XI Corso Nazionale
Società Italiana Banche degli Occhi

Imola, 1 Aprile 2017

Overview sui controlli microbiologici europei: aggiornamenti EEBA 2017.

Dott.ssa P. Santoro
Banca delle Cornee della Regione Piemonte

XXIX. ANNUAL MEETING OF THE EUROPEAN EYE BANK ASSOCIATION

19 – 21 January 2017, Prague Marriott Hotel, Prague – Czech Republic

ABSTRACTS

International Eye Bank of Prague
Organising Committee
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EEBA 2017

XXIX. ANNUAL MEETING OF THE EUROPEAN EYE BANK ASSOCIATION

EUROPEAN EYE BANK ASSOCIATION

SATURDAY 21st JANUARY, 2017

08:30 - 09:30	EEBA Business Meeting / Bohemia II. room
09:30 - 10:00	*Coffee Break and Poster Session*
10:00 - 10:45	**1st Morning session**
	Cornea Storage / Tissue Media
	Chairman: Katerina Jirsova, Thibaud Garcin
10:00 - 10:10	30. Thibaud Garcin - Saint-Etienne, France (page 43) Preclinical controlled study comparing long-term stored human corneas in an innovative bioreactor versus standard organ-culture
10:10 - 10:20	31. Jana D´Amato Tóthová - Monza, Italy (page 44) Evaluation of donor corneas preserved in a new cold storage medium with antimycotic tablet
10:20 - 10:30	32. Ina Wilkemeyer - Berlin, Germany (page 45) Organ Culture Preservation of Human Donor Corneas without Antibiotics
10:30 - 10:40	33. Laura Giurgola - Monza, Italy (page 46) Validation of microbiological test of corneal preservation media according to "Method suitability test" of European Pharmacopoeia (chapter 2.6.1.)
10:40 - 10:45	Domenico Amato - Ponte San Nicolò, Italy Company presentation: AlchiMIA S.r.l. What is a medical device and how to develop it

EEBA 2016

EUROPEAN EYE BANK ASSOCIATION

Saturday, January 23

2nd Morning session

11:30 – 12:20	**Microbiology & safety (Maier & Lauterbach)**
11:30 – 11:40	T. Sorgenfrei, Hildesheim: Relationship between conjunctival swabs and contamination in organ culture of corneas
11:40 – 11:50	Z. Skenderi, Berlin: A New Approach for Sterility Testing of Corneal Organ Culture Medium
11:50 – 12:00	I. Wilkemeyer, Berlin: Validation of Sterility Testing for Cornea Organ Culture Medium Containing Antibiotics using Blood Culture Bottles with Penicillinase as Additive
12:00 – 12:10	D'Amato Tóthová, Monza: Development of a cold storage medium with lyophilized antimycotic
12:10 – 12:20	E. Martinez-Conesa, Barcelona: Five years analysis of microbiological contamination in organ-cultured corneas in Barcelona Tissue Bank
12:20 – 13:00	**Donor selection Workshop (Beele & Trias)**

lecture no 31

Title:

Evaluation of donor corneas preserved in a new cold storage medium with antimycotic tablet

Authors:

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Text:

Introduction
A new cold storage medium (ECS) with an effective antimycotic concentration, fast dissolving tablet was designed to prevent yeast contamination of donor corneas intended for transplantation.

lecture no 33

Title:

Validation of microbiological test of corneal preservation media according to "Method suitability test" of European Pharmacopoeia (chapter 2.6.1.)

Authors:

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Text:

Purpose
The aim of this study was to validate the microbiological test of corneal preservation media according to the "Method suitability test" (EP) using the HB&L (HB&L, Alifax) and BACTEC (Becton Dickinson) automatized systems.

lecture no 32

Organ Culture Preservation of Human Donor Corneas without Antibiotics

Ina Wilkemeyer, Zemra Skenderi, Axel Pruß, Jan Schroeter

Cornea Bank Berlin, University Tissue Bank, Institute for Transfusion Medicine, Charité – Universitätsmedizin Berlin

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Purpose
Since antibiotics in the organ culture medium may inhibit the microbial growth in microbiological testing, and therefore, might lead to false negative results, we started to investigate the organ culture of human donor corneas without antibiotics.

