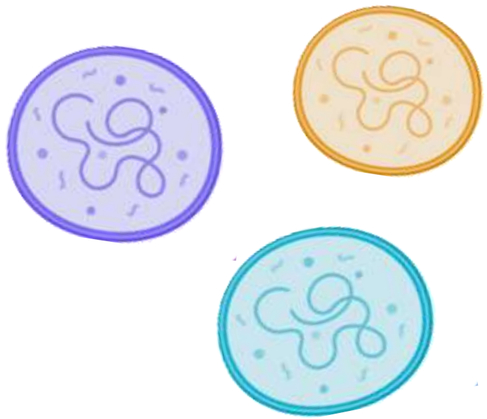
Valutazione del possibile impatto dei micoplasmi sul deterioramento endoteliale



Elena Paveggio

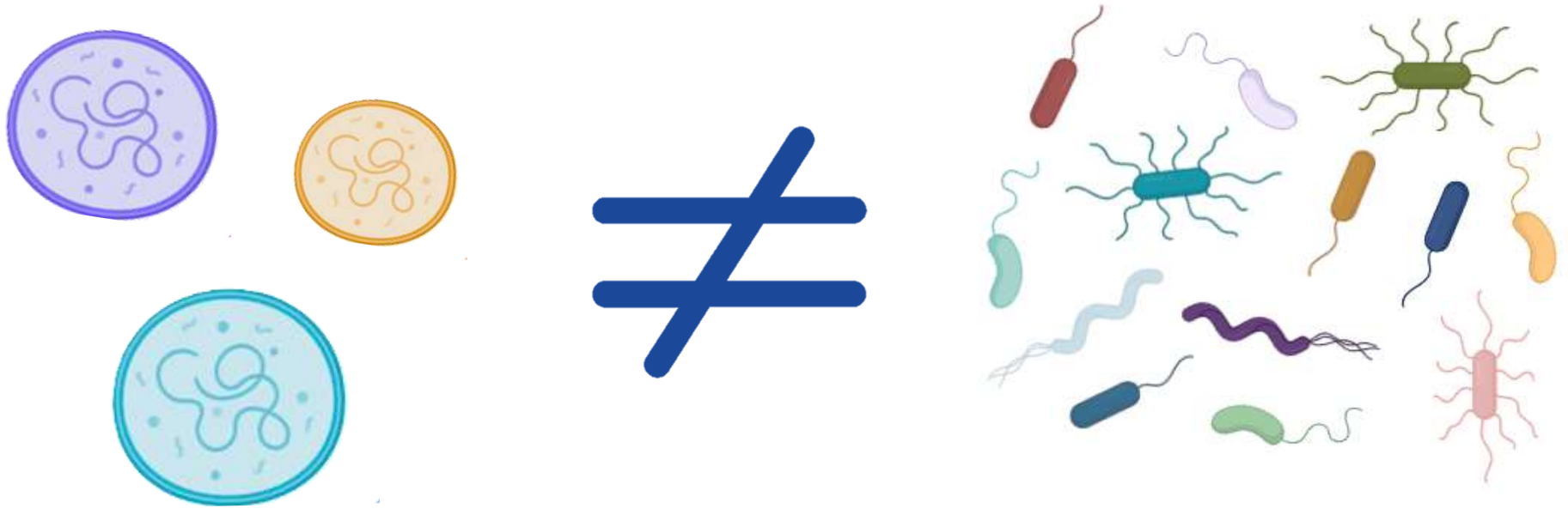
Fondazione Banca degli Occhi del Veneto E.T.S.
Venezia, Italia

EEBA 2026

COI Disclosure

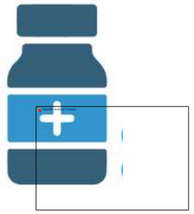
I have no financial conflicts of interest to disclose concerning the presentation.

