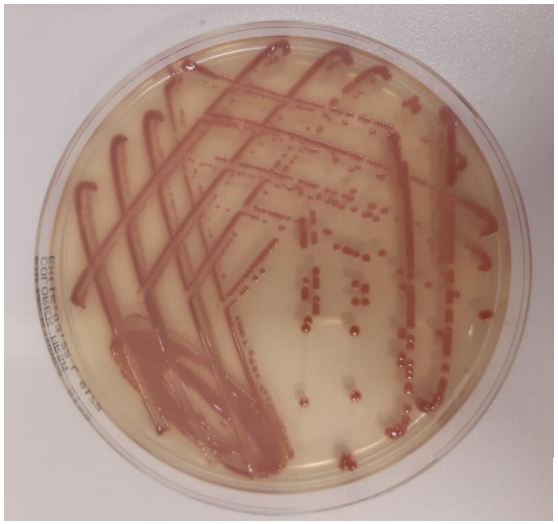
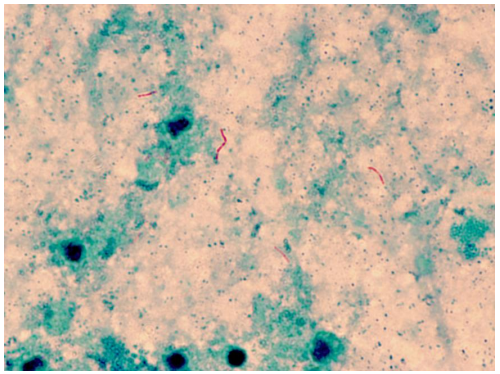
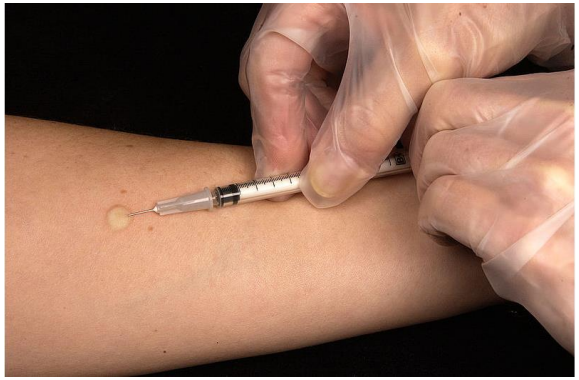
Direct vs. indirect diagnostic methods