Materials and Methods
62 human donor corneas not suitable for transplantation were organ-cultured for 39 hours, on average, with a standard medium (MEM with 2% FCS containing 130 µg/ml Streptomycin, 60 µg/ml Penicillin and 2,5 µg/ml Amphotericin B) at 32 ± 1°C. This was followed by a preservation with MEM with 2% FCS without antibiotics for an average of 28 days at 32 ± 1°C. The medium was changed 3 to 4 times, with an average interval of 9 days, and tested for microbiological contamination by incubating 10 ml of the medium in aerobic and anaerobic blood culture bottles (Bactec automated blood culture system with standard vials, BD). At the end of the organ culture period, the corneas were transferred into Thioglycolate broth for microbiological testing for 14 days at 35 ± 1°C.

Results
3 corneas showed a clear contamination of the organ culture medium after 4-5 days of cultivation, which was confirmed by microbiological testing. All other 59 corneas showed no signs of microbial contamination, and all microbiological tests remained negative.

Conclusion
With a rather low rate of microbial contamination of 4,8% in this study, a continuous antibiotic treatment during organ culture may not be necessary. Further investigation is necessary to determine if this study found addition contaminations when are hidden in media containing antibiotics, or if preservation without antibiotics leads to a higher contamination rate in general.

Technical guidelines 02/2015

1. A minimum storage period is mandatory to allow for proper microbiological testing thus minimizing the risk of contamination

2. The efficacy of this quarantine period and the microbiological testing method should be evaluated and validated due to antibiotics within the storage media.

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

Technical guidelines 02/2017

An inspection of the endothelium is mandatory and the cell loss during storage must be taken into account, except for tissue designated for emergency or anterior lamellar grafting. Due to the short time of storage, it is not possible to wait for the final result of sensitive microbiological testing of the culture medium using traditional microbiological testing (e.g. blood-culture bottles) but there are alternative testing-methods available. However, sampling of the culture medium one day after the start of the storage period, or just before delivery for clinical use is recommended. The efficacy of the used microbiological testing method should be evaluated and validated due to the presence of antibiotics within the storage media. The treating physician/receiving transplanting centre should be informed as quickly as possible in the event of a 'late' positive result. Instruction for surgery-use with recommendation of microbiological testing of corneal storage medium and/or remaining scleral rim at time of surgery should be added.(see 3.7)

- Storage of the corneoscleral button by organ/tissue culture: It is recommended to keep the storage time as short as possible with a maximum of 34 days for selected surgery cases. Inspection of the endothelium is mandatory and should be preferred in any case at the end of the storage period except for tissue designated for emergency or anterior lamellar grafting.
  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 storage medium is at the discretion of the Responsible Person. The efficacy of this quarantine period and the microbiological testing method should be evaluated and validated considering the effectiveness of antibiotics within the storage media.
  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 using aseptic procedures is at the discretion of the Responsible Person and the indications of the manufacturer - taking into consideration that corneal endothelium might be stressed if tissue is to be transferred into other solutions/media.

Technical guidelines 02/2017

3.7 Clinical Use

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

Microbiological testing of medium and/or remaining scleral rim postoperatively is highly recommended, especially due to the fact that tissue is not considered to be sterile (and storage medium is not bactericidal).

Rischio di endoftalmite post trapianto

- Studio delle banche degli occhi tedesche condotto nel 2007: rischio di 0,035%

- Dati di letteratura:

  Birnbaum e Reinhard (2006) e Taban et al. (2005): rischio da 0,1% a 0,38%.

Pels (2008)
- rischio da 0 a 0,1% per cornee di coltura
- rischio da 0,2 a 1,3% per cornee a freddo

Controlli microbiologici nelle Banche degli Occhi Europee

Il rischio microbiologico connesso al trapianto di cornee conservate a caldo si riduce con:

- Disinfezione prima del prelievo
- Presenza di antibiotici e/o antifungini nei liquidi di conservazione
- Presenza di indicatori di pH nei liquidi di conservazione
- Controlli microbiologici durante la quarantena
- Controllo del liquido di trasporto

Test microbiologici

Risposte da 57 banche EEBA

Eye Bank of Munich

1. E-swabs from conjunctiva/anterior chamber after decontamination (at time of retrieval)
2. Sterility testing of medias during storage (hypothermic and organ cultured tissues)
3. Swabs/sterility control of remaining scleral rim/media (AFTER transplantation)
4. Inoculation onto TSB/Thio Sab broth for all testing samples (400 µl/14 days)