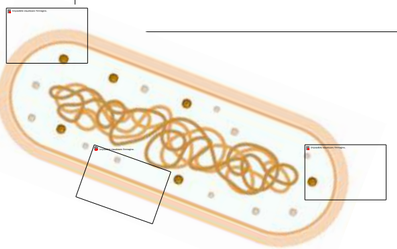
Mycoplasmas peculiarities



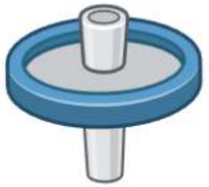
Mycoplasmas peculiarities

Absence of cell wall

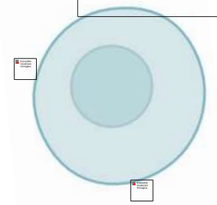




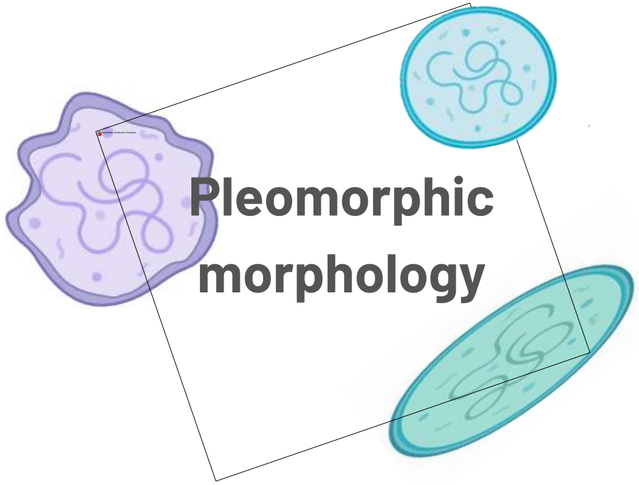
Small size 100-300 nm



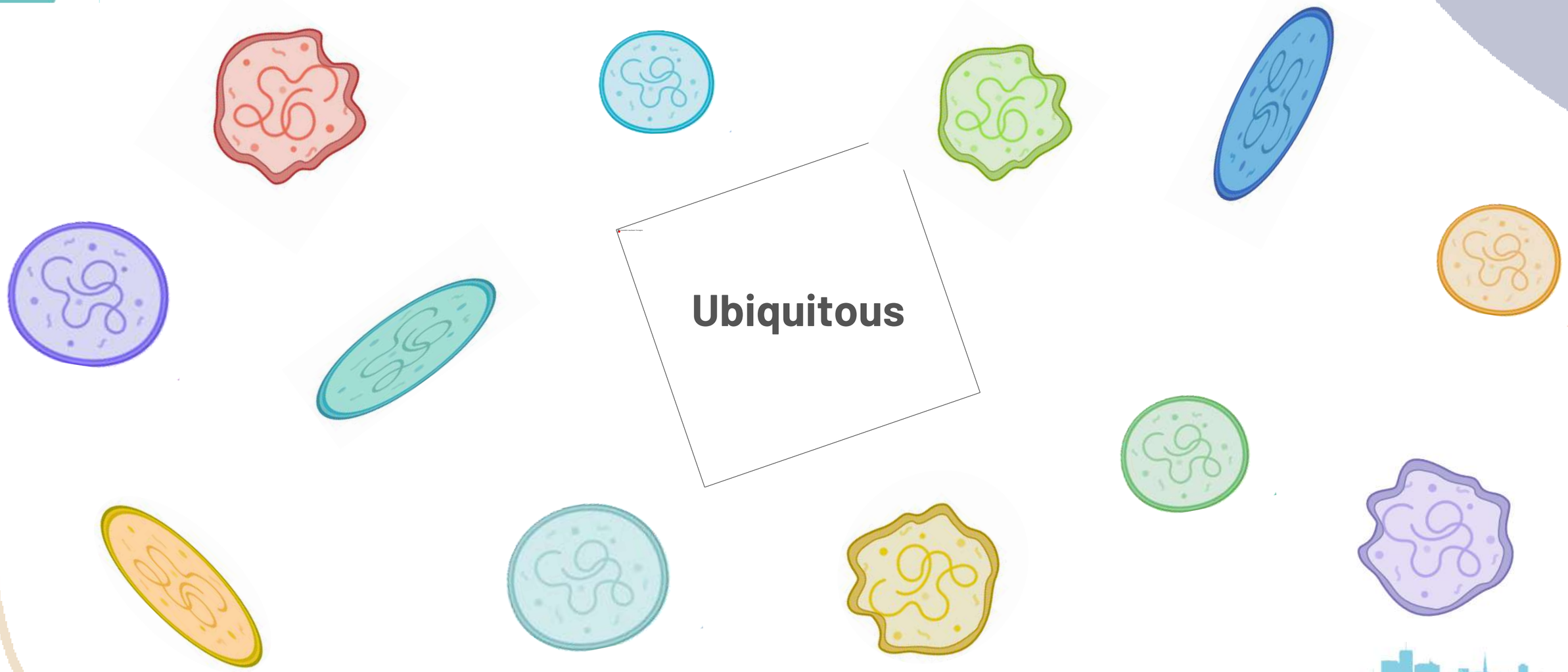
