Direct diagnostics – microscopy II

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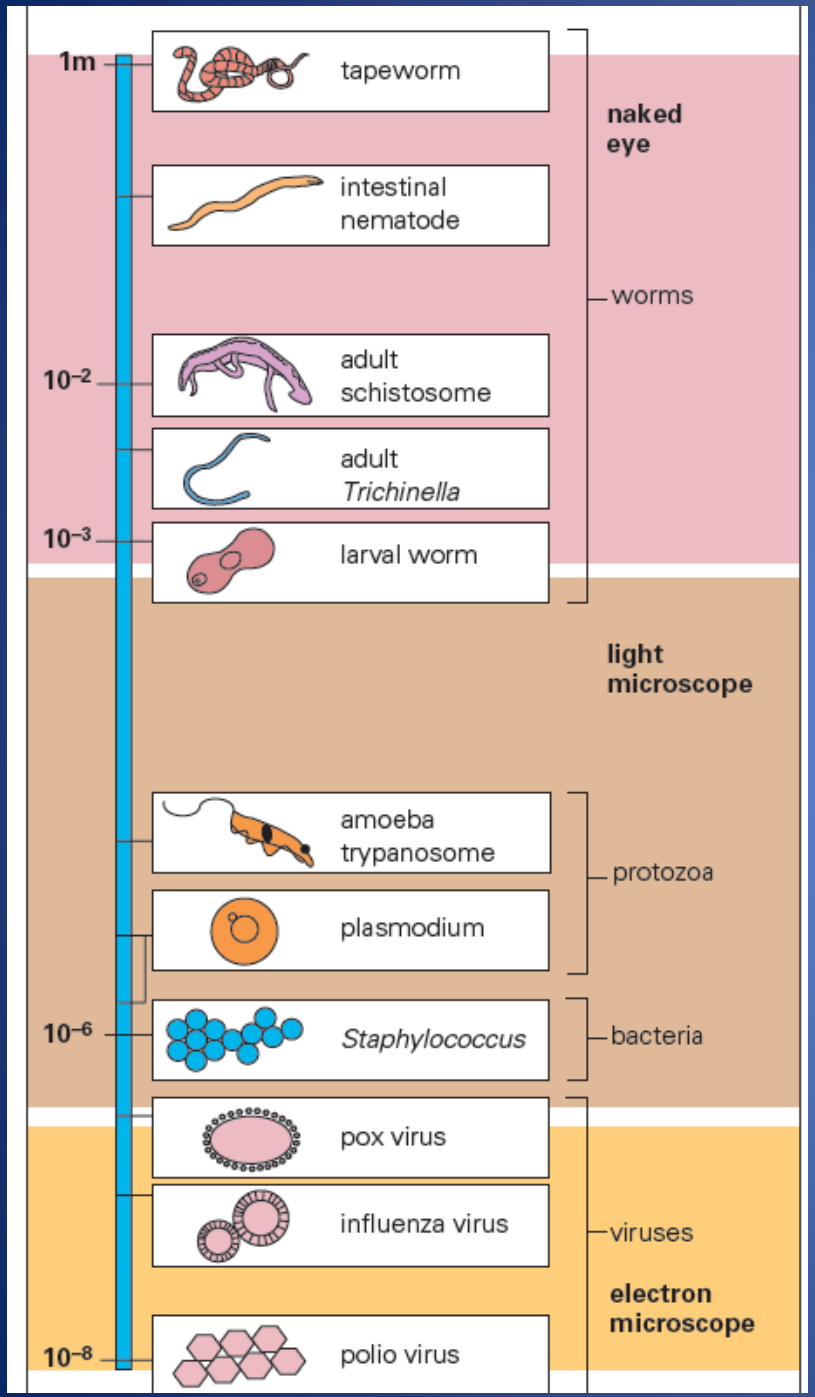
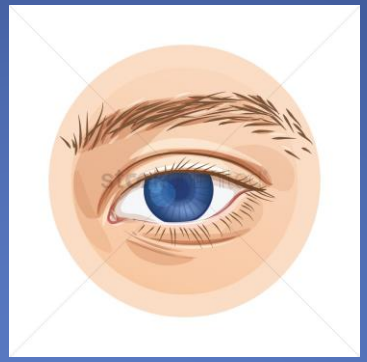
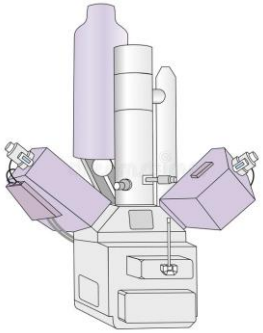
Size of human patogens





Light microscopy
Direct observation of bacteria, yeasts, or protozoa in the sample

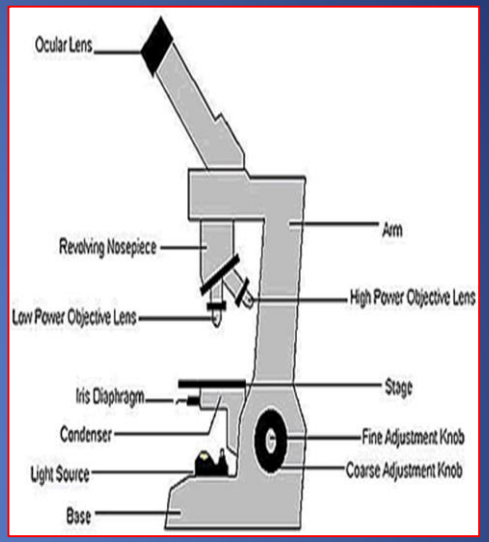
Native x stained sample



Electron microscope

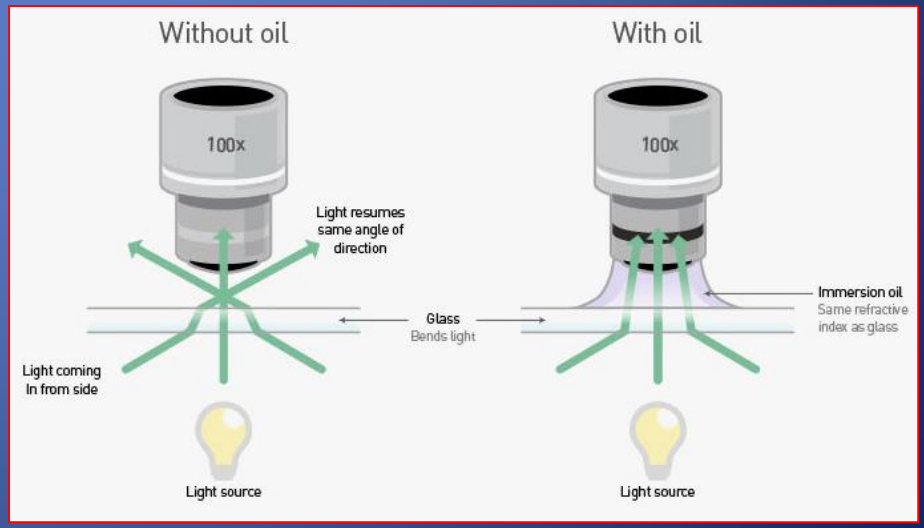
Light microscopy

- Direct observation of microorganisms: yeasts, fungi, parasites, **bacteria!**



- Native (wet mount) slide – 200-400x magnification (protozoa, fungi)

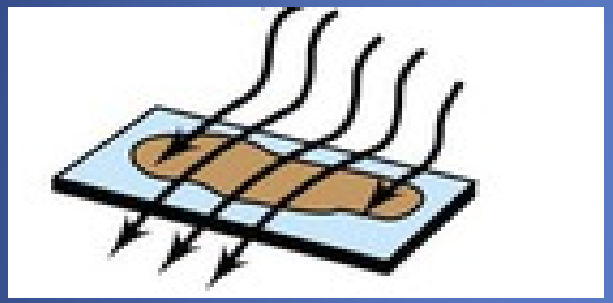
- Imersion oil – up to 1000x magnific. (bacteria)



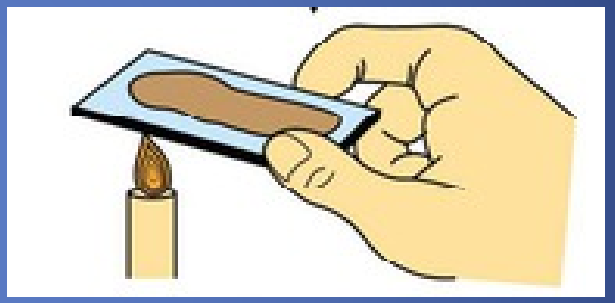
Remember

Before you start:

1. Dry

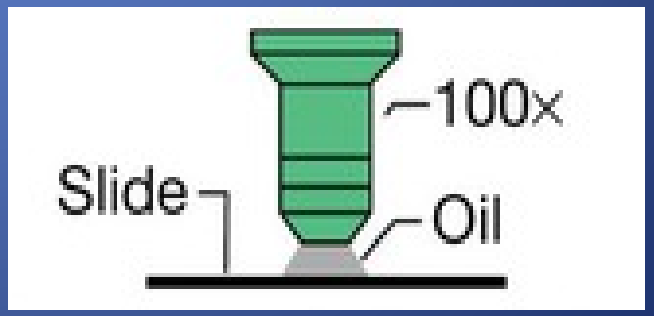


2. Heat fix

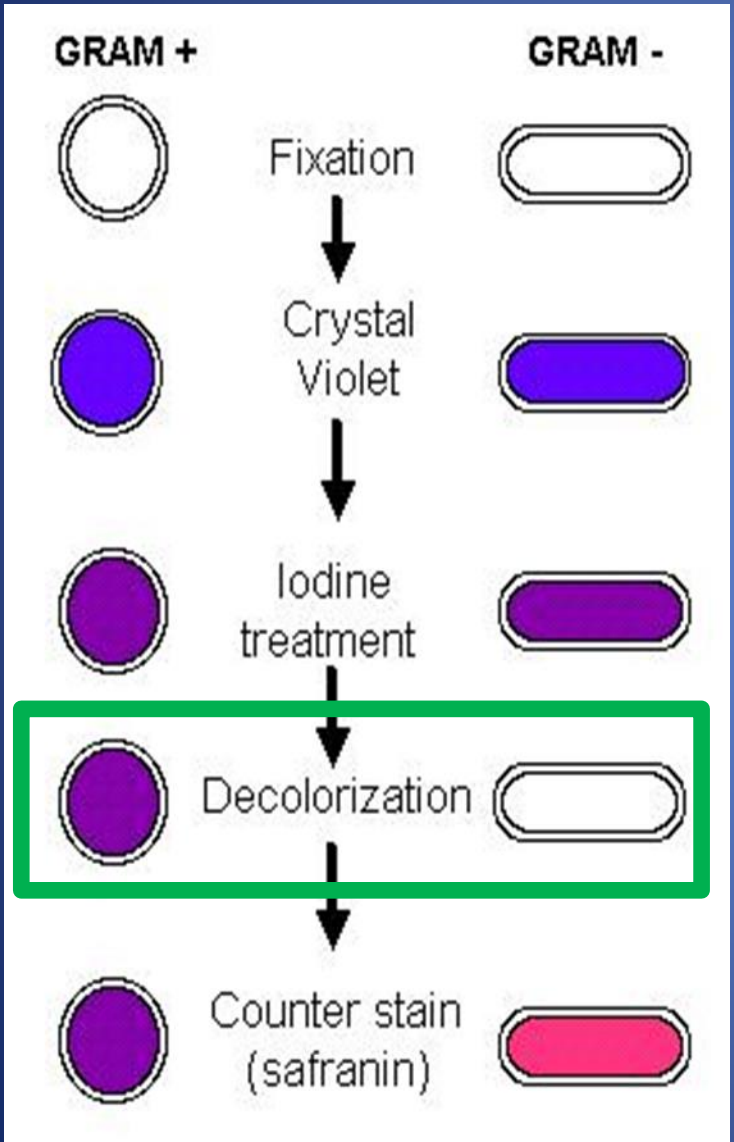


3. Stain

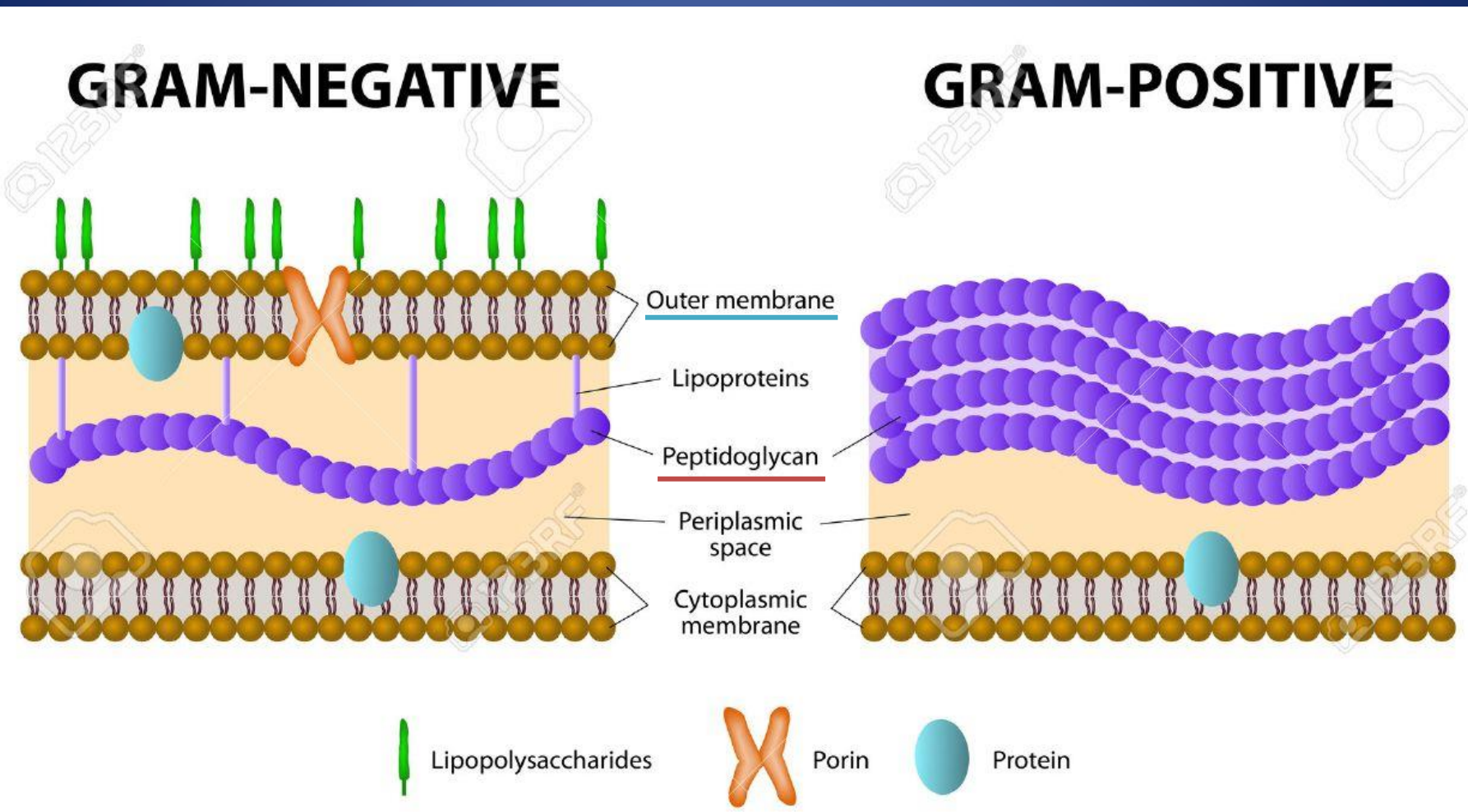
4. Add imersion oil



Gram stain



- 1. crystal violet, rinse with water
- 2. Iodine (Lugol), rinse with water
- 3. decolorization with acetone, rinse with water
- 4. counterstaining with carbolfuchsin/safranin, rinse with water
- 5. air-drying
- 6. observation with immersion oil and 100x magnif.
- G+: blue/violet, G-: pink/red



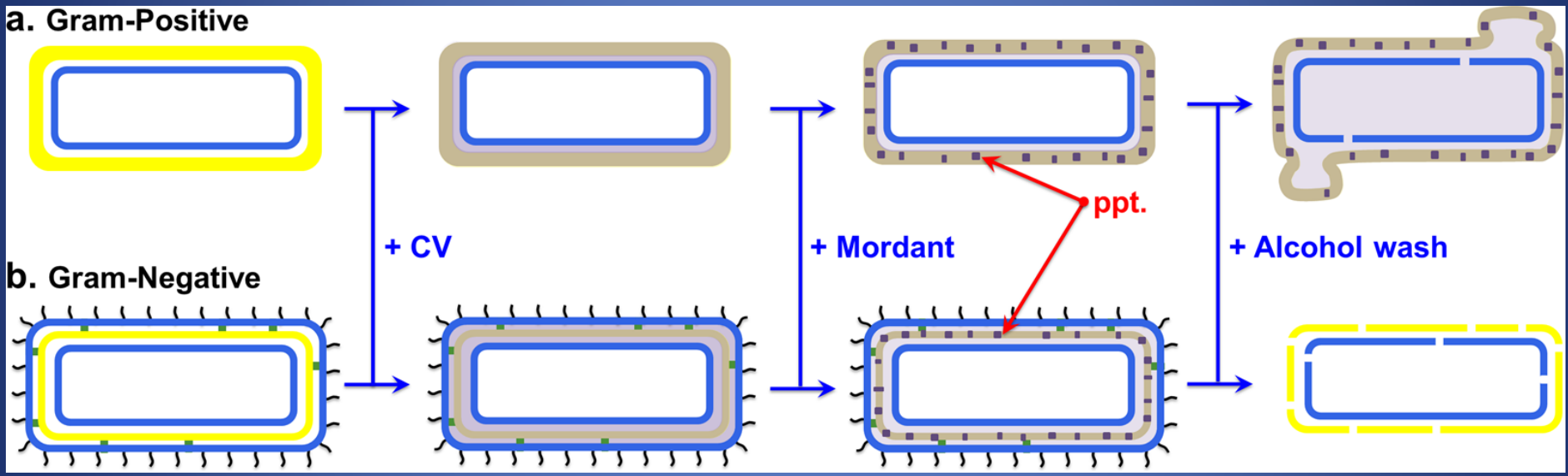
Gram-negative:

- outer membrane (not important for staining)
- **Thin peptidoglycan layer!!!**

Gram-positive:

- **Thick peptidoglycan layer!!!**

Gram - principle

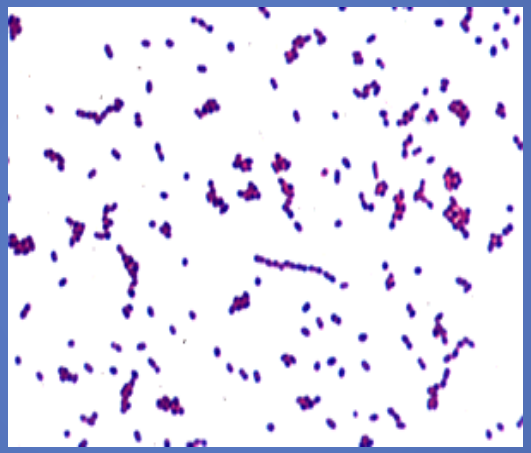


The addition of **Crystal violet** (CV) and mordant **(Lugol=iodine)** results in the generation of a precipitate (ppt.) which is largely isolated in the cell wall. Following the destructive **alcohol wash**, the intact cell wall of the Gram+ cell retains the ppt. within the cell while the perforated cell wall of the Gram− cell allows the ppt. to be washed away.

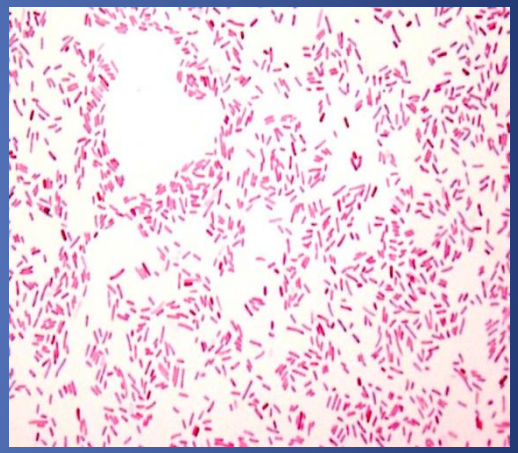
Wilhelm et al 2015 ACS Chem. Biol.

Gram - examples

G+ cocci in clusters
Staphylococcus aureus

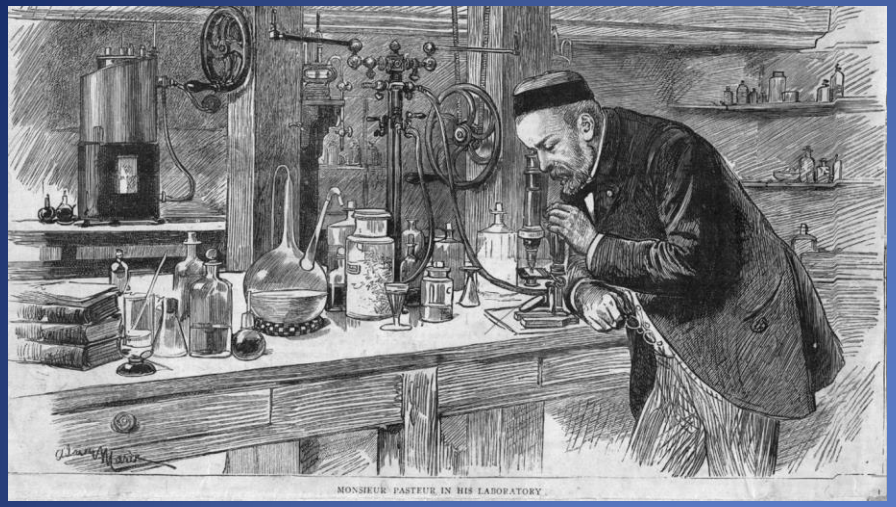


G+ cocci in chains
Streptococcus pyogenes

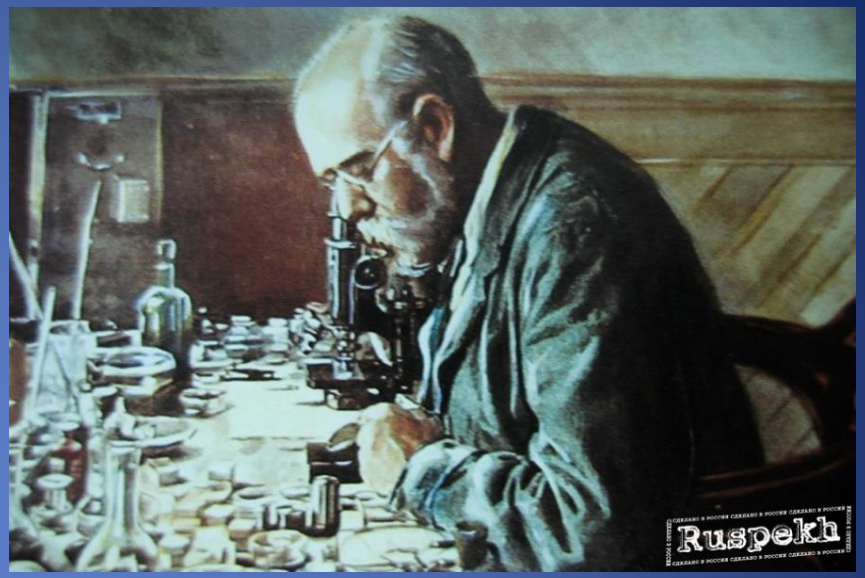


G- rods
Escherichia coli

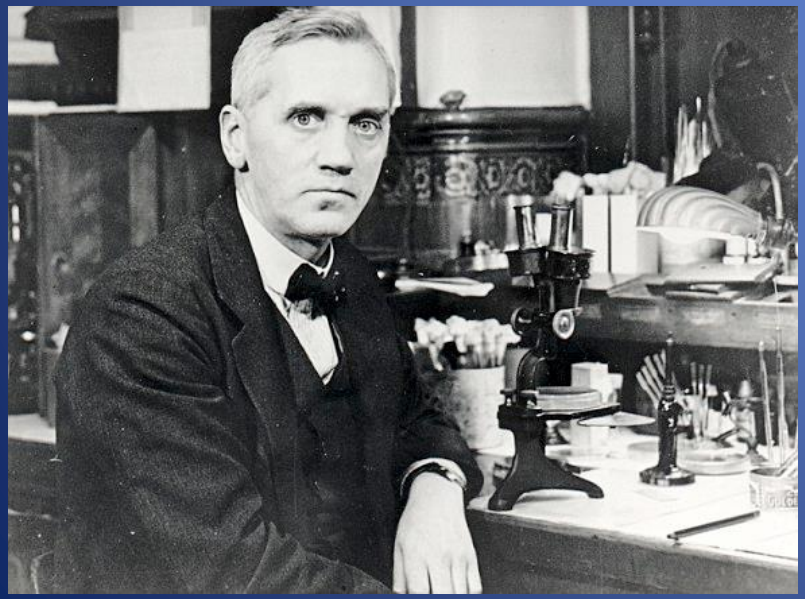
Pasteur



Koch



Fleming



Microscope is basic tool!

Microscopy

Pros and cons

+

- Speed
- Low cost
- Direct observation (bacteria + IS cells)
- Non-cultivable organisms

-

- Subjectivity
- Low reproducibility
- Impossible to tell exact bacterial species
- Low sensitivity – negative result did not rule out infection!