- *Staphylococcus epidermidis*
- *Enterococcus faecalis*
- Rare *Candida*

Controlli microbiologici nelle Banche degli Occhi Europee

Il rischio microbiologico connesso al trapianto di cornee conservate a caldo si riduce con:

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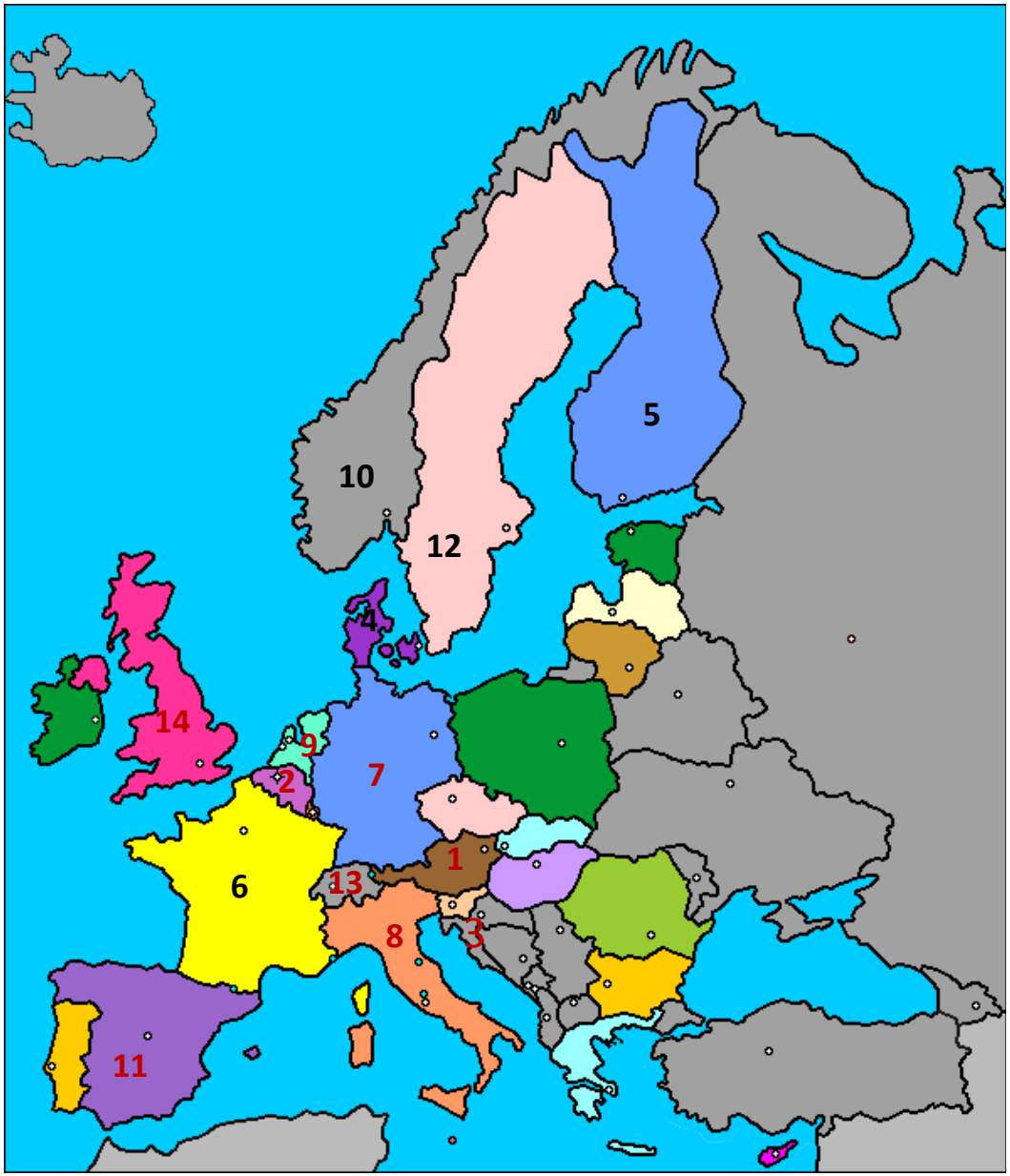
- **Presenza di antibiotici e/o antifungini nei liquidi di conservazione**
- Presenza di indicatori di pH nei liquidi di conservazione
- Controlli microbiologici durante la quarantena
- Controllo del liquido di trasporto



