



ACCREDITATION MANUAL – Standards for Skin

(Chapter H)

EUROPEAN GROUP FOR ACCREDITATION AND
LIAISON OF BLOOD-TISSUES AND CELLS ESTABLISHMENTS



Co-funded by
the Health Programme
of the European Union

This document is part of the project “101056852/EGALITE” funded by the European Union (EU4Health programme 2021-2027). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority (European Health and Digital Executive Agency (HaDEA)) can be held responsible for them.

Contents

INTRODUCTION	1
ABBREVIATIONS	4
DEFINITIONS	6
INDEX/STANDARDS STRUCTURE	8
PART H.1.: SKIN	9
Donor Evaluation	9
Collection	10
Processing, preservation and storage	13
Quality Control	16
Distribution	18
BIBLIOGRAPHY	19

INTRODUCTION

EGALiTE is a voluntary accreditation programme endorsed by the leading European Scientific Societies [European Eye Bank Association (EEBA), European Association of Tissue and Cell Banks (EATCB), and European Society for Blood and Marrow Transplantation (EBMT), and European Blood Alliance (EBA)].

The EGALiTE Standards are designed to provide guidelines for organisations, programmes, facilities, and individuals involved in activities pertaining to Substances of Human Origin (SoHO) - or providing support services for such activities – and who wish to apply for the European/EGALiTE Accreditation Program.

These Standards are designed by experts in the SoHO field for facilities wishing to demonstrate their commitment to quality medical care and best laboratory practice through an EGALiTE accreditation.

EGALiTE Standards are intended to apply to all actions performed by SoHO Entities in the preparation of SoHO, which include donor recruitment, selection, evaluation, testing, and SoHO collection, sampling, quality control, processing, preservation, storage, release and distribution for clinical application. The standards applicable to hematopoietic progenitor cells (HPC) activities are defined by FACT-JACIE (Foundation for the Accreditation of Cellular Therapy and Joint Accreditation Committee of the International Society for Cell and Gene Therapy and the European Society for Blood and Marrow Transplantation (EBMT))^{*} and the World Marrow Donor Association (WMDA)[†], and are not included in this guide.

The EGALiTE Standards were defined taking into account on the good practices defined by:

- European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM) Guide to the quality and safety of tissues and cells for human application¹;
- EDQM Guide to the preparation, use and quality assurance of Blood Components²
- FACT-JACIE³;
- WMDA Standards⁴
- EuroGTP I and EuroGTP II⁵
- And other guidelines, namely related with the Good Manufacturing Practices (GMP)⁶ and quality management.

^{*} The scope of the FACT-JACIE Standards includes:

- HPCs defined as self-renewing and/or multi-potent stem cells capable of maturation into any of the hematopoietic lineages, lineage-restricted pluripotent progenitor cells, and committed progenitor cells, regardless of tissue source (bone marrow, umbilical cord blood, peripheral blood, or other tissue source).
- Nucleated cells or mononuclear cells from any hematopoietic tissue source (marrow, peripheral blood, umbilical cord, and placental blood) collected for therapeutic use other than as HPCs.
- Immune effector cells (IECs), defined as cells, in vitro modified or not, that have differentiated into a form capable of modulating or effecting a specific immune response.
- Genetically modified cells, defined as cells that have been modified by replacing a disease-causing gene with a health copy of the gene, inactivating a disease-causing gene that is not functioning properly, or introducing a new or modified gene into the body to help treat a disease.
- Clinical administration of the cellular therapy products derived from umbilical cord.

[†] WMDA standards cover all aspects of blood stem cell registry operations

These Standards are not intended to establish absolute best practice or include all procedures and practices that an organisation, programme, facility, or individual should implement if the standard of practice in the community or applicable governmental laws or regulations have determined additional requirements. Each organisation, programme, facility, and individual should analyse its practices and procedures to ascertain whether additional standards apply. In addition, compliance with these Standards is not an exclusive means of complying with the required standards of care as dictated by local, national, or international laws or regulations.

Compliance with the Standards does not guarantee compliance with Applicable Law. Governmental regulations must also be followed. It is the responsibility of the individual facility to determine which laws and regulations are applicable. In some cases, regulations of governmental authorities outside of the jurisdiction of the Donation Centre may apply; for example, when a facility is sending or receiving SoHO from outside of its immediate jurisdiction.

Compliance with other organisations' standards or governmental regulations does not ensure that the Standards have been met. Governmental regulations supersede any organisation's standards if those regulations set a higher standard or are inconsistent with a specific standard. However, if a standard is more rigorous than a governmental regulation, that standard must be followed.

