

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

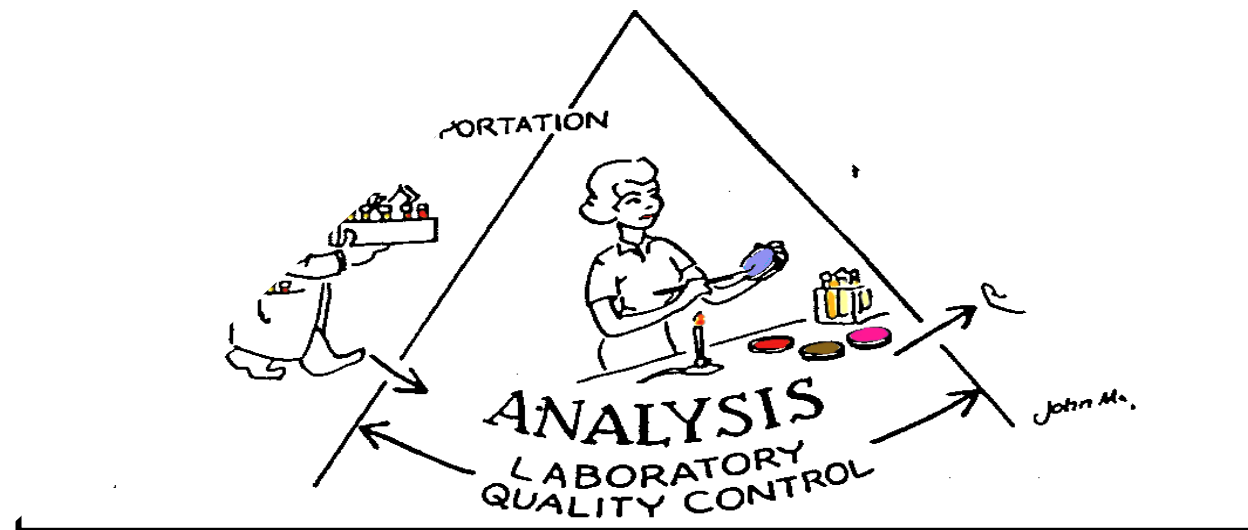
- Gram negative bacilli including *P.aeruginosa*, *Serratia spp*
- *Bacillus spp.*
- *Acanthamoeba spp*