Why to use microscopy:

- Identification of agens in the sample or from pure culture
  - Parasites, fungi
  - Bacteria e.g. TBC, or agens with distinctive morphology patogeny
  - Part of the biochemical identification (G+ or G-) when MALDI-TOF is not available or possible to use
- Sample validation
  - Presence (quantity) of leucocytes
  - Absence of squamous epitelia

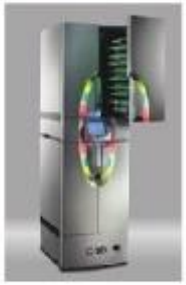
Samples

- Clinical samples
  - liquid
    - CSF
    - Sputum and other respirátory specimen
    - Pus, discharge etc
  - Stool NOT bacteria but parasites and viruses (electron microscopy)
- Pure cultures
- Positive Blood Cultures (BC)

Blood culture diagnosis

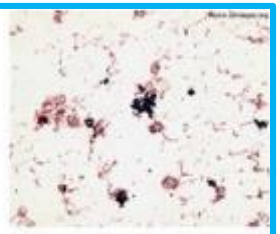
5-24h

Incubation



+18-24h

Microscopy and culture on solid media



Microscopy:

- Confirmation of positivity – we see the bacteria
- Preliminary identification – guidance for a treatment

+18-24h

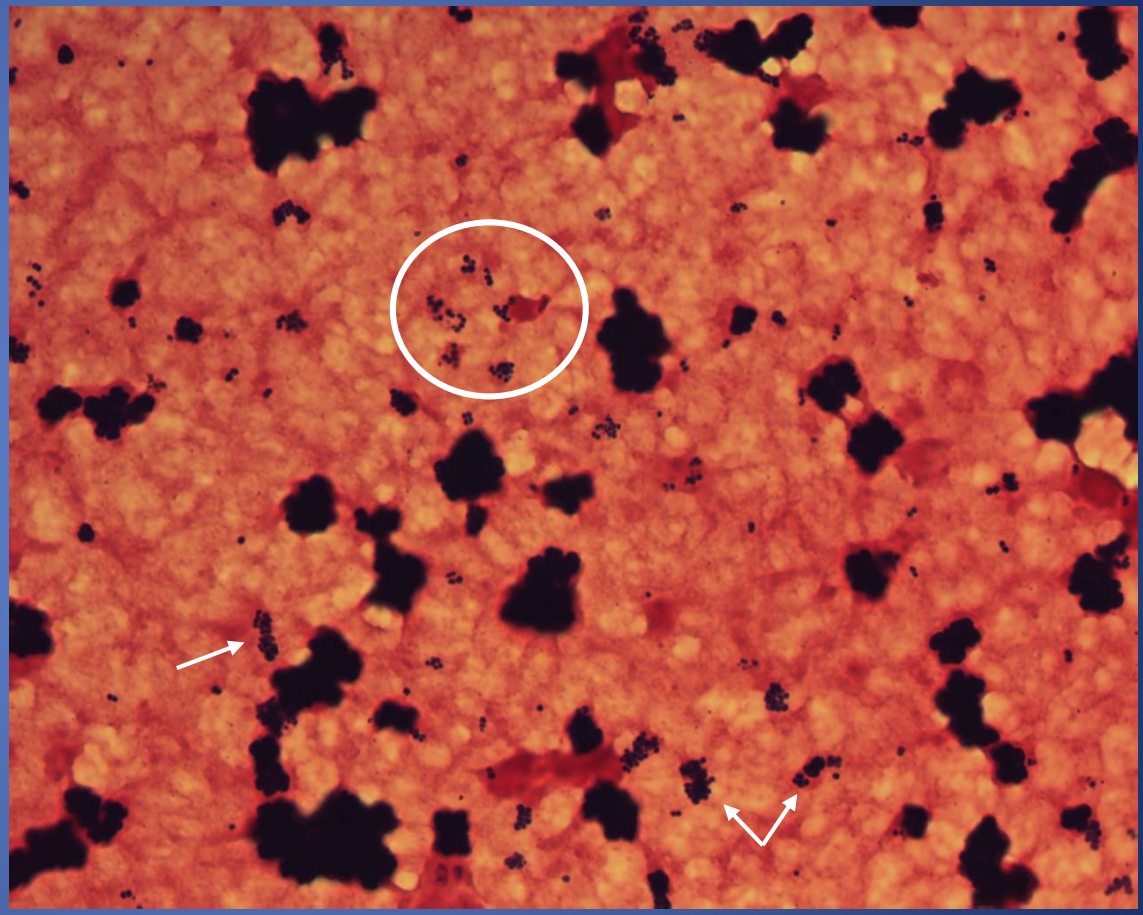
Identification of grown colonies

+ 18-24h

Antimicrobial sensitivity testing

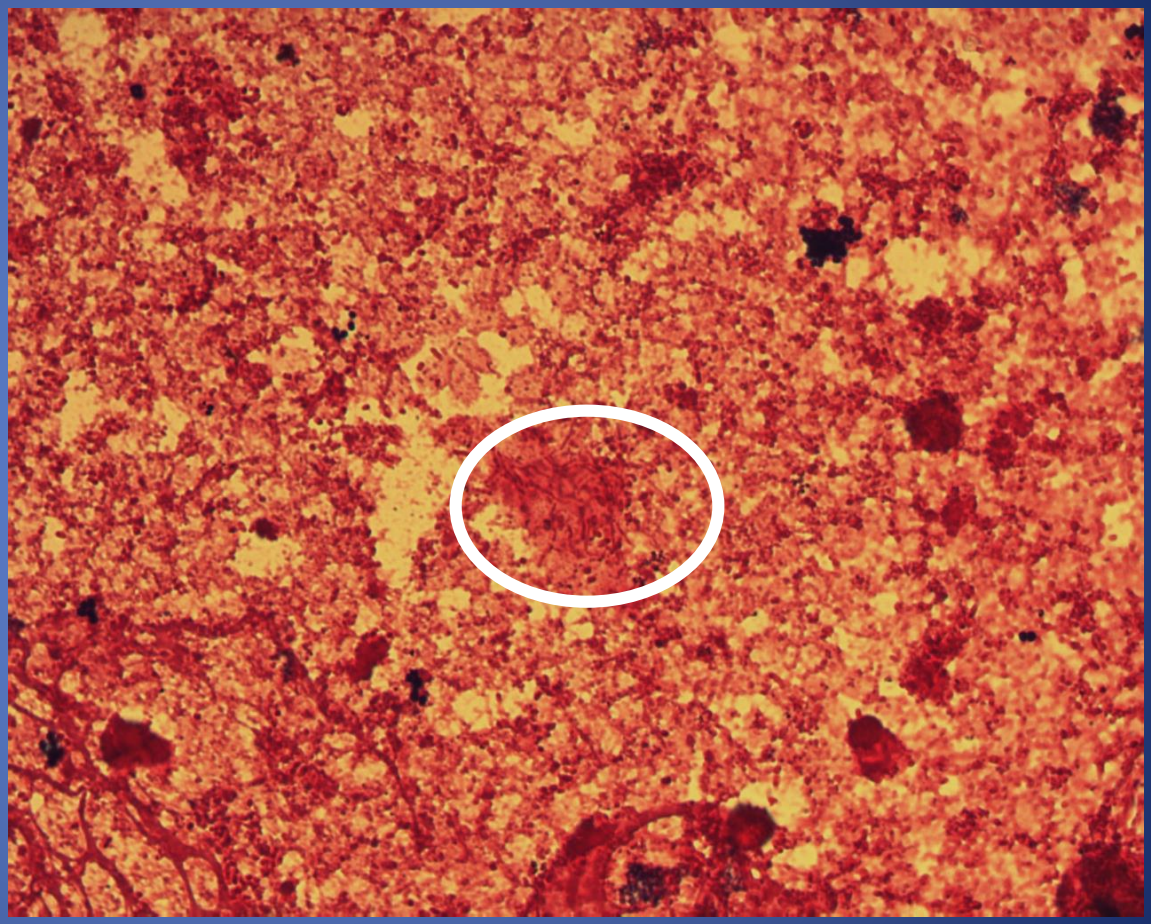
BC: Staphylococcus aureus

Materiál: hemokultura
Pacient: žena 84 let
Dg: endokarditida
Mikroskopie: grampozitivní koky tvořící hrozny



Autor: MUDr. Petra Kabelíková

BC: Escherichia coli
Proteus mirabilis



Materiál: hemokultura
Pacient: žena 89 let
Dg: urosepse
Mikroskopie: gramnegativní tyčinky, v moči nález *E. coli* a *P. mirabilis*

