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## TECHNICAL GUIDELINES FOR OCULAR TISSUE

### GENERAL.

With reference to the following Directives, mandatory in the countries belonging to the EU community:

- Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.
- Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC with regards to certain technical requirements for the donation, procurement and testing of human tissues and cells.
- Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC with regards to certain technical requirements for the coding, processing, preservation, storage and distribution of human cells and tissues.

Suitably trained personnel should perform all tasks according to validated, up-to-date, document-controlled standard operating procedures (SOPs) during:

- 1 Tissue retrieval.
- 2 Tissue processing and storage.
- 3 Tissue selection.

### 1 EYE TISSUE RETRIEVAL.

With reference to the EEBA Donor Medical Assessment and EEBA Contraindications to the use of donor ocular tissue for transplantations:

1.1 Retrieval of the tissue should be performed by qualified personnel.

1.2 Prior to the actual retrieval procedure:

- Identify the donor according to standard procedures.
- Where required, ensure that consent or no objection to donation has been properly obtained (and that it is safe to proceed).
- Perform a gross inspection of the donor's body regarding the medical contra indications.
- Perform a gross inspection of the orbit with special concentration on the corneas with a view to the medical contra indications.
- Record all significant and pathological findings.

1.3 Blood sample:

- Draw a post-mortem blood sample recording the date and time of sampling.
- A suitable ante-mortem blood sample taken up to 7 days before death may be used for donor testing provided identification can be ensured (see EEBA Agreements on Minimum Standards – Donor Medical Assessment and Laboratory tests for donors, EU Directive). The date of sampling should be recorded to indicate that it is an ante-mortem sample.
- In the event that the donor has received blood transfusions, the risk of haemodilution must be assessed.

1.4 Retrieval.

Ensure that the retrieval is performed within the post mortem time limits approved by the Director/Medical Advisor.



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Data submitted for EEBA Directory			
Delay in hours from death to retrieval	mean	min	max
Corneoscleral disc excision in situ (hrs)	11.9	2	33.4
Enucleation (hrs)	13.8	4	48

- Retrieve either the whole eye by enucleation or the corneoscleral disc by in situ excision using validated aseptic procedures.
- Place the whole eye in a fixed position in a moist chamber, or immerse the corneoscleral disc in an appropriate corneal storage solution.
- Indicate the lot number (including expiry date) of all materials used.
- Disposable materials are preferred.  
If re-usable instruments are used, it is recommended that the identification code of instruments or instrument sets is recorded to be able to track which instruments were used with regard to the possibility of slow virus transmission.

1.5 Transport the tissue to the eye bank for further processing as soon as possible by a validated procedure. Every step and time point must be indicated.

## 2 PROCESSING AND STORAGE OF CORNEAL TISSUE.

### 2.1 General.

- Use only reagents and materials from suppliers that meet the documented requirements and specifications approved by the Director.
- All procedures must be documented in SOPs, including method and time point for decontamination, endothelial evaluation and microbiological testing of the tissue.

Data submitted for EEBA Directory 2010		
	nr of banks	
	enucleation	excision in situ
no decontamination	8	0
rinse with saline	5	1
rinse with saline + PVP	2	3
PVP	5	6
antibiotics	5	1
PVP + antibiotics	3	2
chlorohexidine or neosporine	3	0

- Use aseptic techniques while processing the tissue in the eye bank.
- The required air quality standard of the environment during processing of the corneal tissue should be defined and monitored. A direct relation between air quality of the environment and contamination of the corneas has not been proven.
- Considering that:
  - post-mortem eye tissue is generally contaminated,



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- the amount of remaining contaminating microbes is dependent on pre-storage decontamination procedures, antibiotics during storage, and storage procedure,

Each bank should collect data to demonstrate and document that the defined standard of the environment achieves the required quality and safety of the corneal tissue.