Microbiological diagnosis

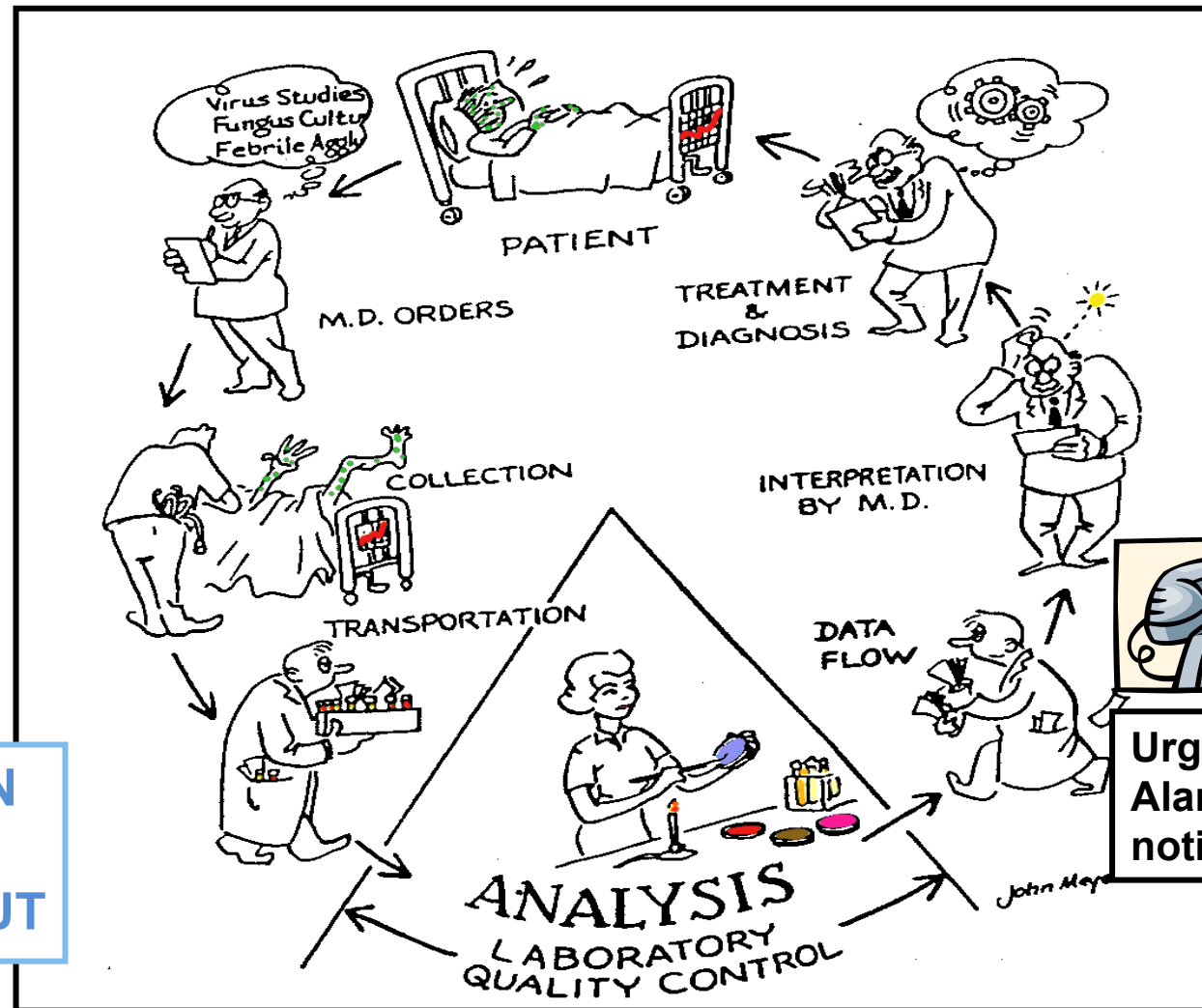
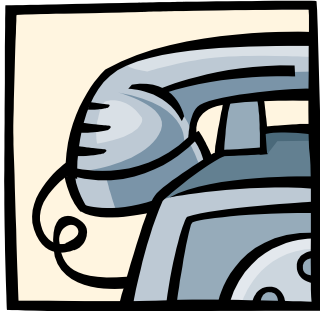
Improve strategies to detect aetiological agents of infectious keratitis

Keys of success:

The best laboratory is not enough !!
Essential close collaboration with micro lab