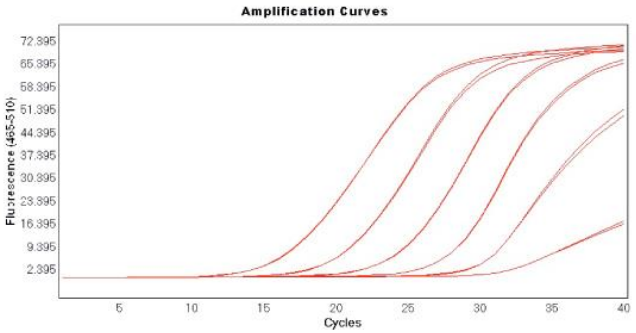
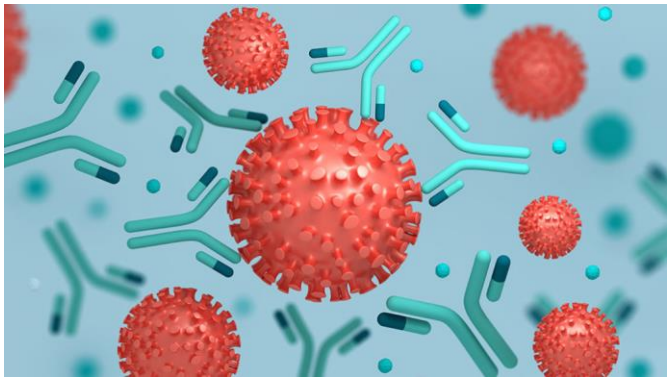
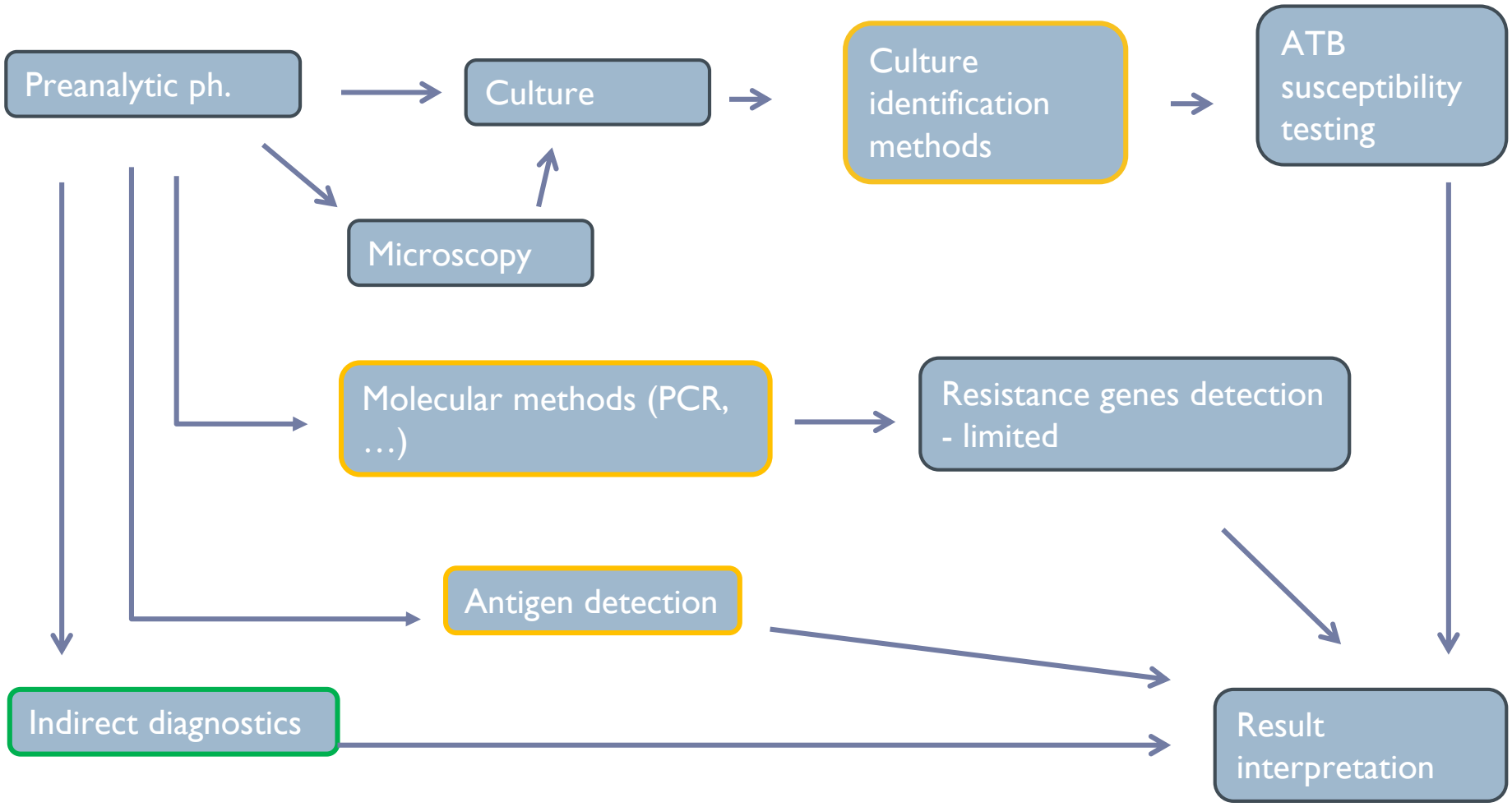


Figure 2. Amplification curves in semi-logarithmic view obtained from serial dilutions of a target DNA. Inset, regression curve obtained from C_T values.



https://cs.m.wikipedia.org/wiki/Soubor:Mantoux_tuberculin_skin_test.jpg
https://www.snexplores.org/article/what-are-antibodies-explainer
https://www.caister.com/highveld/pcr/real-time-pcr-quantification-analysis.html