Data submitted for EEBA Directory 2010		
Processing surroundings		
Room	clean room grade B	16
	clean room grade C	10
	normal room, free access	3
	normal room, limited access	22
	normal room, access via changing room	11
Flow bench	horizontal	16
	vertical	36
	h or v unknown	10

2.2 The following methods for preparation of the cornea are accepted:

- Excision of the corneoscleral button from enucleated whole eyes in vitro.
- Excision of the corneoscleral button from the donor eyes in situ.
- Lamellar tissue preparation on the corneoscleral button obtained in one of the above mentioned ways, using manual or automated methods or lasers

2.3 The following storage methods are generally accepted for the viable cornea:

- Hypothermic storage of the whole eye.  
Recommended storage time is 72 hrs maximum.  
An inspection of the endothelium is mandatory and a cell loss during storage must be taken into account except for tissue designated for emergency or anterior lamellar grafting. The medical director or his designee needs to assure that all the necessary serological tests on the donor are performed within this time period.
- Hypothermic storage of the corneoscleral disc in a corneal storage solution:  
Maximum storage time depends on the storage medium used, see instructions of the manufacturer. It is recommended not to exceed the prescribed storage time.  
An inspection of the endothelium is mandatory and a cell loss during storage must be taken into account except for tissue designated for emergency or anterior lamellar grafting. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining sclera rim at time of surgery should be added.
- Storage of the corneoscleral button by organ culture:  
It is recommended to keep the storage time as short as possible considering the quarantine period with a maximum of 4-5 weeks.  
It is at the discretion of the Director to prolong the storage time provided documentation (evidence or validation of the procedure) is present to support this procedure.  
Inspection of the endothelium is mandatory at the end of the storage period except for tissue designated for emergency or anterior lamellar grafting.



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A minimum storage period is mandatory to allow for proper microbiological testing thus minimizing the risk of contamination. The time period required to perform microbiological tests of the cornea in the storage medium is at the discretion of the Director. The efficacy of this quarantine period should be evaluated and validated.

Microbiological testing of media samples is mandatory, sole visual inspection of the medium for a change in colour or transparency is not acceptable.

Medium change during storage is at the discretion of the Director and the indications of the manufacturer.

- Cryopreservation may be used for non-viable tissue for tectonic grafting.

Data submitted for EEBA Directory 2010				
Storage time corneoscleral button in days		mean	min	max
Hypothermic storage	MK medium	not used in EEBA banks		
	Optisol (GS)	5	< 1	14
Organ culture				
First phase		16	1*	60**
In transport solution		2.3	0.5	10

\*

With relevance to what is stated in the footer:

\*) The SIG emphasizes that a storage time of less than 1 day does not allow the mandatory microbiology testing

\*\*) The SIG emphasizes that according to the technical guide lines a storage time of > 4-5 weeks is not recommended and should require validation.

### 3 CORNEAL TISSUE EVALUATION AND SELECTION FOR TRANSPLANTATION<sup>1</sup>.

#### 3.1 General.

To be able to select the tissue for the specific purpose for which it is intended, it is necessary to check the condition of:

- The epithelium (full-thickness graft, superficial or deep anterior lamellar graft, limbal graft).
- The corneal stroma (full-thickness graft, superficial or deep anterior lamellar graft); transparency is crucial.
- The endothelium, essential for maintaining corneal transparency (full-thickness graft, posterior lamellar graft).

#### 3.2 Macroscopic inspection.

Without optical aid inspect the donor eye for corneal transparency and exclude corneal pathology for:

- Abnormalities of the external globe.
- Signs of previous surgery of the anterior segment.
- Epithelial abrasions, retention of excessive orbital tissue, laceration of the globe.
- Epithelial defects.

<sup>1</sup> GOD Rosenwasser, WJ Nicholson. Introduction to Eye Banking: A Handbook and Atlas Proforma 2003, pp 83-127



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- Stromal opacities. A mild arcus lipoides is acceptable with a defined clear zone. The minimal diameter of the clear zone is at the discretion of the Director.
  - Abnormal corneal shape (keratoconus, micro- or megalocornea).
  - Condition of the anterior chamber (shape, evidence of gross blood).
  - Abnormalities such as pterygium extending to the optical zone.
- 3.3 Slit lamp examination, performed when whole eyes are enucleated or when corneoscleral buttons are excised, is not mandatory, but it might provide additional information.