Pathway to microbiological diagnosis



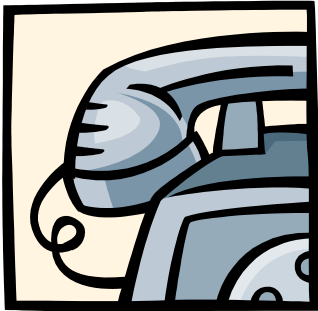
Garbage IN
=
Garbage OUT

Microbiological diagnosis

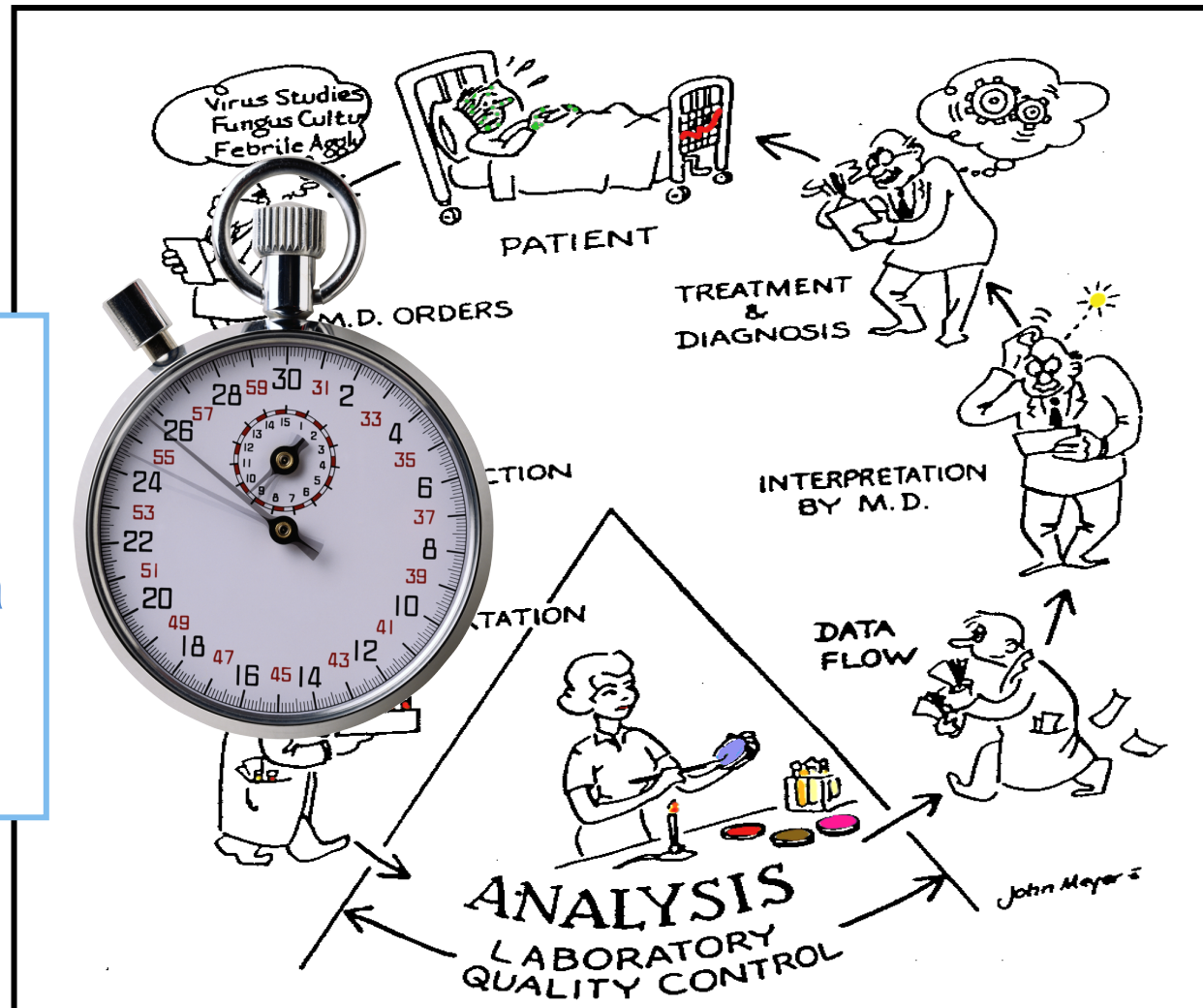
- **Cultures**
 - Bacteria (aerobic, anaerobic & mycobacteria), fungi
 - (Viruses)
- **Direct microscopy**
 - Gram, Giemsa,
 - Immunofluorescence
- **Molecular Biology**
 - Various PCR methods and targets

Minute or scant amount of specimens
Limited viability

Pathway to microbiological diagnosis of ocular infections



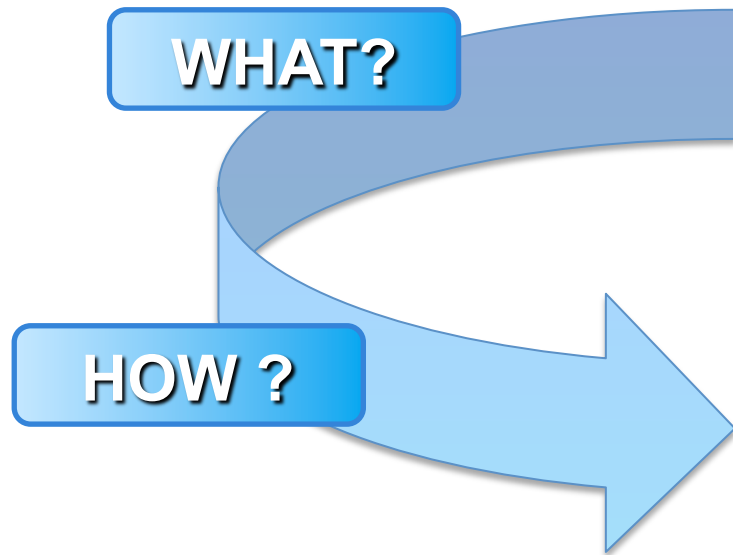
Important for the physician to inoculate culture media at bed- or chair-side



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 conjunctiva

- From both eyes
 - Comparison of microbiological growth from unaffected eye with affected eye
- Lower tarsal conjunctiva
- 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

