: 12/05/14

**Consent in accordance with the Human Tissue Act (2004)****Blood and Transplant**Unique Tissue Number ODT Donor number **CONFIRMATION OF CONSENT****Section 8****I have read and understood** the above and I have had the opportunity to ask and have had my questions answered

Patient/Relationship to patient	
Name Please print	Signed
Date 2 0	Time (24 hr) at :
Address of person giving consent 	Telephone number
	Mobile
Co-signatory Name (Where applicable) Please print	Signed

Healthcare Professional Details (Witness)	
Designation	
Name Please print	Signed
Date 2 0	Time (24 hr) at :

Specialist Nurse – Organ Donation/Nurse Practitioner – Tissue Services/ Tissue Donor Co-ordinator	
Designation	
Name Please print	Signed
Date 2 0	Time (24 hr) at :

	Yes	No
Did the patient give first person consent for the donation via the Organ Donor Register/donor card/expressed wish/will?	☐	☐
Did the patient have a nominated representative?	☐	☐
Was consent obtained from the person ranking highest in the qualifying relationship?	☐	☐

If no, please give details below:

Persons are ranked in the following descending order:

- A)** Spouse or partner (including civil or same sex partner)
- B)** Parent or child
- C)** Brother or sister
- D)** Grandparent or grandchild
- E)** Niece or nephew
- F)** Stepfather or stepmother
- G)** Half-brother or half-sister
- H)** Friend of longstanding

Information leaflets given to family? ☐

OR

Information leaflets to be sent to the family? ☐

FORM FRM4281/3.2

Effective: 12/05/14

**Consent in accordance with the Human Tissue Act (2004)*****Blood and Transplant***

Unique Tissue Number

K							

ODT Donor number

NOTES

Source: National Health Service (NHS), United Kingdom.

Appendix 9. **Sample consent form (women)**

Women's consent to treatment and storage form (IVF and ICSI)

HFEA
WT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit www.hfea.gov.uk.

Who should fill in this form?

Fill in this form if you are a woman and you are having fertility treatment using embryos created outside the body (in vitro) with your eggs. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
 - the different options set out in this form
 - the implications of giving your consent
 - the consequences of withdrawing this consent, and
 - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your eggs, and embryos created using your eggs, to be used or stored (eg, for IVF or ICSI treatment). If you are storing your eggs or embryos, you must also state

in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your eggs and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your eggs and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my eggs and embryos for training purposes?

You may have some eggs and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the eggs and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my eggs and/or embryos?

Unused eggs and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused eggs and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see www.hfea.gov.uk/egg-and-sperm-donors.html. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your eggs' (WD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference

Date embryos were placed in storage

Other relevant forms

Date embryos can remain in storage until



Version 6, 20 April 2015



1	About you	
1.1	Your first name(s)	<i>Place clinic sticker here</i>
1.2	Your surname	
1.3	Your date of birth	1.4 Your NHS/CHI/HCN/passport number (please circle)
2	About your partner	
2.1	Your partner's first name(s)	<i>Place clinic sticker here</i>
2.2	Your partner's surname	
2.3	Your partner's date of birth	2.4 Your partner's NHS/CHI/HCN/passport number (please circle)
3	Your treatment	
3.1	Do you consent to your eggs being used to create embryos outside the body for your treatment (eg, through IVF treatment)? In order to create embryos for your treatment you must provide your consent by ticking the yes box below. Please note that the sperm provider also has to give his consent for embryos to be created. ☐ Yes	
4	Storing embryos	
4.1	Do you consent to the embryos (created outside the body with your eggs) being stored? Please note that embryos can only be stored if the sperm provider has also given his consent. ☐ Yes ► after signing the page declaration below, continue on the next page. ☐ No ►► now sign the page declarations on this page and the next page then go straight to section five.	
►►► Continues on the next page		
Page declaration		
Your signature		Date

For clinic use only (optional)

Patient number

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4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ► go to 4.3.

☐ Yes ► go straight to 4.4.

4.3 For how long do you consent to store your embryos?

You can consent to store your embryos for up to 10 years. Please note that the sperm provider also has to give his consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ► specify the number of years:

years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ► **Now sign the page declaration below and go straight to section five.**

4.4 Premature infertility

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years.

►►► Continues on the next page

Page declaration

Your signature

Date

For clinic use only (optional)

Patient number

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4 Storing embryos *continued*

When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met. The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using eggs and embryos for training

5.1 Do you consent to your eggs being used for training purposes?

☐ Yes ☐ No

5.2 Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?

Please note that embryos can only be used if the sperm provider has also given his consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your eggs, or embryos created outside the body with your eggs, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note your embryos may only be used within the storage period you consented to above. **If you do not give your consent in the below section, your eggs or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.**

6.1 Do you consent to your eggs being used for training purposes?

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

6.2 Do you consent to embryos (already created outside the body with your eggs) being used for training purposes?

Please note that embryos can only be used if the sperm provider has also given his consent.

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

Other uses for your eggs or embryos

If you wish your eggs or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following: • 'Your consent to donating your eggs' (WD form), • 'Your consent to donating embryos' (ED form), or • 'Women's consent to the use and storage of eggs or embryos for surrogacy' (WSG form).

Page declaration

Your signature



Date

For clinic use only (optional)

Patient number

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7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
 - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
 - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
 - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of eggs or embryos in training, or the eggs or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature

X

Date

DD MM YY

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because she is unable to sign for herself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with her direction.

Representative's name

Representative's signature

X

Relationship to the person consenting

Date

DD MM YY

Witness's name

Witness's signature

X

Date

DD MM YY

D

For clinic use only (optional)

Patient number

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Appendix 10. **Sample consent form (women)**

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 at the moment of birth. From puberty onwards, during each normal menstrual cycle, groups of oocytes undergo maturation phenomena that results in the release by the ovary of an oocyte that is mature and can therefore be fertilised.

In certain clinical situations - when essential treatments threaten the survival of reproductive cells, for example cryopreservation of oocytes has been proposed in an attempt to protect future fertility.

The cryopreservation of oocytes may also be justified in other clinical situations.

A number of key points should be highlighted:

- Cryopreservation of oocytes is a technique the global implications of which are not yet a matter of consensus in the scientific world.
- Cryopreservation of oocytes does not guarantee that a pregnancy will be obtained; it only guarantees a reserve of female reproductive cells that can be used in future. Currently, the rate of pregnancy achieved with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) of cryopreserved oocytes is lower.
- Given the small number of children born as a result of the use of cryopreserved oocytes there is no reliable information as regards a possible increased risk of foetal abnormalities.
- Cryopreservation of oocytes does not establish any obligation on this centre in relation to the future use of those cells, nor does it grant the person from whom they originated any type of preferential treatment; at all times, the clinical criteria of good clinical practice appropriate to each situation will be applied.
- You alone, and no one else, have the right to use the cryopreserved oocytes.
- 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.

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 the situations that could arise in the future.
 - I have understood and I accept that the 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 am aware that I alone have the right to use these frozen oocytes and/or ovarian tissue.
 - I have understood that, under the prevailing legislation, at the end of this five-year period, I will have to travel to the centre to sign a consent form for this freezing 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 hereby express authorisation for their use for scientific purposes. In those circumstances (write **Yes** or **No**):
- I consent to use of my oocytes in scientific research projects
- I fully understand and accept the conditions, risks and limitations set out above.



Therefore, having been fully informed, I freely assume the obligations arising from conclusion of this agreement and give my consent for the use of this technique in preserving my oocytes.

NAME _____

SIGNATURE _____

CIVIL ID/PASSPORT NO. _____

Clinician: _____ / ____ / ____

Appendix 11. **Sample consent form (men)**

Men's consent to treatment and storage form (IVF and ICSI)

HFEA
MT form

About this form

This form is produced by the Human Fertilisation and Embryology Authority (HFEA), the UK's independent regulator of fertility treatment and human embryo research. For more information about us, visit www.hfea.gov.uk.

Who should fill in this form?

Fill in this form if you are a man and your partner is having fertility treatment using embryos created outside the body (in vitro) with your sperm. This may be in vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI).

What do I need to know before filling in this form?

Before you fill in this form, you should be certain that your clinic has given you all the relevant information you need to make fully informed decisions. This includes:

- information about:
 - the different options set out in this form
 - the implications of giving your consent
 - the consequences of withdrawing this consent, and
 - how you can make changes to, or withdraw, your consent.
- an opportunity to have counselling.

If you are unsure, or think that you have not been given all of this information, please speak to your clinic. There is a declaration at the end of this form which you must sign to confirm you have received this information. If you do not receive this information before filling in this form, your consent may be invalid.

If you are unable to complete this form because of physical illness, injury or disability you may direct someone else to complete and sign it for you. However, if you are consenting to being registered as the legal father of any child born as a result of treatment after your death (see section 6.5), you **must** sign the form yourself.

Why do I have to fill in this form?

By law (the Human Fertilisation and Embryology Act 1990 (as amended)), you need to give your written consent if you want your sperm, and embryos created using your sperm, to be used or stored (eg, for IVF or ICSI treatment). If you are

storing your sperm or embryos, you must also state in writing how long you consent to them remaining in storage.

You are also legally required to record what you would like to happen to your sperm and embryos if you were to die or lose the ability to decide for yourself (become mentally incapacitated). While this is perhaps not something you have considered, your clinic needs to know this so that they only allow your sperm and embryos to be used according to your wishes. If you are unsure of anything in relation to this, please ask your clinic.

Why are there questions about using my sperm and embryos for training purposes?

You may have some sperm and embryos left after treatment which you do not wish to use (eg, because you do not want future treatment or the sperm and embryos are not viable for treatment). On this form, you can consent to donate these for training purposes to allow healthcare professionals to learn about, and practice, the techniques involved in fertility treatment.

What if I want to donate my sperm and/or embryos?