Intra- and extra-cellular infection



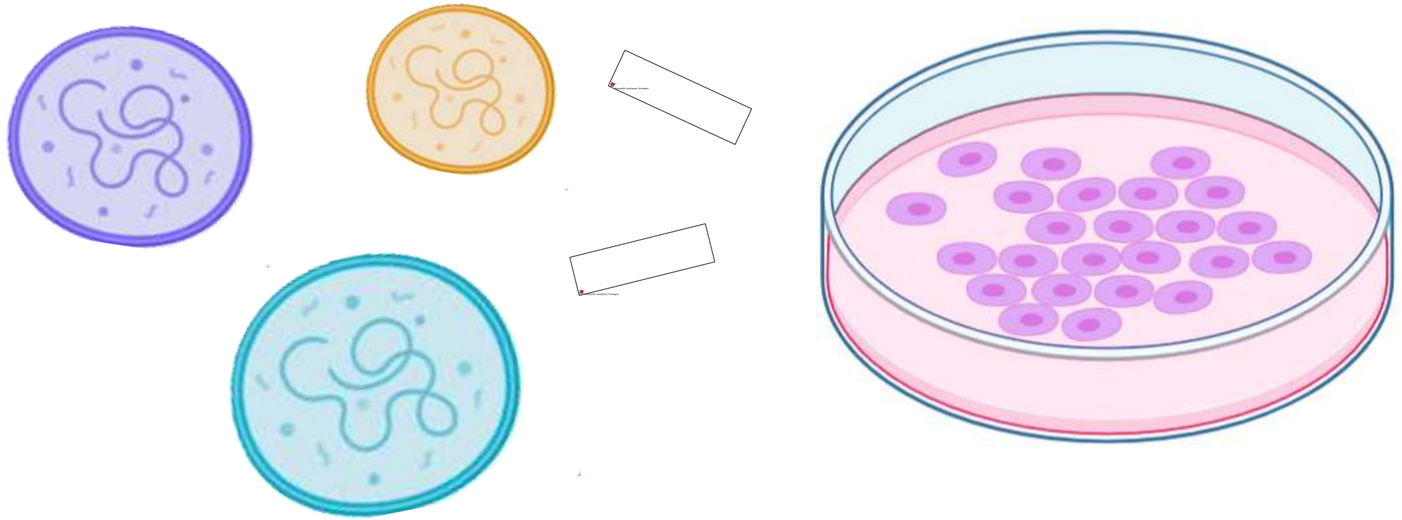
Pleomorphic morphology



Mycoplasmas peculiarities

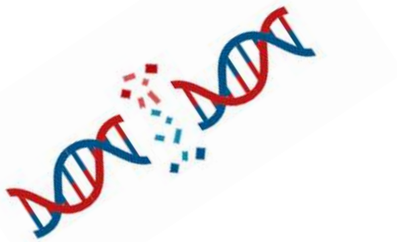


Mycoplasmas effect on cell cultures



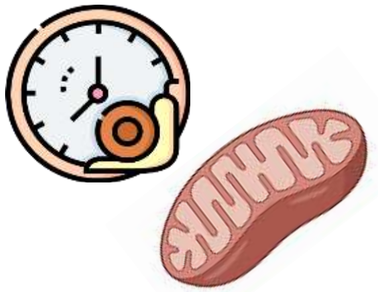
OXIDATIVE STRESS (↑ ROS)