Disclaimer

EGALiTE expressly disclaims any responsibility for setting maximum standards and additionally expressly disclaims any responsibility, liability, or duty to member programmes, directors, staff, or programme donors or patients for any such liability arising out of injury or loss to any person by the failure of member programmes, directors, or staff to adhere to the Standards or related guidance.

While the EGALiTE Accreditation Programme encourages the sharing of best practices and recommendations, it does not assume the role of a regulatory body or authority. The EGALiTE Accreditation Programme involves a peer review process designed to assess and evaluate the compliance of SoHO organisations with standards and criteria established in the present document, where experts in the field conduct assessments based on predetermined criteria. The peer review process is conducted with the utmost independence and impartiality to ensure a fair and unbiased evaluation, and the compliance with the best practices.

The EGALiTE Accreditation Programme is not responsible for informing National Competent Authorities of any non-compliance identified during the peer review process, including major non-compliances.

Participants voluntarily engage in this process to demonstrate their commitment to quality and best practices, and organisations participate in the EGALiTE Accreditation Programme voluntarily, acknowledging that the outcomes of the process are not intended for regulatory purposes.

ABBREVIATIONS[‡]

ADM	Acellular dermal matrix (ADM)
AM	Amniotic Membrane
aW	available water
CAPA	Corrective and Preventive Actions
CFU	Colony-forming units
CMV	Cytomegalovirus
CV	Cardiovascular
DALK	Deep anterior lamellar keratoplasty
DCD	Deceased donors after circulatory death
DMEK	Descemet membrane endothelial keratoplasty
DMSO	Dimethyl sulphoxide
DSAEK	Descemet stripping automated endothelial keratoplasty
EATCB	European Association of Tissue and Cell Banks
EBA	European Blood Alliance
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein–Barr virus
ECDC	European Centre for Disease Prevention and Control
EDQM Blood	Guide to the preparation, use and quality assurance of Blood Components (20 th Edition)
EDQM Guides	<i>Guide to the quality and safety of tissues and cells for human application</i> and <i>Guide to the preparation, use and quality assurance of Blood Components</i>
EDQM Tissue and Cells	Guide to the quality and safety of tissues and cells for human application (5 th Edition)
EDQM	European Directorate for the Quality of Medicines and Healthcare
EEBA	European Eye Bank Association
EK	Endothelial keratoplasty
EM	Electron microscopy
EU	European Union
EuroGTP II	Good Practices for demonstrating safety and quality through recipient follow-up - Good Practices for evaluating quality, safety and efficacy of novel tissue and cellular therapies and products (EU Project 709567)
FMEA	Failure mode and effects analysis
FMECA	Failure mode, effects and criticality analysis
FMT	faecal microbiota transplantation
FTSG	Full-thickness skin graft

[‡] For practical reasons, the present list includes the abbreviations and acronyms utilized in other chapters of the EGALiTE Standards.

GAPP JA	Facilitating the Authorisation of Preparation Process for blood, tissues and cells, Joint Action
GMP	Good Manufacturing Practice
HACCP	Hazard analysis and critical control points
HM	Human Milk
HMB	human milk bank
HoP	Holder pasteurisation
HV	Heart valve
IDM	infectious disease marker
JACIE	Joint Accreditation Committee of the ISCT (International Society for Cellular Therapy) and the EBMT
MiRCA	Microbiological Risk of Contamination Assessment tool for tissues and cells
MSK	Musculoskeletal Tissues
ORHA	Organisations for Human Application
PK	Penetrating keratoplasty
PPA	Preparation Process Authorisation
PQR	Product Quality Review
PRF	Platelet-Rich fibrin
PRP	Platelet-Rich Plasma
QM	Quality Manager
rCDI	<i>Clostridioides</i> (formerly <i>Clostridium</i>) <i>difficile</i> infection
RP	Responsible Person
SARE	Serious Adverse Reaction and Event
SED	Serum Eye Drops
SMF	Site Master File
SoHO	Substances of Human Origin
SOP	Standard Operating Procedure
TED	Tissue Establishment Dossier
TGOT	Technical Guidelines for Ocular Tissue
TNC	Total nucleated cell
UCB	Umbilical Cord Blood
URS	User-requirement specifications
VMP	Validation Master Plan
WMDA	World Marrow Donor Association

DEFINITIONS[§]