Unused sperm and embryos can also be donated for research purposes, helping to increase knowledge about diseases and serious illnesses and potentially develop new treatments. Your clinic can give you more information about this and provide you with the relevant consent form(s).

You could also think about donating viable unused sperm and embryos to another person for use in their treatment. Before doing this, there are lots of issues to consider. For more information, see www.hfea.gov.uk/egg-and-sperm-donors.html. If you decide to donate, you will need to complete a separate form: 'Your consent to donating your sperm' (MD form).

When filling in this form, make sure you sign the declaration on every page to confirm that you have read the page and fully agree with the consent and information given. When you have completed the form you may request a copy of it from your clinic.

For clinic use only (optional)

HFEA centre reference

Date embryos were placed in storage

Other relevant forms

Date embryos can remain in storage until



Version 4, 1 April 2015

1 About you

1.1 **Your first name(s)** *Place clinic sticker here*

1.2 **Your surname**

1.3 **Your date of birth** 1.4 **Your NHS/CHI/HCN/passport number (please circle)**

2 About your partner

2.1 **Your partner's first name(s)** *Place clinic sticker here*

2.2 **Your partner's surname**

2.3 **Your partner's date of birth** 2.4 **Your partner's NHS/CHI/HCN/passport number (please circle)**

3 Your treatment

3.1 **Do you consent to your sperm being used to create embryos outside the body for your partner's treatment (eg, through IVF treatment)?**
 In order to create embryos for your partner's treatment you must provide your consent by ticking the yes box below. Please note that the egg provider also has to give her consent for embryos to be created.
☐ Yes

4 Storing embryos

4.1 **Do you consent to the embryos (created outside the body with your sperm) being stored?**
 Please note that embryos can only be stored if the egg provider has also given her consent.
☐ Yes ► after signing the page declaration below, continue on the next page.
☐ No ► now sign the page declarations on this page and the next page then go straight to section five.

►►► Continues on the next page

Page declaration

Your signature **Date**

For clinic use only (optional)

Patient number

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4 Storing embryos *continued*

Embryo storage periods

You may wish to store any embryos left after treatment so they can be used in future treatment. To be stored, embryos are frozen or 'vitrified'. When considering how long to store for, you may want to think about how far in the future you might want/be able to use your stored embryos and the costs of storing – ask your clinic if you are unsure. The law permits you to store for any period up to 10 years but in cases where you or your partner are prematurely infertile, or likely to become prematurely infertile, you may store for longer, up to 55 years.

Please note that any arrangements you need to make regarding the practicalities of storage with your clinic or funding body are separate from this consent. For example, your clinic may only continue to store your embryos for the period you have specified in this form if you, or your funding provider, continue to pay the storage fees.

4.2 Have you, or your partner, been diagnosed as prematurely infertile or likely to become prematurely infertile?

Causes of premature infertility can include chemotherapy treatment and early menopause. Please speak to your clinic if you are unsure. If your circumstances change and either you or your partner become prematurely infertile, or are likely to become prematurely infertile, you and your partner can change your consent to store your embryos for up to 55 years.

☐ No ► go to 4.3.

☐ Yes ► go straight to 4.4.

4.3 For how long do you consent to store your embryos?

You can consent to store your embryos for up to 10 years. Please note that the egg provider also has to give her consent to storage.

☐ For 10 years

☐ For a specific period (up to a maximum of 10 years) ► specify the number of years:

years

The consent period will start from the date of storage. Remember you can always change the time period you consent to by completing this form again and specifying the new total time period you would like your embryos to be stored for. For example, if you consented to five years' storage on the original form and wish to consent for a further five years (10 years in total), you should complete another copy of this form but tick the box for 10 years. This second form would supersede the first form you completed. ► **Now sign the page declaration below and go straight to section five.**

4.4 Premature infertility

If you or your partner are prematurely infertile, or likely to become prematurely infertile, you can consent to store your embryos for up to 55 years. Although you can consent up to a maximum of 55 years on this form, after the first 10 years your medical practitioner will need to certify in writing that the medical criteria for premature infertility have been met for storage to continue for more than 10 years. When the criteria have been met, the storage period will be extended by 10 years from the date the criteria are met.

►►► Continues on the next page

Page declaration

Your signature

X

Date

For clinic use only (optional)

Patient number

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4 Storing embryos *continued*

The storage period can then be extended by further 10 year periods (up to a maximum of 55 years) at any time within each extended storage period if it is shown that the criteria continue to be met. For more information about this, please ask your clinic.

For how long do you consent to store your embryos?

Please specify the number of years you consent to store your embryos for (up to a maximum of 55): years.

Clinic staff: please attach all relevant medical practitioners' statements to this form.

5 Using sperm and embryos for training

5.1 Do you consent to your sperm being used for training purposes?

☐ Yes ☐ No

5.2 Do you consent to embryos (already created outside the body with your sperm) being used for training purposes?

Please note that embryos can only be used if the egg provider has also given her consent.

☐ Yes ☐ No

6 In the event of your death or mental incapacity

As part of your consent, you also need to decide what you would like to happen to your sperm, or embryos created outside the body with your sperm, if you die or lose the ability to decide for yourself (become mentally incapacitated). Please note that if you would like your partner to use your sperm or embryos in the event of your death or mental incapacity, your partner should be named on this form. Your embryos may only be used within the storage period you consented to above.

If you do not give your consent in the below section, your sperm or embryos must be allowed to perish in the event of your death or mental incapacity and cannot be used for treatment.

6.1 Do you consent to your sperm being used to create embryos outside the body for your partner's treatment?

Please note that the egg provider also has to give her consent for embryos to be created.

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

6.2 Do you consent to embryos (already created outside the body with your sperm) being used for your partner's treatment?

Please note that embryos can only be used if the egg provider has also given her consent.

If you die

☐ Yes ☐ No

If you become mentally incapacitated

☐ Yes ☐ No

»»» Continues on the next page

Page declaration

Your signature

X

Date

For clinic use only (optional)

Patient number

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6	In the event of your death or mental incapacity <i>continued</i>
6.3	Do you consent to your sperm being used for training purposes? If you die ☐ Yes ☐ No If you become mentally incapacitated ☐ Yes ☐ No
6.4	Do you consent to embryos (already created outside the body with your sperm) being used for training purposes? Please note that embryos can only be used if the egg provider has also given her consent. If you die ☐ Yes ☐ No If you become mentally incapacitated ☐ Yes ☐ No Other uses for your sperm or embryos If you wish your sperm or embryos to be used in someone else's treatment if you die or become mentally incapacitated, please speak to your clinic for more information. Depending on your circumstances, you will need to complete one of the following: • 'Your consent to donating your sperm' (MD form) • 'Your consent to donating embryos' (ED form), or • 'Men's consent to the use and storage of sperm or embryos for surrogacy' (MSG form). Consent to birth registration Complete this part of section six if you consented to your sperm, or embryos created outside the body with your sperm, being used in your partner's treatment after your death. If you have given your consent to your sperm or embryos (to be created outside the body with your sperm) being used after your death, you may also wish to consent to being registered as the legal father of any child that is born as a result of your partner's treatment.
6.5	Do you consent to being registered as the legal father of any child born as a result of your partner's treatment after your death? By ticking yes, you consent to the following: • I consent to my name, place of birth and occupation being entered on the register of births as the legal father of any child born from my partner's treatment. This register is kept under the Births and Deaths Registration Act 1953, or the Births and Deaths Registration (Northern Ireland) Order 1976, or the Registration of Births, Deaths and Marriages (Scotland) Act 1965. • I also consent to information about my or my partner's treatment being disclosed to my partner and one of the following registrars: – the Registrar General for England and Wales – the Registrar General for Scotland – the Registrar for Northern Ireland. Please note that being recorded in the register of births as the legal father of a child born from your partner's treatment does not transfer any inheritance or other legal rights to the child. ☐ Yes ☐ No

Page declaration	
Your signature	Date
X	DDMMYY

For clinic use only (optional)

Patient number MT page 5 of 6
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7 Declaration

Please sign and date the declaration

Your declaration

- I declare that I am the person named in section one of this form.
- I declare that:
 - before I completed this form, I was given information about the different options set out in this form, and I was given an opportunity to have counselling
 - the implications of giving my consent, and the consequences of withdrawing this consent, have been fully explained to me, and
 - I understand that I can make changes to, or withdraw, my consent at any point until the time of embryo transfer, use of sperm or embryos in training, or the sperm or embryos have been allowed to perish.
- I declare that the information I have given on this form is correct and complete.
- I understand that information on this form may be processed and shared for the purposes of, and in connection with, the conduct of licensable activities under the Human Fertilisation and Embryology Act 1990 (as amended) in accordance with the provisions of that act.

Your signature



Date

If signing at the direction of the person consenting

If you have completed this form at the direction of the person consenting (because he is unable to sign for himself due to physical illness, injury or disability), you must sign and date below. There must also be a witness confirming that the person consenting is present when you sign the form. However, if the person consenting consented to being registered as the legal father after his death (that is if he ticked yes to question 6.5), he **must** sign the form himself.

Representative's declaration

I declare that the person named in section one of this form is present at the time of signing this form and I am signing it in accordance with his direction.

Representative's name

Representative's signature



Relationship to the person consenting

Date

Witness's name

Witness's signature



Date

For clinic use only (optional)

Patient number

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Appendix 12. **Sample donor assessment form**

Patient Assessment Form

Directions for completion

- 1 This six-page form must be completed in **black or dark blue ink** by the SN-OD/NP/ANP/Tissue Transplant Co-ordinator and signed where required.
- 2 The original copy should be retained by the **SN-OD/NP/ANP/Tissue Co-ordinator** for the donor file.
- 3 A copy should be made for the patient's medical records.
- 4 In the event of organ and tissue donation, a legible photocopy should be sent to the relevant **Tissue Establishment/CTS Eye Bank**, where required.

