

# Chapter 5.2

## **Organ culture preservation for corneal tissue. Technical and quality aspects.**

In: Bredehorn-Mayr T, Duncker GIW, Armitage WJ (eds): Eye Banking.  
Dev Ophthalmol. Basel, Karger, 2009, vol 43, pp 31-46.

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## **Abstract**

### **Purpose**

The technical and quality aspects of organ culture as a storage method for human donor corneas are described.

### **Materials and Methods**

Data electronically stored since 1989 of >41,000 corneas, processed in the Cornea Bank Amsterdam, are analyzed. The technical information of eye banks collected in the Directory of the European Eye Bank Association (EEBA) is used as comparison. European Union (EU) directive for tissue banking and EEBA technical guidelines are references for the quality aspects.

### **Results**

Organ culture allows the storage of donor corneas up to 4-5 weeks. The storage phase is followed by a generally much shorter phase of 1-7 days, to reverse the corneal swelling occurring in the first phase and to transport the tissue to the clinic. Selection of the corneas based on inspection of the endothelium after storage as well as microbiological testing of the storage solution after a quarantine period are mandatory for this technique. General agreement exists about the outline of the method, but technical variations are applied to suit local circumstances and preferences of corneal surgeons. Agreement exists about a minimum endothelial cell count as selection criterion in case the donor endothelium is meant to be grafted. The use and cutoff points of other selection parameters for the cornea, e.g. the endothelial cell mosaic, are varying. According to EU regulations, a quality management system should be installed. This way each bank is able to issue a standardized product, while the production process is monitored with quality registrations.

With the clinical outcome of the graft, the quality of the selection and storage procedures is verified. With the notification of adverse reactions such as primary graft failure and endophthalmitis, minimum risks will be assessed.

### **Conclusion**

The organ-cultured cornea is a well-documented product concerning microbiological safety and quality of the tissue. However, variations in performance and materials and no definite cut-off points for selection do not make an organ-cultured cornea a generally standardized product. The corneal surgeons have to ascertain themselves of the safety and quality of the followed procedure. It is up to an organization such as the EEBA to formulate tissue-specific additions to the EU regulations such as training opportunities, technical guidelines and criteria based on science.

## Technical aspects

### General

Summerlin was the first to store a cornea by organ culture in 1973. Doughman et al.<sup>1</sup> however adapted it for eye banking to allow a storage time for corneas of 4-5 weeks before grafting. In his turn, Sperling<sup>2</sup> modified the technique of Doughman et al. and introduced organ culture in Europe in 1978. He added dextran T500 to the original storage solution. This addition prevented the corneal swelling in vitro and facilitated a closed system with microbiological testing of the storage medium before grafting. Sperling supposed that the high molecular weight of this dextran,<sup>10</sup> times higher than the dextran used in the media for cold storage, prevented its uptake by the cornea and the corneal cells. Grafting results were good; nevertheless, electron microscopy revealed that this dextran was also taken up and ingested by all corneal cells.<sup>3</sup> Another modification followed, and from that time onwards until today organ culture consists of two successive phases: storage in Doughman's medium, the longest phase, and a subsequent much shorter phase to reverse swelling in Sperling's medium, which is also used for transport at room temperature.<sup>4</sup> Sperling's other contribution to the storage of donor corneas by organ culture, the evaluation of the endothelium by light microscopy after swelling of the intercellular space, became inherent to this storage technique.<sup>5</sup> In addition to the medium-term storage period, it provides the advantage of delivering corneal tissue with a defined endothelial quality determined after storage.

Also a quarantine period to allow microbiological testing of the storage solution is inherent to the organ culture procedure. In this way, the vulnerability of organ cultures for microbes was exploited – microbiological contamination will be more readily evident – to reduce the risk of grafting contaminated corneal tissue.<sup>6</sup>

Technical details, like storage temperature, composition of the basal medium, concentration of serum and dextran, antibiotics used, medium change and maximum storage period, differ between eye banks applying organ culture in Europe (Directory European Eye Bank Association, EEBA). The documented results and experiences demonstrate the common denominator of organ culture and the variations possible to adapt the technique to local circumstances and preferences of the involved corneal surgeons.

The organ culture storage technique performed by the Cornea Bank Amsterdam (CBA) is described below as an example to show the outline with the relatively uniform steps of the procedure. The performance of the steps aiming at the same result may differ however between banks, and references are made to these other methods.