Mycoplasmas effect on cell cultures



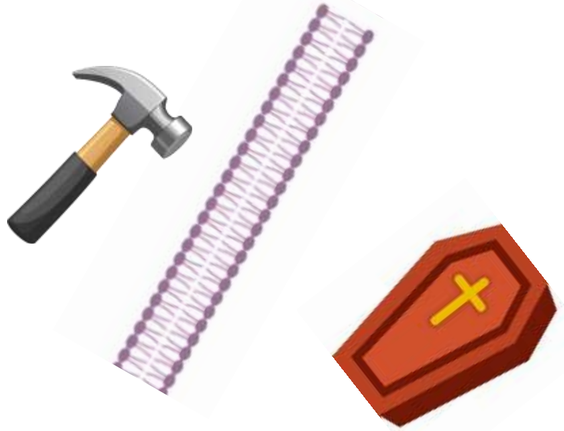
Chromosomal aberrations

Altered cell metabolism



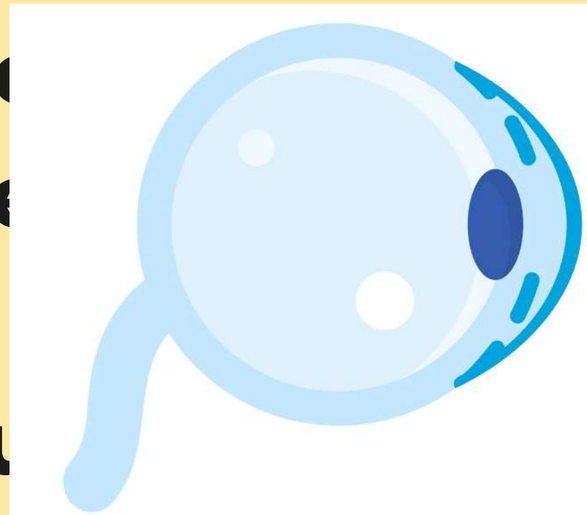
Nutrient depletion

Altered cell morphology



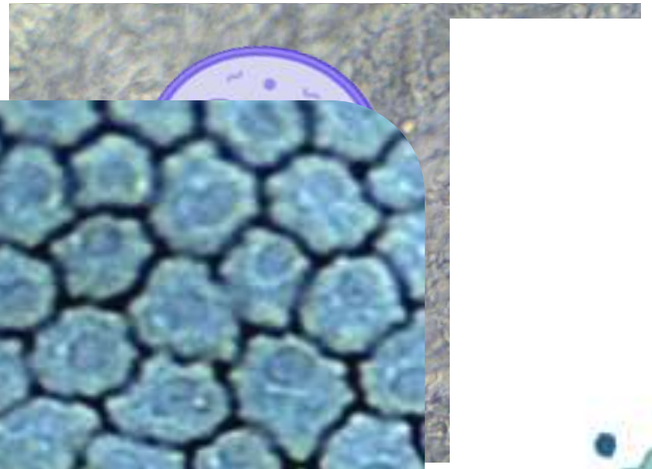
OXIDATIVE STRESS (↑ ROS)

Hypothesis

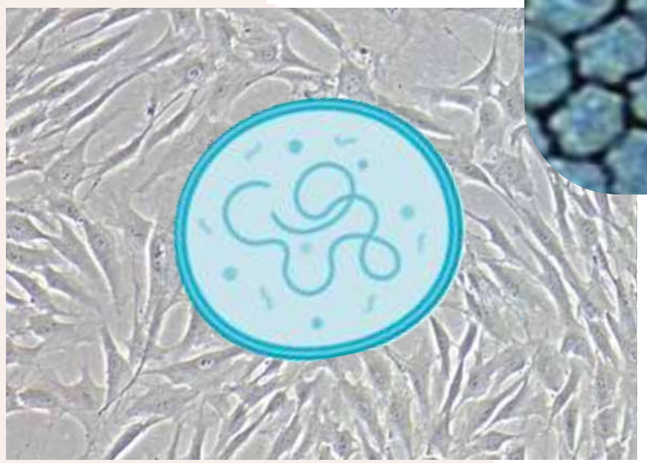


Hypothesis

Altered endothelial morphology

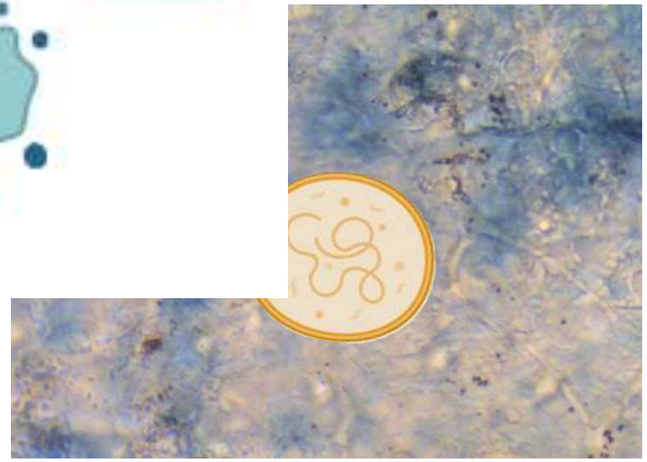


Visible altere keratocyte



responsible f endotelial unsuitability?