NOTE: The term patient is used throughout the form to refer to the potential donor.



ODT Donor number

PATIENT ASSESSMENT

(Information obtained from relative/significant other)

Patient's name	Please print				
Donor hospital					
Hospital/CHI number					Cause of death:
Patient date of birth (dd/mm/yyyy)					

In order to proceed with organ and tissue donation it is necessary for us to ask you some questions - which should be answered to the best of your knowledge - about your relative's medical and behavioural history. All information will be treated with the strictest confidence.

For paediatric patients under the age of 18 months or those who have been breast-fed in the past twelve months the mother is required to answer these questions with regard to both her own and her child's health.

For children: has your child been breast-fed in the last twelve months? Yes ☐ No ☐ Not applicable ☐

NOTE: For all donors under the age of 18 months and any baby or child who has been breastfed in the last 12 months, a blood sample is required from the mother of the donor

For ALL female patients aged between 13 and 53 years of age
Is there a possibility that your relative could be pregnant? Yes ☐ No ☐ Unknown ☐

GENERAL HEALTH INFORMATION

1	Has your relative visited his/her general practitioner in the last two years? Was he/she currently seeing or waiting to see their general practitioner or any other healthcare professional?	Yes ☐	No ☐	Unknown ☐
	If YES, give details			
2	Did your relative have diabetes?	Yes ☐	No ☐	Unknown ☐
	If yes, were they on insulin?	Yes ☐	No ☐	Unknown ☐
	Is there a family history of diabetes?	Yes ☐	No ☐	Unknown ☐
	If yes, is it insulin dependent diabetes?	Yes ☐	No ☐	Unknown ☐
3	Did your relative take regular medication?	Yes ☐	No ☐	Unknown ☐
	If YES, give details			
4	Did your relative ever undergo any investigations for cancer or have they ever been diagnosed with cancer?	Yes ☐	No ☐	Unknown ☐
	If YES, give details			
5	Did your relative recently suffer from any significant weight loss?	Yes ☐	No ☐	Unknown ☐
	If YES, give details			
6	Did your relative have any signs of recent infection, eg colds, flu, fever, night sweats, swollen glands, diarrhoea, vomiting or skin rash?	Yes ☐	No ☐	Unknown ☐
	If YES, please specify			
7	Did your relative come into contact with any infectious disease recently or have any immunisations within the last eight weeks?	Yes ☐	No ☐	Unknown ☐
	If YES, give details			
8	Did your relative ever have hepatitis, jaundice or liver disease?	Yes ☐	No ☐	Unknown ☐
	If YES, give age and any diagnosis			

Patient Assessment Form		ODT Donor number
GENERAL HEALTH INFORMATION continued		
9	Did your relative have a history of ocular disease or previous eye surgery or corrective laser treatment?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
10	Did your relative ever suffer from any bone, joint, skin or heart disease, eg rheumatic fever?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
11	Did your relative ever have any operations or illnesses including an organ or tissue transplant? <i>If no go to question 13.</i>	Yes ☐ No ☐ Unknown ☐
If YES, give details		
12	Did your relative ever have neurosurgical operations for tumour or cyst of the spine/brain or implantation of dura mater, before August 1992?	Yes ☐ No ☐ Unknown ☐
If YES, please specify		
13	Did your relative receive a blood, or blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) at any time (particularly since 1980)?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
14	Was your relative ever told never to donate blood?	Yes ☐ No ☐ Unknown ☐
If YES, give details of where, when and the reason		
15	Did your relative suffer from any chronic or autoimmune illness or disease of unknown cause, eg inflammatory bowel disease, multiple sclerosis, sarcoidosis?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
16	Did your relative suffer from any type of brain disease such as Parkinson's disease, Alzheimer's disease, recent memory loss, confusion or unsteady gait?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
17	Did your relative have a family history of prion disease or do you know if they were ever told that they were at risk of CJD, vCJD, GSS or FFI?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
18	Did your relative ever receive human pituitary extracts, eg growth hormones, fertility treatment or test injections for hormone imbalance?	Yes ☐ No ☐ Unknown ☐
If YES, give details		
19	Did your relative ever have any other serious infection such as tuberculosis, malaria, West Nile virus, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme disease or brucellosis?	Yes ☐ No ☐ Unknown ☐
If YES, give details, and any treatment received		
20	Did your relative have any acupuncture, tattooing, body piercing, botox, injections or cosmetic treatments that involves piercing the skin in the last six months?	Yes ☐ No ☐ Unknown ☐
If YES, give details		

Patient Assessment Form

ODT Donor number

GENERAL HEALTH INFORMATION continued

21	In the last twelve months has your relative been in close contact with a bat anywhere in the world or been bitten by an animal whilst abroad?	Yes ☐	No ☐	Unknown ☐
If YES, give details of animal and place of treatment				
22	Did your relative ever have a sexually transmitted infection e.g. syphilis, gonorrhoea, genital herpes, genital warts?	Yes ☐	No ☐	Unknown ☐
If YES, give details of diseases, dates and treatment				

TRAVEL RISK ASSESSMENT

23	Did your relative ever travel outside the UK? (If NO or UNKNOWN, proceed to 26b then continue with Behavioural Risk Assessment. If YES, continue with questions below)	Yes ☐	No ☐	Unknown ☐
24	Has your relative travelled outside the UK in the last 12 months?	Yes ☐	No ☐	Unknown ☐
If yes, give details of date of visit/return and destination.				
25	Ever had a fever or received treatment for an illness whilst abroad or within six months of leaving an area where there is malaria or West Nile Virus?	Yes ☐	No ☐	Unknown ☐
If YES, give date of fever/illness and places visited				
26	(a) Ever live or work in rural Central or South America for a continuous period of four weeks or more?	Yes ☐	No ☐	Unknown ☐
If YES, specify place and date of last visit, and details of living conditions				
26	(b) Was your relative or their mother born in Central or South America?	Yes ☐	No ☐	Unknown ☐
	(c) Was your relative ever given a blood transfusion in that country?	Yes ☐	No ☐	Unknown ☐
27	(a) Ever spend a continuous period of six months or longer in an area where there is malaria at any time during his/her life?	Yes ☐	No ☐	Unknown ☐
	(b) If YES, have they travelled to a malaria area since then?	Yes ☐	No ☐	Unknown ☐
If YES, give details of where				

BEHAVIOURAL RISK ASSESSMENT

28	Did your relative:			
	(a) consume alcohol?	Yes ☐	No ☐	Unknown ☐
If YES, approximately how many units per week				
	(b) smoke tobacco or any other substance?	Yes ☐	No ☐	Unknown ☐
If YES, give details				
29	Is it possible that any of the following apply to your relative?			
	(a) is, or may be infected with HTLV, HIV or hepatitis B or C?	Yes ☐	No ☐	Unknown ☐
	(b) has ever injected or been injected with non-prescriptive drugs, including body building drugs, even if it was a long time ago or only once?	Yes ☐	No ☐	Unknown ☐
	(c) has ever been given payment for sex with money or drugs?	Yes ☐	No ☐	Unknown ☐
	(d) (for male patients only) ever had sex with another man with or without a condom?	Yes ☐	No ☐	Unknown ☐

Patient Assessment Form

ODT Donor number

BEHAVIOURAL RISK ASSESSMENT continued

(e) (for female patients only) had sex in the last 12 months with a man who has had sex with another man with or without a condom?	Yes ☐	No ☐	Unknown ☐
(f) been in prison or a juvenile detention centre for more than three consecutive days within the last 12 months? NB: This excludes those who have been in a police cell for <96 hours.	Yes ☐	No ☐	Unknown ☐
(g) had sex in the last 12 months with:			
(i) anyone who is HIV or HTLV positive?	Yes ☐	No ☐	Unknown ☐
(ii) anyone who has hepatitis B or C?	Yes ☐	No ☐	Unknown ☐
(iii) anyone who had a sexually transmitted disease?	Yes ☐	No ☐	Unknown ☐
(iv) anyone who has ever been given payment for sex with money or drugs?	Yes ☐	No ☐	Unknown ☐
(v) anyone who has ever injected drugs?	Yes ☐	No ☐	Unknown ☐
(vi) anyone who may ever have had sex in any part of the world where AIDS/HIV is very common (this includes most countries in Africa)?	Yes ☐	No ☐	Unknown ☐

Having answered all the previous questions is there anyone else who you think may provide more information?

Yes ☐ No ☐

If YES, please specify

Question number	Relevant additional information

Information discussed with

Name	Please print	Relationship	Please print
Signature of healthcare professional obtaining information		Please print name	
Designation of healthcare professional obtaining information			
Date of interview	/ / 20	Time of interview	:

Rationale document for patient assessment form PA1 (v03)

Introduction

The purpose of patient assessment is firstly to determine if a potential donor is suitable to donate *any* organ or tissue and then to determine *which* organs and tissues can be donated. Whilst the donor may 'in general' be acceptable for donation, not all organs or tissues may be suitable due to 'system specific' medical problems. This document aims to provide a rationale for specific information that is required to assess a potential donor's suitability for organ/tissue donation and should be used in conjunction with the NHS Blood and Transplant **FRM4211** Patient Assessment Form (PA1).

The purpose of risk assessment is to determine risk factors for the transmission of disease from donor to recipient. It is the responsibility of the Specialist Nurse – Organ Donation (ODT), Nurse Practitioner/Assistant Nurse Practitioner (Tissue Services) and Tissue Transplant Co-ordinator (SNBTS) to collect comprehensive information on medical, behavioural and travel history and relay all of the information obtained to the organ recipient and tissue procurement centres. In addition, for organs, it is the responsibility of the implanting surgeon to assess the risk of transplant for their individual patients. For tissues, it is the responsibility of the tissue establishment to make the final decision on donor suitability.

Risk is relative to the risks of not receiving a transplant.