Autor: MUDr. Petra Kabelíková

Neuroinfection
CSF examination

Cytology and biochemistry: **indicators of bacterial infection**

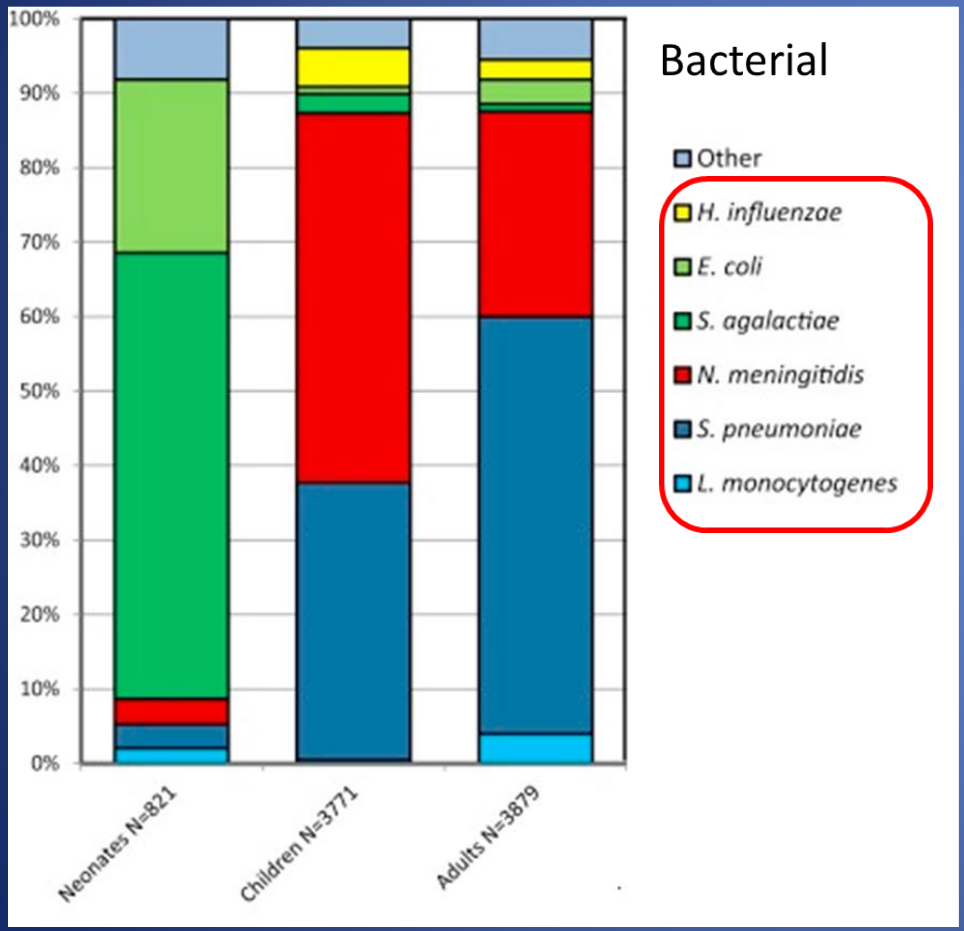
- **turbidity**
- **↑WBC count (PMN)**
- **↓glucose**
- **↑protein**