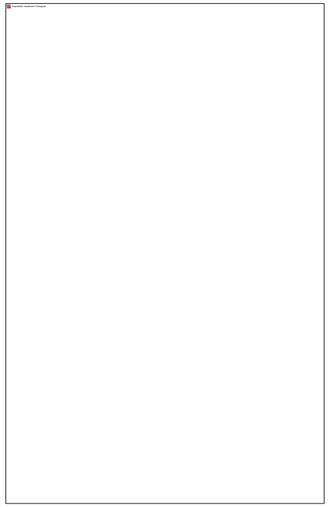
othelial rtality

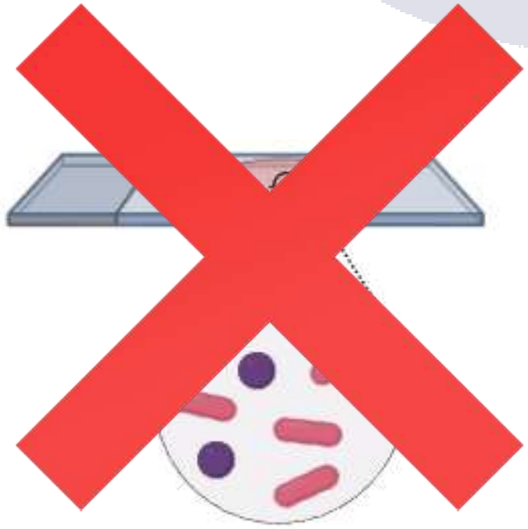


Microbiological tests

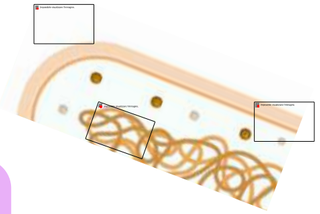
Cultural tests

No turbidity





Gram staining



Too small

Immunochromatographic strip tests

Negative

Positive

- Rapid and Simple

- Isothermal PCR

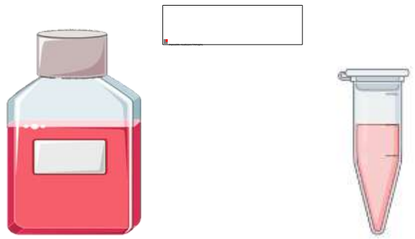
Mycoplasmas

16S rRNA gene

- Sensitive (10-100 CFU/ml)
- No special lab equipment required

Strip tests procedure

1 **Sample preparation**



Resuspended Pellet

However…

Debris

Dirty sample

Epithelial cells

Endothelial cells

Mycoplasmas can be intracellular

Solution?

Strip tests procedure

1

Sample preparation

DMEK preparation

water for injections

2h oscillation

Hypotonic shock

Cell lysys

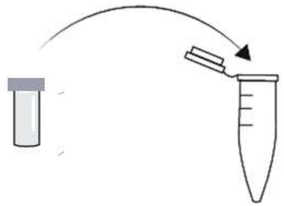
More Mycoplasmas **free in solution**

Unlocking the surgical potential of Descemet's membrane: a standardized decellularization protocol

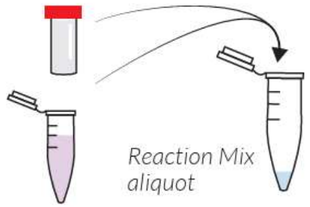
Stefania D'Agostino [1], Elena Daniele [2], Diego Ponzin [2], Stefano Ferrari [2]

Strip tests procedure

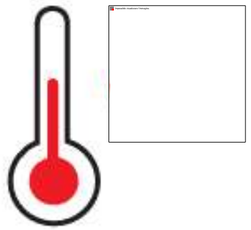
2 **Assay**



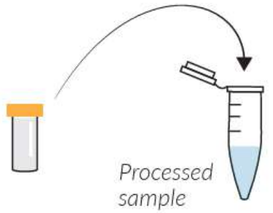
Add 15 µl reaction mix



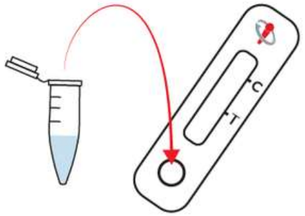
Add:
- 5 µl Reaction buffer
- 5 µl prepared sample



Incubate
65 °C
40 min



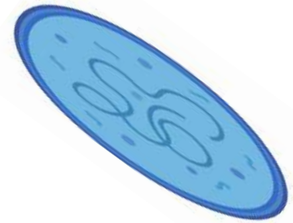
Add migration buffer



Add processed sample to the cassette

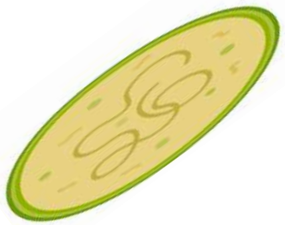
Mycoplasmas
16S rRNA gene Amplification

Preliminary results



Sample	Reactive	Non reactive	Total
Suitable corneas	0	14	14
Unsuitable corneas	6	49	55

In some cases, *Mycoplasmas* seem to be involved in endothelial impairment



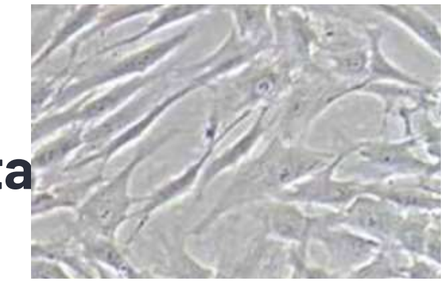
Conclusions

NEXT STEPS?

