



S.I.T.R.A.C.
SOCIETÀ ITALIANA TRAPIANTO DI CORNEA
E SUPERFICIE OCULARE

VENEZIA
21-22-23 febbraio 2019

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«Validazione di metodi colturali per la ricerca di batteri anaerobi e sporigeni in terreni di coltura, conservazione e trasporto di tessuti oculari»

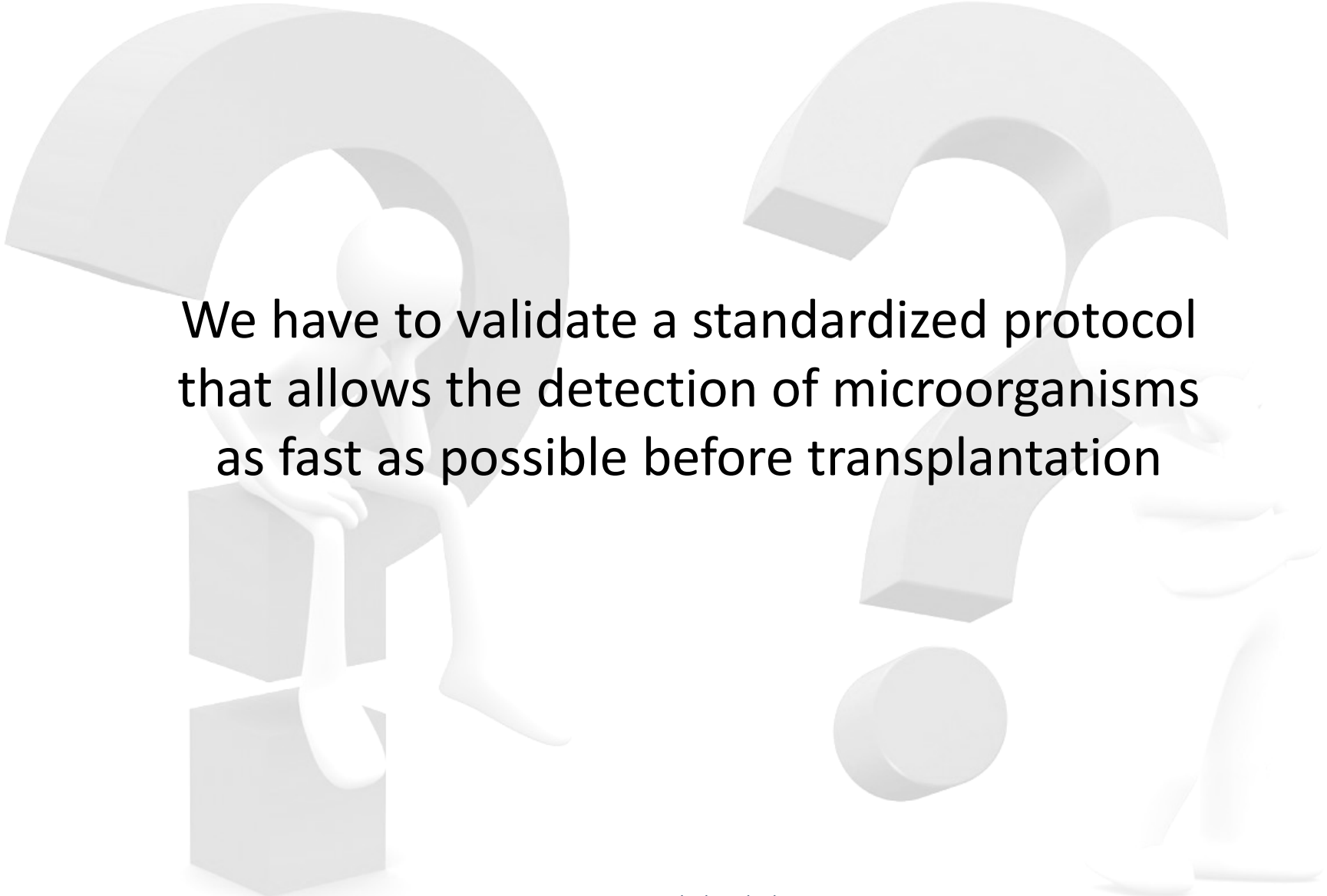
“Validation of culture methods for the detection of anaerobic and sporigenic bacteria in culture media, storage and transport of eye tissues”

Piera Santoro
Banca degli occhi di Torino

S.I.T.R.A.C. VENEZIA, Giovedì 21 Febbraio
SIMPOSIO SOCIETÀ ITALIANA BANCHE DEGLI OCCHI



WHAT DO WE HAVE TO DO?

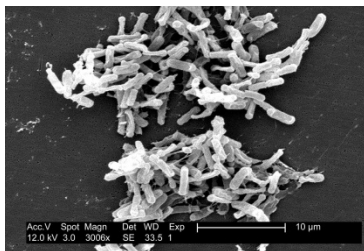


We have to validate a standardized protocol
that allows the detection of microorganisms
as fast as possible before transplantation

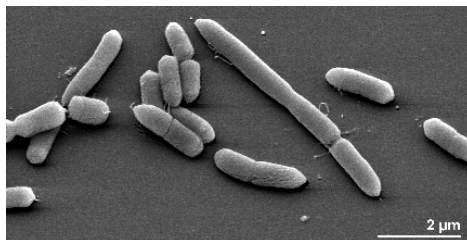
Increase of infections by anaerobic bacteria in clinical infections

- Anaerobic bacteria were detected in 30% of children with conjunctivitis

-clostridi spp



-bacilli anaerobi gram negativi



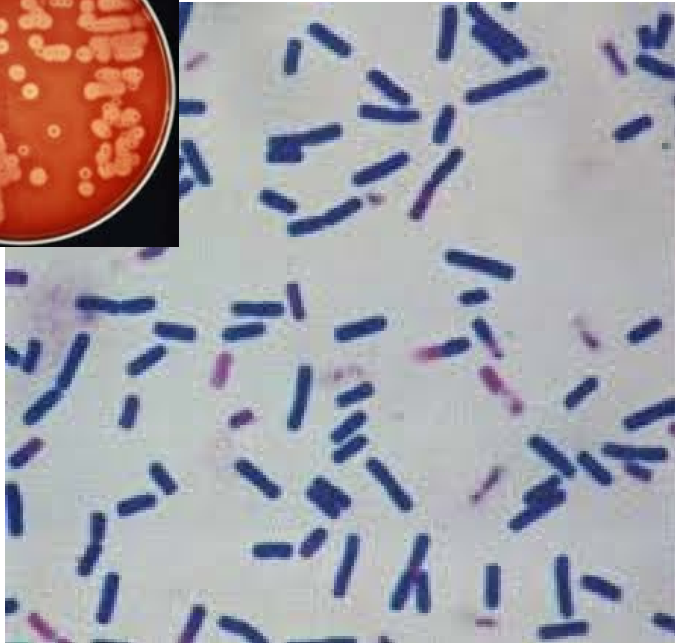
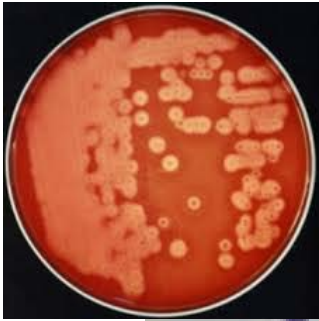
-peptostreptococcus



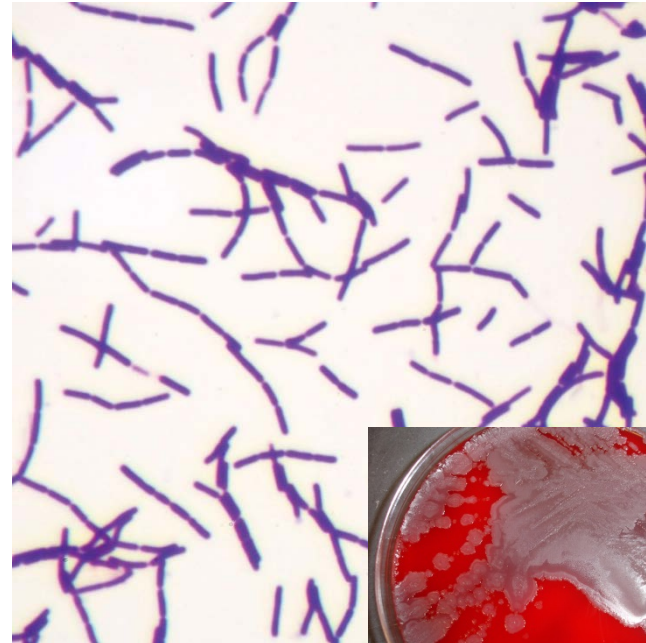
- Anaerobic bacteria are frequently detected in contact lens wearers



Clostridi and *Bacilli*: endospore-forming bacteria; spores makes them extremely resistant even to marked thermal changes or disinfectants.