Audit	means a systematic and independent examination to determine whether activities and the related results of such activities comply with legislation and planned arrangements and whether such arrangements are applied effectively and are suitable to achieve the objectives ⁷
Collection	means a process by which SoHO are obtained from a person, including any preparatory steps, such as hormone treatment, needed to facilitate the process at, or under the supervision of, a SoHO entity ⁷
Donation Centre	A SoHO Entity that carries out one or more of the activities: SoHO donor history review and eligibility assessment; SoHO testing of donors for eligibility or matching purposes; collection of SoHO from donors or patients.
Donor recruitment	means any activity aimed at informing persons about activities related to SoHO donation or at encouraging them to donate SoHO ⁷
Must (or shall)	Indicates an absolute requirement. Compliance with the statement is mandatory, and failure to meet this requirement is considered a violation of the standard.
Processing	All operations involved in the preparation, manipulation, preservation, storage and packaging of SoHO intended for human application. ¹ means any operation involved in the handling of SoHO, including, but not limited to, washing, shaping, separation, decontamination, sterilisation, preservation and packaging, except for the preparatory handling of SoHO for immediate human application during a surgical intervention, without the SoHO being removed from the surgical field before they are applied ⁷ .
Qualification	According to European Union Good Manufacturing Practice (EU GMP) ^{6,8} , the action of proving that any equipment works correctly and actually leads to the expected results. More generally, qualification is applied to the inputs to a process, i.e. equipment, facilities, materials and software (and their suppliers), as well as to operators and the relevant written procedures ¹
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 ¹
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 ¹ .

^{§ §} For practical reasons, the present list includes the definitions referred in other chapters of the EGALiTE Standards.

Should	Indicates a recommendation. While it is not mandatory, adherence to this statement is strongly advised to ensure the best outcomes. Non-compliance is not a violation of the standard but may affect the overall effectiveness or quality of the implementation.
SoHO Entity	Means an organisation legally established in the Union that carries out one or more of the SoHO activities that have a direct impact on the quality, safety or effectiveness of SoHO, as follows: (i) SoHO donor registration; (ii) SoHO donor history review and medical examination; (iii) testing of SoHO donors or of persons from whom SoHO are collected; (iv) collection; (v) processing; (vi) quality control; (vii) storage; (viii) release; (ix) distribution; (x) import; (xi) export; (xii) human application; (xiii) clinical-outcome registration. ⁷
SoHO establishment	means a SoHO entity that carries out any of the following SoHO activities: (a) both processing and storage; (b) release; (c) import; (d) export. ⁷
SoHO preparation	means a type of SoHO that: (a) has been subjected to processing and, where relevant, one or more other SoHO activities referred to in Article 2(1), point (c) of the EU SoHO Regulation; (b) has a specific clinical indication; and (c) is intended for human application to a SoHO recipient or is intended for distribution. ⁷
SoHO Regulation	Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024, on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC.
Substance of Human Origin (SoHO)	means any substance collected from the human body, whether it contains cells or not and whether those cells are living or not, including SoHO preparations resulting from the processing of such substance ⁷
Validation	Documented evidence giving a high degree of assurance that a specific process or system, including pieces of equipment or the environmental conditions, will perform consistently to deliver a product meeting its pre-determined specifications and quality attributes, based on intended use (EU Directive 2006/86/EC⁹)¹. or Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (ISO9000:2015¹⁰)¹
Verification	Preferred term for the validation or qualification of IT systems/software. Or, Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled (ISO9000:2015 ¹⁰) ¹

INDEX/STANDARDS STRUCTURE

GENERIC STANDARDS FOR SoHO ACTIVITIES:	A. DONOR SELECTION & DONATION:	A.1. LIVING DONATION	F. OCULAR TISSUES
		A.2. DECEASED DONATION	G. AMNIOTIC MEMBRANE
	B. COLLECTION		H. SKIN
			I. CARDIOVASCULAR
	C. PROCESSING, STORAGE AND RELEASE OF SoHO		J. MUSCULOSKELETAL
			K. UMBILICAL CORD BLOOD
	D. DISTRIBUTION		L. ADIPOSE TISSUE
			M. HUMAN MILK
	E. REGISTRIES AND VIGILANCE		N. BLOOD COMPONENTS FOR TOPICAL USE OR INJECTION
			O. INTESTINAL MICROBIOTA

PART H.1.: SKIN

Donor Evaluation

STANDARD:

H.1.001 Skin must be visually checked for mechanical damage, the results must be recorded and taken into account.

Evidence:

Criteria should be detailed in an Standard Operating Procedure (SOP).