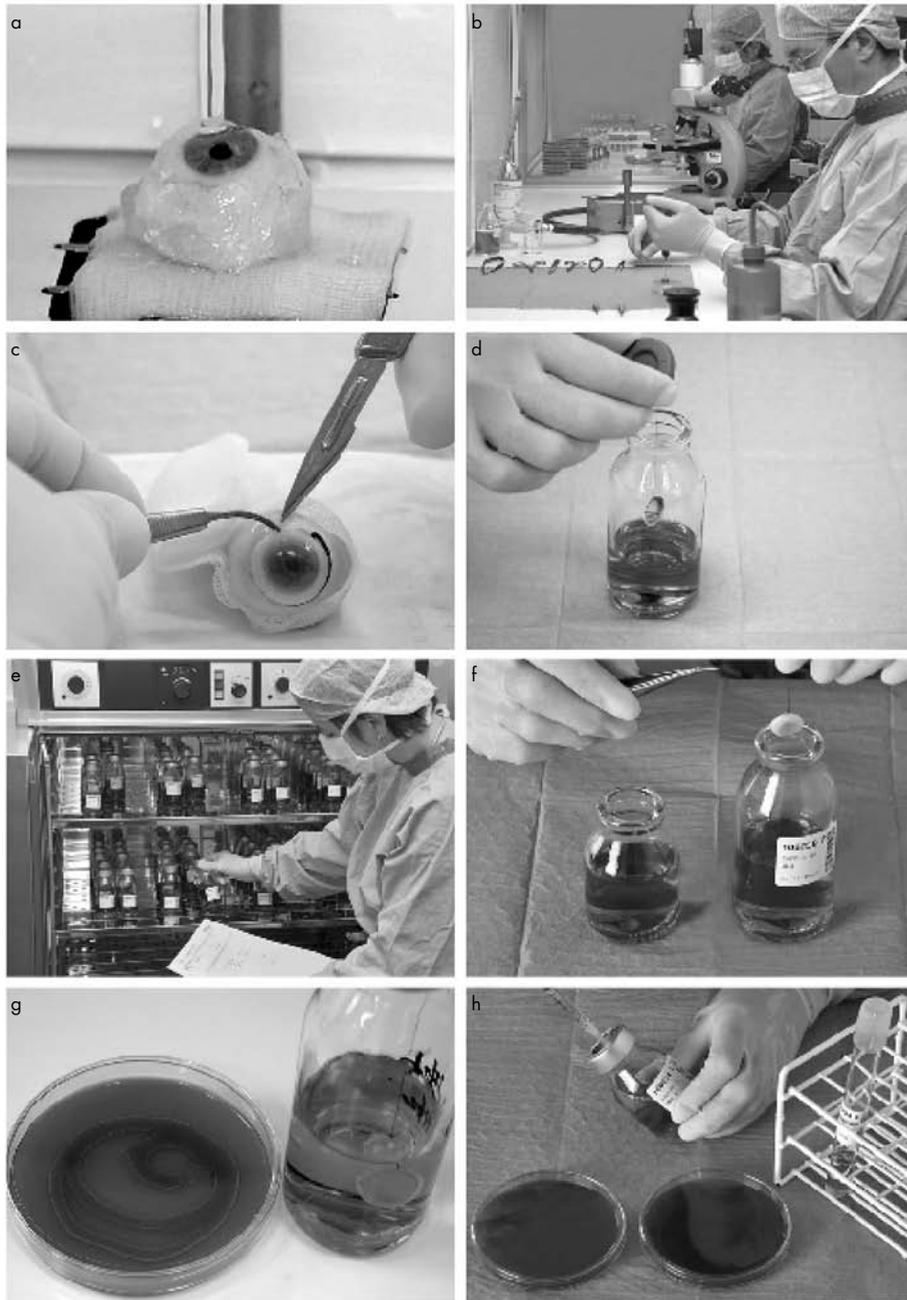


Figure 1

## Decontamination

Aim: Reduction of contaminating microbes from the donor cornea.

Retrieval of the donor tissue may occur by removal of the corneoscleral button in situ or by enucleation of the globe. In case of retrieval of globes, the bulbi are decontaminated on arrival in the bank.

With a running solution (fig. 1a), the number of microbes is significantly reduced. In the past, in the old location, tap water was used by the CBA. Since 1999, when the microbiological quality of the tap water turned out to be no longer standard and became a risk, it has been replaced by a sterile saline solution.

Tap water is still used by some banks, while others prefer sterile solutions.

A further reduction of the contaminating microbes is obtained by immersion in 0.5% polyvinylpyrrolidone-iodine solution for 2 min, followed by rinsing steps for 1 min in 0.5% thiosulphate solution and finally buffered saline. There are banks that prefer only the first or only the final step or the use of antibiotics in addition to the polyvinylpyrrolidone-iodine. The polyvinylpyrrolidone-iodine concentration may vary from 0.5 to 5%.

In case of removal of a corneoscleral button in situ, comparable variations in decontamination regimens are applied before the excision.

In both situations the decontamination is continued with the antibiotics in the storage solution.

### **Figure 1: Storage by organ culture.**

- a Decontamination of the globe by rinsing.
- b Handling in the laminar airflow cabinet using aseptic techniques.
- c Excision of the corneoscleral button.
- d Corneoscleral button hanging in the culture medium by a suture attached to the stopper.
- e Storage in the incubator at 31°C (first phase).
- f Transfer of the corneoscleral button from the storage medium to the transport medium.
- g A blood agar plate showing growth of microbes in a sample of the medium while the storage medium concerned is still clear.
- h Sampling of the transport solution for microbiological testing.