1. Penicillina G/Streptomicina/Amfotericina B (Alchimia)

2. - Penicillina/Streptomicina/Amfotericina (MEM FBS 2 %)
   - Amukina/Tazocin/Nistatina (MEM FBS)

3. Penicillina/Streptomicina/Amfotericina B (Eagle's MEM FBS 2%)

4. Biklin/Piperacillina/Fungizone (MEM FBS)

5. Penicillina/Streptomicina (Eurobio Cornea Prep, Max e Jet FBS)

6. Penicillina/Streptomicina (Eurobio Cornea Prep, Max e Jet)

7. Penicillina/Streptomicina/Amfotericina B (EMEM, MEM, Biochrom Culture Medium I, II, DMEM, Cornea Max Eurobio, Alchimia)

8. Penicillina/Streptomicina/Amfotericina (Alchimia, MEM FBS)

9. - Penicillina/Streptomicina/Amfotericina B (MEM FBS)
   - Penicillina/Streptomicina (Cornea Max Eurobio)

10. Garamicina/Vancomicina/Amfotericina B (MEM)

11. Penicillina/Streptomicina/Amfotericina B (Alchimia)

12. - Biklin, Piperacillina/Tazobactam,Fungizone (MEM FBS 8%)
    - Biklin,Piperacillina/Tazobactam, Nistatina (MEM FBS 8%)

13. Penicillina/Streptomicina/Amfotericina B (Eurobio e Stem alpha)

14. Penicillina/Streptomicina/Amfotericina B (Eagle's MEM FBS 2%)

Antibiotici e antifungini presenti nei terreni di conservazione utilizzati in Europa

Antibiotici	Antifungini
Penicillina - Streptomicina	Amfotericina
Penicillina - Streptomicina	
Amukina – Tazocin*	Nistatina
Amikacina – Tazocin*	Nistatina
Amikacina – Tazocin*	Fungizone**
Garamicina** - Vancomicina	Amfotericina

*Amikacina – Tazocin = Biklin + Piperacillina/Tazobactam
**Amfotericina
***Gentamicina

Quantità di antibiotici e antifungini presenti nei terreni di conservazione utilizzati in Europa

Antibiotici	Quantità
Penicillina	100 u 1.000 u 10.000 u 100.000 u
Streptomicina	0,1 mg 1 mg 10 mg
Biklina (Amukina)	100 µg 200 µg
Tazocin (piperacillina+tazobactam)	0,5 mg 0,2 mg 2 mg
Garamicina (gentamicina)	50 µg
Vancomicina	100 µg

Antifungini	Quantità
Amfotericina (Fungizone)	0,25 µg 0,50 µg 2,50 µg 25 µg 250 µg 2500 µg 5 mg
Nistatina	

Siero Bovino Fetale	Quantità
FBS	2 %
FBS	8 %

Percentuale di Cornee eliminate per controlli microbiologici positivi in EEBA	
2014	3,1 %
2015	2,6 %

Percentuale di Cornee eliminate per controlli microbiologici positivi in Torino	
2014	3,5 %
2015	2,3 %
2016	1,7 %

Tipi di microrganismo rilevati presso la Banca di Torino

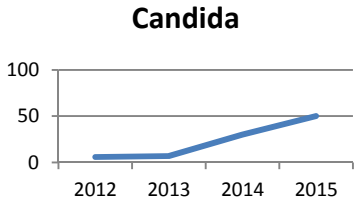
2014		2015		2016	
GRAM -	20 %	**GRAM -**	20 %	**GRAM -**	37 %
GRAM +	30 %	**GRAM +**	30 %	**GRAM +**	53 %
FUNGHI *	50 %	**FUNGHI** **	50 %	**FUNGHI**	10 %

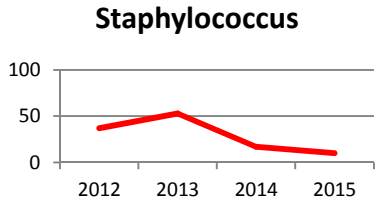
* 33 % contaminazioni rilevate sul terreno di trasporto