Microbiological analysis in bacteriology



Microscopy in bacteriology

MUDr. Anežka Gryndlerová

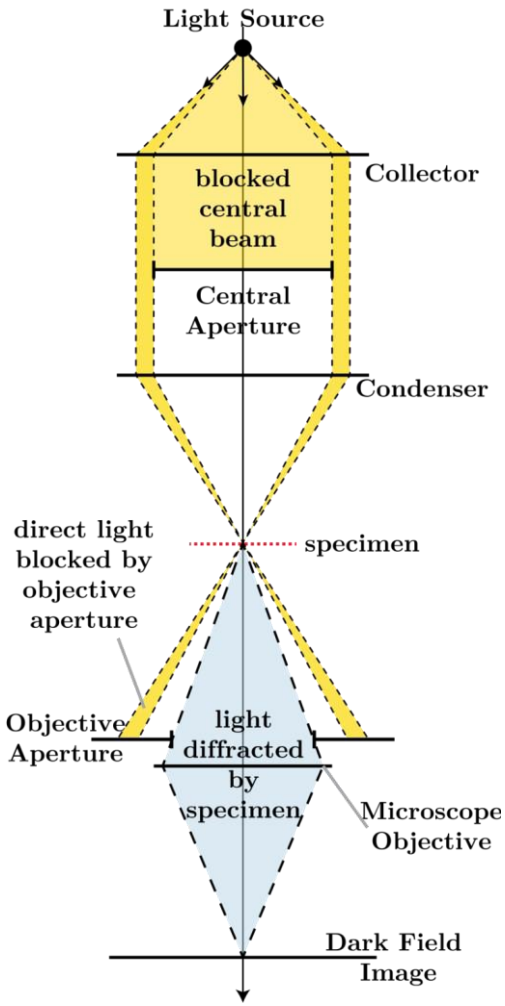
Content

- Theory
  - Microscope types
  - Stain types used in bacteriology
- Practicals

Compound microscope

- Bacteriology
  - Size of bacteria ~ µm
  - Objective lens 100x
  - Immersion oil
- Mycology
- Parasitology
- Virology?

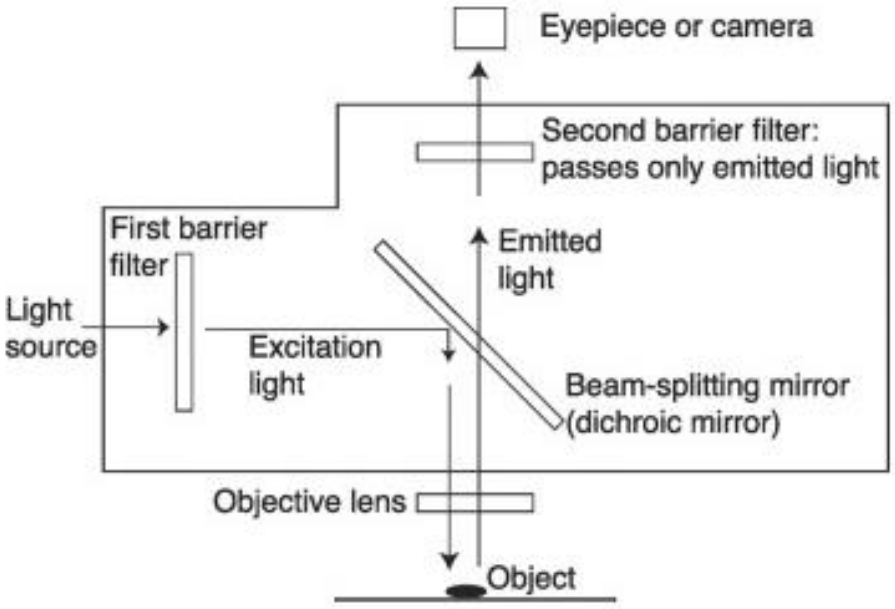
Dark field microscopy



- https://www.youtube.com/watch?v=X9NQ8xJLy3E

Other microscope types

- Fluorescent microscope – mycology, TBC



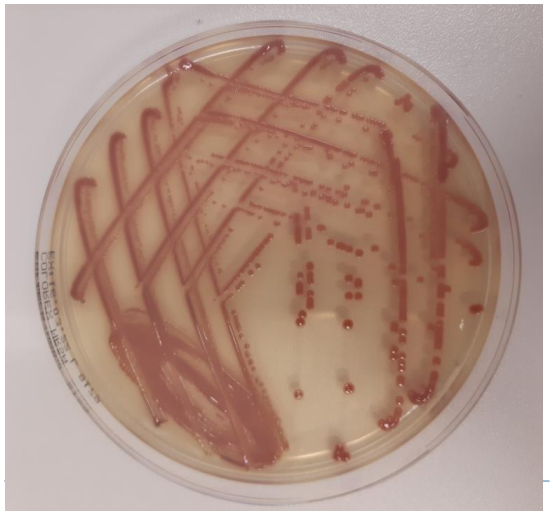
- Electron microscopy
  - Not in routine practice