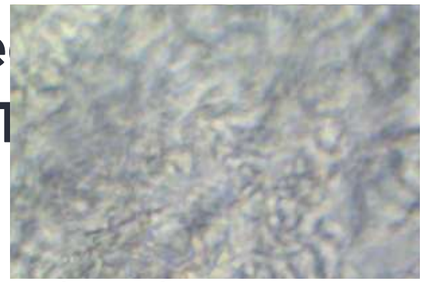
Future perspectives

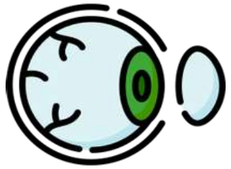
Qualitative test

Quantita



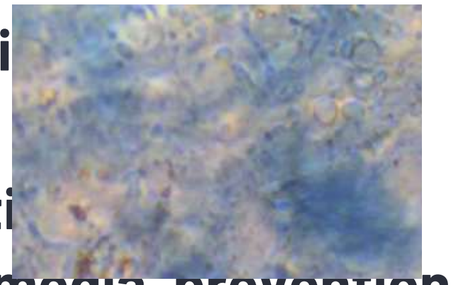
Mass spe
(MALDI-T

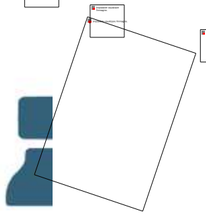




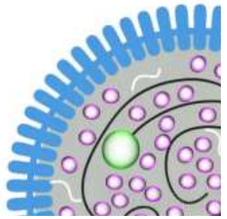
Few corneas to test

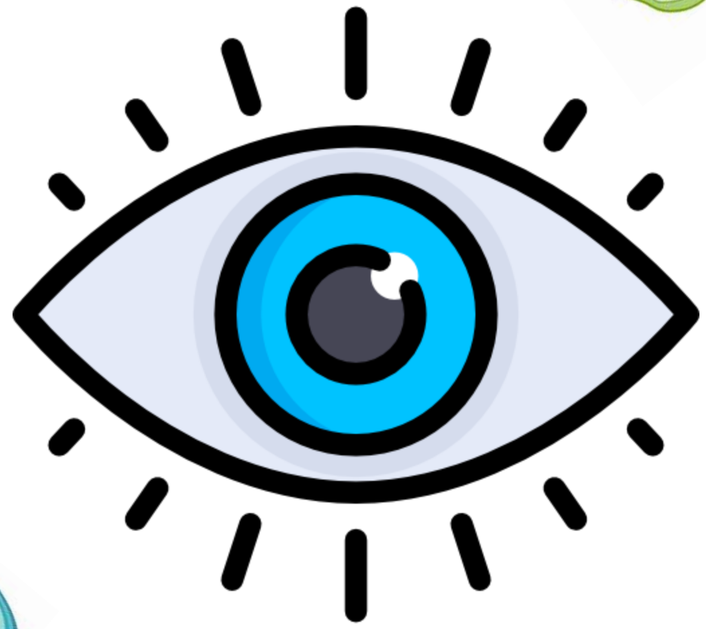
Further validation on more corneas i





Antibioti
culture media, prevention strategies





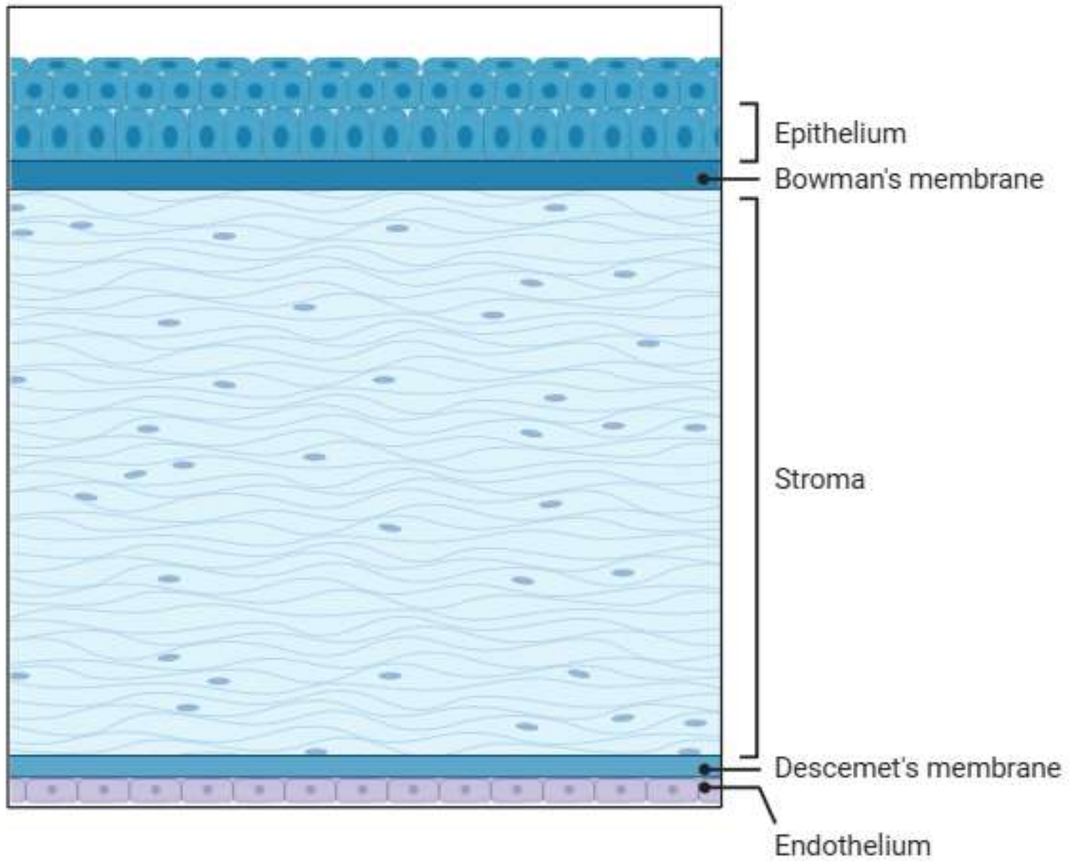
Let's keep an eye up for *Mycoplasmas*!