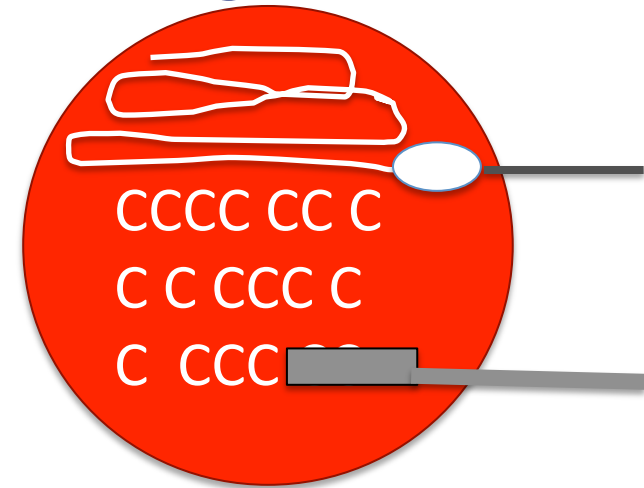
Specimen collection

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

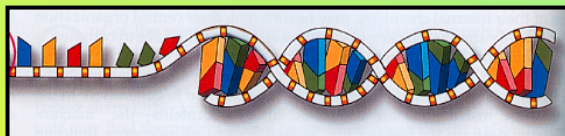
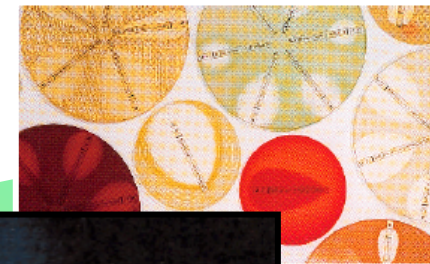
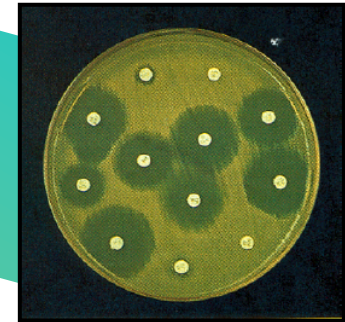
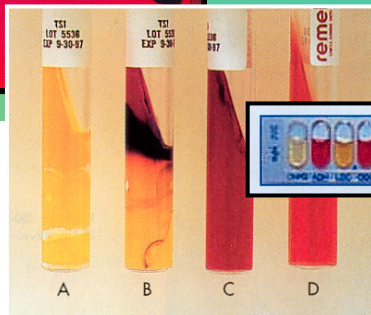
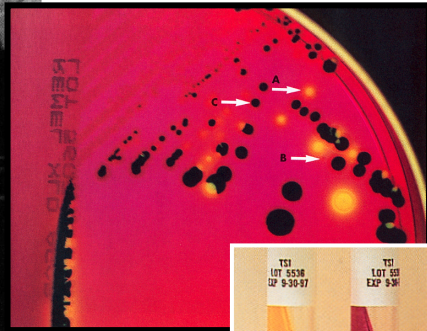
- Identification of plates
- Inoculation of each set of scrapings onto appropriate media
 - By successive « C » imprints
 - (Or Zig-zag with swab)
- Preparation of smears
 - By applying scrapings in a gentle circular motion over clean identified glass slides
 - Immersion for 5'-10' in methanol (fixing)
 - *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 → bacterial infection?
 - Presence of mononuclear cells → viral infection
 - Bacteria
 - **Calcofluor white**
 - Fungi; *Acanthamoeba*
 - **Immunofluorescence**
 - Viruses