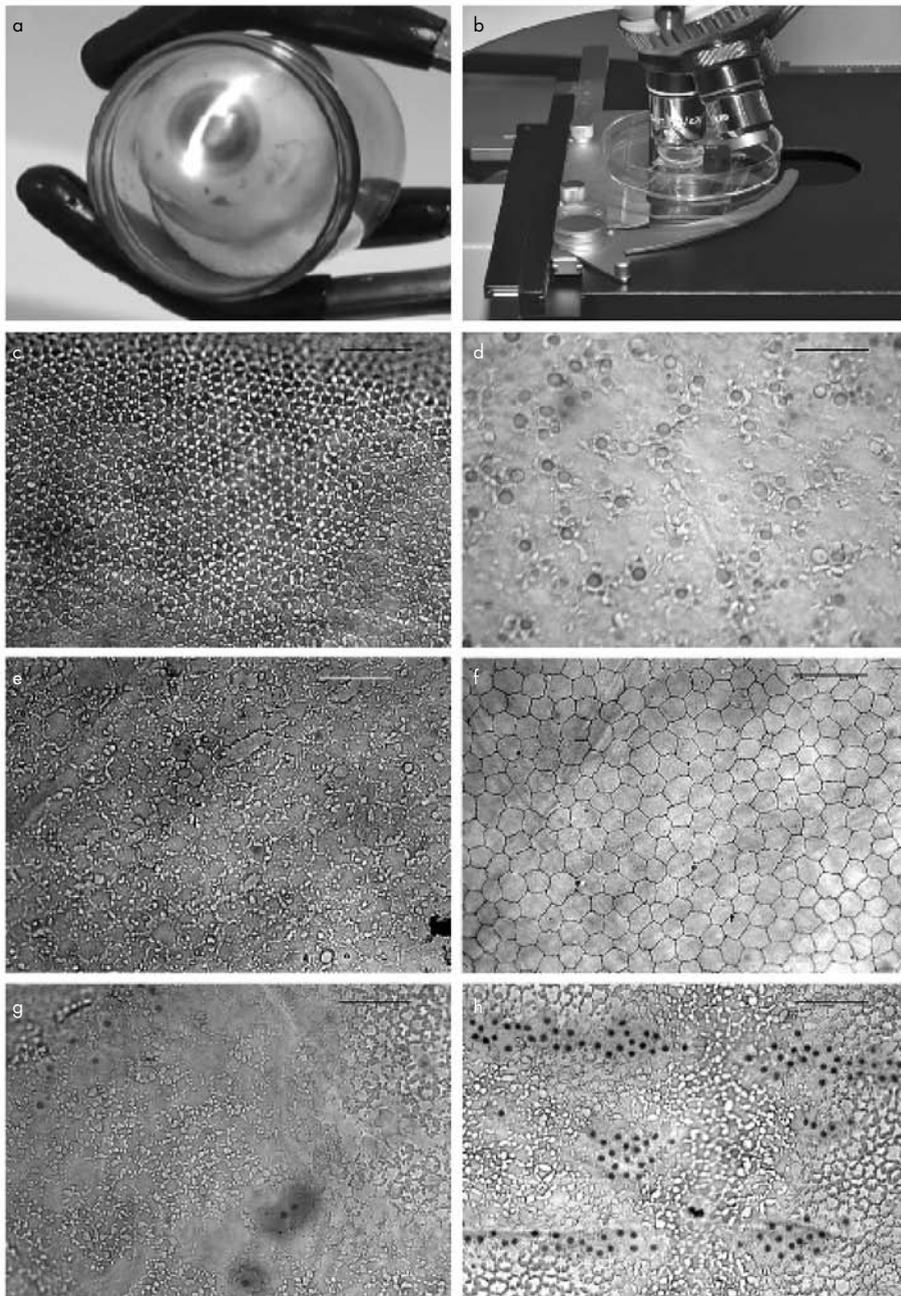


Figure 2

A combination of penicillin, streptomycin and nystatin is preferred by the CBA for various reasons. The loss of corneas due to a contamination is <2%. This is considered acceptable by the CBA. Further reduction with more stable and wide-spectrum antibiotics has been balanced against the possible induction of multi-resistant microbes in an organ culture environment. Also it is considered as an advantage that stronger antibiotics are available in the clinic in case a contamination has passed undetected.

Most other banks use penicillin as well as streptomycin but amphotericin B instead of nystatin. A few banks prefer another class of antibiotics.

## Evaluation of the Tissue

Aim: Selection of the tissue according to tissue-specific criteria to prevent graft failure.

At first the cornea is macroscopically examined for clarity, epithelial integrity, foreign objects, opacities and colour of the sclera (such as jaundice). This is followed by slitlamp examination (fig. 2a).