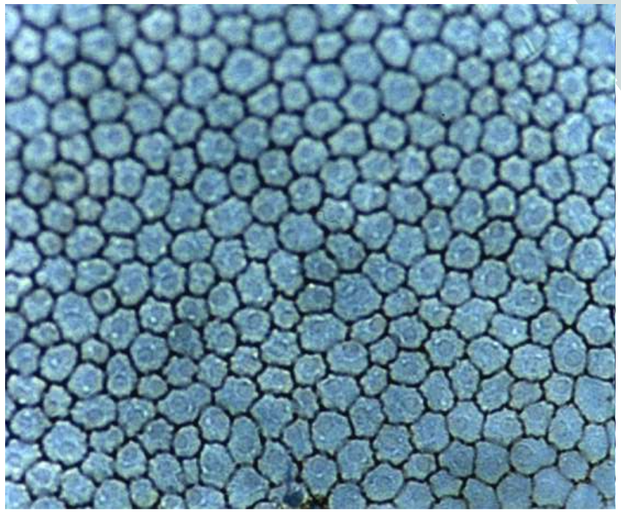
FONDAZIONE BANCA DEGLI OCCHI DEL VENETO - ETS

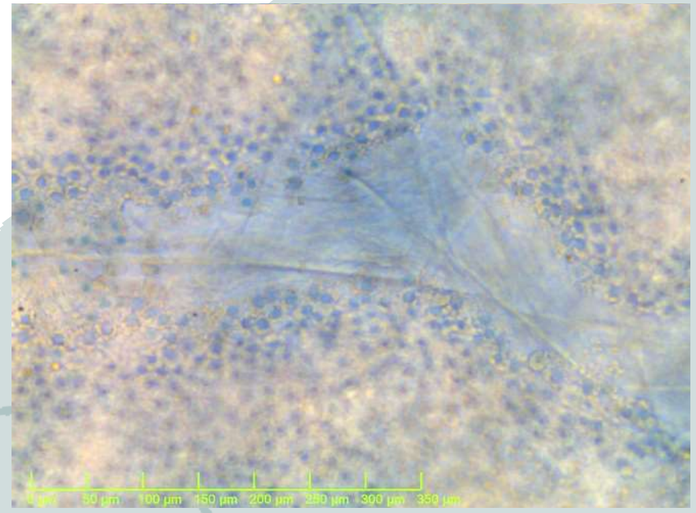
SIBO Società Italiana Banche degli Occhi

thank you for your attention!

Corneal structure

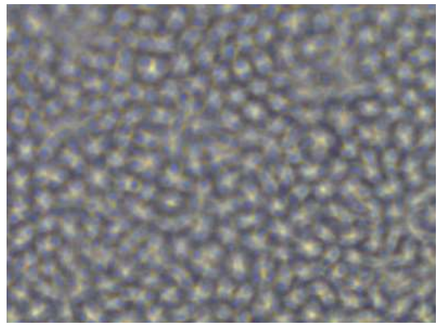


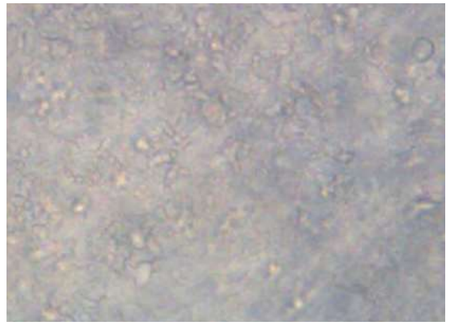




Test samples

- Initial good density but later discarded
- Not assessable endothelial quality
- Markers of endothelial suffering



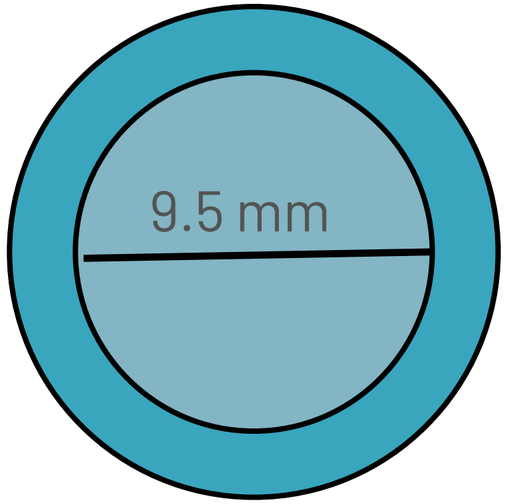


Unexplainable

Corneas unsuitable for transplantation

Negative control samples

- **Residual endothelium from DMEK preparation**



- **Unsuitable donors**
  - Positive blood tests (Hepatitis, HIV…)
  - Lymphoma, myeloma
  - Neurodegenerative diseases
  - Unknown cause of death
  - Unknown/problematic social anamnesis

Practically, elimination of mycoplasma is almost impossible with antibiotics.