Example(s):

Open wounds, multiple (> 100) or dysplastic naevi, dermatitis, local infections, scars and ectoparasites are examples of aspects which should be checked and recorded.

STANDARD:

H.1.002 The following conditions contraindicate skin donation:

- a. autoimmune diseases and systemic connective tissue diseases affecting skin;*
- b. diseases affecting the dermis (e.g. dermal mucinosis, nephrogenic fibrosing dermopathy, porphyria, lupus erythematosus);*
- c. toxicity of the skin as a result of the presence of toxic agents or poisons;*
- d. systemic use of corticosteroids or Cushing disease, inducing severe skin atrophy*

Evidence:

Criteria should be detailed in an SOP.

STANDARD:

H.1.003 The following relative contraindications for skin donation must be considered case by case, and eventually require risk assessment:

- a. extensive lacerations, haematoma or scars;*
- b. skin diseases with extensive involvement (e.g. psoriasis, eczema);*
- c. relevant skin ulcers, pressure ulcers, stoma, pyoderma or mycoses;*
- d. skin disorders interfering with procurement or aesthetically not acceptable for patients (e.g. extensive tattoos, jaundice);*
- e. diabetes mellitus with skin complications (e.g. ulcers, amputation, neuropathy);*
- f. pre-malignant conditions such as actinic keratoses and Bowen's disease;*
- g. mechanical, thermal or microbial damage where skin is to be procured;*
- h. extreme peripheral oedema, high body mass index (BMI) (≥ 40) or poor nutritional status affecting procurement or body reconstruction.*

Evidence:

Criteria should be detailed in an SOP.

STANDARD:

H.1.004 Other criteria (e.g. age and weight limits), are determined by the medical responsible for SoHO Establishment activities, according to characteristics and quality of tissues.

Explanation:

The common practice is not to procure skin from donors aged < 15 years but many SoHO Establishments do not indicate any.

Some SoHO Establishments define a minimum body weight criterion for procuring skin.

Collection

STANDARD:

*H.1.005 Skin must be procured within a period of 12 h, should the body not be refrigerated, or up to 24h after death if the body has been cooled or refrigerated within 6 h of death.
It may be permissible to extend procurement times to >24h after death if skin processing has been validated to guarantee quality and microbiological safety; in these cases, the blood samples for serological testing should be taken just prior to death or within 24 h after death (to avoid extensive haemolysis)*

Explanation:

Refrigeration of the deceased donors after circulatory death (DCD) before procurement will reduce skin contamination and facilitate skin procurement due to the harder consistency of the subcutaneous tissue.

STANDARD:

H.1.006 In case of living donation, potential donors are evaluated similarly to deceased donors to determine donor suitability according to standard exclusion criteria for skin donation and absence of adverse physical, psychological or emotional outcome before, during or following the donation.

Explanation:

Skin can also be obtained from living donors if there is a shortage of post mortem donors, from patients having abdominoplasty or mammoplasty procedures who consent to tissue donation.

STANDARD:

H.1.007 In case of living donation, the procurement area is prepared by depilation and disinfection and the tissue is processed to obtain full-thickness skin grafts.

Evidence:

Technique defined in an SOP.

STANDARD:

H.1.008 Skin-procurement teams should consist of at least two people operating under aseptic conditions and appropriately clothed for the type of procurement

Evidence:

Details shall be defined in an SOP

STANDARD:

*H.1.009 In the case of multi-tissue procurements, 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.
Procurement of skin before ocular tissue is recommended to avoid eye bleeding from the sockets, if the donor has to be placed in a prone position following enucleation of the eyes*

Explanation:

In the case of multi-tissue procurements, the order in which the tissues are removed should be standardised and predefined and, in the case of multiple procurement teams, should be agreed between the teams beforehand so that risks of cross- contamination between tissues are minimised

Studies show that, whether the skin procurement is done before or after bone procurement, the contamination rate of skin is not different if the procurement process is controlled and standardised.

Evidence:

Technique defined in the SOP.

STANDARD:

H.1.010 Skin is procured under aseptic conditions after shaving of the donor areas and appropriate pre-operative scrubbing and disinfection of the donor skin.

Explanation:

This aims to remove the transient, and reduce the resident, microbial flora.

The procedure should aim to reduce the bioburden, which can significantly decrease the microbial positivity rate of processed skin samples. Therefore, suitable disinfectants, such as povidone- iodine or chlorhexidine, should be chosen. Their concentrations and the durations of exposure should also be evaluated and validated. Thorough cleaning of the skin with a surgical detergent prior to application of disinfectants will enhance their efficacy.