### Figure 2: Evaluation of the corneal tissue.

- a. *Slit-lamp examination of a cornea with an advanced arcus lipoides.*
  - b. *Examination of the corneal endothelium by light microscopy. Objective  $\times 10$ , oculars  $\times 12.5$ .*
  - c. *Male, 24 years, 3,260 cells/mm<sup>2</sup>, absence of polymegathism and pleomorphism.*
  - d. *Female, 79 years, about 1,818 cells/mm<sup>2</sup>, swelling of the intercellular space is incomplete, protrusions of Descemet's membrane, cornea guttata, stained with trypan blue.*
  - e. *Male, 68 years, 1,003 cells/mm<sup>2</sup> after cataract extraction and placement of an anterior chamber lens.*
  - f. *Male, 68 years, 1,003 cells/mm<sup>2</sup> after cataract extraction and placement of an anterior chamber lens.*
  - g. *Female, 29 years, 3,072 cells/mm<sup>2</sup>, cells with stained nuclei and stained denuded areas of Descemet's membrane.*
  - h. *Female, 59 years, 2,727 cells/mm<sup>2</sup>, stained nuclei lying in rows indicating extrinsic damage due to folding; note the absence of the intercellular swelling in the neighbourhood of the stained cells.*
- c-h: *Images of the corneal endothelium. Scale bars = 0.1 mm.*
- c-e, g, h: *After staining with trypan blue and artificial swelling of the intercellular space.*
- f: *After staining of the intercellular borders with alizarin red.*

The status concerning the presence of an arcus lipoides and the diameter of the clear zone, damage or erosions of the epithelium, scar(s) and stromal opacities with size, depth and their location in or out of the optic centre, number and depth of Descemet's folds, snail tracks and striae, signs of previous operations of the anterior segment such as cataract extraction, glaucoma surgery, refractive surgery, signs of pathology, e.g. cornea guttata, or inflammation are recorded.

Following excision of the corneoscleral button (fig. 1c), the endothelium is inspected by light microscopy after staining with trypan blue and swelling of the intercellular space with a hypotonic solution at a magnification of  $\times 125$  (fig. 2b) (see chapter on the evaluation of the donor corneal endothelium for corneal organ culture by Schroeter and Rieck, this vol., pp.47-62). The whole surface of the endothelium is inspected, and relevant images are made. Cell density is estimated with the help of a graticule in one of the oculars and a nomogram. This is a graph where the cell density, assessed on images by manual counting according to Gundersen's method, is plotted against the number of cells determined on the lines of the graticule. Findings are recorded, such as the swelling pattern on and outside Descemet's folds, the presence of dead cells with nuclei stained by trypan blue, degenerating cells with granules in the cells, the distribution of these dead and dying cells indicative of intrinsic (postmortem damage) or extrinsic (enucleation, transport and excision) damage (fig. 2g,h) and the cell mosaic (fig. 2d). The status of the epithelium and keratocytes is also described. Mean corneal thickness of 5 spots in the centre is estimated by use of the micrometre screw of the microscope. The difference between the micrometre readings when first the epithelial side of the cornea is focused and then the endothelial side is correlated with the thickness of the cornea.

Before reversal of the corneal swelling by transferring the cornea to the transport medium, the evaluation of the endothelium by light microscopy is repeated. Special attention is paid to the condition of the endothelium on the preservation folds, the loss of cells during storage and the presence of reformation patterns indicative of cell loss.<sup>7</sup> Inspection of the endothelium after reversal of the swelling is not preferred by the CBA. The advantage of a cornea with fewer folds and a flatter surface that requires less focusing has been balanced against an additional handling of the cornea and extra recovery time before the transport after the induced intercellular swelling.

Other methods are used to visualize the endothelium. Trypan blue is not always used. Dead and degenerating cells can also be recognized without staining of the nuclei. But because trypan blue also stains the denuded Descemet's membrane, it helps to discriminate between areas without endothelium and areas where the endothelium is present but not visible because the intercellular space does not swell.

Different solutions are applied to visualize the endothelium by provoked swelling of the intercellular space. The pattern of this intercellular swelling may vary but each bank is accustomed to its own images. Staining of the cell membranes with alizarin red on a regular basis in experimental circumstances (fig. 2e,f) may be a great help for the interpretation of the images and the training of the staff. The CBA prefers to include slit-lamp examination to inspect the general condition of the anterior segment of the donor eye. Inspection of the cornea with reflected light in addition to microscopic evaluation with transmitted light may be helpful, in particular to detect stromal opacities.

There are also banks that solely rely on microscopic evaluation after the first storage phase. Some prefer to inspect the endothelium after the reversal of the swelling.

In accordance with the EEBA Technical Guidelines, the condition of the epithelium, the stroma and the endothelium is checked. The vitality of the endothelium has been proven to be essential for graft transparency. Selection parameters are dependent on the surgical procedure for the intended kind of grafting: anterior or posterior lamellar; penetrating, either scheduled or emergency procedure; endothelium or Descemet's membrane. In the case of the CBA, definite cut-off points are agreed on with the Dutch corneal surgeons. Criteria for the endothelium are:  $>2,300$  cells/mm<sup>2</sup>, no or minimal polymegathism or pleomorphism, cell loss  $<20\%$  at the end of the first-phase storage period and normal cell morphology.

General agreement exists about the use of morphometric parameters of the endothelium for selection. As the specific influence of them on graft outcome remains uncertain, cut-off points are however at the discretion of the director. As a result the cut-off points may vary between banks, e.g. for cell count from 2,000 to 2,500 (EEBA Directory).