Clostridium spp



Bacillus spp



CONJUNCTIVITIS

- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*
- *Haemophilus parainfluenzae*
- Group b *Streptococcus*
- *Enterobacteriaceae* (*Klebsiella pneumoniae* and *Proteus mirabilis*)
- *Pseudomonas aeruginosa*

KERATITIS

- *Staphylococci*
- *Streptococci*
- *Pseudomonas*
- *Enterobacteriaceae*
- *Corynebacterium*
- *Acantameba moraxella*
- *Serratia*
- *Neisseria gonorrhoea*
- *Acantameba*
- *Aspergillus*
- *Candida*
- *Fusarium*
- *Propionibacterium*

ENDOPHTHALMITIS

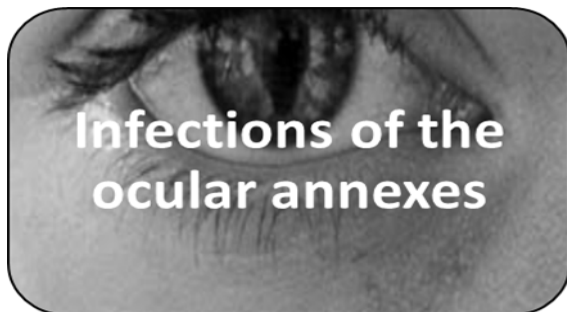
- *Coagulase negative Staphylococcus*
- *Staphylococcus aureus*
- *Streptococci*
- *Propionibacterium acnes*
- *Yeasts and molds*
- POSTOPERATIVE ACUTE ENDOPHTHALMITIS
- *Enterobacteriaceae*
- *Pseudomonas aeruginosa*
- *Propionibacterium Acnes*
- CHRONIC ENDOPHTHALMITIS
- *Mycobacterium spp*
- POST-TRAUMATIC ENDOPHTHALMITIS
- *Bacillus cereus*
- *Funghi*
- *Streptococci*
- *Clostridium spp*
- *Microsporidia spp*
- ENDOGENOUS ENDOPHTHALMITIS



Conjunctivitis

Keratitis

Endophthalmitis



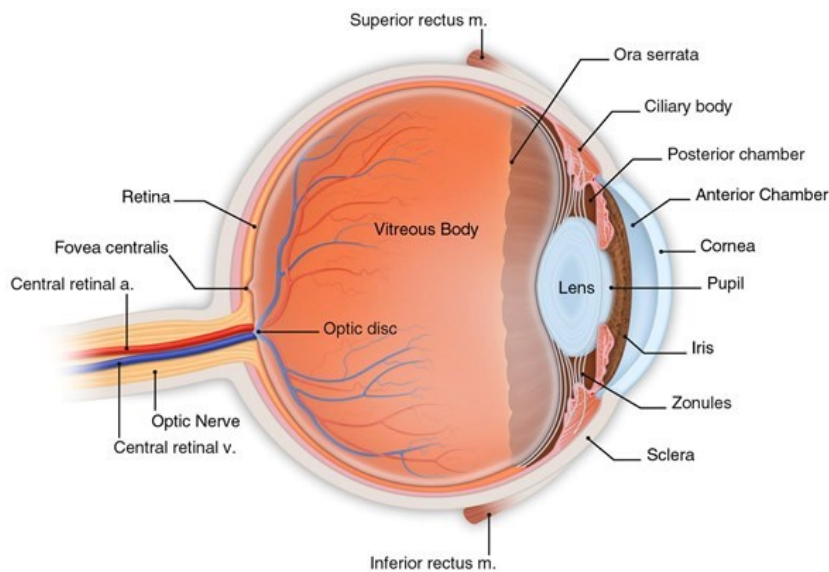
Dacryoadenitis

Dacryocystitis

Canaliculitis

Preseptal and orbital cellulitis





INTERNAL COMPARTMENT

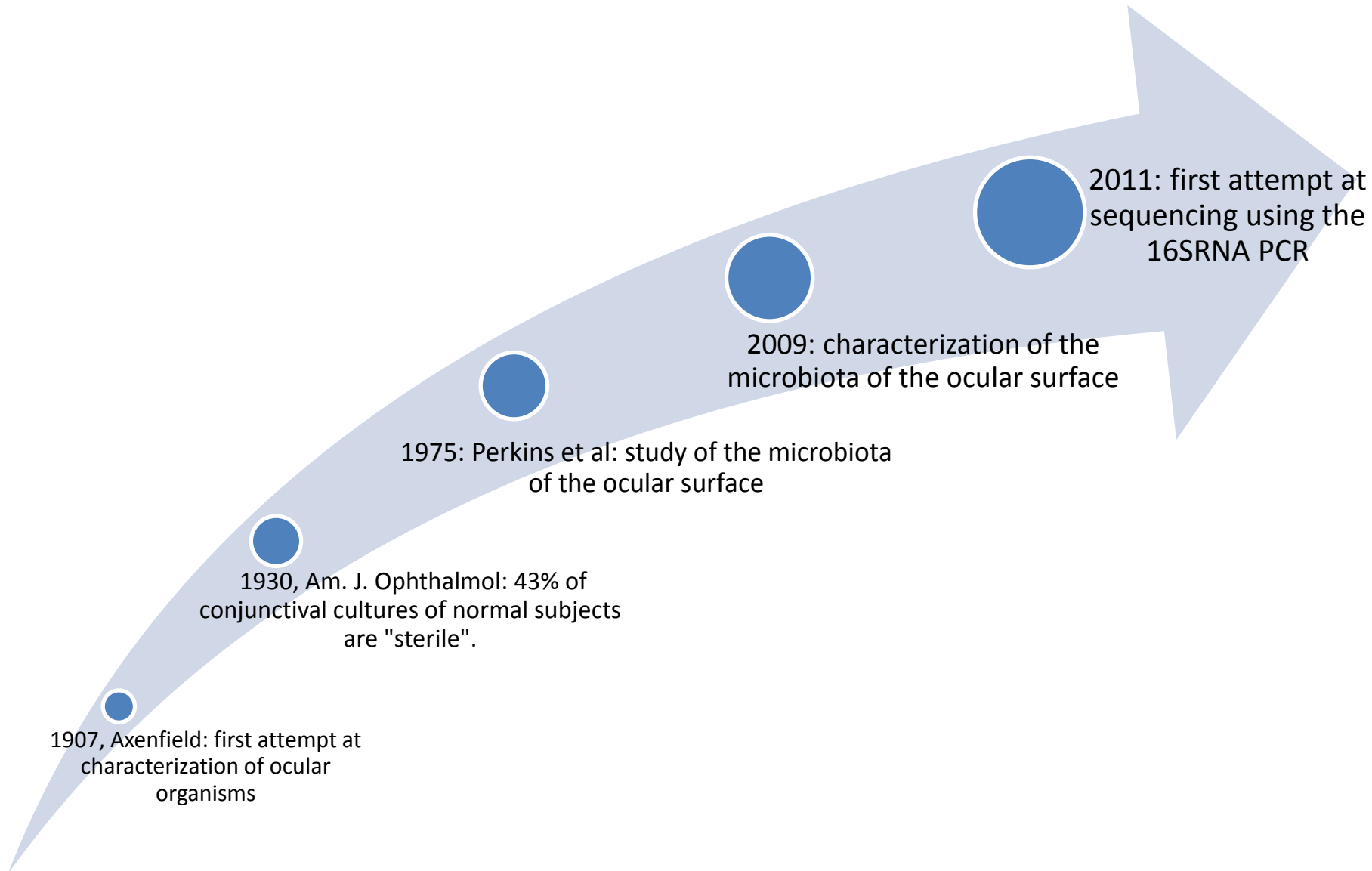
(sterile environment, physically separated from the immune system by the blood retinal barrier):