Evidence:

An effective and validated procedure for skin disinfection should be established by the SoHO Establishment and applied to all procurement sites.

STANDARD:

H.1.011 A local sterile field using sterile drapes must be used prior to procurement to effectively prevent microbial contamination.

Evidence:

Technique defined in an SOP.

STANDARD:

H.1.012 Skin grafts can be procured by manually, electrically, compressed-air or battery-operated dermatomes from areas of the body that are typically not exposed, particularly from the posterior trunk and the lower limbs.

Explanation:

Grafts should be obtained as homogeneously as possible.

Evidence:

Technique defined in an SOP.

STANDARD:

H.1.013 Full-thickness skin grafts (FTSG) can be procured using a scalpel instead of a dermatome.

Evidence:

Technique defined in an SOP.

STANDARD:

H.1.014 Staged separate procurement of particular body areas, with placement of procured material from each area into separate containers, is preferred in order to reduce eventual cross-contamination of the procured tissue

Evidence:

Technique defined in an SOP.

STANDARD:

H.1.015 For aesthetic reasons and with a view to a respectful reconstruction of the donor, it is not acceptable to take skin from the neck, face and other typically exposed areas of the body that might be visible when people pay their last respects to the donor, unless specific authorisations are obtained

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.016 Conditions which may affect body reconstruction after procurement such as extreme oedema or a high BMI (≥ 40) should be evaluated prior to procurement; body reconstruction should ensure that loss of fluid that accompanied skin procurement is not noticeable.

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.017 Appropriate dressing of the donation areas should be used to prevent leaking and oozing.

Evidence:

Criteria defined in an SOP.

Example(s):

This could include the use of sealing agents, padding and/or appropriate garments.

STANDARD:

H.1.018 Immediately after procurement, skin samples for initial microbiological control should be obtained and the recovered tissue must be stored in sterile, pre-labelled containers filled with a suitable refrigerated transport medium (e.g. PBS at pH 7.2-7.3).

Explanation:

If samples are not taken immediately after procurement, or before the start of processing, the testing process should be validated to demonstrate that post-procurement transport/storage does not impact its accuracy.

Evidence:

Technique defined in an SOP.

STANDARD:

*H.1.019 The containers must be sealed securely, refrigerated to 2-8 °C and transported to the processing facility or SoHO Establishment.
If skin grafts are to be glycerolised (i.e. they are non-viable grafts), refrigerated transportation may not be required and the recovered skin can be stored and transported at room temperature in 50 % glycerol solution.*

Explanation:

Transportation at low temperatures prevents proliferation of most bacteria and fungi, and maintains skin viability (if viable grafts are required). Antibiotics (whose stability at the transport temperature should be defined) can be added to the transport medium, taking into account that the optimal operating temperature of most antibiotics is much closer to 37 °C than to 2-8 °C and, at refrigerated temperature, antibiotic cocktails can fail to decontaminate skin grafts.

Evidence:

Technique defined in an SOP.

The incubation temperature and the composition of decontamination cocktails should be defined, after validation, in written procedures by each SoHO Establishment, considering the initial tissue microbial contamination.

STANDARD:

H.1.020 After procurement, skin grafts should be transferred to the processing SoHO Establishment as soon as possible; immediately in the case of cryopreserved skin grafts, and no longer than 72 hours after procurement for other types of graft.

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.021 Before processing, the recovered skin should be kept in a temperature-controlled refrigerator at 2-8 °C, without interruption throughout the refrigeration process.

Explanation:

It is recommended that the cell nutrient medium used for viable grafts be changed shortly after receipt of skin grafts, or that the medium be validated for the storage time (i.e. adequate buffering capacity).

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.022 All the manipulations where the transport containers are going to be exposed and the media changed must be performed in a controlled-air-environment (safety cabinet in a clean room of at least Grade D), or otherwise justified through a risk assessment exercise.

Evidence:

Criteria defined in an SOP and risk assessment (when applicable) available for evaluation.

STANDARD:

H.1.023 The procurement report must contain a description and identification of the procured skin (including samples for testing) as well as any incident during procurement.

Evidence:

Requirements defined in an SOP.

Processing, preservation and storage

STANDARD:

H.1.024 Skin grafts destined for cryopreservation should be processed as soon as possible after procurement in order to maintain cell viability and structural integrity.

Evidence:

Criteria defined in an SOP.