The Specialist Nurse – Organ Donation (ODT), Nurse Practitioner/Assistant Nurse Practitioner (Tissue Services) and Tissue Transplant Co-ordinator (SNBTS) must be familiar with the purpose of each question and must recognise when to expand the question in order to obtain more details and what additional information might be required. The conditions which will cause the deferral of a potential donation vary significantly between organs, ocular tissue and other tissues. For potential tissue donors further detailed information regarding the deferral criteria for each type of tissue can be found in the current version of the UKBTS Tissue Donor Selection Guidelines for Deceased Donors (TDSG-DD). Due to the avascular nature of corneal grafts, many of the deferral criteria for other tissues do not apply to cornea.

For all paediatric donors under the age of 18 months, and any infant donor over the age of 18 months but who has been breast-fed in the past 12 months, the mother is required to answer the questions in the patient assessment document with regard to both her own and her child's health.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
For paediatric donation: has your child been breast-fed in the last 12 months?	There is a risk of vertical transmission of some viral infections from the mother to her child via breast milk. This may be determined by the mother's medical and behavioural history that is used as a surrogate for the infant's history. If yes, the medical history of the mother will need to be assessed and a maternal blood sample must be taken for virology testing.	Not an absolute contraindication; inform recipient centres and ensure the following sampling takes place: All babies and children who have been breastfed within 12 months of donation should have maternal sampling. Neonates (less than 2 months) – maternal only sampling. Babies greater than 2 months not breastfed should have samples from the infant with maternal samples as a fallback position if required. Babies greater than 18 months not breastfed should only require infant sampling.	Provided the mother's blood sample is found to be negative for markers of viral infection, this is not a contraindication to donation. Ensure the following sampling takes place: All babies and children who have been breastfed within 12 months of donation should have maternal sampling. Neonates (less than 2 months) – maternal only sampling. Babies greater than 2 months not breastfed should have samples from the infant with maternal samples as a fallback position if required. Babies greater than 18 months not

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
			breastfed should only require infant sampling.
For all female patients between 13 and 53 years of age Is there a possibility that your relative could be pregnant?	If there is a possibility that the patient could be pregnant then a pregnancy test should be performed, to determine whether the foetus is viable. This would have a direct effect upon whether donation is able to proceed or not.	If the foetus is not determined to be viable there is no contraindication to donation.	Donation acceptable.
General health information Did your relative/significant other:			
1. Visit his/her general practitioner in the last 2 years? Was he/she currently seeing or waiting to see their general practitioner or any other healthcare professional?	1.1. These are broad questions to quickly ascertain if the donor has on-going health problems. If the answer to either is yes, it is important to obtain as much information as possible. 1.2. Note: It is important to obtain accurate information on past medical history. Therefore it is a requirement that the GP be contacted to complete the NHSBT GP questionnaire (FRM1602).	Donation acceptable. For organ donation this should be done pre-donation. Attempts should always be made to contact the GP before retrieval of organs. If these attempts do not enable contact with the GP this must be completed within 3 working days.	A positive answer is not itself a contraindication to donation: however each condition must be assessed for its acceptability as per the current version of TDSG-DD. For tissue only donation this is usually done post-donation.
2. Have diabetes? If yes, were they on insulin?	Due to the effect diabetes can have on a number of organs particularly the kidneys additional tests/information relating to function may be necessary.	Not an absolute contraindication except for pancreas; inform recipient centres.	Donation acceptable except for pan-creatic tissue.
3. Take regular medication?	3.1. Very few drugs are themselves contraindications to donation but knowledge of the donor's drug therapies may indicate an underlying disease that is itself a contraindication to donation for some tissues. It is useful to know why the medication was being taken although doses and frequency are not required.	Not an absolute contraindication; inform recipient centres.	Certain drugs may exclude the donation of specific tissues, e.g. long-term steroid therapy may affect the quality of bone and skin. See TDSG-DD and seek advice from tissue establishment.
	3.2. A small number of drugs may cause birth defects in babies exposed to them while in the womb (consider potentially pregnant organ recipients). It is important to allow time for the drugs to be cleared from the donor. It takes longer to clear some drugs than others.	Not an absolute contraindication; inform recipient centres.	For tissue donation other than cornea; isotretinoin (roaccutane), acitretinate (neotigason), etretinate (tigason) used to treat acne and dutasteride (avodart) and finasteride (proscar) used to treat prostatic hyperplasia all have specified deferral periods. See current version of TDSG-DD.
	3.3. Individuals being treated with immunosuppressive drug therapy, such as transplant recipients may not be eligible to donate; as the serology test may be misleading in addition any infection may be masked.	Not an absolute contraindication; inform recipient centres.	Must not donate if immunosuppressed.
4. Ever undergo any inves-	4.1. The presence, or previous history, of cancer	Not an absolute contraindication; inform	Depending on the type cancer can

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
tigations for cancer or ever been diagnosed with cancer?	poses a risk of transmission of cancer cells to a recipient. If yes, obtain further information regarding dates and treatments.	recipient centres. It is important to assess the type and grade of cancer. Please refer to Council of Europe document (1997) for more information.	very often be acceptable for corneal tissue donation but usually not for other tissues. See current version of TDSG-DD.
5. Recently suffer from any significant weight loss?	5.1. Recent weight loss may be an indication of illness, which includes cancer. It is important therefore to obtain the reason for the weight loss.	Not an absolute contraindication; inform recipient centres.	Not an absolute contraindication – depends on underlying cause.
6. Have any signs of recent infection, e.g. colds, flu, fevers, night sweats, swollen glands, diarrhoea, vomiting and skin rash?	6.1. Bacterial, viral and protozoal infections can all be transmitted by transplantation. Successful antibiotic treatment may make donation acceptable.	Not an absolute contraindication; inform recipient centres. However it is important to ascertain specific information about the organism so that appropriate antibiotic/antifungal treatment may be administered to the recipient.	Active systemic infection is a contraindication to most tissue donation but cornea donation may be possible. Localised infection may be acceptable. Each condition must be assessed for its acceptability as per the current version of TDSG-DD.
	6.2. Many of these symptoms can also be signs of underlying malignancy.	Not an absolute contraindication; inform recipient centres.	Each condition must be assessed for its acceptability as per the current version of TDSG-DD.
7. Come into contact with any infectious diseases recently or have any immunisations within the last 8 weeks?	7.1. Potential donors who have been in recent contact with an infectious disease (for which they have no history of previous infection) may be in the asymptomatic stage of developing an infection at the time of donation.	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.
	7.2. Immunisations with live vaccine may cause severe illness in people who are immunosuppressed. By 8 weeks any infection caused by the immunisation should have been controlled and so should not be passed on through donated material. There are special rules for BCG and smallpox immunisations.	Not an absolute contraindication; inform recipient centres.	Unacceptable for tissue donation if less than 8 weeks since receiving a live immunisation.
8. Ever have hepatitis, jaundice or liver disease?	8.1. Viral hepatitis is readily transmitted by all types of transplantation. Any history of jaundice or hepatitis must therefore be investigated. Testing alone may not exclude all infectious donors and the donor history may suggest the need for additional testing. However, jaundice can be caused by many non-infectious conditions, e.g. gallstones, obstruction of the bile ducts, congenital biliary atresia or neonatal jaundice.	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.
9. Have a history of ocular disease or previous eye surgery or corrective laser treatment?	9.1. This question is specifically designed to assess the suitability of ocular tissue.	Not an absolute contraindication; inform recipient centres.	Corneal disease and infections, e.g. herpes, ocular inflammation, retinoblastoma and malignant tumours of the anterior segment are contraindications to eye donation. Laser refractive surgery (e.g. LASIK) to the cornea is also a contraindication. However, other existing eye disease or previous eye surgery does not necessarily exclude corneas from transplantation. See current version of TDSG-DD or where appropriate seek

