Microbiological diagnosis of infectious keratitis

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- **Infectious keratitis**
  - Inflammation of underlying corneal stroma caused by replicating organisms
    - Bacteria
    - Viruses
    - Fungi
    - Protozoa
  - Acute presentation – significant pain and distress
  - Rapid initiation of aggressive treatment needed
    - To halt disease process
    - To limit extent of corneal scarring and loss of vision

Sight-threatening condition
Infectious keratitis
Primary pathogens

- **Corneal trauma/ulcer**
  - *P.aeruginosa*
  - *S.aureus*
  - *S.pneumoniae*
  - Viridans group streptococci
  - *Moraxella spp*
  - AFB-rapid growers (*M.ch*)
  - *Nocardia spp*
  - Herpes simplex & Varicella zoster viruses

- **Contact lens associated**
  - Gram negative bacilli including *P.aeruginosa, Serratia spp*
  - *Bacillus spp.*
  - *Acanthamoeba spp*
Microbiological diagnosis

Improve strategies to detect aetiologicaal agents of infectious keratitis

Keys of success:
The best laboratory is not enough !!
Essential close collaboration with micro lab
Pathway to microbiological diagnosis

Urgent Alarming notification

Garbage IN = Garbage OUT
Microbiological diagnosis

- **Cultures**
  - **Bacteria** (aerobic, anaerobic & mycobacteria), fungi
  - (Viruses)
- **Direct microscopy**
  - Gram, Giemsa, ....
  - Immunofluorescence
- **Molecular Biology**
  - Various PCR methods and targets

Minute or scant amount of specimens
Limited viability
Pathway to microbiological diagnosis of ocular infections

Important for the physician to inoculate culture media at bed- or chair-side
Material

- **Instructions (+ training !)**
- **Fresh media**
  - *Schedule to replace expiring media*
    - Blood agar, chocolate agar
    - Thio Broth or TSB
    - Media for anaerobic, fungal and mycobacterial cultures
- **Slides**
- **Specimen collection & Transport devices**
- **Topical anesthetic**
  - (proparacaine hydrochloride 0.5%)
SPECIMEN COLLECTION, TRANSPORT, AND HANDLING
Specimen collection

1. Instillation of 1 or 2 drops of proparacaine HCL
   *Some topical anesthetics and topical dyes: inhibitory to a variety of microorganisms*

2. Specimens from the conjonctiva
   - From both eyes
     - Comparison of microbiological growth from unaffected eye with affected eye
   - Lower tarsal conjonctiva
   - Gentle scraping with a Kimura spatula
     - Or Dacron/Flocked swabs moistened with Thio or TSB
     - Not cotton or calcium alginate swabs
     - To avoid touching eyelid or eyelid margin
3. Corneal scrapings

- From the advancing edge of ulcer
  - By scraping multiple areas of ulceration and suppuration
  - With a Kimura spatula (short firm strokes in one direction)
  - To avoid touching eyelashes
  - 3 to 5 scrapings per cornea
Specimen processing

- **Identification of plates**
- **Inoculation** of each set of scrapings onto appropriate media
  - By successive « C » imprints
  - (Or Zig-zag with swab)
- **Preparation of smears**
  - By applying scrapings in a gentle circular motion over clean identified glass slides
  - Immersion for 5’-10’ in methanol (fixing)
    - Gram, Giemsa, Calcofluor, immunofluorescence, …
Specimen handling and transport

To identify and transfer to the microbiology lab without any delay! (<30’ – 2h)

- Inoculated identified plates
- Collection device with transport media
  - if specimens not inoculated at bedside
- Specific transport media for PCR tests
- Slides for smear staining
- For research of *Acanthamoeba spp.*
  - Call the lab
Interpretation

- **Smears**
  - **Gram, Giemsa**
    - Presence of PMN $\rightarrow$ bacterial infection?
    - Presence of mononuclear cells $\rightarrow$ viral infection
    - Bacteria
  - **Calcofluor white**
    - Fungi; *Acanthamoeba*
  - **Immunofluorescence**
    - Viruses
Interpretation of cultures

- Identification / antimicrobial susceptibility testing of significant organisms

- False positive cultures
  - Contamination of specimen with skin microbiota

- False negative: 35-60%
  - Scanty sample material
  - Delay in performing investigations
  - Prior use of antimicrobial agents or of certain corneal stains (e.g., Rose bengal, ...)
  - Lack of viability in vitro
    → improved by PCR methods (under development)
Acanthamoeba sp

Calcofluor white

Culture track left behind by amebae
Take home messages
Summary

- Various infectious agents
  - Variety of methods
- Minute amount of specimen
  - To target (priority) analysis to perform
- Essential close collaboration with microbiologists
- Crucial quality of pre-analytic issues
  - Short time from collection to inoculation
  - Direct inoculation by ophthalmologist