Anterior and posterior chambers, iris, lens, vitreous cavity, retina, ciliar body, choiroid, intrinsic ocular muscles

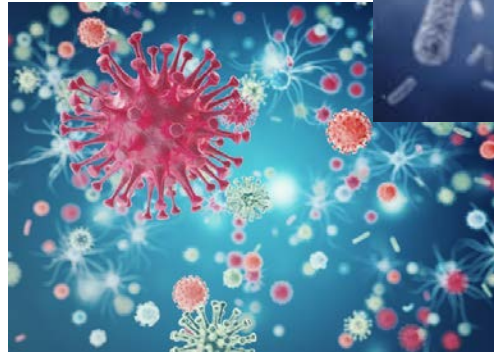
EXTERNAL COMPARTMENT

(exposed to microorganisms in the environment):

Conjunctiva, cornea, sclera, tear film



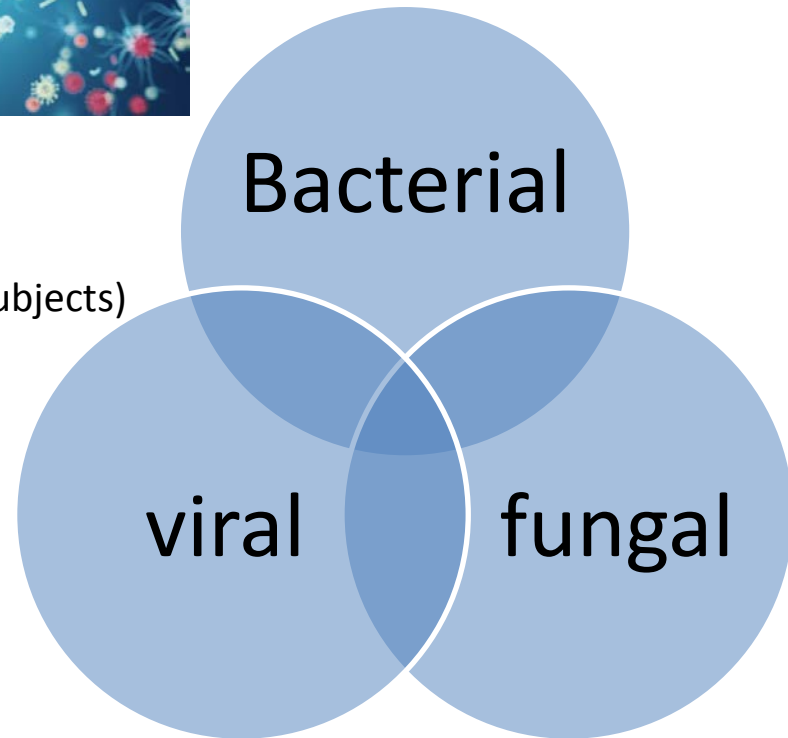
Microbiome:



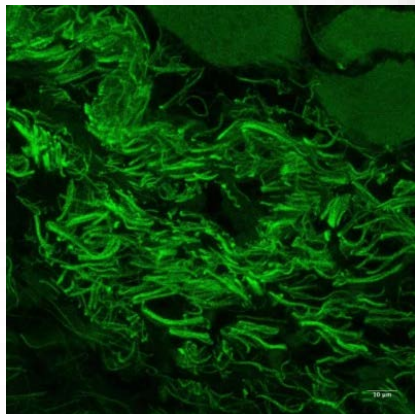
Bacteria

Viruses (herpes simplex in 30% of healthy subjects)

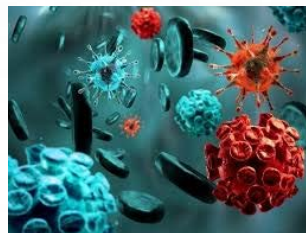
Fungi (30% of children)



The MICROBIOMA, together with epithelial cells of the ocular surface, has a protective role in preventing the colonization of pathogens



The MICROBIOMA of the ocular surface can be altered by some factors of the host and the environment, by pathological conditions, by antibiotics, by infections and by the presence of contact lenses

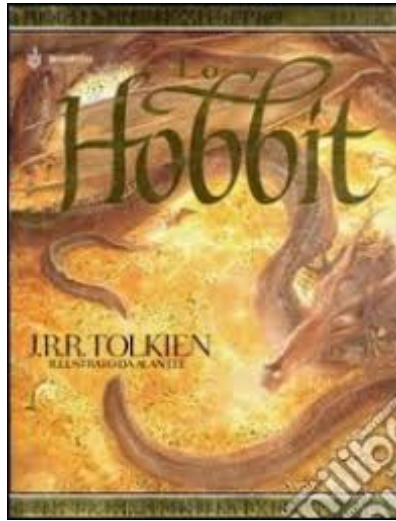




What happens in the donors?

There's Nothing Like Looking, If You Want To Find Something

(J.R.R. TOLKIEN – Lo Hobbit)



What you find depends on what you were looking for

Storage and transport medium

1. Donor corneal tissues



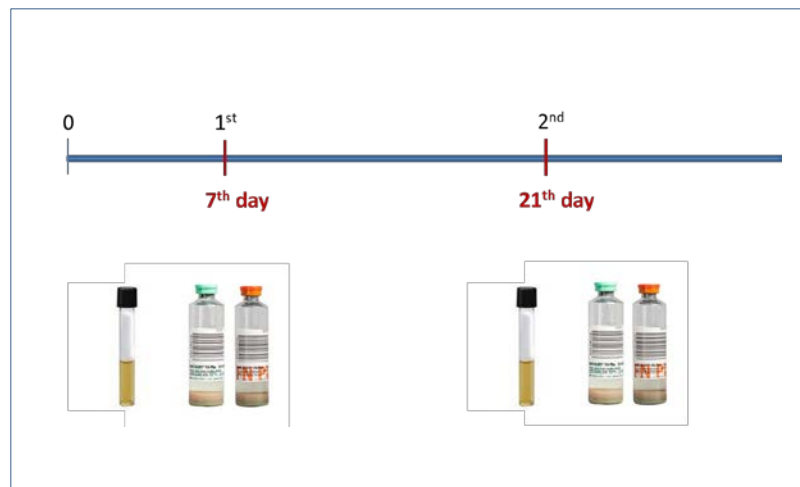
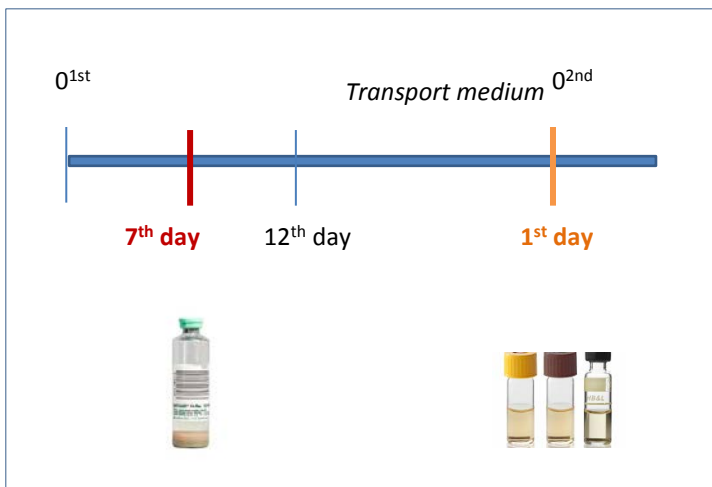
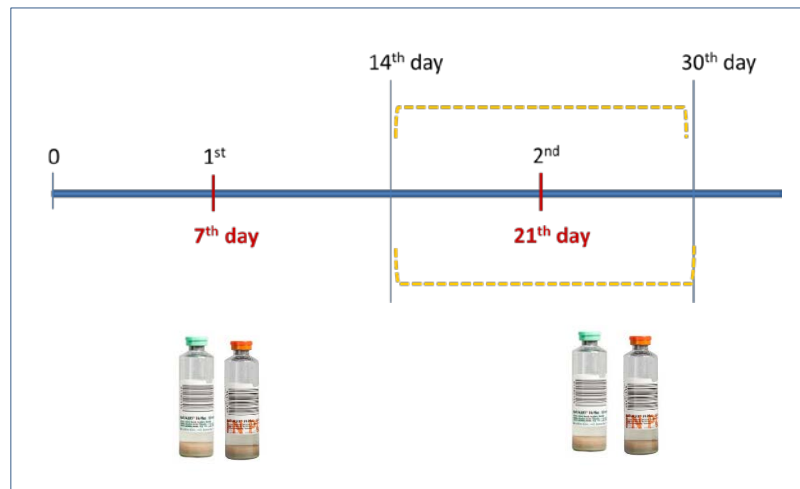
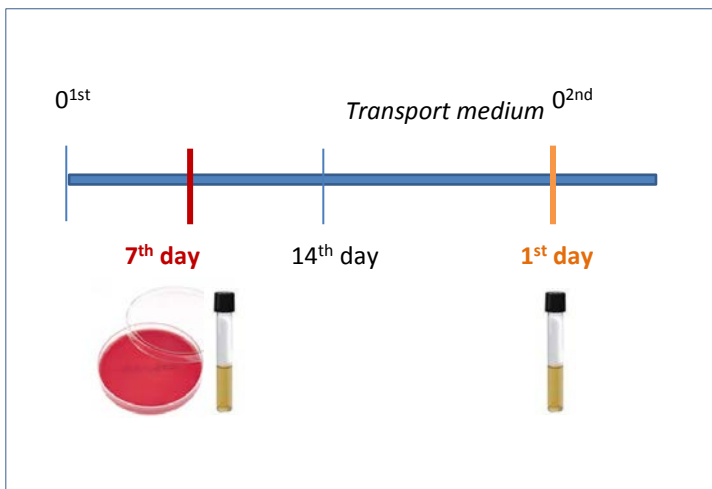
2. Donor scleral tissues