## Storage

Aim: Maintenance of corneal viability, in particular of the endothelium, for a medium term period of up to 4-5 weeks.

This storage period allows sufficient time for the performance of microbiological testing for safety, tissue typing and matching, recovery of reversible post-mortem damage and scheduling of the transplantation.

Under aseptic conditions (fig. 1b) with the help of a trephine of 15-16 mm as preferred by the CBA, scissors, forceps and knife, the corneoscleral button is carefully excised (fig. 1c). After evaluation of the endothelium, the cornea is suspended in 50 ml culture medium by a suture attached to the inside of the impermeable stopper of a 100-ml glass vial (fig. 1d). It is stored in an incubator at 31°C for minimally 6 (quarantine period) and maximally 28-35 days (fig. 1e).

During storage, the cornea swells to about twice its normal thickness in 6-10 days. The swelling is more pronounced in tissue derived from younger donors.<sup>4</sup>

In general the survival of the endothelium and the keratocytes is not affected, although exceptions may occur. Organ culture is considered as a stress test, and tissue with irreversibly affected vitality reveals itself by significant endothelial cell loss and necrosis of cells. The epithelial layer renews itself but is reduced to 2-3 cell layers. The superficial layers are shed off and are found as cellular debris at the bottom of the glass vial. This is the reason why the cornea is suspended in the medium and is not lying at the bottom.

Because the storage medium contains a pH indicator and the pH of the medium changes from 7.4 to 7.0 during storage, the colour of the medium changes from red orange to yellow orange.

Some banks prefer permeable stoppers or plastic vials and use a CO<sub>2</sub> incubator to maintain the pH. The nourishing conditions for the cornea are improved by renewal of the medium and larger volumes of medium.

With the increasing interest in lamellar grafting, the optimal storage conditions may be different dependent on the requirements: optimal survival of keratocytes, epithelium or endothelium. Storage conditions may also have to be adapted for corneoscleral buttons provided with a section plane by the bank, handmade, made with a microkeratome or intralase.

### **Reversal of the swelling and transport**

Aim: Delivery of tissue that is sufficiently clear for surgical handling and regains its function as soon as possible after grafting.

Minimally 3 and maximally 7 days before surgery and after inspection of the endothelium, the cornea is transferred to the transport medium containing 5% dextran T500 (fig. 1f).

Reversal of the swelling is complete within 12-24 h. The time needed for the reversal is independent of the dextran concentration whereas the final thickness is determined by it. The handling of the cornea during inspection and transfer may restimulate the growth of remaining microbes. A quarantine period for another microbiological test at the expense of some accumulation of dextran in the cornea and the corneal cells has to be balanced against a short time between transfer and grafting to prevent these microbes to become a risk by growing. The preference is affected by the expected time of transport and the distribution area of a bank.

Transport occurs at room temperature. The dextran in the medium protects the endothelium from damage due to the lower than physiological temperature and fast movements of the cornea due to its viscosity.

The toxicity of dextran for the endothelium and the corneal cells is judged differently by banks. Whether this is caused by the source of the dextran, its purity and by-products, is not known. It has however consequences for the limits set for the transport phase. They vary from 1 to 7 days. Studies are performed for alternatives such as hydroxyethyl starch and poloxamers. The CBA considers the adherence of cellular debris from the epithelium to the endothelium a larger risk than the uptake and possible toxicity of the dextran and accepts 7 days in transport medium as a maximum. Whether the dextran might interfere with the adhering capacity of posterior corneal lamellae is however currently studied. Therefore the time period in dextran containing medium is currently limited to 3 days in those cases.

### **Microbiological Testing of the Storage Solutions**

**Aim:** Reduction of the risk of grafting a contaminated cornea, which might cause an adverse reaction in the graft of increasing severity, infection of the anterior segment, ocular infection and endophthalmitis.

Despite all decontamination procedures, contaminating microbes remain. Some will reveal themselves by a change in colour or clarity of the medium, while others might be present without a sign (fig. 1g).

A sample of the medium is taken after 3 days of storage. It is expected that the antibiotics should have done their job before they get instable and inactive. The medium sample is cultured for 7 days on blood agar plates at 35°C and room temperature and in tryptic soy broth at 35°C. This time period turned out to be too short to be fully safe; not all (about 84%) of the contaminants are detected. Therefore an additional test is performed on the day of transfer, after minimally 6 days of storage. The CBA tests the transport medium again 1 day after transfer of the cornea (fig. 1h). The handling of the storage medium and the cornea as well as the transfer to a new medium might stimulate remaining, still undetected microbes. As the transport time may increase up to 7 days, these microbes are a possible risk. Other banks prefer to reduce this time period to 1-2 days with the toxicity of the dextran as reason but also to reduce the microbiological risk that way. All test results should be negative on the day of shipment and the day of grafting (minimally 2 and 3 days after transfer, respectively).

The frequency of delivering a contaminated graft that needs additional treatment in the clinic has been less than 0.023%. One out of 22,019 grafts lost transparency.