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
			specific specialist advice.
10. Ever suffer from any bone, joint, skin or heart disease, e.g. rheumatic fever?	10.1. This question relates to the suitability of specific tissues. Whilst the donor may 'in general' be acceptable for donation not all tissues may be suitable due to 'system specific' medical problems. This question is aimed at identifying some of these medical diseases.	Inform recipient centres of details of specific diseases. Inform recipient centres of details of specific diseases.	The presence of disease in any of these systems may preclude donation of that specific tissue. See current version of TDSG-DD. See current version of TDSG-DD.
	10.2 Note however that some tissue specific symptoms may be part of a systemic disease, e.g. SLE, and therefore a general deferral for the donation of tissues.	Inform recipient centres of details of specific diseases.	See current version of TDSG-DD.
11. Ever have any operations or illnesses, including an organ or tissue transplant?	11.1. The first part of this question is to quickly ascertain if the donor has had previous significant health problems. If the answer is yes, it is important to obtain as much information as possible. Surgery may be related to underlying malignancy.	Not an absolute contraindication; inform recipient centres.	Each condition must be assessed for its acceptability as per the current version of TDSG-DD.
	11.2. The question regarding transplantation is a SaBTO requirement. There is the risk of viral or prion transmission when someone has received a tissue transplant. There has been one definite and one probable case of CJD transmission by corneal transplants.	Individual assessment is required.	If dura mater or ocular tissue was transplanted no tissue donations can be accepted. A history of receipt of other tissue transplants since 1980 is a contraindication for most types of tissue donation, with the exception of skin or heart valve donation in some circumstances refer to TDSG-DD and seek advice from tissue establishment.
	11.3. There is the risk of viral or prion transmission when someone has received an organ transplant. Individuals being treated with immunosuppressive drug therapy, such as transplant recipients may not be eligible to donate, as the serology test may be misleading. In addition any infection may be masked.	Individual assessment is required.	A history of receipt of an organ is a contraindication for all types of tissue donation.
12. Ever have neurosurgical operations for a tumour or cyst of the spine/brain or implantation of dura mater, before August 1992?	12.1. This is to ascertain if the donor may have been given a dura mater graft as part of a neurosurgical procedure. This material is known to have transmitted CJD in around 200 cases. Brain surgery often required dura mater repair. Neurosurgeons may use different materials for this but before 1992; dura mater from cadaveric donors was used in brain and spinal surgery. Spinal fusion and burr holes did not usually involve using dura mater.	Not an absolute contraindication; inform recipient centres.	If yes, tissue donation can only be accepted if it can be shown that dura mater was not used.
13. Receive a blood transfusion/blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) in the last 12 months or at any	13.1. Blood or blood product/component transfusion (such as Fresh Frozen Plasma (FFP), Platelet, Cryoprecipitate or Immunoglobulins) transfusions have transmitted bacterial, viral, protozoan and prion infections. Testing of blood donors for markers of infection varies by country and also by date. A complex set of criteria exist for tissue donor acceptability depending on	Not an absolute contraindication; inform recipient centres.	See current version of TDSG-DD.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
other time (particularly since 1980)?	when and where the transfusion took place and also for the type of tissue to be donated. To date there have been 4 cases of vCJD and 2 cases of asymptomatic prion transmission by blood transfusion. The question regarding transfusion is a SaBTO requirement. See TDSG-DD for detailed guidance.		
	13.2. If there has been significant blood loss and replacement of fluids with blood components and/or colloids within the 48-hour period prior to obtaining the donor's blood sample there may be significant haemodilution of the sample. This may result in 'false negative' results when testing the donor for viral markers of infection. If no pre-transfusion sample is available, a detailed assessment of all intravenous fluid intakes during the 48-hour period before sampling is required to enable the haemodilution calculation to be performed.	Not an absolute contraindication; inform recipient centres.	If there is > 50% haemodilution no tissue donations can be accepted.
	13.3. The reason for the blood transfusion should be obtained as this may itself be a contraindication to donation.	Inform recipient centres of details of specific diseases.	See current version of TDSG-DD.
14. Ever told never to donate blood?	14.1. Must establish reason why person told never to give blood. There are a number of individuals who have been informed that they are classified as being at "increased risk" of CJD/vCJD for public health purposes because they have been exposed to possible risk through blood transfusion, surgery, or tissue transplantation. The individuals have all been informed that they should not donate blood, tissues or organs. Examples are: - Individuals who had surgery using instruments that had been used on someone who developed CJD. - Individuals who have given blood to someone who later developed vCJD. - Individuals who have received more than 80 units of blood or blood components.	Not a contraindication to donation; inform recipient centres.	Contraindication if person told never to give blood owing to CJD risk.
	14.2. Individuals may have been told not to donate for other reasons, for example HCV infection or for a haematological disorder.	Not a contraindication to donation; inform recipient centres.	See current version of TDSG-DD.
15. Suffer from any autoimmune illnesses or disease of unknown aetiology, e.g. inflammatory bowel disease, multiple sclerosis and sarcoidosis?	15.1. Some diseases of unknown aetiology may have an infectious origin and may be transmissible.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD. For example multiple sclerosis is an absolute contraindication for all tissues whilst for sarcoidosis ocular tissue can be donated provided there is no actual ocular involvement.
	15.2. Inflammatory bowel disease can also increase the risk of bacteria entering the blood stream.	Not an absolute contraindication; inform recipient centres.	Crohn's disease and ulcerative colitis are exclusions for tissue donation except for corneal donation.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
	15.3 Autoimmune disease is caused by the body attacking itself and can be either limited to a single organ, e.g. thyroid disease or affect multiple systems, e.g. rheumatoid disease. Severe systemic disease may adversely affect the quality of a number of tissues. In addition treatment to suppress the condition may be with steroids, immunosuppressive drugs, anti-metabolites or antibodies directed against part of the immune system. This may well make the donor more susceptible to certain types of infection and also make some infections more difficult to diagnose.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD. For example multiple sclerosis is an absolute contraindication for all tissues whilst for sarcoidosis ocular tissue can be donated provided there is no actual ocular involvement.
16. Suffer from any type of brain disease such as Parkinson's disease, Alzheimer's disease, motor neurone disease, recent memory loss, confusion or unsteady gait?	16.1. CNS disease may be: • of suspected infective origin, e.g. multiple sclerosis or CJD, or • a neurodegenerative condition of unknown aetiology, e.g. Parkinson's disease or Alzheimer's disease. There are concerns that Alzheimer's disease may mask symptoms of CJD. In the event of confusion or memory loss the risk of CJD has to be excluded.	Not an absolute contraindication; inform recipient centres.	Contraindication unless confusion and/or memory loss has an underlying clinical reason that is itself not a contraindication to transplantation.
17. Have a family history of CJD, vCJD, Gerstmann-Straussler-Schienker disease, or Fatal Familial Insomnia?	17.1. These are all varieties of prion-associated disease. 10-15% of classical CJD cases are associated with gene mutations (familial CJD). Individuals at familial risk of prion-associated disease are those who have 2 or more blood relatives with a prion-associated disease or where the family has been informed it is at risk following genetic testing and counselling.	Contraindication.	Contraindication.
18. Ever receive human pituitary extracts, e.g. growth hormones, fertility treatment or test injections for hormone imbalance?	18.1. Prior to 1985 treatment with growth hormone, infertility treatment and some thyroid diagnostic tests used material derived from the pituitary glands of untested cadavers, some of whom may have died from CJD. Around 200 recipients of this material subsequently developed CJD.	Not an absolute contraindication; inform recipient centres.	Contraindication.
19. Ever have any other serious infection, e.g. tuberculosis, malaria, West Nile virus, SARS, typhoid fever, toxoplasmosis, rabies, encephalitis, Lyme disease or brucellosis?	19.1. This question picks up a range of infections which could be transmitted by transplantation. It is important to obtain as much information as possible to determine the degree of risk of transmission to a recipient. Please refer to the Advisory Committee on the Safety of Blood Tissues and Organs (SaBTO) (February, 2011) for further information. West Nile virus, which has been identified as a disease that is a potential risk to organ and tissue recipients, must be identified where possible. However, where some patients are asymptomatic, relevant travel risks must also be noted (Question 25). Note: West Nile virus is active during certain seasons, and migrates across the world; therefore healthcare professionals must remain vigilant as to areas of recent outbreaks.	Not an absolute contraindication; inform recipient centres. <i>Active Tuberculosis</i> Or treatment within 6 months is not an absolute contraindication to donation. If the donor has still to complete a course of chemotherapy the recipient patient will require appropriate drug treatment. A previous history of tuberculosis is not a contraindication to donation other than at the original site of infection, e.g. liver. <i>Malaria</i> Contraindication to donation if there is a known active infection and no curative chemotherapy has been given. <i>West Nile virus</i> Contraindication to donation if there is a	Each condition must be assessed for its acceptability as per the current version of TDSG-DD. In all cases active infection is a contraindication to donation. There are a variety of deferral periods relating to either the date of cessation of symptoms or the date of termination of treatment. Some infections are a permanent contraindication to donation, whilst for malaria it is also dependant on the results of antibody testing.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
		known active infection. Incubation is up to 14 days; therefore relevant travel history is a requirement.	
20. Have any acupuncture, tattooing, body piercing, Botox injections or cosmetic treatment that involves piercing the skin in the last 6 months?	20.1. This question aims to identify donors who may be at risk of having been exposed to reused needles. Acupuncture, tattooing, body piercing, Botox injections or cosmetic treatment that involves piercing the skin all carry a low risk to transmit viral disease. Most tattooist and piercers work to high standards, using disposable needles, but not all do. In the UK there have been occasional large outbreaks of both HBV and HCV as a result of poor hygienic standards. None of these activities are reasons to reject a donor if they were carried out more than 6 months prior to donation. It is helpful, if possible, to know where and when the treatment was carried out.	Not an absolute contraindication; inform recipient centres.	For tissue donors the deferral period has been reduced from 12 months to 6 months since all tissue donors are tested for anti-HBc.
21. In the last 12 months either been in close contact with a bat any-where in the world or bitten by any mammal outside the UK?	21.1. Animal bites may result in many different infections. This question aims to identify donors who may be at risk of having been exposed to rabies. There have been 2 recent cases where organ donors transmitted rabies to all recipients of their organs and to the recipient of a blood vessel. Historically there have been a small number of cases of rabies transmitted by corneal transplantation. In the UK the only risk of rabies comes from contact with infected bats whilst outside the UK bites from infected mammals, especially dogs, are also major routes of infection.	Contraindication.	Contraindication – see TDSG-DD. In addition, bites from a non-human primate at any time are a permanent contraindication to tissue donation.
22. Ever have a sexually transmitted infection, e.g. syphilis, gonorrhoea, genital herpes, genital warts?	22.1. A history of sexually transmitted infection is often not immediately forthcoming from relatives when enquiring about someone's general health. This question is to specifically raise this topic in isolation to evoke either a positive or negative response. If the answer to it is yes, it is important to obtain as much information as possible. Untreated STDs can eventually cause damage to many organs and tissues.	Not an absolute contraindication; inform recipient centres.	Acceptance criteria are specific for each condition, see TDSG-DD.
Travel risk assessment This group of questions is designed to establish the risk of donated organs and tissues transmitting a number of serious infections which are not found within the UK. Due to the forever changing pattern of infections worldwide, when a history of travel abroad has been obtained it is necessary to consult both the TDSG-DD and the Geographical Disease Risk Index (GDRI) for up-to-date information on the specific deferral criteria current at the time. These can be obtained by accessing the JPAC (Joint Professional Advisory Committee) website www.transfusionguidelines.org.uk which is updated as information becomes available. (If access to this website is not available to the SN-OD the NHSBT Duty Office will access this for you.) Did your relative/significant other:			
23. Ever travel outside the UK?	23.1. This opening question, if negative for travel, allows rapid progression to the next set of questions without the need to answer further travel questions. If the answer is yes, it is important to obtain as much information as possible based on the subsequent questions.	Not an absolute contraindication; inform recipient centres.	Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
24. Travel outside the UK in the last 12 months? If yes, please give details of date of visit/return and destination.	24.1. Twelve months is referred to as this is the longest temporary deferral period for tropical infections. Other infections (both tropical and non-tropical) have shorter deferral periods. Cor-neal tissue is treated differently from other tissues as it is avascular not considered to be a risk of transmitting protozoal infections such as malaria or Trypanosoma cruzi infection.	Not an absolute contraindication; inform recipient centres.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue, 'visitors' to a malarial area < 6 months ago are not acceptable, 6-12 months ago require a malaria antibody test, > 12 months ago are acceptable. Refer to the TDSG-DD and GDRI.
25. Ever have a fever or treatment for an illness whilst abroad or within 6 months of leaving an area where there is malaria or West Nile virus?	25.1. Malaria and other endemic infections such as West Nile virus can be transmitted by blood, viable organs, tissues and cells therefore it is important to determine the nature of the illness. Note: A malaria antibody test is of no use if taken prior to 6 month incubation period.	<i>Malaria</i> Not an absolute contraindication; inform recipient centres. Ensure blood sample for malaria screen is sent to the appropriate reference laboratory for all high risk patients. <i>West Nile virus</i> Contraindication.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.
26a. Ever live or work in rural Central or South America for a continuous period of 4 weeks or more?	26a.1. Individuals who have ever lived in Central or South America are at risk of Trypanosoma cruzi infection, which is caused by a parasite transmitted by an insect vector, which bites humans and animals at night time. Those at most risk are trekkers, backpackers and soldiers on manoeuvres in jungle areas as they may have been living in primitive areas and/or sleeping out in the jungle.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.
26b. Was the deceased or their mother born in Central or South America?	26b.1. T. cruzi infection can be passed vertically from mother to child so that a child born outside this area and who has never travelled to this area is still at risk of infection.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.
26c. Given a blood trans-fusion in that country?	26c.1. As T. cruzi is endemic in this area and individuals remain asymptomatic for years after infection many blood donors are infected by this organism. T. cruzi is readily transmitted by blood transfusion from an infected donor. Screening and treatment of blood in this area is becoming more widespread but is still not universal. See also Question 13.	Not an absolute contraindication; inform recipient centres.	For corneal tissue T. cruzi is not a deferral criterion. For other tissues a T. cruzi antibody test is required. Refer to the TDSG-DD and GDRI.
27a. Ever stay for 6 months or longer in an area where there is malaria, at any time in his/her life?	27a.1. This question is designed to make it possible to establish whether a potential donor meets the required criteria of malaria area 'resident'. Individuals who have lived in a malaria affected area for more than 3 months before the age of 5 years develop a partial immunity to malaria through repeated exposure. Partial immunity means that people may be infected with the malaria parasite but show no symptoms, sometimes for years. These individuals were classified as 'residents' as opposed to 'visitors' and, as they pose a much higher risk of transmitting infection, were managed in a different way to people who had simply visited a malaria area.	Not an absolute contraindication; inform recipient centres. Ensure a blood sample for malaria screen is sent to the appropriate reference laboratory for all high risk patients.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
	More recently the definition of 'resident' was extended to include all individuals who have resided in a malaria area for a continuous period of 6 months at any time in their lives. Note: A malaria antibody test is of no use if taken prior to 6 month incubation period.		
27b. If yes, ever travelled outside the UK since then?	27b.1. An individual who is classified as 'resident' is managed differently from a non-resident for each subsequent visit to a malaria area no matter how short the visit. A malaria antibody test is required for all non-corneal tissue even if it > 12 months since the last visit.	Not an absolute contraindication; inform recipient centres. Ensure a blood sample for malaria screen is sent to NHSBT Colindale for all high risk patients.	For corneal tissue malaria is not a deferral criterion. For non-corneal tissue a malaria antibody test is required. Refer to the TDSG-DD and GDRI.
Behavioural risk assessment To the best of your knowledge did your relative:			
28a. Consume alcohol?	28a.1. The effect of alcohol can impact on the quality of liver tissue. If yes, it is important to obtain as much information as possible.	Not an absolute contraindication; inform recipient centres.	Not a contraindication.
28b. Smoke tobacco or other substances?	28b.1. The effect of smoking can impact on the quality of lung tissue. If yes, it is important to obtain as much information as possible.	Not an absolute contraindication; inform recipient centres.	Not a contraindication.
Behavioural risk assessment Based on information obtained from blood donors who tested positive and epidemiological data from larger populations, it is known that certain groups of people may be at increased risk of infection by HIV, HCV and HBV. Unfortunately it is not possible to exclude all cases of infection by relying on blood testing alone as infected donors may be missed in the very early stages of infection, commonly referred to as the 'window period'. This refers to the period between being infected and the appropriate test being able to detect the infection. It takes around 10-12 days to start to form antibodies and a number of weeks before the antibody levels are high enough to be detected by a test that is based on antibody detection. Tests that are based on antigen detection will pick up the infection earlier but it still takes 10-20 days (depending on the specific virus) for adequate numbers of viral particles to be present in the blood to be detected. During all this period the potential 'negative' donor is highly infectious and any organ or tissue transplant will transmit the infection. For this reason donors found to be in any of the known high risk groups must be excluded from tissue donation on the basis of history alone. To the best of your knowledge, is it possible any of the following applies to your relative:			
29a. Is or may be infected with HTLV, HIV or hepatitis B or C?	29a.1. HIV/hepatitis B or C can be transmitted via organ/tissue donation therefore it is vital to identify anyone who is known to be, or thinks that they may be infected with the viruses.	HIV disease is an absolute contraindication, however HIV infection is not. Hepatitis B or C are not absolute contraindications: inform recipient centres.	Contraindication.
29b. Ever injected or been injected with non-prescribed drugs, including body-building drugs, even if it was a long time ago or only once?	29b.1. People with a history of intravenous drug use remain the largest group with HCV infection in the UK. They also have a higher rate of HIV and HBV infection. It is important to obtain as much information as possible to assess possible risk behaviour. Viral infection can be transmitted by sharing equipment used to inject drugs.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29c. Ever received payment for sex with money or drugs?	29c.1 People who receive payment for sex have a higher risk of contracting HIV/hepatitis B or C and other sexually transmitted diseases due to the high number of sexual partners and the promiscuity of these partners. In addition this group of people often sell sex to fund a drug habit. This further increases the risk of infection within this group.	Not an absolute contraindication; inform recipient centres.	Contraindication.