Preserved Sclera

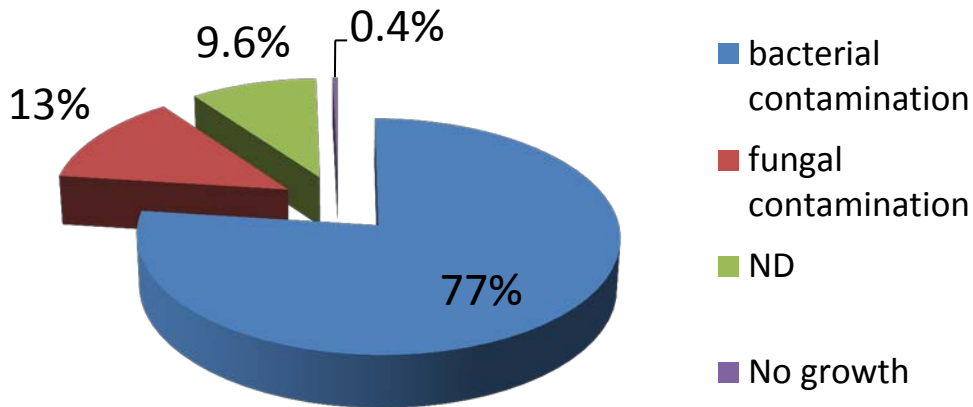
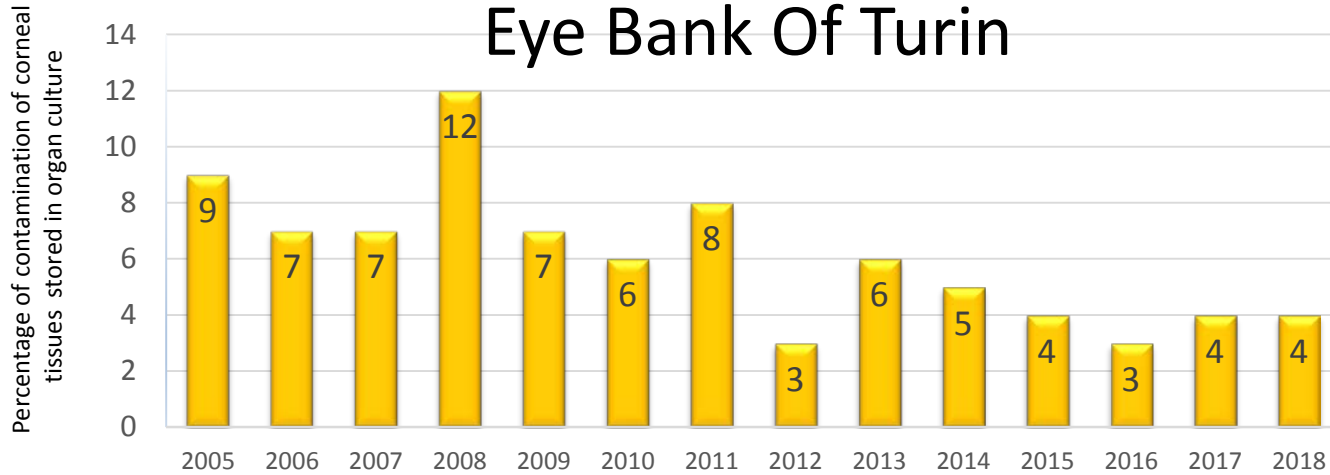


Analysis of ocular tissues

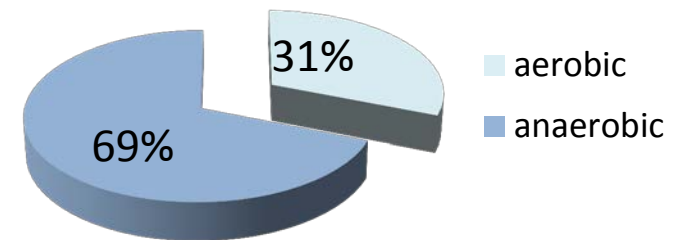


Corneal Tissues Contamination 2005 – 2018

Eye Bank Of Turin



Bacterial Contamination



Contaminations of organ cultured corneas in 2005 - 2018

AEROBIC

GRAM POSITIVE

- 1. Corynebacteriaceae**
(*Corynebacterium spp.*)
- 2. Nocardiaceae**
(*Nocardia otidiscaviarum*)

GRAM NEGATIVE

- 1. Moraxellaceae**
(*Acinetobacter spp*)
- 2. Pseudomonadaceae**
(*Pseudomonas aeruginosa, P. stutzeri*)
- 3. Comamonadaceae**
(*Comamonas acidovorans, Delftia acidovorans*)
- 4. Flavobacteriaceae**
(*Elizabethkingia meningoseptica*)
- 5. Neisseriaceae** (*Neisseria polysaccharea*)
- 6. Xantomonadaceae**
(*Stenotrophomonas maltophilia, Xanthomonas maltophilia*)
- 7. Alcaligenaceae**
(*Alcaligenes xilosidans*)

ANAEROBIC (FACULTATIVE)

GRAM POSITIVE

- 1. Streptococcaceae**
(*Streptococcus viridans*)
- 2. Enterococcaceae**
(*Enterococcus faecali, E. faecium, E. raffinosus*)
- 3. Micrococcaceae**
(*Micrococcus spp*)
- 4. Staphylococcaceae**
(*Staphylococcus aureus, S. epidermidis, S. haemolyticus, S. capitis, S. intermedius, S. xilosus, S. simulans*)
- 5. Paenibacillaceae**
(*Paenibacillus spp*)

GRAM NEGATIVE

- 1. Enterobacteriaceae**
(*Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Morganella morganii*)
- 2. Burkholderiaceae**
(*Burkholderia pickettii*)