Example(s):**STANDARD:**

H.1.025 The dimensions and area of the final graft must be recorded and displayed on the label.

Explanation:

The final graft sizes are measured with a ruler or calipers.

Evidence:

Technique defined in an SOP.

Example(s):**STANDARD:**

H.1.026 When skin tests positive for microbiological contaminants, it can be sterilised by gamma or electron beam irradiation.

Explanation:

Sterilisation methods should be validated for the initial estimated level of bioburden prior to application of the sterilisation method to skin allografts

Sterilisation can be used to 'rescue' skin that has been initially processed as cryopreserved, viable skin but is not considered suitable for transplantation due to microbial contamination. Sterilisation with irradiation

will destroy viable donor cells, but if performed appropriately does not compromise the structure and mechanical properties of the graft, meaning it can serve as a biological dressing.

Example(s):

Research has shown that a maximum dose of 25 kGy irradiation of deep-frozen skin in radio-protective solutions sterilises tissue without relevant histomorphological or physical alterations (such as pliability) compared with normal cryopreserved skin.

STANDARD:

H.1.027 If there are positive microbiology results from cryopreserved skin, the skin can be processed in 85 % glycerol as a recovery procedure

Explanation:

Skin-cell viability, referred to as the mean percentage of cell viability after 10-20 days of storage, is reported to be between 40 and 50 % that of the fresh skin.

When cell viability is required for clinical use, it must be validated and can be assessed by various methods, including vital dye staining, oxygen consumption, and enzymatic and metabolic assays

STANDARD:

H.1.028 For processing Cryopreserved and deep-frozen skin allografts, a controlled-rate freezing procedure is recommended to preserve cell viability.

Explanation:

Skin-cell viability, referred to as the mean percentage of cell viability after 10-20 days of storage, is reported to be between 40 and 50 % that of the fresh skin.

When cell viability is required for clinical use, it must be validated and can be assessed by various methods, including vital dye staining, oxygen consumption, and enzymatic and metabolic assays

STANDARD:

H.1.029 After cryopreservation, skin allografts must be stored at a temperature of < -140 °C in liquid nitrogen tanks (vapour or liquid phase) or ultralow temperature mechanical freezers.

Explanation:

Storage of grafts immersed in liquid nitrogen is not recommended. If stored below -140 °C, a storage period of up to 5 years is permissible; longer storage periods should be supported by validation studies. Storage at higher temperature (-60 °C to -80 °C) is a method applied for medium-term (maximum of 2 years) preservation of viable skin allografts. Inappropriate storage compromises the potential to restore normal metabolic activity and, thus, physiological functioning after transplantation.

Evidence:

Requirements defined in an SOP.

Example(s):

STANDARD:

H.1.030 For the processing and storage of Lyophilised skin allografts; A maximum limit for residual water content should be established and measured (ideally < 5 %), and skin grafts can be stored at ambient temperature for 3-5 years.

Explanation:

Processing of grafts by freeze-drying devitalizes the grafts while maintaining their structure. .

STANDARD:

H.1.031 Acellular dermal matrix (ADM) grafts must comply with the following critical proprieties:

- a. Absence of donor cells (and genetic material).*
- b. Graft thickness ranging 0.2-0.8 mm (thin), 0.8-1.2 mm (medium thickness) and 1.3 mm or higher (thick).*

- c. *Preserved structural integrity.*
- d. *Flexibility/pliability.*
- e. *Resistance to mechanical stress and suturability.*
- f. *No evidence of microbial growth.*

Explanation:

The main biological characteristics of an optimal ADM are biocompatibility (the ability to take after engraftment and the absence of rejection/inflammatory reaction due to induction of pro- inflammatory cytokines), integrity of the matrix (the maintenance of integrity of elastic fibres and collagen physiologically identified in the tissue), sterility (absence of Gram+/- bacteria, fungi and bacterial endotoxins), malleability and suturability (handling; mechanical resistance with or without stitches) and storage options (the ability to be stored by different methods e.g. cryostorage in nitrogen vapours, storage at -80 °C, freeze-drying, dehydration, room- temperature storage, storage in high- percentage glycerol).

STANDARD:

H.1.032 The processing of ADM grafts may include sterilisation techniques to provide a higher level of safety.

Explanation:

There are significant advantages in combining decellularisation and sterilisation processes to ensure a clinically safe ECM, minimising the effect on its ultrastructure.