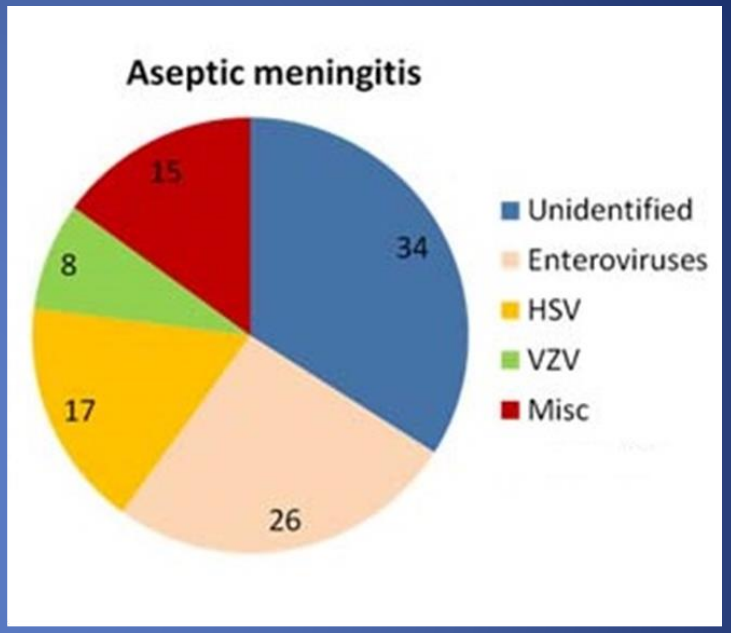
Microbiology

- Sample centrifugation
- Microscopy
- Antigen detection
- PCR
- Culture

CSF = cerebrospinal fluid

Neuroinfection

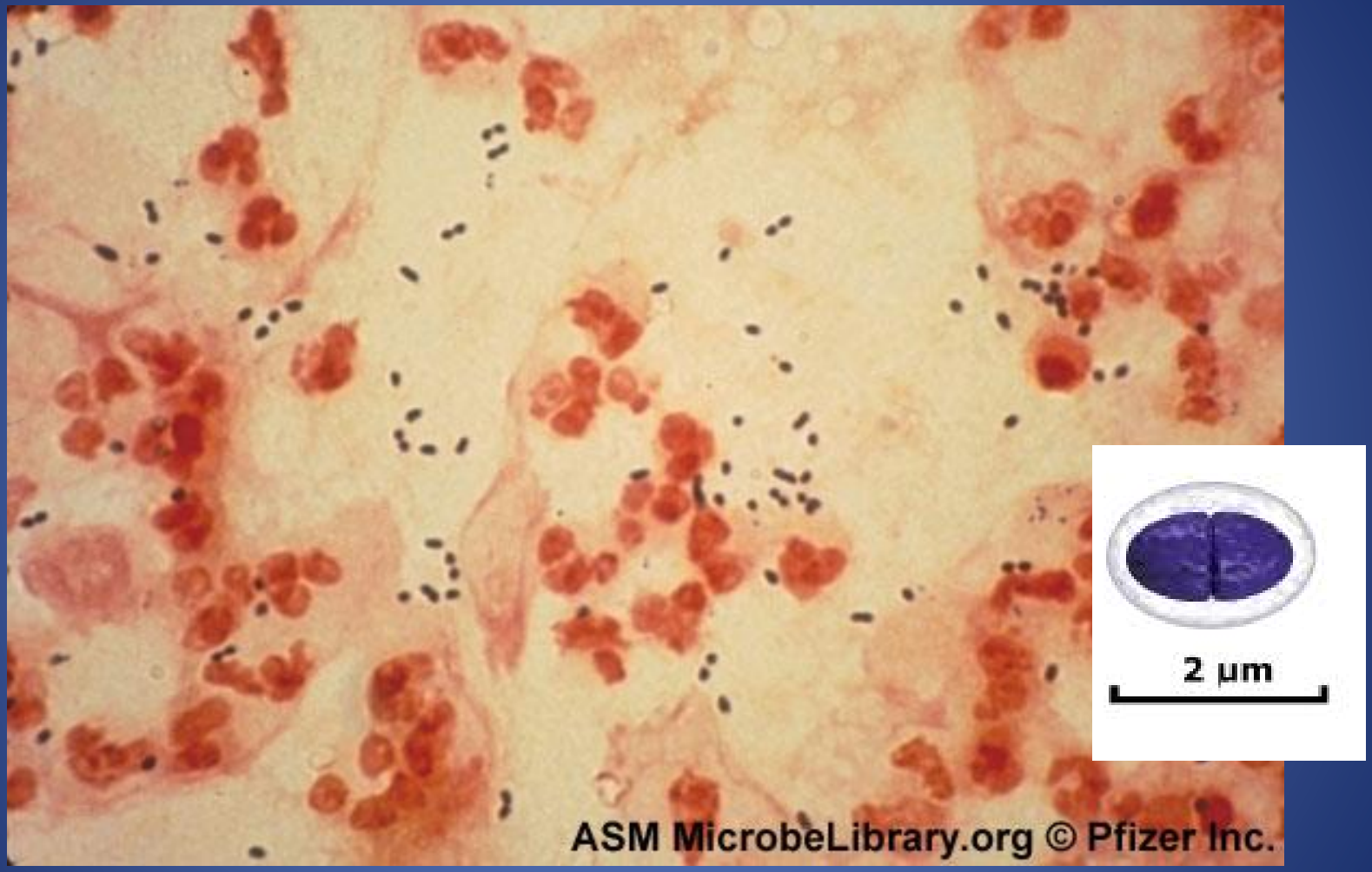




Viral agents are not visible by light microscopy

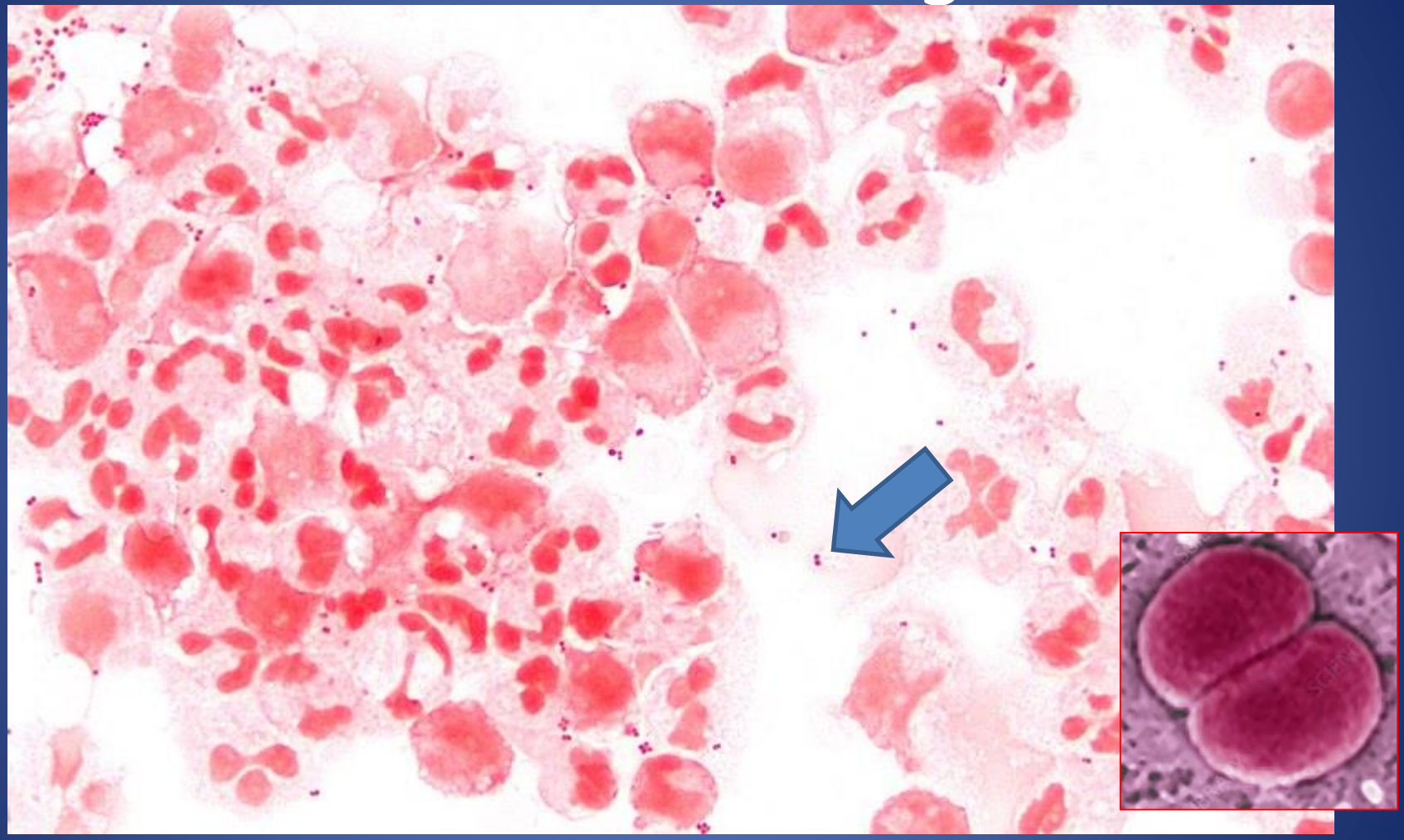
Typical morphology of main agents of septic/bacterial meningitidis

CSF: Streptococcus pneumoniae



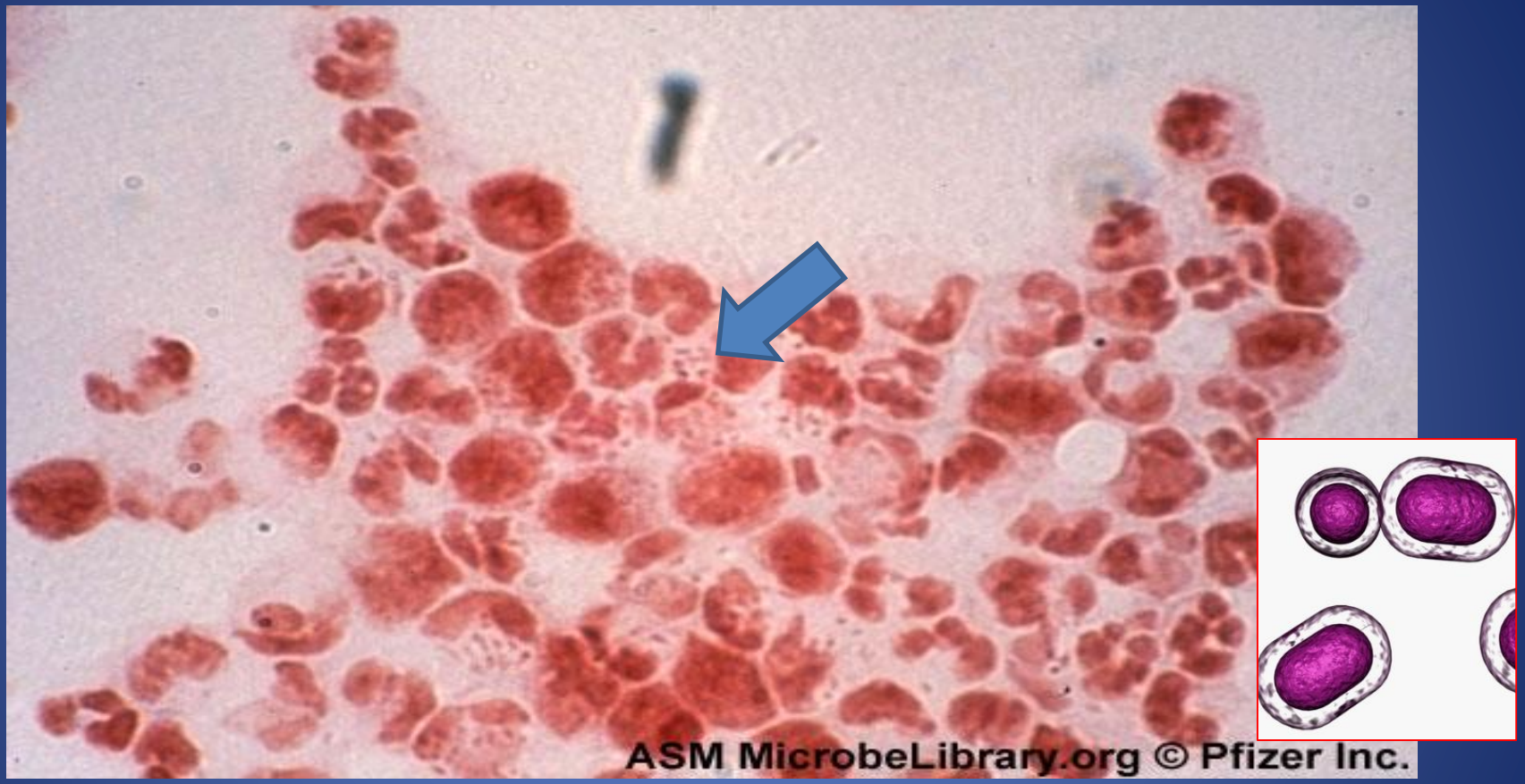
Neutrophils and oval gram-positive cocci singly and in pairs (diplococci)