The time schedule for the microbiological testing of the CBA is historically grown. Tests were added in the past whenever the test system needed improvements (1993 and 1999).

Other banks prefer other time schedules for sampling and other microbiological test methods, methods designed or adapted to their circumstances and conditions. In all cases a quarantine period that is documented to be safe and actual microbiological testing of the solutions are mandatory. Relying only on a change of colour and turbidity of the medium is not acceptable (fig. 1g).

## **Quality aspects**

### **General**

Banks originated as supporting units to facilitate grafting. They have been transferred to production units to comply with European Union (EU) legislation. Corneal tissue should be a documented safe and standardized product. Quality management systems are nowadays mandatory. This means e.g. that process steps are documented in standard operating procedures. They are monitored, measured and analyzed with the help of quality registrations. The results may be incentives for improvement.

According to current legislation, each eye bank shall deliver a cornea for grafting in a standardized way. The technical details of the production process are however not standard for all banks (see above) but adapted to local preferences. Selection criteria are described (EEBA Minimum Technical Guidelines) but definitive cut-off points are not available as links with graft outcome are not clearly demonstrated. Because the scientific support for only one ideal procedure or specific selection criterion is lacking, the corneas delivered for grafting by different banks do not necessarily have a standardized quality and safety. Corneal surgeons should be aware of these differences when accepting tissue from other sources than usual. The most important aspects are discussed below.

### **Microbiological Safety**

The screening of the donor tissue for transferable diseases will be described in another chapter. Other aspects of safety are discussed here.

On the one hand the microbiological safety is affected by decontamination and microbiological testing procedures in the bank. On the other hand additional measures may be taken in the clinic.

According to EU regulations, the air quality during the processing of the cornea is considered a key factor in tissue processing.

In the CBA the handling of the corneoscleral button using aseptic techniques is performed under aseptic conditions provided by a laminar airflow cabinet with an air quality comparable to good manufacturing practice (GMP) grade A. The background environment is grade C as is the case in many eye banks.

The CBA performs a check for aseptic handling once each week. A cornea discarded for grafting at the second evaluation with no contaminants found in the first-phase medium is transferred to an antibiotic-free medium. Medium samples for microbiological tests are collected after 1 week. In 2 of >590 corneas, growth has been observed indicating a risk of less than 0.34% of contaminating the cornea by handling.

Since 1995 the air quality of the environment has been assessed by particle counting (large, >0.5  $\mu\text{m}$ , and small, <0.5  $\mu\text{m}$ ). The number of colony-forming units has been assessed since 2000. Settle plates have been used since 2003. No correlation is observed with the percentage contamination (fig. 3a, b).

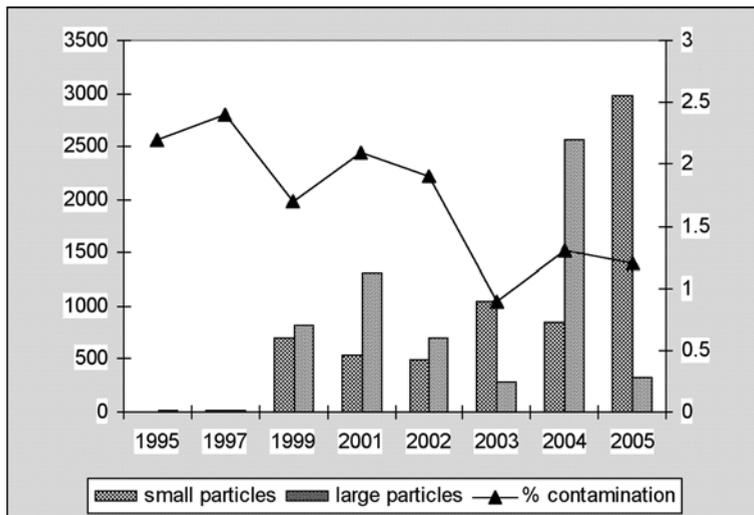


Figure 3a

The results demonstrate and document that the current environment of GMP class C quality does not affect the microbiological quality and safety of the cornea. In this way the environment chosen by the CBA achieves the quality as prescribed by the Commission Directive 2006/86/EC.

Other banks claiming to work in clean rooms with GMP class A critical areas and GMP class B background do not always have less contamination (Directory EEBA 2007). This shows that other factors play a larger role.

The post-mortem retrieved cornea is generally contaminated. The effectiveness of the decontamination procedure and the microbiological testing should therefore be documented.