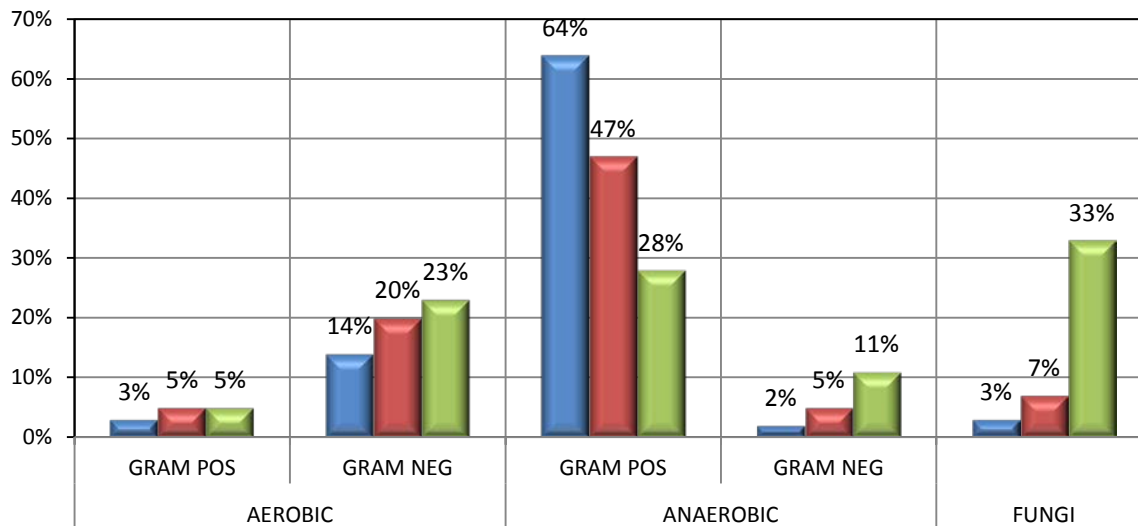
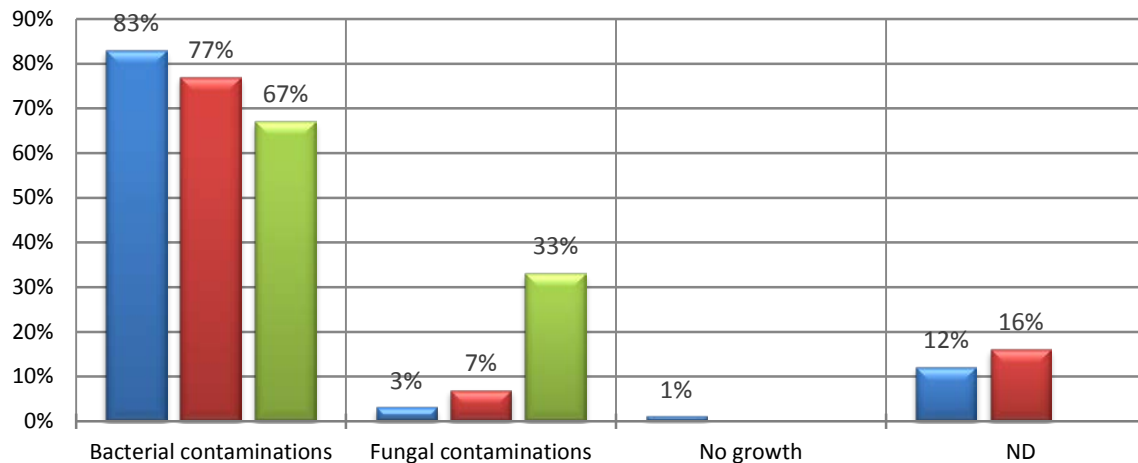
FUNGI

- 1. Saccharomycetaceae**
(*Candida albicans, C. glabrata, C. guilliermondii, C. krusei, C. parapsilosis, C. tropicalis, Torulopsis glabrata*)
- 2. Netriaceae** (*Fusarium*)
- 3. Filamentous fungi**

OTHERS

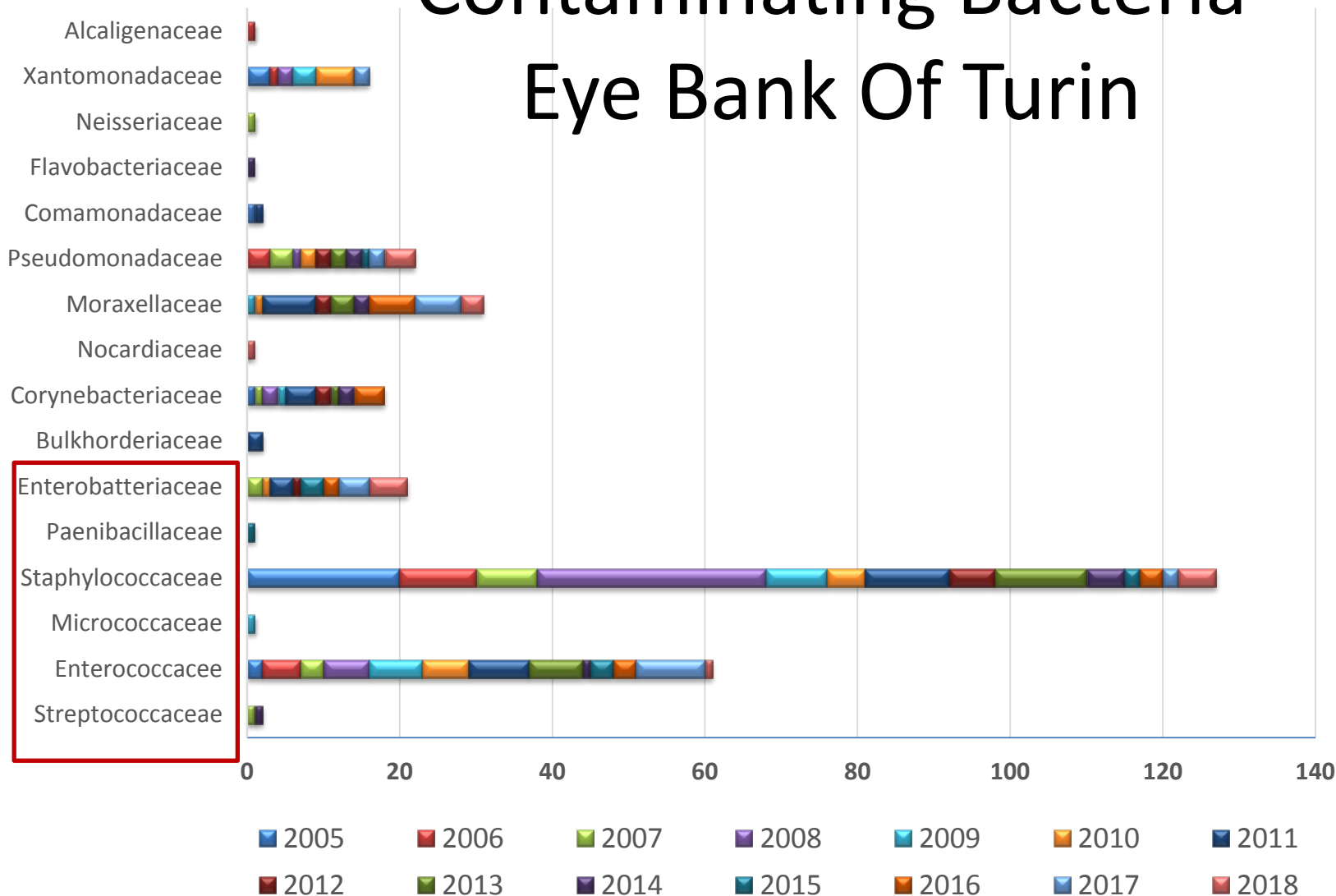
- 1. Polymicrobial flora Gram +**
- 2. Polymicrobial flora Gram -**
- 3. Molds in the medium**
- 4. Change in the medium**
- 5. ND**