CSF: Neisseria meningitidis



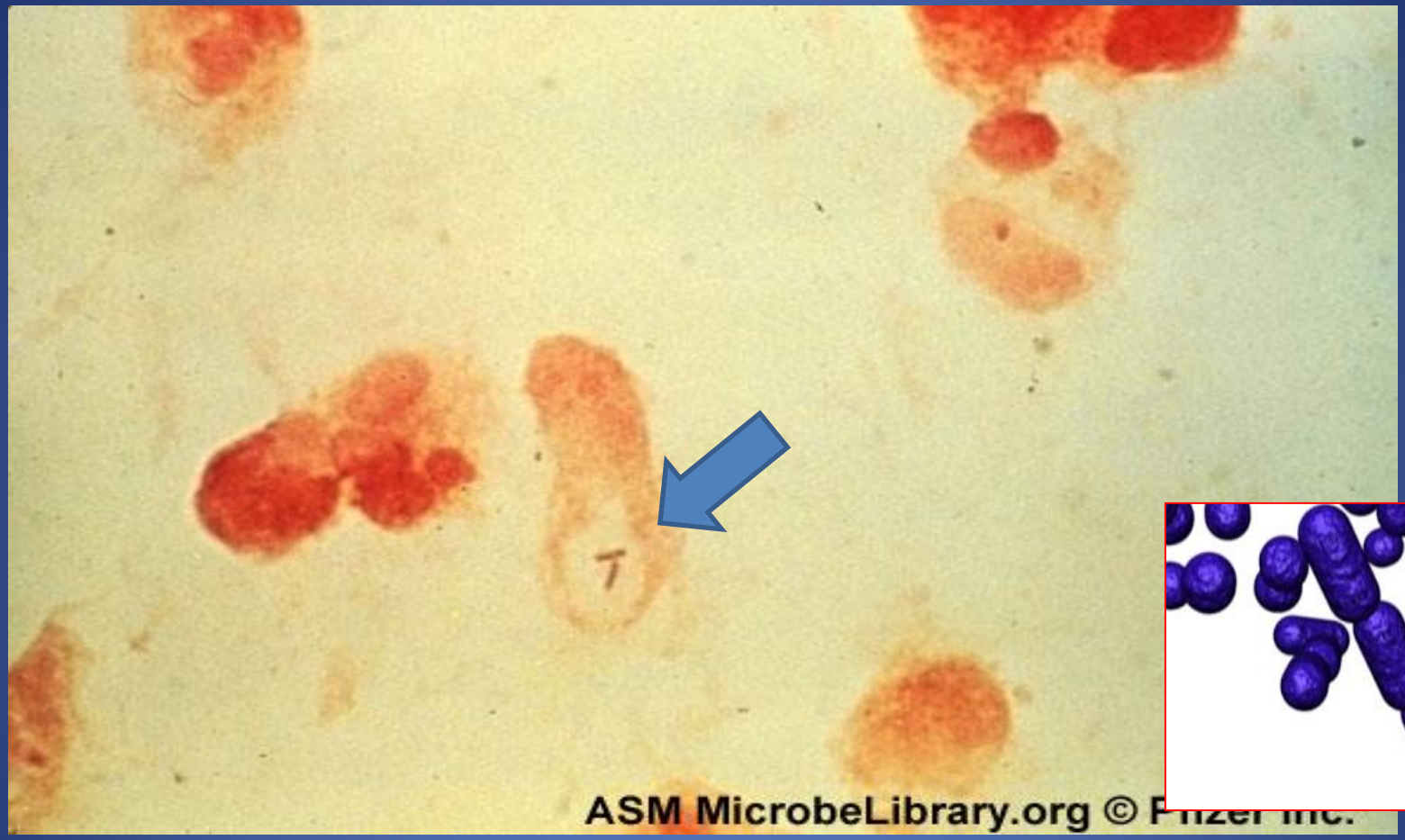
gram-negative diplococci (shape of the coffee beans)

CSF: Haemophilus influenzae



gram-negative coccobacilli, primarily in the cytoplasm of the white cells

CSF: Listeria monocytogenes



Typicaly few neutrophiles and few bacteria – grampositive rods (bacili)

Case report culture negative *N. meningitidis* CSF infection

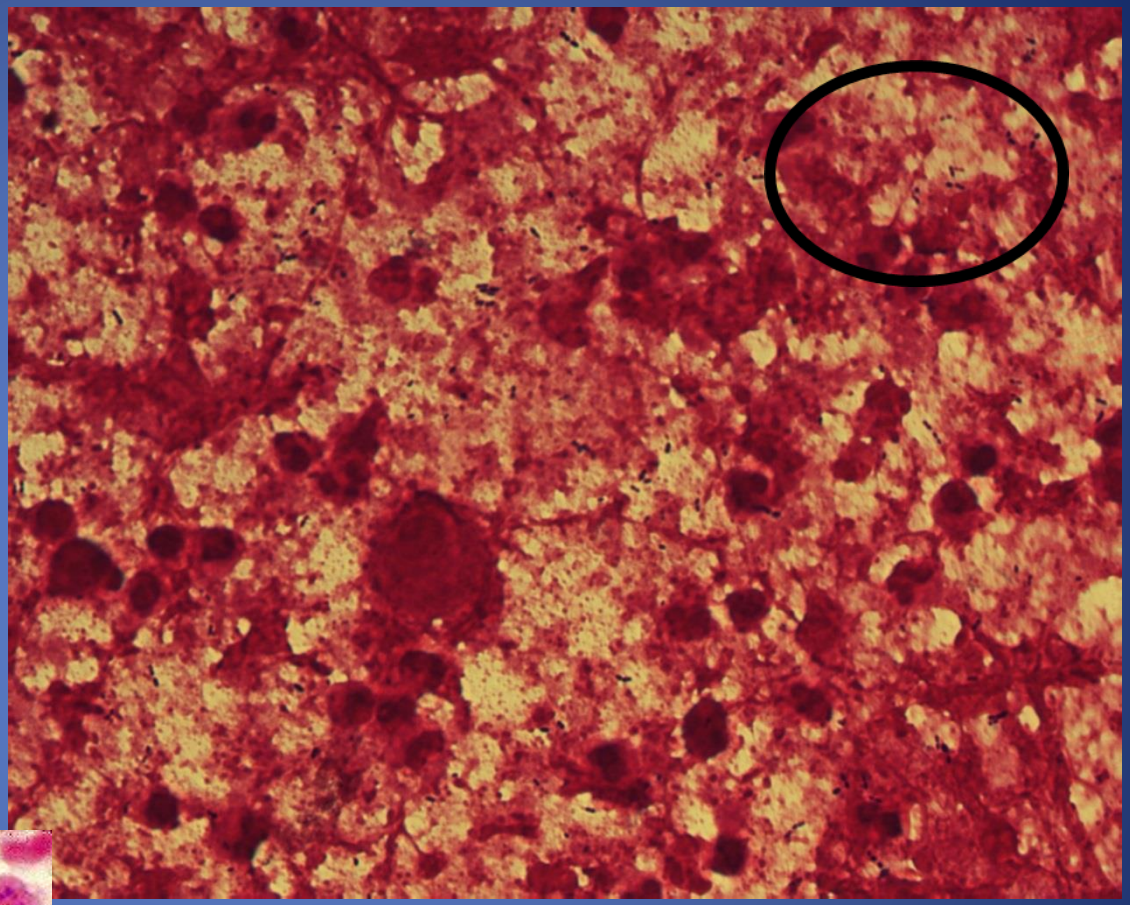
- Young girl, 5 months, visiting Emergency with fever and spreading rash (purpura fulminans) – suspicion of memingococcal sepsis/meningoencephalitis
- Empirical therapy with Ceftriaxon (cephalosporin) started imediately
- Bloodculture and CSF samples taken after administration of ATB
- Microbiology :
  - BC negative, CSF culture negative (due to therapy)
  - Diplococci in blood and CSF – *N. meningitidis*
  - PCR confirmed *N. meningitidis*

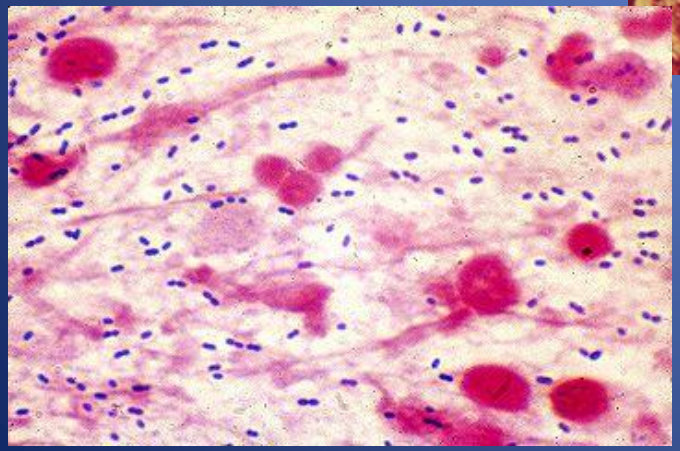
Microscopy of respiratory samples

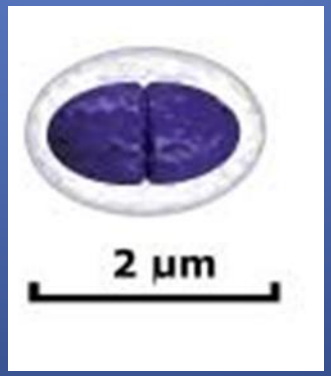
- sputum and aspirates
- We are looking for microbes but also for neutrofiles (markers of the inflamation)
- Presence of high number of squamous epitelia (oral cavity) indicates invalide sample
- Be aware that contaminating bacteria could be present e.g. CoNS