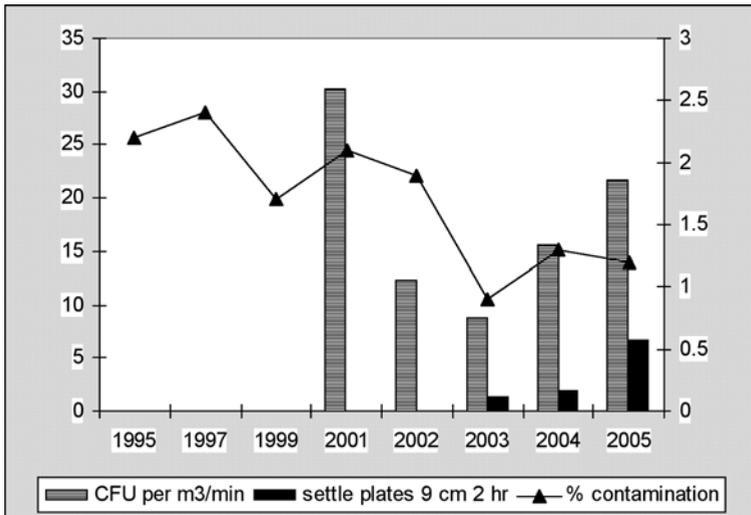


Figure 3b

Figure 3: Contamination of the cornea and the air quality of the process environment. The percentage of corneas detected as contaminated versus all stored corneas (right y-axis in the graphs) is plotted for consecutive years, in addition to the number of large and small particles per cubic foot per minute assessed in the air (a) and the number of colony-forming units (CFU) in the air with the number of colonies observed on settle plates (b).

The effectiveness of the used decontamination procedures has been studied.<sup>8</sup> In addition the percentage of contaminated corneas is plotted for the four quarters of the year (fig. 4). A significant effect of season was not observed. The gradual decrease in contaminated tissue might reflect the use of standard operating procedures by the banks since 1995 and the tissue retrieval organization since 1998. On 3 occasions, a deviation of the general pattern was observed:

In 1993 one specific contaminant, *Bacillus*, was prominent and the observed frequency increased dramatically. It turned out that some of the bottles with nystatin suspension while delivered as sterile were contaminated. This contamination did not change colour or clarity of the medium, so it would not have been detected without microbiological tests of the medium (fig. 1g).

Less significant in this graph because of the scale of the y-axis, but requiring attention at that time, is the contamination in the second quarter of 1999. It is increased compared to the previous time period. In addition the presence of slowly growing microbes was remarkable. Evaluation of the water system showed that the microbiological quality of the tap water was seriously affected by another kind of processing of the water destined for the laboratories. The tap water was replaced by sterile phosphate-buffered saline (see Technical Aspects).

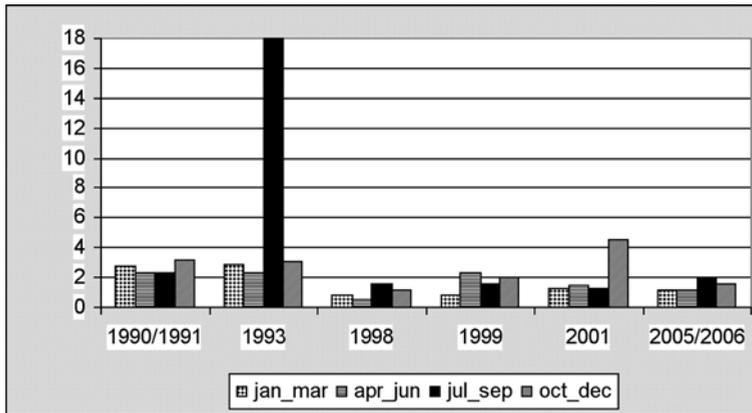


Figure 4: Percentage of contaminated corneas over the years. The percentage of corneas detected as contaminated versus all stored corneas is plotted for the different quarters of the year.

In 2001 the increase in contamination consisted of different types of bacteria belonging to the flora on donor eyes. An increased transport time with insufficient cooling of the donor tissue turned out to be the cause.

Banks should collect this kind of information and make it available for corneal surgeons.<sup>9,10</sup> In this way they can judge the microbiological safety. Additional measures may be considered, e.g. microbiological tests of the corneoscleral rim and transport medium, extended storage of the transport medium for tests later on when judged necessary, additional preventive antibiotic treatment.

By September 2007 Commission Directive 2006/86/EC shall be brought into force by the member states. This requires notification of serious adverse reactions to the competent authority. Grafting of a contaminated cornea resulting in affected graft outcome should be reported. Collection of this kind of information will show the actual risks.

### **Safety and Quality Affected by Storage Solutions**

The storage solutions are critical materials and require documented specifications according to EU legislation (Commission Directive 2006/86/EC part C).

After the introduction of organ culture as a storage method, it took a long time before storage solutions became commercially available. In addition the used storage solutions differ in composition. Banks may therefore have a long history of producing these materials.

Since 1995 the CBA has produced its own storage solutions in a documented and well-controlled way. Produced batches are stored frozen until release and final use. Each batch consists of about 100 bottles with the fully composed storage solution, ready for use after thawing. Only the nystatin, being a suspension, has to be added. Before release each batch is extensively tested for microbiological safety. In addition the quality is tested with at least 5 human corneas not suitable for grafting. Experience has shown that minor changes in the composition or the origin of different basic substances may affect the vitality of the cornea. These modifications passed the quality control of the manufacturer unnoticed.

Since 1981 attention has been paid to the origin of the bovine serum to reduce the risk of prion disease. Serum batches have always been tested before use to exclude toxicity.