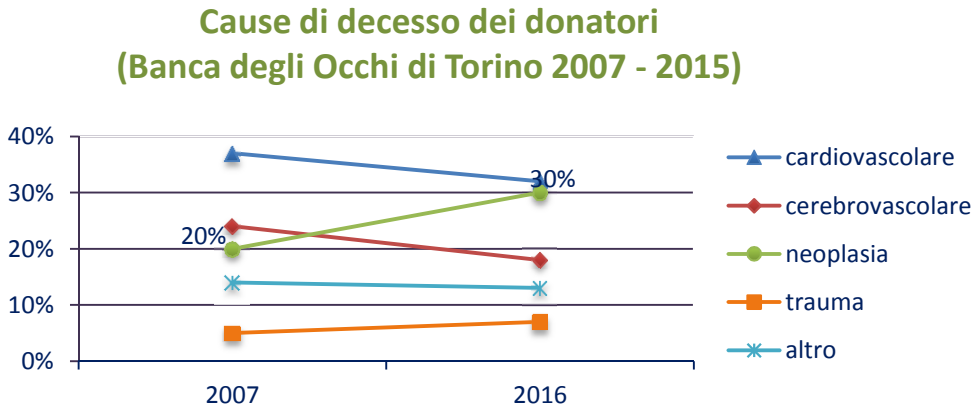
** 40 % sul terreno di trasporto

Alcune considerazioni…..

CORSO SIBO 2016
Palazzo delle Arti di Napoli – 23 Aprile 2016







2012		2013		2014		2015		2016	
Batteri	94 %	**Batteri**	90 %	**Batteri**	50 %	**Batteri**	50 %	**Batteri**	90 %
Funghi	6 %	**Funghi**	10 %	**Funghi**	50 %	**Funghi**	50 %	**Funghi**	10 %

Classificazione secondo il TIPO D'AZIONE

L'azione dell'antibiotico può essere:

Batteriostatica:
l'antibiotico blocca la riproduzione dei batteri

Battericida:
l'antibiotico determina la morte dei batteri. Si definisce battericida l'antibiotico il quale dopo 24 h di contatto "in vitro"determina una sopravvivenza uguale o inferiore allo 0,01%.

AMINOGLICOSIDI

STREPTOMICINA **GENTAMICINA** **BIKLINA (AMIKACINA)**

La **streptomicina** è un antibiotico batteriostatico a dosi terapeutiche, a dosi superiori diventa battericida, il primo ad essere scoperto di una famiglia chiamata amminoglicosidi, uno dei primi rimedi contro la tubercolosi.

Principalmente attivi contro batteri aerobici Gram - come *Pseudomonas*, *Acinetobacter* ed *Enterobatteri.*

Inefficaci contro gli anaerobi.

β-LATTAMICI

PENICILLINA **PIPERACILLINA**

La piperacillina appartiene alla classe dei β-lattamici, del gruppo delle penicilline (penicillina semisintetica, dotata di attività battericida). Normalmente associata ad un inibitore della β-lattamasi, in particolare nella combinazione piperacillina/tazobactam. Il tazobactam può prevenire la resistenza di alcuni batteri verso la piperacillina.
Ciò significa che quando la piperacillina e il tazobactam vengono somministrati insieme, più tipi di batteri vengono uccisi.

Spettro batterico ampio, attiva verso batteri aerobici Gram + *(Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis)*
e batteri Gram -
(Pseudomonas aeruginosa, Serratia, marcescens, Enterobacteriaceae, Klebsielle pneumoniae, E. coli) e diversi anaerobi (tra cui *Bacteroides fragilis).*

GARAMICINA → **GENTAMICINA**

GLICOPEPTIDI

↓

VANCOMICINA

Effetto battericida, inefficace contro i batteri Gram -, ad eccezione dei *Flavobacterium.*

La somministrazione contemporanea di vancomicina e di gentamicina e streptomicina potenzia l'azione nei confronti di *Enterococcus faecium* ed *Enterococcus faecalis;* attivo contro gli *Stafilo* produttori di *b*-lattamasi e resistenti alla meticillina, alla nafcillina e alla oxacillina.

Gentamicina e kanamicina sono marginalmente efficaci contro i micoplasmi, contro i quali si preferisce utilizzare TIAMULIN, MINOCICLINA e **VANCOMICINA.**

ANTIMICOTICI

AMFOTERICINA B
(0,2 μg/mL – 2 μg/mL)

NISTATINA
(100 U/mL e 250 U/mL)

L'amfotericina è un fungicida e fungistatico, a seconda del microrganismo e della concentrazione del farmaco. È efficace contro un'ampia varietà di funghi, come: *candida albicans*, *coccidioides*, *aspergillus* e *blastomices*

Grazie !!!