Question	Reason for asking the question	Action to take regarding organ donation	Action to take regarding tissue donation
29d. (for male patients only) Ever had sex with another man, with or without a condom?	29d.1. Men who have sex with men have a much higher prevalence of HIV infection and this activity remains the leading cause of HIV infection within the UK.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29e. (for female patients only) Had sex in the last 12 months with a man who has ever had sex with another man, with or without a condom?	29e.1. As these infections can be transmitted sexually; there is also a higher risk of infection for the sexual partners of individuals who fall into any of these above categories. A temporary deferral for 12 months from the time of the last exposure is used to prevent the risk of any 'window period' infections from being transmitted.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29f. Has been in prison or a juvenile detention centre for more than 3 consecutive days within the last 12 months? Note: This excludes those who have been in a police cell for < 96 hours.	29f.1. It is known that there is a higher risk for individuals who are in prison of being exposed to transmissible viruses through sexual contact and intravenous drug abuse. For a living donor these questions would be asked directly but for a deceased donor this is not possible. It is felt that relatives, who sometimes do not even normally reside with the donor, are unlikely to be able to answer these questions especially relating to the period in prison. As it is essential to rely on virology testing only, the possibility of a 'window period' infection must be excluded by use of a deferral period. It is therefore important to identify individuals who have been exposed to this environment.	Not an absolute contraindication; inform recipient centres.	Contraindication.
29g. Had sex in the last 12 months with: i. Anyone who is HIV or HTLV positive? ii. Anyone who has hepatitis B or C? iii. Anyone who had a sexually transmitted disease? iv. Anyone who has ever had payment for sex with money or drugs? v. Anyone who has ever injected drugs? vi. Anyone who may ever have had sex in a part of the world where AIDS/HIV is very common (this includes most countries in Africa)?	29g.1. There is a higher risk of contracting HIV through heterosexual intercourse in some parts of the world where the virus is endemic. It is therefore important to identify individuals who fall within this category. As these infections can be transmitted sexually, there is also a higher risk of infection for the sexual partners of individuals who fall within the above categories. A temporary deferral for 12 months from the time of the last exposure is used to prevent the risk of any 'window period' infections from being transmitted.	Not an absolute contraindication; inform recipient centres.	Contraindication.
Having answered all the previous questions is there anyone else who you think may provide more information?		<i>This question provides the opportunity to suggest others who may have alternative knowledge of any aspects of the patient's history. For example parents for past medical history or close friends for behavioural history.</i>	

Source: National Health Service (NHS), United Kingdom.