STANDARD:

H.1.033 Deep-frozen skin allografts comply with the following critical proprieties:

- a. *A minimum of 20 % of residual cell viability should be achieved. The mean percentage after 10-20 days of storage is reportedly 30-51 % (deep-frozen skin grafts) compared to that of the fresh skin, according to different viability assays.*
- b. *No evidence of microbial growth.*
- c. *Preserved structural integrity (normal epidermal/dermal structure).*
- d. *Graft thickness ranging 0.2-0.8 mm.*
- e. *Mechanical resistance.*
- f. *Capable of engraftment.*

STANDARD:

H.1.034 Glycerol-preserved skin allograft comply with the following critical proprieties:

- a. *Graft thickness ranging 0.2-0.8 mm.*
- b. *Plain or meshed.*
- c. *No evidence of microbial growth*

STANDARD:

H.1.035 Cryopreserved skin allografts comply with the following critical proprieties:

- a. *Cell viability: it is maintained by cryopreserved skin grafts; to consider*
- b. *a graft as viable, a minimum of 20 % of residual cell viability should be*
- c. *achieved. The mean percentage after 10-20 days of storage is reportedly*
- d. *20-60 % for cryopreserved skin grafts, compared to that of the fresh skin,*
- e. *according to different viability assays. • No evidence of microbial growth. • Preserved structural integrity (normal epidermal/dermal structure).*
- f. *Graft thickness ranging 0.2-0.8 mm. • Mechanical resistance.*
- g. *Capable of engraftment. • Long-term storage*

STANDARD:

H.1.036 Skin grafts are stored in various conditions depending on processing method:

- a. Glycerolised skin: Refrigerators (2 to 8 °C)*
- b. Lyophilised (freeze-dried) skin/dermis: Room temperature (15 to 25 °C)*
- c. Cryopreserved skin: Vapour phase of liquid nitrogen or mechanical freezer (< -140 °C)*
- d. Deep-frozen skin: Mechanical freezers (-80 to -60 °C)*

Quality Control

STANDARD:

H.1.037 Microbiological testing should be done before the start of processing and on post-processed samples of skin (after rinsing to remove as much disinfection and processing reagent as possible), before the skin is approved for clinical use.

Specimens of a representative sample of finished product should be sent for microbiological testing to check for aerobic and anaerobic bacteria and fungi using appropriate culture media

Explanation:

These approaches are based on the microbiological test methods of *Ph.Eur.* and cover the minimum standards to control the microbiological safety of preparations of human tissues.

Deviations from such standards should be justified and the suitability of the intended alternative test methods must be demonstrated and validated.

Acceptance criteria for microbial load and types of contaminant in processed tissues should be defined in advance and reported in written procedures

Evidence:

Technique defined in an SOP.

Example(s):

(e.g. a predefined number of pieces of skin allografts that have undergone all stages of production)

STANDARD:

H.1.038 Skin allografts may be accepted for clinical use, without sterilisation, when bacteriological and mycological assessment reveal only low bioburdens of inherent inhabitants of the residential skin flora.

Explanation:

Refer to the EDQM Tissue and Cells Guide.

If a positive microbiology result is obtained at the initial stage or at an intermediate stage of processing, a risk-assessment analysis should be conducted to assess the suitability of the skin tissue (and other tissues from the same donor), taking into account the micro-organism(s) detected

For terminally sterilised skin, an analysis should be carried out, taking into consideration the capacity of the sterilisation process as demonstrated through validation

The surgeon must be informed of the skin-graft bioburden before the use of the graft.

Specimens contaminated by (endo)spore-forming micro-organisms such as *Bacillus* (anthracis or cereus) and *Clostridium* spp. or any of the pathogens listed in the EDQM Tissue and Cells Guide, at any stage of the process (even if negative at the end of processing), unless otherwise validated should be discarded without corrective actions in order to remove potentially unsuitable tissue from the transplantation process, unless a terminal sterilisation process is to be applied

Evidence:

SOP describing the algorithm for decisions and risk assessment procedure and bioburden calculation methods shall be made available

STANDARD:

H.1.039 The presence of micro-organisms in finished product samples results in a definite rejection of the donor tissue if no validated sterilisation or decontamination method is applied

Explanation:

The EDQM Tissue and Cells guide provides an Algorithm for acceptance or rejection of skin after bacteriological assessment.

In exceptional cases, skin grafts could be released with low bioburdens of skin commensals “on board”, after appropriate risk assessment and consultation with surgeons (e.g. in urgent cases).