Inspect the cornea and limbal area for features which may preclude use of tissue e.g. signs of corneal pathology or post mortem artefacts, taking into account:

- The state of the epithelium and epithelial irregularities.
- The presence of stromal opacities (macula, nebula).
- The number of Descemet folds (increasing with post mortem time).
- Endothelial precipitates.
- Corneal guttae.
- Abnormal corneal shape (keratoconus, micro- or megalocornea).
- Also pay attention to the following items (in case of corneoscleral button excision, this is difficult to detect):
  - Condition of the anterior chamber (shape, evidence of gross blood).
  - Signs of surgery in the anterior segment (glaucoma, cataract extraction, refractive surgery), see medical standards. The presence of synechiae (anteriores, posteriores). This may only be detected by slit lamp examination.

Data submitted for EEBA Directory 2010:

Out of 65 banks providing these data for EEBA Directory 2010, 28 reported to use a slit lamp.

3.4 Other microscopic examinations are mandatory by one of the following methods:

- Specular microscopy.  
The appearance of endothelial cells with specular microscopy varies with temperature, type and time of preservation and media. Evaluation of corneas at room temperature would be recommended.
- Transmitted light microscopy (bright field, phase-contrast).  
For light microscopy, it is necessary to make the endothelial cells visible by induction of swelling of the intercellular space with a hypotonic solution. The induction of the swelling and the swelling pattern is dependent on type of medium and time of preservation. The use of a vital stain (e.g. trypan blue) may help to recognize dead or necrotic cells and denuded Descemet's membrane.

• Data submitted for EEBA Directory 2010			
Number of banks using vital stain Trypan blue (no other vital staining used)			
concentration	nr of banks	concentration	nr of banks
unknown	4	0.2%	1
0.06%	1	0.25%	5
0.1%	1	0.3%	16
0.15%	1	0.4%	12



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Data submitted for EEBA Directory 2010			
Swelling of intercellular borders			
	nr of banks		nr of banks
BSS	14	saline / sucrose	5
PBS	1	sucrose 0.36%	1
glucose 10%	1	sucrose 0.9-1.8%	1
hypotonic solution	1	sucrose 1.4%	2
saline	11	sucrose 1.8%	10
The other banks did not specify this			

### 3.5 Areas of interest during microscopic examination:

- Endothelium:
  - Appearance of swelling pattern of the intercellular space if applicable.
  - Quantity of Descemet's folds.
  - Presence and distribution of dead cells resulting from trauma or post-mortem cell decay
  - Density, size and shape of endothelial cells.
    - The endothelial cell density should be assessed according to a validated and regularly checked method, either counting directly with help of a graticule or afterwards on a photograph or with a calibrated software program.  
Caution is warranted for automatically obtained cell counts as in most cases interactive manipulation of the image is required for a reliable cell count and reliable results of the morphometric analysis for cell size, variation in cell size, % hexagonals and other shape parameters.
    - Cell counting should be done at different areas, centrally and para-centrally up to 5-6 mm from the centre.
    - Polymegatism refers to variation in cell sizes and is graded from trace, mild, moderate to severe.
    - Pleomorphism refers to variation in cell shape and the deviation from the normal hexagonal shape.
    - Presence of corneal guttae.
  - Signs of significant cell loss.
  - Morphological characteristics of endothelial cells (e.g. granulation).
- Stroma: presence of stromal opacities, abnormal morphology of fibroblasts.
- Epithelium.

### 3.6 Exclusion criteria for the endothelium of the cornea, in case the endothelial layer is included in the graft:

- Tissue where the viability is severely affected by trauma, post-mortem effects, indicated by dead and necrotic cells and/or inflammation (presence of inflammatory cells).
- While the specific influence of morphometric parameters for the endothelium on graft outcome remains uncertain, cut off points are at the discretion of the Director. Based on literature a cell density of less than 2000 cells/mm<sup>2</sup>, moderate to severe signs of polymegatism and pleiomorphism, signs of significant cell loss during organ culture or the presence of dead cells are generally considered as



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contraindications for a long term graft survival.