Appendix 13. **Sample donor physical assessment form**

Identification

Name stated on Consent (Authorization) : _____

Age: _____ ☐ days ☐ months ☐ years Recovery Agency ID#: _____Sex/gender: ☐ Male ☐ Female Race: _____ ID#: _____Weight: _____ ☐ lbs. ☐ kgs Weight is: ☐ estimated/team ☐ reported (source: _____) ☐ actualHeight: _____ ☐ ft. ☐ in. ☐ cm. Height is: ☐ estimated/team ☐ reported (source: _____) ☐ actualManner identified by: ☐ hospital ID band ☐ toe tag ☐ other (describe) _____**Identification Band/Tag**

ID re-created as closely as possible,

or check here ☐ if N/A (not present)

Personnel confirming donor identification: _____ Date/time: _____

Evidence of Donation/AutopsyEye donor: ☐ whole eyes ☐ corneas only ☐ N/A ; Organ donor: ☐ Yes ☐ No UNOS#: _____Autopsy: tissue recovery is ☐ pre or ☐ post autopsy (☐ full ☐ limited); ☐ no autopsy planned;
or, ☐ plan for autopsy unknown**Recovery Team Assessment:**

Is there evidence of:

Jaundice _____ ☐ Yes _____ ☐ NoGenital lesions _____ ☐ Yes _____ ☐ NoEnlarged lymph nodes _____ ☐ Yes _____ ☐ NoTattoo/piercing _____ ☐ Yes _____ ☐ NoWhite spots in the mouth _____ ☐ Yes _____ ☐ No _____ ☐ Unable to visualizeNon-medical injection sites _____ ☐ Yes _____ ☐ NoEnlarged liver (hepatomegaly) _____ ☐ Yes _____ ☐ NoInsertion trauma/perianal lesions _____ ☐ Yes _____ ☐ NoRash/scab/skin lesion (non-genital) _____ ☐ Yes _____ ☐ NoBlue/purple (gray/black) spots/lesions _____ ☐ Yes _____ ☐ NoTrauma/infection to potential retrieval sites _____ ☐ Yes _____ ☐ NoAbnormal ocular finding (e.g., icterus, scarring) _____ ☐ Yes _____ ☐ No _____ ☐ Unable to visualize

Notes/Explain if "unable to visualize", or if any answers are "Yes": _____

General AppearanceCleanliness: ☐ Good ☐ Poor; Describe if "poor" _____

Personnel performing physical assessment: _____ Date/time: _____

Name of Person Completing Form (Print) _____

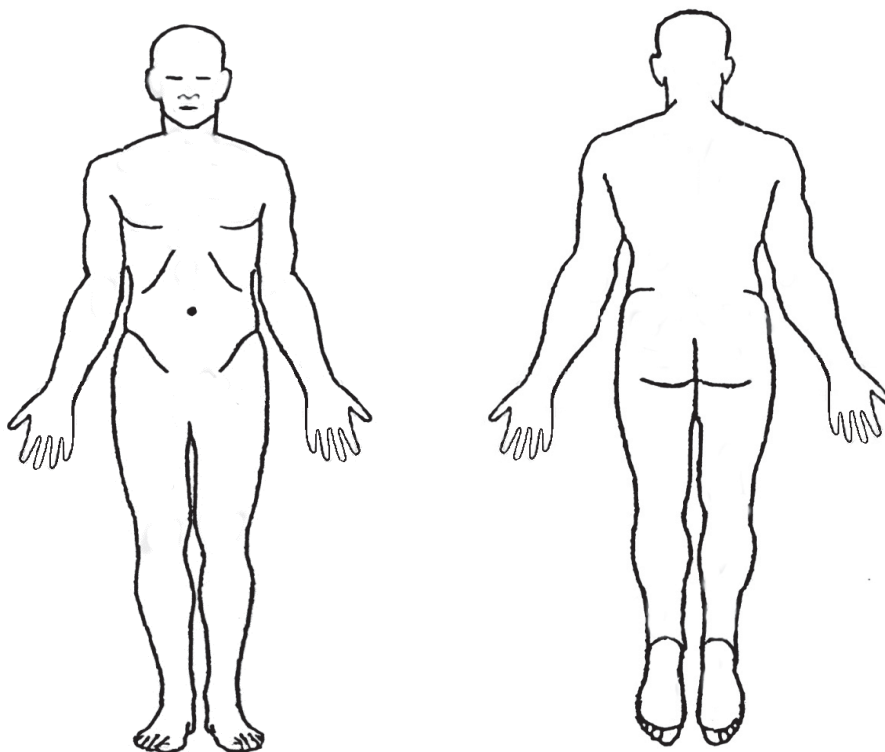
Signature _____

Initials _____

Date _____

Sample Tissue Donor Physical Assessment Form

Recovery Agency ID#: _____

Recovery Team Assessment: (continued)**Key to schematics:**

(A) Abrasion	(J) Team blood draw site	(T) Tattoo – requires description
(B) Bruise/Contusion	(L) Laceration/Wound	(U) Urethral catheter
(C) Cast/Ortho device	(M) ID band/tag	(V) Skin lesion
(D) Dressing/Bandage	(N) Needle entry site	(W) Scab
(E) ET tube/NG tube	(O) Organ recovery incision	() _____
(F) Fracture/Dislocation	(P) Body Piercing – requires description	() _____
(H) Hematoma	(R) Rash	
(I) IV/Arterial line	(S) Scar (surgical/trauma)	

Summary

A review of available medical records & physical assessment findings were completed & found to be acceptable/not acceptable prior to recovery. _____

(Circle one)

(Responsible person)

(Date/time)

Source: American Association of Tissue Banks (AATB), United States.

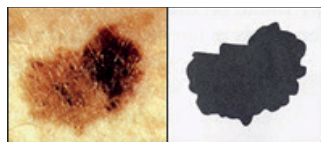
Appendix 14. Practical guidance for the evaluation of pigmented skin lesions and differential diagnosis of melanoma

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 4).

The 'ABCDE rule' is an easy guide to detecting usual signs that may be indicative of melanoma [1-2].

A. Asymmetry

If one half is not identical to the other half, suspect melanoma.



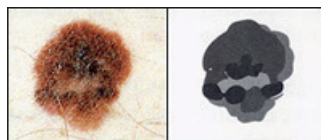
B. Border irregularity

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 more or less regular aspect but among them 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.dermatology.ucsf.edu/skincancer/general/types/melanoma.aspx and www.skincancer.org/skin-cancer-information/melanoma.

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. 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* 35(3):130-151, 1985.
2. Whited JD, Grichnik JM: The rational clinical examination. Does this patient have a mole or a melanoma? *JAMA* 279(9):696-701, 1998.
3. Gachon J *et al.*: First prospective study of the recognition process of melanoma in dermatological practice. *Arch Dermatol* 141(4):434-438, 2005.

Appendix 15. **Sample haemodilution algorithm**

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) x 70 mL/kg

PV = donor's plasma volume

Calculated plasma volume = donor's weight (kg) / 0.025 OR
donor's weight (kg) x 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

Source: Appendix 2 at www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm091345.pdf, accessed 12 January 2017.

Appendix 16. **Validation of screening for infectious disease assays for use with blood from deceased donors**

The reliability of the results of screening for infectious diseases on 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 to screen blood donors are considered suitable to use for 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 most important because a false-negative result is the major threat in regard to the safety of the tissue or cell transplant. In general, specificity is not as important because algorithms can be employed to effectively discriminate 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 5.3.1.1);
- 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. sample storage methods (i.e. 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 (i.e. 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.

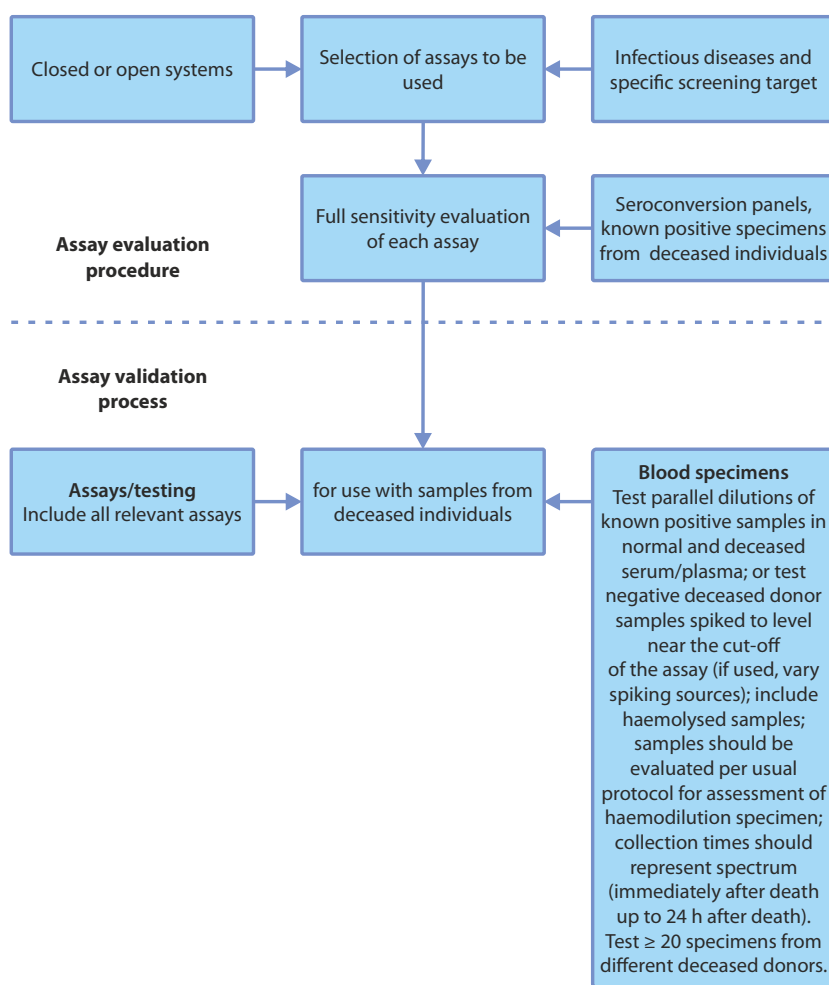
Validation of assays for use with blood from deceased individuals is critical, both for serological assays that detect antigens or antibodies and for molecular assays that detect viral nucleic acid. However, simultaneously, this is problematic due to key issues stated above, and a lack of suitable blood 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 Dr 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 eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:327:0024:0025:en:PDF, accessed 13 January 2017.

Appendix 17. **Sample form to assess the suitability of the working environment**

(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 18. **Sample donor identification form**

(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 (NHS), United Kingdom.