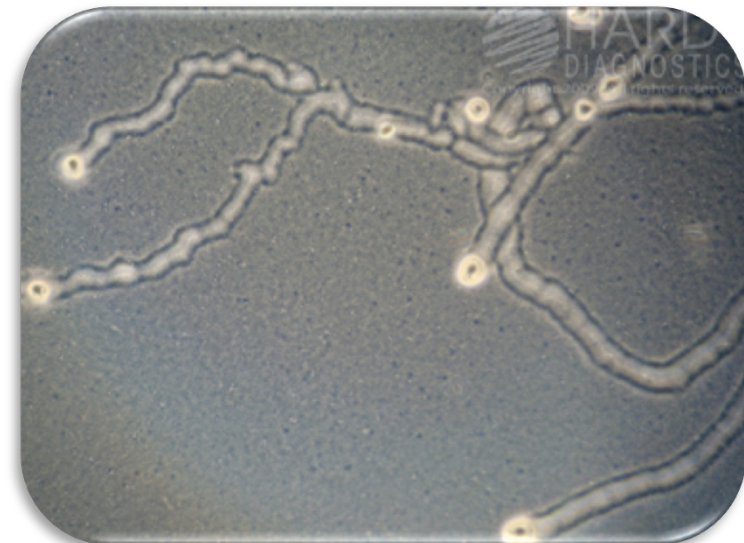
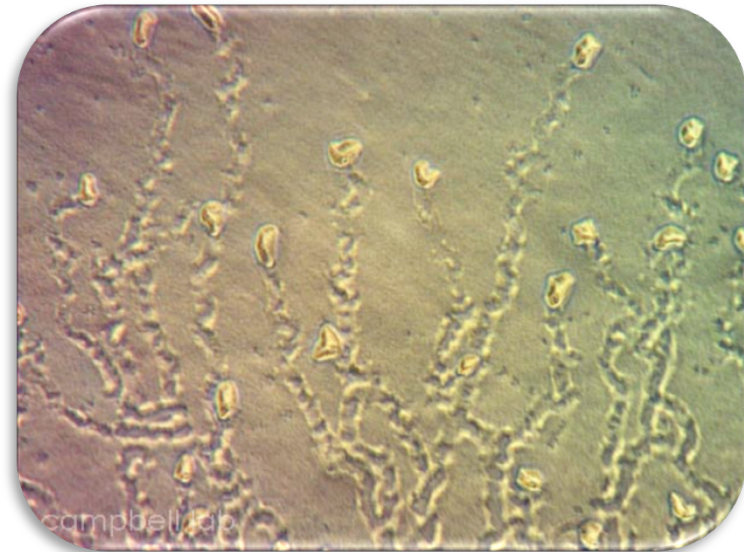
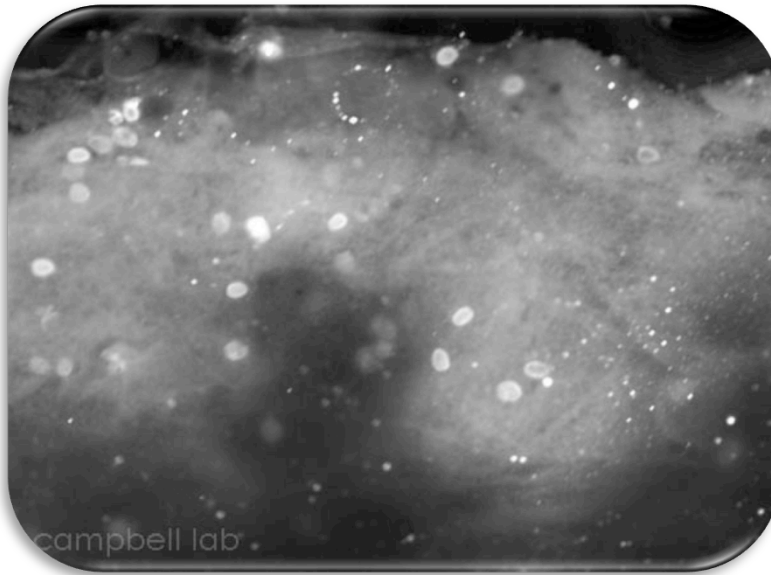
Interpretation of cultures

- **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 (eg. Rose bengal, ...)
 - Lack of viability in vitro

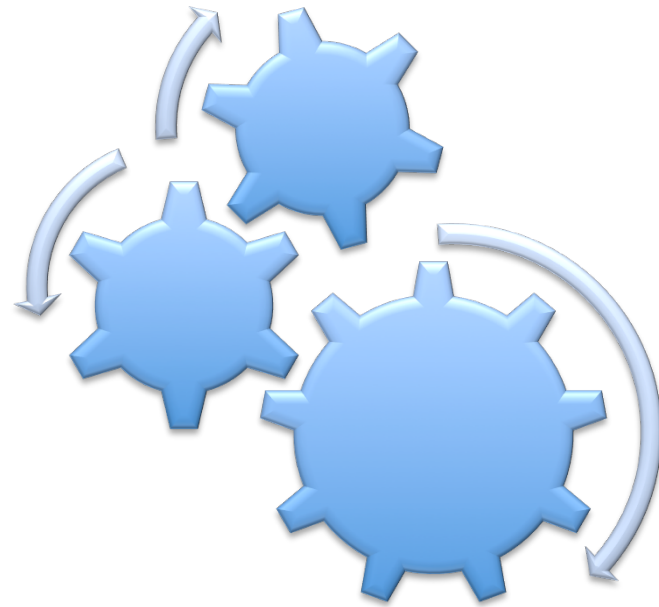
→ improved by PCR methods (under development)

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**