■ 2005-2008 ■ 2009-2013 ■ 2014-2018

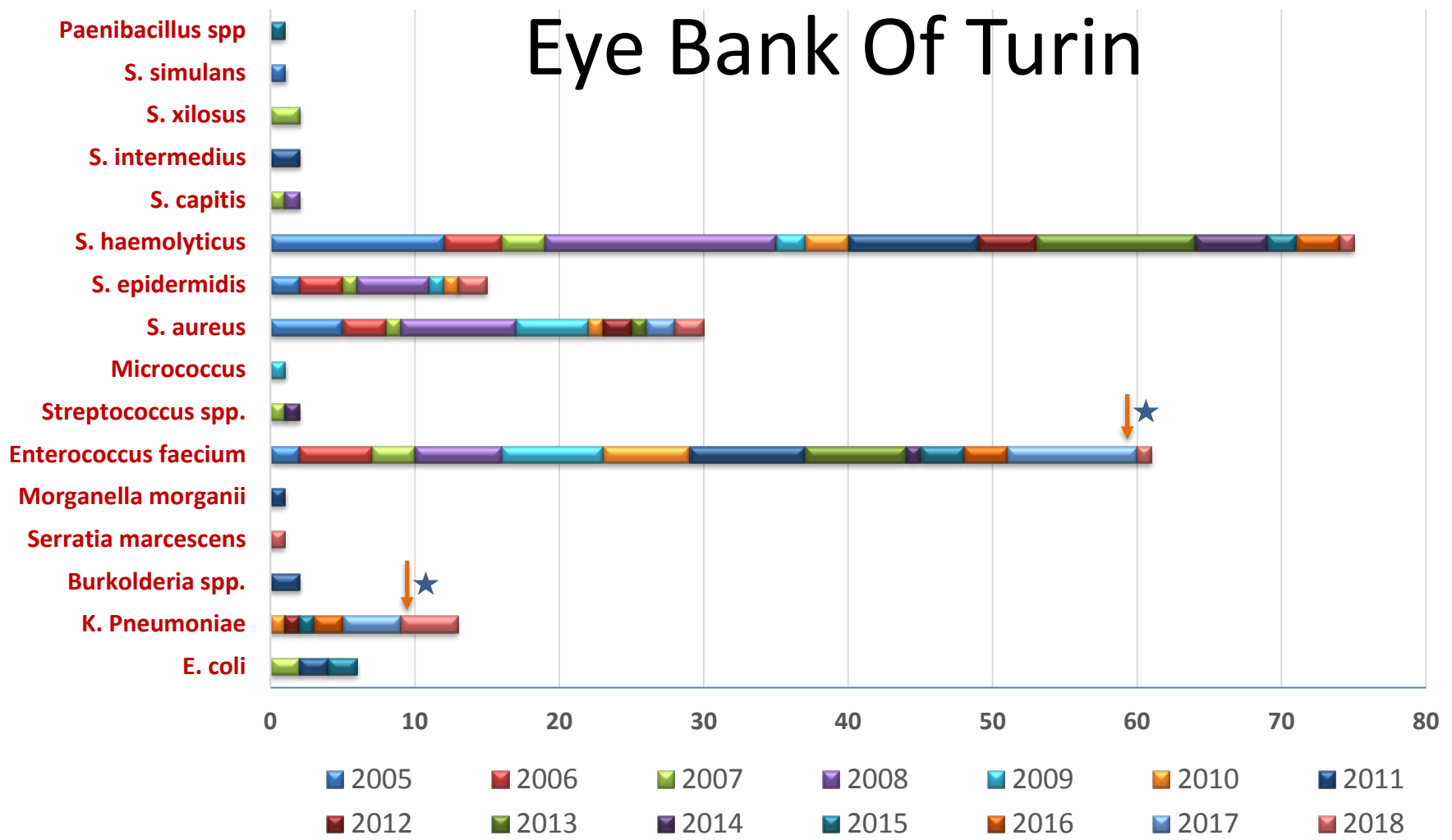


Contaminating Bacteria Eye Bank Of Turin

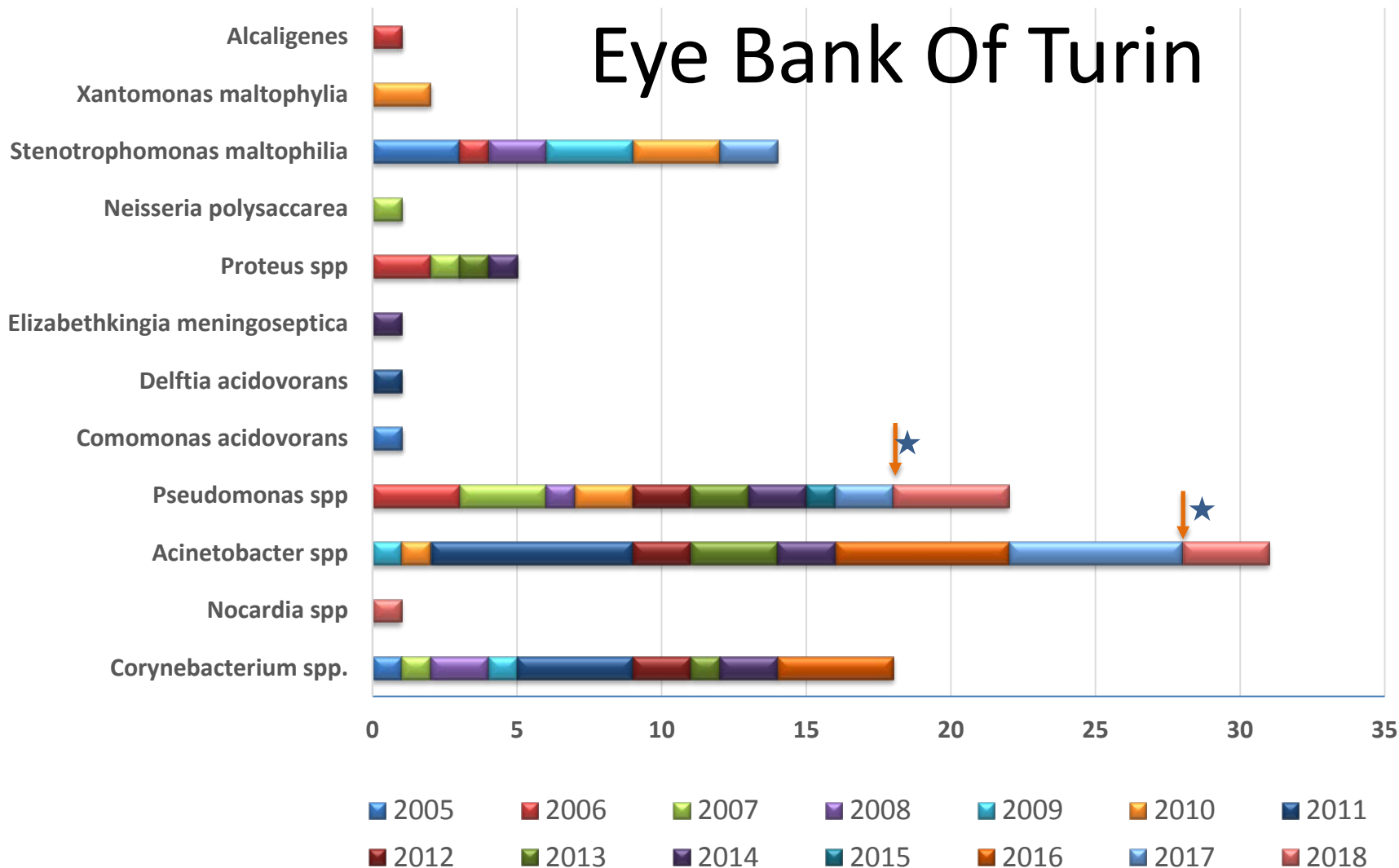
ANAEROBI



Contaminating Anaerobic Bacteria Eye Bank Of Turin

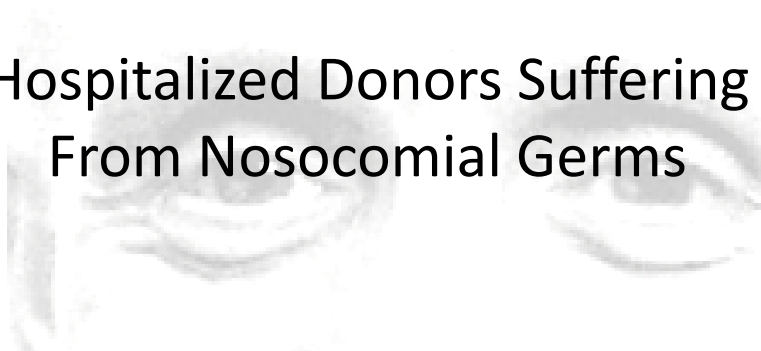


Contaminating Aerobic Bacteria Eye Bank Of Turin

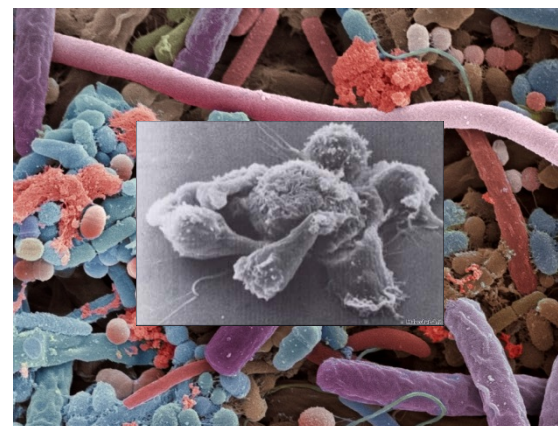




Hospitalized Donors Suffering From Nosocomial Germs



Alterations Of The Immune System That Can Favour Microbial Colonization



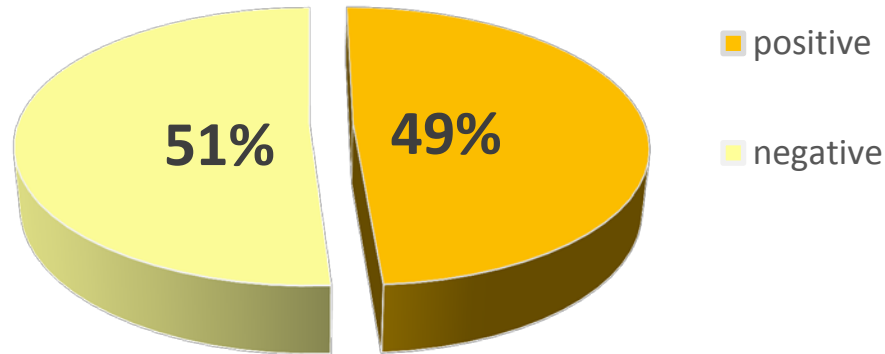


What contaminants are there?

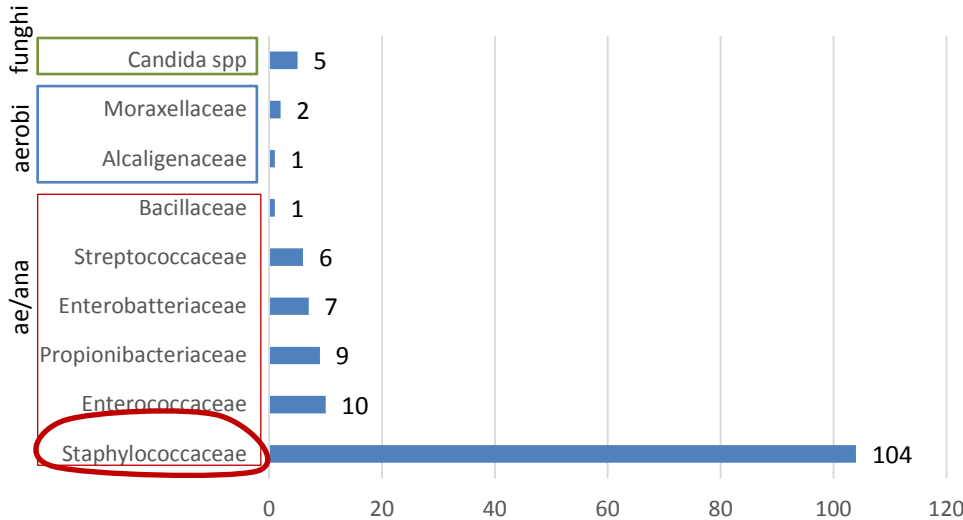


Biopsy from 265
corneal tissues in
Tioglycollate medium

Results of biopsies



RESULTS	POSITIVE	NEGATIVE	TOTAL
NUMBER OF TISSUES	129 (49%)	136 (51%)	265
PREVIOUS INFECTION OF THE DONOR	27 (21%)	30 (22%)	57 (21,5%)



CONTAMINANT	PERCENTAGE
Candida spp.	3,4
Moraxellaceae	1,4
Alcaligenaceae	0,7
Bacillaceae	0,7
Streptococcaceae	4,1
Enterobacteriaceae	4,8
Propionibacteriaceae	6,2
Enterococcaceae	6,9
Staphylococcaceae	71,7

Conclusion 1