Appendix 19. Checklist for internal (or external) 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, 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	

7. Information security	Comments
☐ Is access to the system (and login) associated with a certificate of authorisation? ☐ Who decides on, and keeps records of, access to the system? ☐ Are records for access to the system updated (i.e. access removed when not needed any more)?	
☐ 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?	

Source: 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 20. **Serious adverse reaction or event: impact assessment tool**

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.

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 →					
	1 Rare	2 Unlikely	3 Possible	4 Likely	5 Certain/ almost certain
Impact of recurrence ↓					
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: SOHO V&S Guidance for Competent Authorities.

Appendix 21. **European report of serious adverse reactions and events associated with the clinical application of tissues and cells**

Extracts from the 2015 report covering EU and EEA member states (data for 2014)*

Serious adverse reactions

A total of 190 serious adverse reactions (SAR) were reported for 2014, of which 109 were related to non-reproductive tissues and cells and 81 to reproductive tissues and cells.

There were 12 and 10 countries that reported SAR for non-reproductive and reproductive tissues and cells, respectively. According to the data, when compared with the total amount of tissues and cells distributed, SAR occurred in only 0.16 % of cases. The data should be interpreted with caution because a few countries could not provide data for SAR denominators. In some cases, it was acknowledged that data submitted were only partial because of difficulties in collection of information from some end users (hospitals/clinics).

In addition, 14 countries reported that no SAR related to the human application of human tissues and cells was recorded in their country in 2014.

Of the 190 SAR reported:

- 109 SAR (57 %) were related to the transplantation of non-reproductive tissues and cells;
 - 42 of these SAR were related to haematopoietic progenitor cell (HPC) transplants (including bone marrow 8; blood peripheral stem cells 30; cord blood 4);
 - 67 of these SAR were related to transplantation of replacement tissues (general musculo-skeletal tissue 1, bone 8, cartilage 4, ocular tissues 49, amniotic membranes 1, heart valves 2, other tissues 2);
- 81 SAR (43 % of all reported SAR) were related to the human application of reproductive cells and tissues (sperm, oocytes, embryos).

No SAR were reported for a few types of tissues and cells, including skin, hepatocytes, pancreatic islets and reproductive tissues (e.g. testicular tissue, and other reproductive tissues).

For the clinical application of reproductive cells, most of the reported SAR were genetic diseases for which the transmission from the gamete donors was considered at least 'possible'.

As in previous years, serious adverse reactions in donors were also included in the annual report. In 2014, 19 countries reported 620 SAR occurring in donors. Recognising the importance of all donor adverse reactions, including those not influencing the quality and safety of tissues and cells which are

* The summary report compiled from information submitted by member states and EEA countries is available at http://ec.europa.eu/health/sites/health/files/blood_tissues_organs/docs/2015_sare_summary_en.pdf.

reportable under the pharmacovigilance systems, the Commission continues to collect such data on a voluntary basis in agreement with the Tissues and Cells Competent Authorities. These figures were, however, calculated separately, and are not included under the total number of SAR.

Serious adverse events

Data came from 16 reporting countries regarding the number of tissues and cells processed in 2014. For the purpose of this reporting, the term ‘tissues and cells processed’ refers to tissues and cells processed in the tissue establishments, but not necessarily distributed to end users. Overall, 1 433 828 units of tissues and cells were reported to have been processed in 2014.

Serious adverse events (SAE) were recorded by 19 reporting countries. The total number of SAE reported for 2014 was 551, most of which occurred during the processing, procurement and storage stages and were attributed mainly to human error.

Of the 551 reported SAE:

- 129 SAE (23 %) were linked to ‘procurement’;
- 46 SAE (8 %) were linked to ‘testing’;
- 20 SAE (4 %) were linked to ‘transport’;
- 134 SAE (24 %) were related to ‘processing’;
- 78 SAE (14 %) were linked to ‘storage’;
- 42 SAE (8 %) were linked to ‘distribution’;
- 16 SAE (3 %) were linked to ‘materials’;
- 86 SAE (16 %) were included in the category ‘Other SAE’.

The 551 SAE were attributed to one of four specifications:

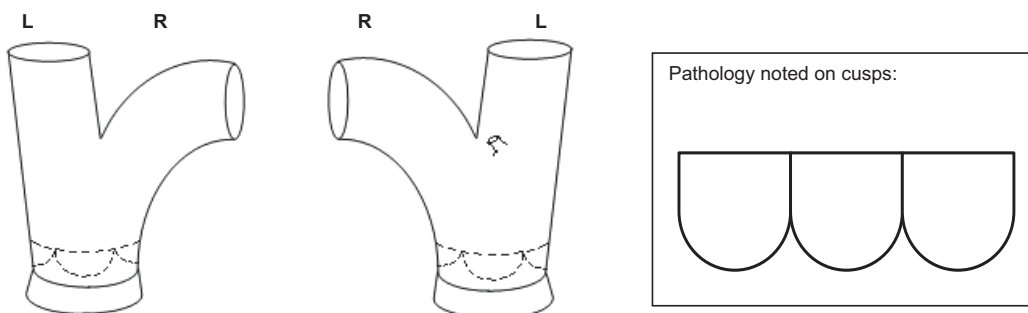
- Tissue and cell defects: 79 SAE (14 %);
- Human error: 263 SAEs (48 %);
- Equipment failure: 81 SAE (15 %);
- Other: 128 SAEs (23 %).

Overall, the completeness and accuracy of the EU reports improved over time as reporting countries developed their systems for SARE data collection in the tissue and cell sector.

Appendix 22. **Sample form 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



Condition: Excellent / Good / Fair

Key (please annotate on the diagram above)
Atheroma = ...
Fenestration = 000
Fibrosis = XXXX
Other:

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:
Syphilis:	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

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 on 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:	

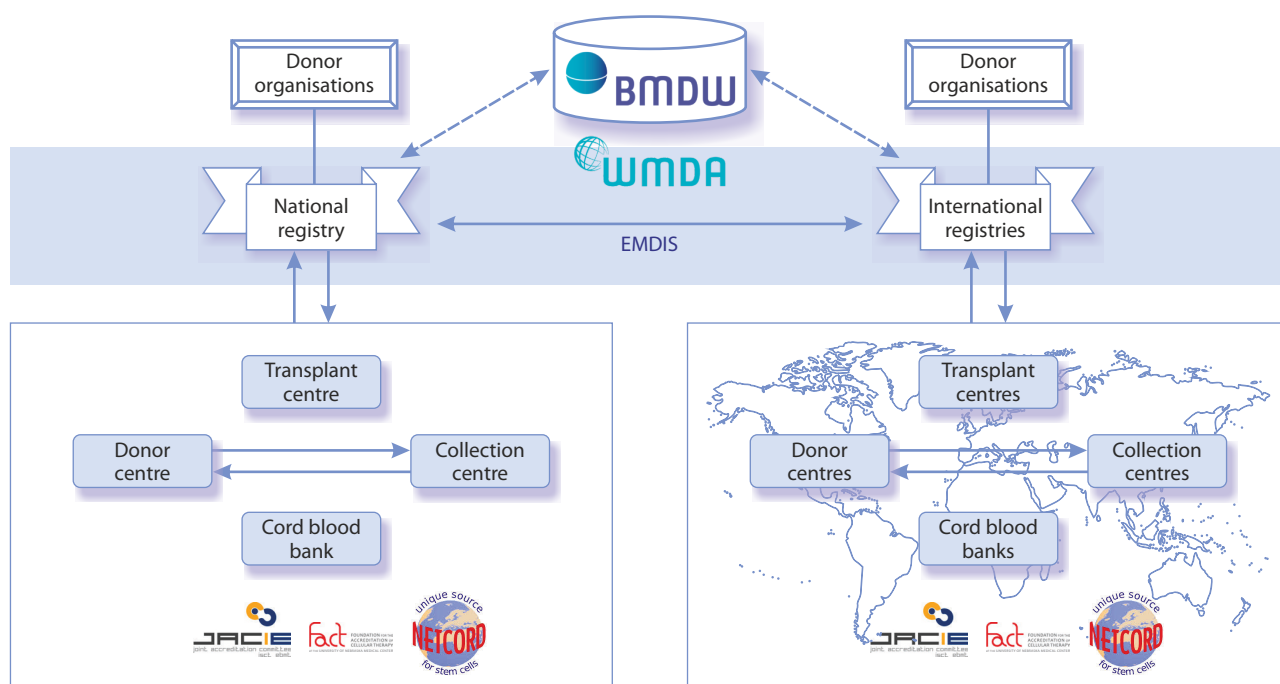
SEROLOGY / BACTERIOLOGY STATUS		
Hepatitis B:	Hepatitis B Core Antibody:	Hepatitis C:
HIV I and II:	HIV PCR:	HCV PCR:
HBC PCR:	Syphilis:	HTLV:
Mycobacteria:	Microbiology Culture:	
ABO Blood group (if known):	Other:	

DONOR INFORMATION	
Age	Sex:
Date of Death:	Cause of Death

Information Entered By:	Date	Signature

Source: National Health Service (NHS), United Kingdom.

Appendix 23. Donor search through registries for haematopoietic progenitor cells transplantation



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Human tissues and cells can save lives or restore essential functions, but their use also raises questions of safety and quality since 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 diseases. In addition, 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 the donation and human application of human tissues and cells in compliance with the principles of non-commercialisation and voluntary donation of materials of human origin.

This 3rd 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 identifying potential donors, transplant co-ordinators managing the process of donation after death, bone marrow and cord blood collection centres, fertility clinics, tissue establishments processing and storing tissues and cells, testing laboratories, organisations responsible for human application, inspectors auditing any of these establishments and Health Authorities responsible for tissues and cells for human application.

For matters dealing with the use of organs and blood components, 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.

ENG

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