Sputum: Streptococcus pneumoniae

Pneumonia



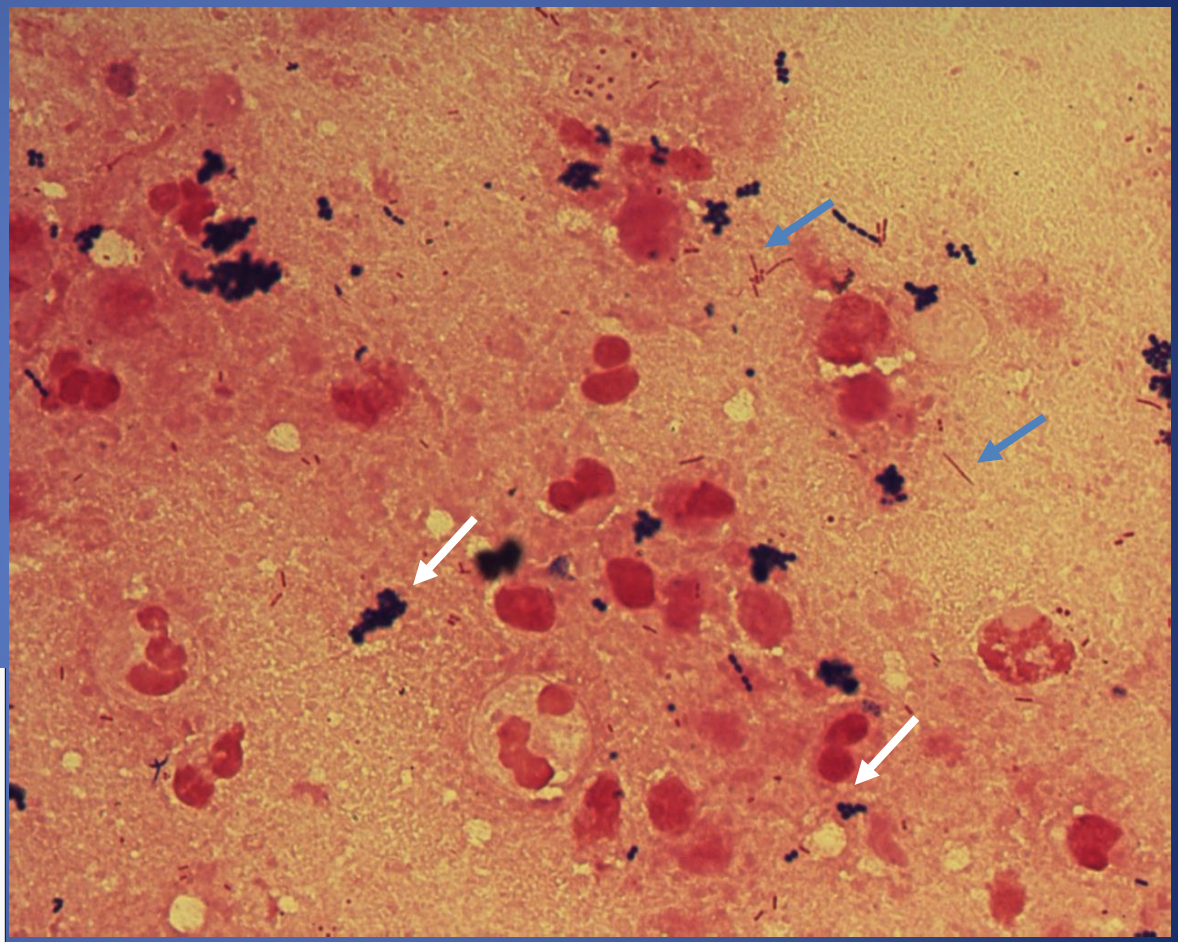




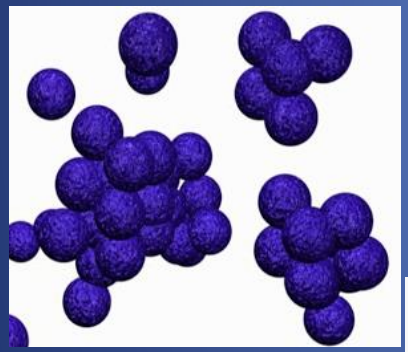
Autor: MUDr. Alena Píšová

Sputum:
Staphylococcus aureus, Pseudomonas aeruginosa

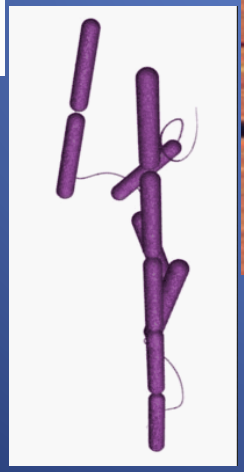
Patient with cystic fibrosis



Staph. aureus



P. aeruginosa

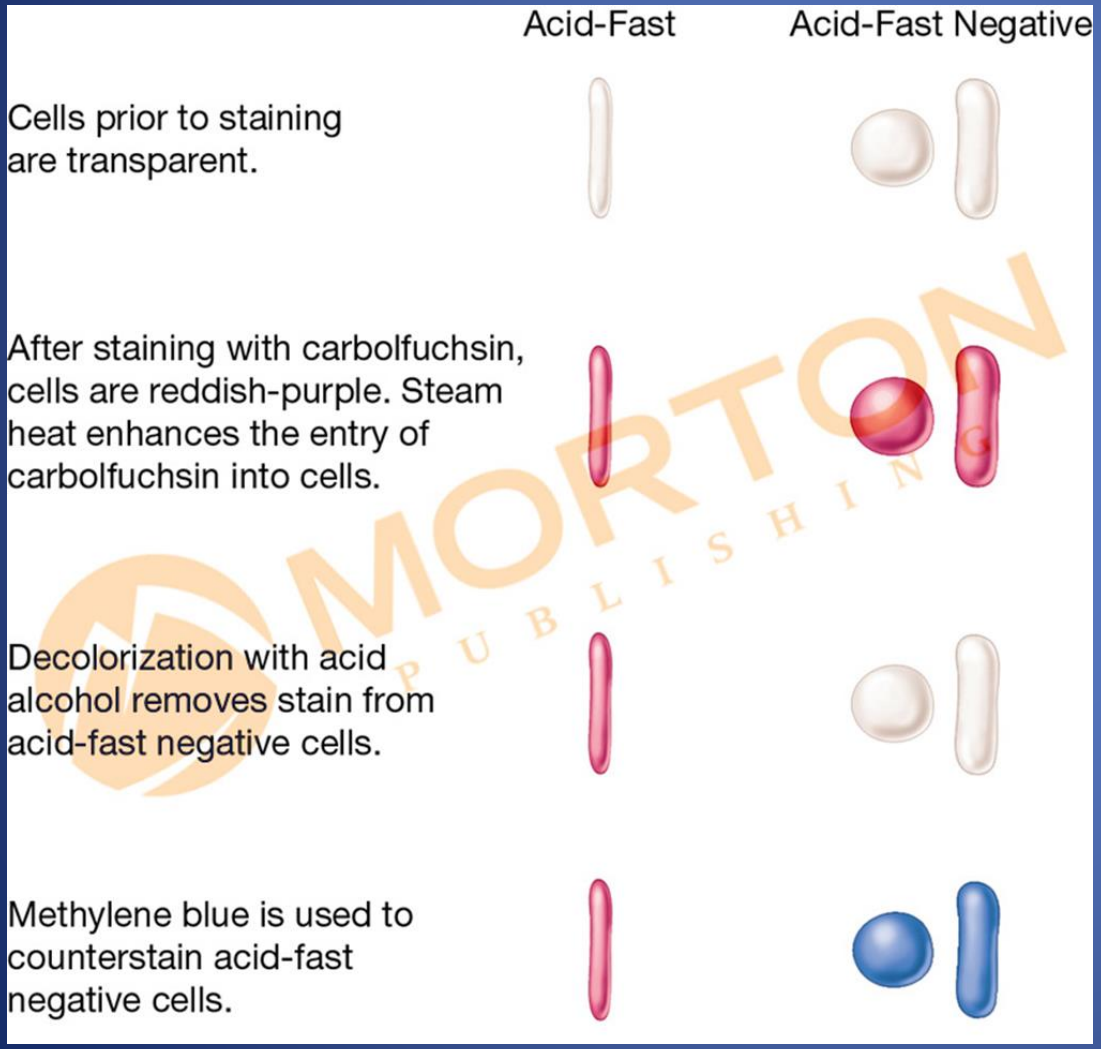


Autor: doc. MVDr. Otto Melter, PhD.

Tuberculosis

- *Mycobacterium tuberculosis* – extremely long cultivation up to 6 weeks
- Diagnosis– microscopy (Ziehl-Neelsen) or PCR

Mycobacteria stain (Ziehl-Neelsen)



Capsule stain

- ***indirect visualization of capsules***
- 1. a drop of India ink is placed on a side of a slide
- 2. loopful of bacteria is resuspended in ink
- 3. spread (as with blood smear)
- 4. air-dry
- 5. stain with crystal violet 1 min
- 6. rinse with water (gently, thoroughly)
  - capsules are not stained
  - Bacteria are violet
  - background is black (ink)