• Data submitted for EEBA Directory 2010		
Mimimum accepted cell count for grafts including the endothelium	Number of banks	Cell loss accepted after preservation
1800	2	5-20% not dependent on minimal cell count before preservation One bank before 1800 accepts 20%, two banks 2300 accept only 5%
2000	43	
2100	2	
2200	9	
2300	5	
2400	1	
2500	1	

### 3.7 Clinical Use

A preservation and expiry date for the cornea must be indicated. If medium changes are performed, indicate these dates as well. Indicate the date of transfer to transport medium.

## 4 SCLERAL TISSUE.

### 4.1 Tissue selection, contra indications for sclera donation:

- Age is at the discretion of the Medical Director.
- Malignancies.
- Pathology of the eye: pterygium, abnormal shape, staphyloma.
- Previous surgery (Cryosurgery (pterygium), Ablation surgery (cerclage surgery).

### 4.2 Processing:

Prepare the sclera after removal of the corneoscleral button; remove the remaining contents (vitreous, lens, iris, choridal and retinal tissue) and adnexa (remnants of muscles, conjunctiva). If requested cut the tissue in pieces. Use aseptic techniques.

### 4.3 Storage (complete or in separately packed pieces).

Generally accepted storage methods are:

- At room temperature:
  - Dehydrated in 70% ethanol or higher, glycerin.
  - Fixed in formalin.
  - Freeze dried or frozen
- In the refrigerator in Optisol-GS or saline with antibiotics

### 4.4 Microbiological control.

Decontamination in a gentamicin bath for 20 minutes before storage in glycerine or a quarantine period in ethanol 70% for 14 days before renewal of the ethanol 70% is recommended in addition to the performance of microbiological tests before storage.

### 4.5 Clinical use:

A preservation and expiry date for scleral tissue shall be indicated. A suitable reconstitution protocol for further surgical use must be documented and made available to the surgeon or surgical centre.



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## 5 AMNION TISSUE.

### 5.1 Tissue selection:

Amniotic membrane is procured from living donors and thus implies a different procurement system including a written informed consent and an extensive medical questionnaire system. A second serology testing of the living donor is necessary after six months of quarantine unless NAT testing has been performed.

### 5.2 Processing:

The amniotic membrane has to be processed in a sterile manner under laminar air flow. The whole placenta is rinsed several times and the amnion and chorion are mechanically separated according to a documented standard operating protocol. The amnion is then placed on a nitrocellulose carrier and divided in smaller pieces.

### 5.3 Storage:

Generally accepted storage methods are in a medium or glycerol:

- In a freezer at - 80° C
- In liquid nitrogen, vapour phase.

### 5.4 Microbiological testing:

During the entire procedure samples of the different rinsing solutions and finally also pieces of tissue are taken for microbiological testing.

### 5.5 Clinical use:

A preservation and expiry date for amniotic tissue shall be indicated. A suitable reconstitution protocol for further surgical use must be documented and made available to the surgeon or surgical centre.

## 6 TISSUE DISTRIBUTION

6.1 Data concerning the donor and in case of the cornea the microscopic evaluation should accompany the donor tissue.

6.2 Surgeons should fill in the form accompanying the graft with the name of the recipient and all other requested data in order to facilitate tracking of donor tissue.

6.3 Surgeons should report to the eye bank serious adverse reactions and events, such as primary graft failure, post-operative endophthalmitis or disease transmission, as these may be related to the quality of the transplanted tissue. All such incidents must be investigated by the Director and, where necessary, appropriate corrective and preventive actions must be taken. If other ocular tissue from the same donor has been transplanted the concerning surgeon must be informed.

Eye banks are required to seek this information on a regular basis. This in addition to the notification of serious adverse reactions and serious adverse events to the competent authority as required by EU regulations.