Considering standardization in general, commercially available products should be preferred. Agreement about the ideal composition does however not yet exist. So may the dextran concentration vary in the transport solutions (EEBA Directory). This affects the appearance of the corneal tissue, more or less swollen. It also affects the induction of the artificial swelling necessary to visualize the endothelium and by this the interpretation of the images. On the other hand, the production of storage solutions is not the core business of the manufacturers as it is for the eye banks that can test their products with the tissue itself.

In different centres media are developed and tested which are free of bovine serum. The development of these media is very important. From a safety point of view the risk of prion disease is reduced due to the replacement of the bovine products, provided the origin of the replacements is known. From a qualitative point of view, the replacement of the biological component serum by chemically better-defined products is an improvement.

### **Quality of Tissue Affected by Selection**

For the transparency of the graft, a functioning endothelium is essential. A rationale has been presented for the setting of minimum donor cell densities by eye banks. This means that proper calibration of the microscope as well as evaluation of the counting results should be essential, irrespective of whether the counts are obtained manually or in a computer-assisted or fully automated manner.<sup>11,12</sup>

In the CBA the cell density is manually counted, and consistency is assessed at documented time intervals. The interobserver variation of 6 staff members is 3.2%. Lacking scientific support for other morphometric selection criteria, the evaluation of the endothelium should at least be standardized within the bank. Consistency in the judgement of the endothelial

cell mosaic needs regular consultation between colleagues in the CBA. Documented training of staff is a key factor. Providing training courses might be a challenge for the EEBA.

Monitoring of the selection result, the percentage of corneas judged suitable for grafting versus the total number of donated corneas, has been another way in the CBA to test the consistency of selection (fig. 5)

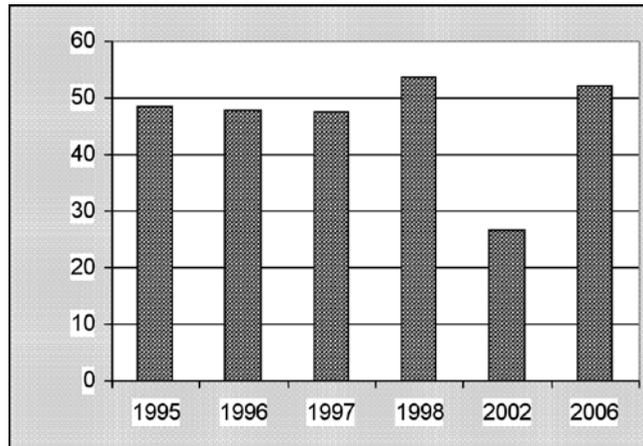


Figure 5: Selection results. The percentage of selected corneas versus the donated corneas is plotted for a consecutive time period.

In 1998 the results have been improved by the introduction of a maximum age of 80 years for the donor because donor age has been shown to be an important factor.<sup>13,14</sup> When in 2002 the selection result dropped, investigation showed that the vitality of the tissue was affected. After measures had been taken to improve tissue retrieval and transport, the selection result returned to the original level. A similar phenomenon has been described by another bank.<sup>15</sup> With the growing interest in lamellar grafting, it is expected that the results will change because tissue judged not suitable for a penetrating keratoplasty might be suitable for an anterior lamellar graft.

Utmost caution is warranted when selection results of individual banks per se are compared. They are dependent on many factors and preselections and are not indicative of the quality of tissue issued by a bank.

The final test for storage and selection is the clinical outcome. Clinical information about the corneas of patients grafted in the Netherlands has been collected and stored in a computer database since 1995. Of the 7,243 corneas transplanted up to July 2006, 4,424 have been followed minimally once (follow-up percentage 61%).

Eighteen corneas (0.41%) got cloudy within 1 month. Only 1 never cleared after grafting and is considered a primary graft failure. The others cleared firstly.

Other complaints are also collected as they may be a trigger for improvement. For example, corneal surgeons started to complain about the presence of an arcus lipoides. A study of the graft size revealed that the arcus had not been overlooked in the bank but that the mean graft size in general was 7.5 mm. A clear diameter of less than 8 mm is nowadays judged as a contra-indication.

Banks in other countries do have their own follow-up registration (see EEBA Directory) and have published their results.<sup>16,17</sup> In Australia<sup>18</sup> and Sweden, national graft registries are available collecting graft results of tissue processed by different banks. The presence of such a register is an important tool for a bank in quality management. The storage and selection can be monitored. Results stimulate improvement.

According to EU legislation by 2008 all banks should have procedures in place to collect adverse reactions without delay. They have to notify nationally installed competent authorities of these events such as primary graft failure and endophthalmitis.

Evaluation results should be reported as well. In this way a minimum level will be ensured.

## **Conclusion**

The organ-cultured cornea is a well-documented product concerning microbiological safety and quality of the tissue. General agreement exists about the outline of the storage technique and selection parameters. However, variations in performance and materials, and the absence of definite cut-off points during selection, make the organ cultured cornea not yet a fully standardized product. Training of the staff by the EEBA may stimulate the standardization insofar as a living cornea may be considered a standard product.

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