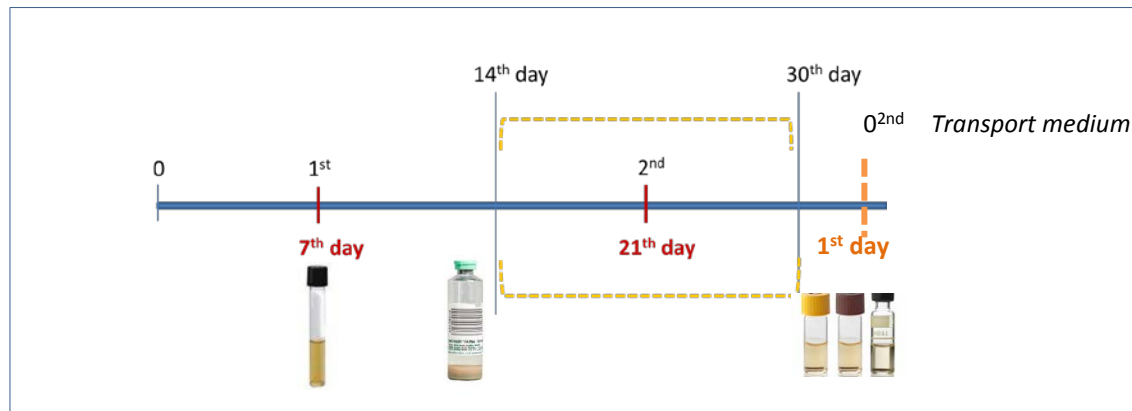
In all the analysed samples, a positive result was detected only in FA Plus or both FA Plus-FN Plus bottles.

Some slow-growing microorganisms can be detected later in the storage medium, as well as *Candida* or *Enterococcus*, two emergent nosocomial pathogens

We have not found microorganisms that grow only on BactAlert FN PLUS



Any anaerobes are sensitive to the antibiotics of the storage medium
The permanence of the in vitro tissue inhibits the growth of anaerobes due to the conditions of the system itself





Document	Technical Guidelines for Ocular Tissue (TGOT)
Revision	10
Page	9 of 11
Operative from	01/02/2019

Technical Guidelines for Ocular Tissue

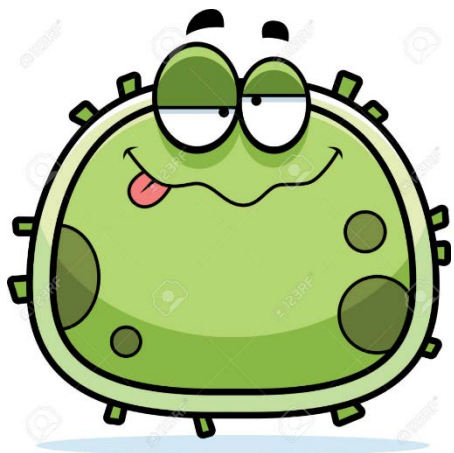
4. Scleral Tissue

4.4 Decontamination And Microbiological Control:

Decontamination in an antibiotic 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 of storage solution and/or piece of tissue before final storage and release for surgery.**

The efficacy of the microbiological testing method should be evaluated and validated.