STANDARD:

H.1.040 Unless justified by the result of a risk assessment the following quality controls must be performed in ADM grafts, either in routine or in the context of validation of the process:

- a. Microbiological testing (aerobic and anaerobic bacteria, fungi).*
- b. Graft thickness measurement. Biocompatibility test (cytotoxicity tests).*
- c. Mechanical resistance test.*
- d. Morpho-structure evaluation by histological staining (e.g. orcein, Masson, haematoxylin-eosin) or electron microscopy (EM).*
- e. Decellularisation process by specific histological staining e.g. DAPI, Hoersch*
- f. DNA quantification assay (ng DNA/mg dry tissue). • Residual water test ($\leq 5\%$) – for lyophilised ADM*

STANDARD:

H.1.041 Unless justified by the result of a risk assessment the following quality controls must be performed in Deep-frozen skin allografts:

- a. Microbiological testing (aerobic and anaerobic bacteria, fungi).*
- b. Cell viability assessment (if required, depending on the intended application).*

Example(s):

Different methodologies are used to assess cell viability in skin grafts before and after thawing:

1. quantitative, e.g. tetrazolium salt assay (MTT), neutral red test (NRT), resazurin test, oxygen or lactate consumption assay
2. and /or qualitative, by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

STANDARD:

H.1.042 Unless justified by the result of a risk assessment the following quality controls must be performed in Glycerol-preserved skin allograft

- a. Intact epidermis and upper dermis (normal morphological structure).*
- b. Microbiological testing (aerobic and anaerobic bacteria, fungi).*

STANDARD:

H.1.043 Unless justified by the result of a risk assessment the following quality controls must be performed in Cryopreserved skin allografts

- a. Cell viability assessment (if required, depending on the intended application).*
- b. Microbiological testing (aerobic and anaerobic bacteria, fungi).*

Example(s):

Different methodologies are used to assess cell viability in skin grafts before and after thawing:

1. quantitative, e.g. tetrazolium salt assay (MTT), neutral red test (NRT), resazurin test, oxygen or lactate consumption assay
2. and /or qualitative, by histological staining, e.g. orcein, Masson, haematoxylin-eosin.

Distribution

STANDARD:

H.1.044 The release and acceptance criteria and specifications of processed skin/dermal allografts should be defined, validated, documented and approved.

Explanation:

The criteria should be based on validated protocols and reported in the SoHO Establishment's written procedures.

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.045 There should be a defined procedure for exceptional release of non-standard skin/dermal allografts under a planned non-conformity system. The decision to allow such release should be documented clearly, and traceability should be ensured.

Evidence:

Criteria defined in an SOP.

STANDARD:

H.1.046 The distribution procedures associated with the distribution of skin grafts depend on its processing method, and must be validated and preserve the proprieties of the tissues

Explanation:

Refer to the EDQM Guide - Skin Monographs to determine the transport conditions of the different skin grafts.

Evidence:

SOPs

Example(s):

Deep-frozen skin grafts are stored in ultra-low-temperature refrigerators, which generally maintain a temperature between – 60 ° and – 80 °C. • Transport in dry ice.

Glycerol-preserved skin allograft: The graft is stored in glycerol 85 % solution to keep the tissue preserved before use.

The graft is stored at refrigerator temperature (2-8 °C); storage at room temperature (15-25 °C) during transportation is possible.

BIBLIOGRAPHY

1. EDQM; Council of Europe. Guide to the quality and safety of Tissues and Cells for human application. 5th edition. (2022).
2. EDQM. 20th Edition of the Guide to the preparation, use and quality assurance of blood components. European Committee on Blood Transfusion (CD-P-TS). (2020).
3. FACT & JACIE. 8th edition of the International Standards for Hematopoietic Cellular Therapy Product Collection, Processing, And Administration. 465 (2021).
4. World Marrow Donor Association (WMDA). International standards for unrelated hematopoietic stem cell donor registries. 1–26 (2021).
5. EuroGTPII (709567)- Good Practices for demonstrating safety and quality through recipient follow-up. Euro GTP II Guide - Good Practices for evaluating safety, quality and efficacy of tissue and cellular therapies and products.
6. European Commission. EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines.
7. Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024, on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC.
8. European Medicines Agency (EMA). Guidance for applicants/MAHs involved in GMP, GCP and GVP inspections coordinated by EMA; EMA/274221/2021 Version: 3.1. (2021).
9. Commission Directive 2006/86/EC of 24 October 2006, implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements. (2006).
10. International Organization for Standardization (ISO). (2015). ISO 9000:2015 Quality management systems — Fundamentals and vocabulary.