- some mycoplasma species can escape from elimination (when internalized in cells)
- *Mycoplasmas* easily develop antibiotic resistance
- Since they lack a cell wall, they are naturally resistant to beta-lactam antibiotics like penicillin
- Long treatment periods (7-14 days)
- The efficiency of antibiotics in elimination of mycoplasmas is between 66% and 85%. These percentages include the cultures in which the growth of eukaryotic cells was inhibited → 3-11% of cells are lost after antibiotic treatment

Antibiotic resistance of mycoplasma from infected cell cultures

Antibiotic	Resistance
Chloramphenicol	30%
Chlortetracycline	11%
Ciprofloxacin	15%
Erythromycin	98%
Gentamicin	80%
Kanamycin	73%
Lincomycin	28%
Neomycin	86%
Spectinomycin	14%
Streptomycin	88%
Tetracycline	14%
Tylosin	21%

Table 5: Effective anti-mycoplasma antibiotics

Brand name	Generic name	Antibiotic category
BM-Cyclin	Tiamulin (BM-Cyclin 1) Minocycline (BM-Cyclin 2)	Macrolide Tetracycline
Ciprobay	Ciprofloxacin	Quinolone
Baytril	Enrofloxacin	Quinolone
Zagam	Sparfloxacin	Quinolone
MRA	unknown	Quinolone
Plasmocin	unknown unknown	Tetracycline? Quinolone

Prevention and Detection of Mycoplasma Contamination in Cell Culture

Laleh Nikfarjam, Ph.D.*, Parvaneh Farzaneh, Ph.D.

Human and Animal Cell Bank, Iranian Biological Resource Center, ACECR, Tehran, Iran

Good afternoon.
Is there a mycoplasma lysis step in the procedure? How can the reagents access and amplify mycoplasmas DNA if the microorganisms are still intact and/or alive?

Thank you so much in advance,
Kind regards,
Elena Paveggio

Dear Elena.

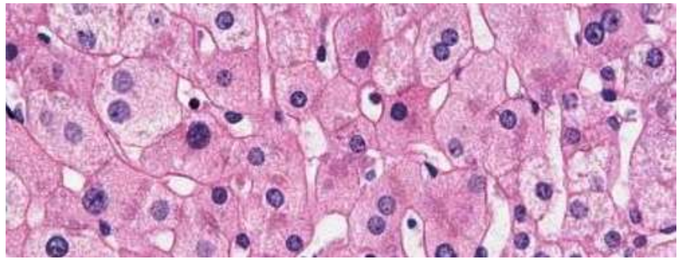
You are right.
With the mycostrip there is no need of a lysing step.
The few mycoplamsa nucleic acids available after the spinning are largely enough for being detected .
Mycostrip is a very sensitive PCR actually.

Histology slides to confirm cell lysis:

We only found nuclei and pieces of cell membrane, but not whole cells.

Hematoxylin-Eosin Stain (H&E):

Hematoxylin: nuclei → violet
Eosin: cytoplasma → pink



Sources of *Mycoplasmas* contamination

- Collection procedures
- Operator-induced contamination
- Laboratory equipment
- Media
- Incubators
- Laminar Hoods

Solutions:

- Better aseptic techniques
- Cultural tests on surfaces and air flows
- Antibiotics (not suggested)
- Routine testing of corneas

Immunochromatographic test principle

- Sample Preparation: DNA is extracted from a sample.
- Amplification: The 16S rRNA gene is amplified using Polymerase Chain Reaction (PCR)
- Labeling: During or after amplification, the 16S rRNA amplicons are labeled with tags, most commonly biotin (for capture) and a hapten like FAM (fluorescein) or Digoxigenin (DIG) (for detection).
- Sample Application: The labeled, amplified DNA is applied to the sample pad of the strip.
- Migration: The sample migrates along the membrane by capillary action.
- Detection: In the conjugate pad, the FAM/DIG-labeled DNA binds to gold nanoparticles (or latex beads) conjugated with anti-FAM/DIG antibodies.
- Capture: The complex moves to the test line, where it is captured by streptavidin immobilized on the membrane.
- Visualization: The accumulation of gold nanoparticles creates a colored line, indicating a positive result for the 16S rRNA gene

.