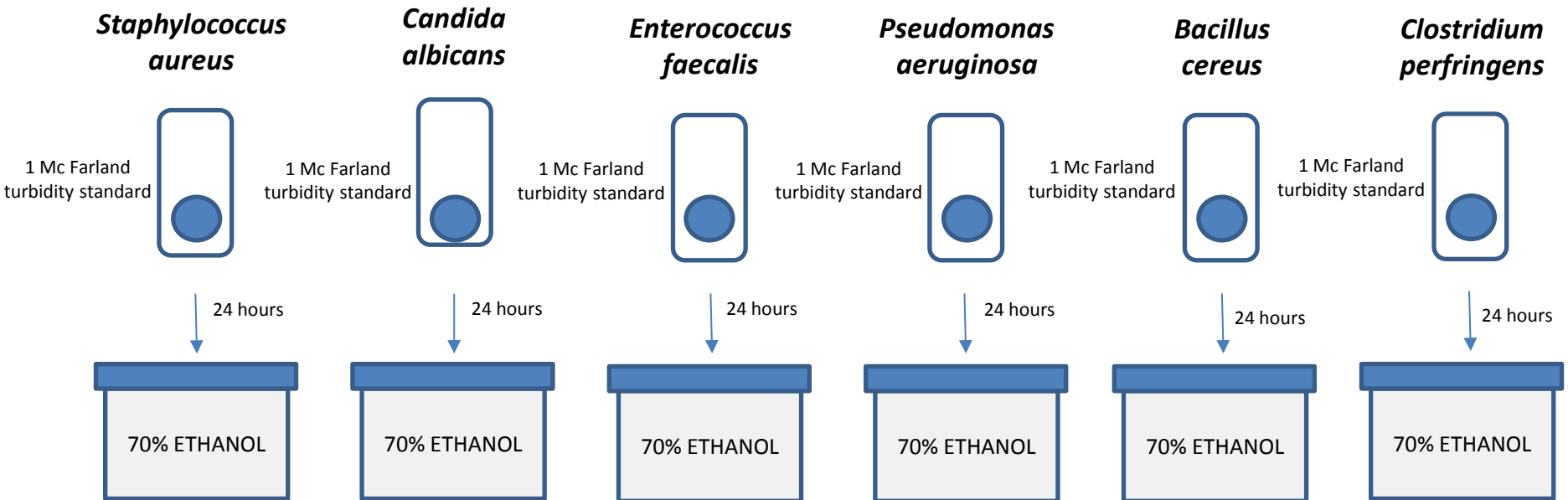
Purpose of the study:



Determine the ability of bacteria to survive
in sclera preserved in 70% ethanol

Materials and Methods

12 fresh human sclerae were submitted to several cuts and 96 scleral patches were obtained. They were divided in 6 groups, and placed in saline solutions containing:



	CONTROLLI POSITIVI			CONTROLLI NEGATIVI		
	FTM	BACT/Alert	HB&L	FTM	BACT/Alert	HB&L
<i>Staphylococcus aureus</i>	POS	POS	POS	NEG	NEG	NEG
<i>Candida albicans</i>	POS	POS	POS	NEG	NEG	NEG
<i>Enterococcus faecalis</i>	POS	POS	POS	NEG	NEG	NEG
<i>Pseudomonas aeruginosa</i>	POS	POS	POS	NEG	NEG	NEG
<i>Bacillus cereus + spore</i>	POS	POS	POS	NEG	NEG	NEG
<i>Clostridium perfringens + spore</i>	POS	POS	/	/	NEG	NEG

All cultures were positive, as expected, to demonstrate that scleral tissues were effectively contaminated by selected bacteria.

Change of alcohol

		2 hours	24 hours	48 hours	72 hours	96 hours	7 days	10 days	14 days	22 days	29 days	36 days	43 days
<i>Staphylococcus aureus</i>	HB&L	-	-	-	-	-	-	-	-	-	-	-	-
	BacT/Alert	-	-	-	-	-	-	-	-	-	-	-	-
	Thyoglicollate	-	-	-	-	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	HB&L	-	-	-	-	-	-	-	-	-	-	-	-
	BacT/Alert	-	-	-	-	-	-	-	-	-	-	-	-
	Thyoglicollate	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterococcus faecalis</i>	HB&L	-	-	-	-	-	-	-	-	-	-	-	-
	BacT/Alert	-	-	-	-	-	-	-	-	-	-	-	-
	Thyoglicollate	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	HB&L	-	-	-	-	-	-	-	-	-	-	-	-
	BacT/Alert	-	-	-	-	-	-	-	-	-	-	-	-
	Thyoglicollate	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bacillus cereus + spores</i>	HB&L	-	-	-	-	-	-	-	-	-	-	-	-
	BacT/Alert	-	-	+	-	-	-	-	-	-	-	-	-
	Thyoglicollate	+	+	+	+	+	+	+	+	+	+	+	+
<i>Clostridium perfringens + spores</i>	HB&L	-	-	-	-	-	-	-	-	-	/	/	/
	BacT/Alert	-	-	+	+	-	+	+	-	+	/	/	/
	Thyoglicollate	+	+	+	-	-	+	+	-	-	-	-	/

None of the bacterial species was isolated from storage solution, neither from biopsy at all periods tested

B. Cereus was recovered from contaminated scleral patches at all immersion period tested, from 2 hours of immersion until the 43nd day.

C. Perfringens was recovered from contaminated scleral patches at 2H, 24H, 48H, until 10D of immersion.

Bacillus cereus + spores			
INCUBATION TIME	FTM	BACT/Alert	HB&L
2H	+	-	-
24H	+	-	-
48H	+	+	-
72H	+	-	-
96H	+	-	-
7 days	+	-	-
10 days	+	-	-
14 days	+	-	-

Bacillus cereus + spores				
INCUBATION TIME	FTM pre-alcohol change	FTM post-alcohol change	BACT/Alert post-alcohol charge	HB&L post-alcohol change
17 days	+	+	-	-
22 days	+	+	-	-
29 days	+	+	-	-
36 days	+	+	-	-
43 days	+	+	-	-

Clostridium perfringens + spores			
INCUBATION TIME	FTM	BACT/Alert	HB&L
2H	+	-	-
24H	+	-	-
48H	+	+	-
72H	-	+	-
96H	-	-	-
7 days	+	+	-
10 days	+	+	-
14 days	-	-	-
17 days	-	+	/
22 days	-	/	/
29 days	-	/	/
36 days	-	/	/



BIOPSY
THIOGLYCOLLATE



3 mL
BACT/ALERT



0,5 mL / each
HB&L
(aerobic, Sabouraud, anaerobic)

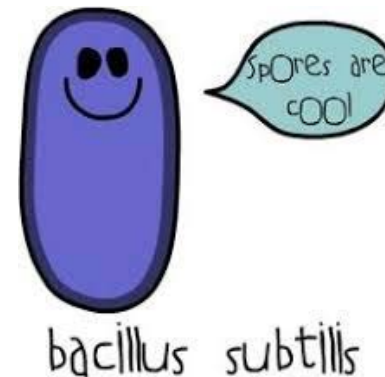
Discussion

Cases of scleral infection are often difficult to resolve, mainly due to problems related to antibiotic penetration. Thus, the prevention of infections by means of the use of optimally preserved tissues is crucial.



Ethanol 70% solution is an excellent medium for preserving human scleral tissue, due to its germicidal activity and easy handling in preparation and storage of human scleral grafts.

Nevertheless, resistant microorganisms can survive in scleral tissues preserved with 70% ethanol.



Conclusion 2

Our study suggests that a routine biopsy of the preserved scleral tissue before its releasing for clinical use is needed and advantageous, as a direct and reliable investigation for tissue contamination.



At the moment, direct inoculation of a fragment of scleral patch in thioglycollate medium seems to be the only method able to detect a possible spore contamination, and this test offers the possibility, beyond reasonable doubt, to transplant a microbiologically safe tissue.



Eye Bank of Turin

Tissue Bank and Biobank

Microbiology and Virology

Department of Laboratory Medicine

AOU Città della Salute e della Scienza di Torino – Molinette Hospital