https://www.sciencedirect.com/topics/chemistry/fluorescence-microscopy

Light microscopy in bacteriology - indications

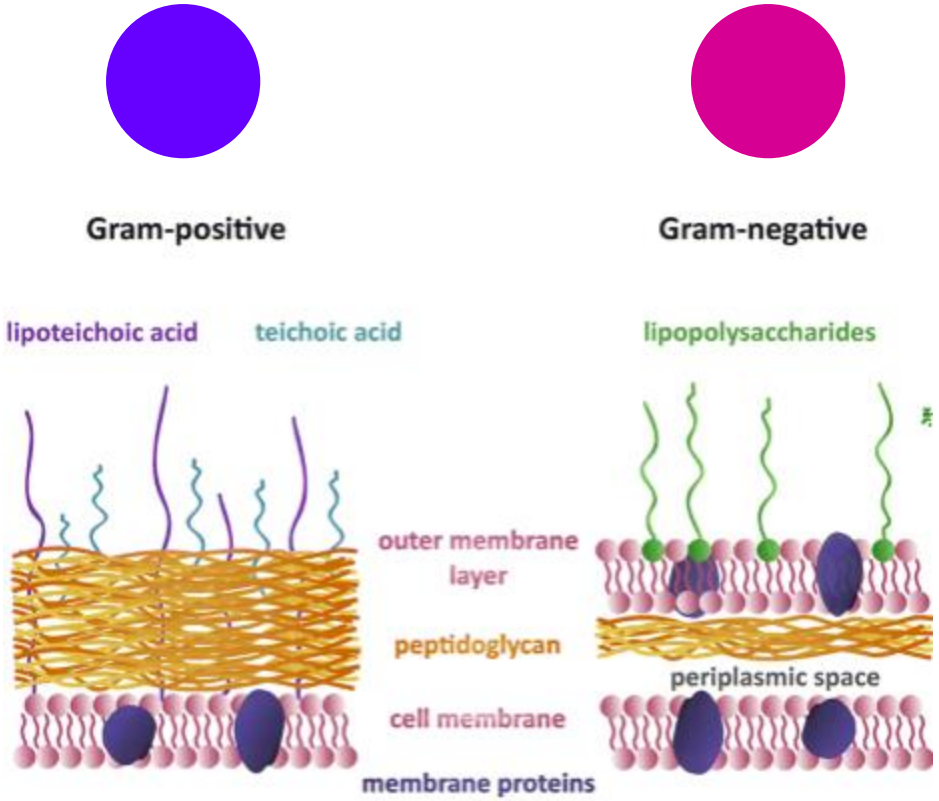
- Liquid samples from primarily sterile locations (CSF, synovial fluid, peritoneal cavity content, …)
- Tissue
- Blood culture – when positivity is detected
- Sputum, …

- (Grown culture microscopy)



Stain types used in bacteriology

Gram stain

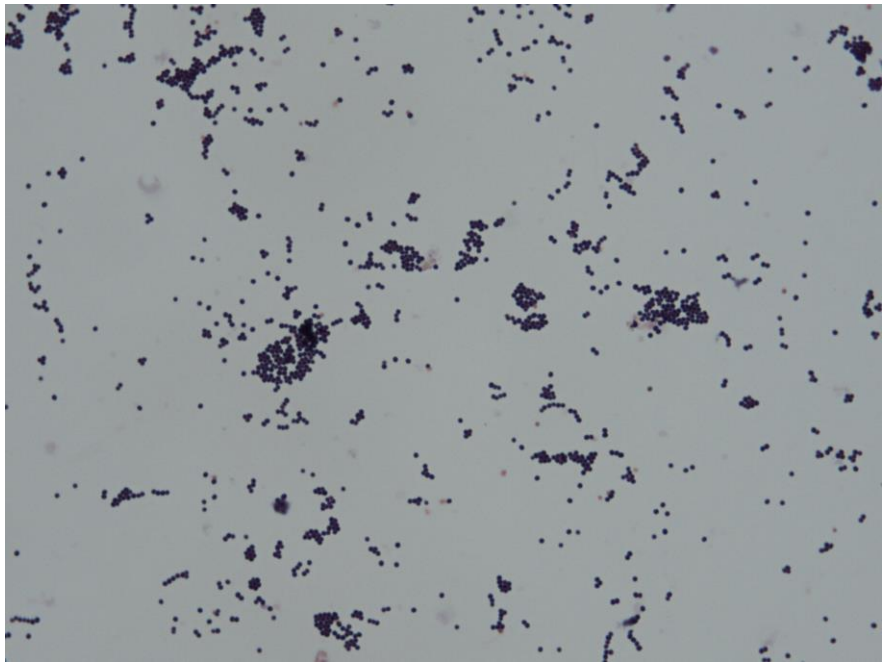


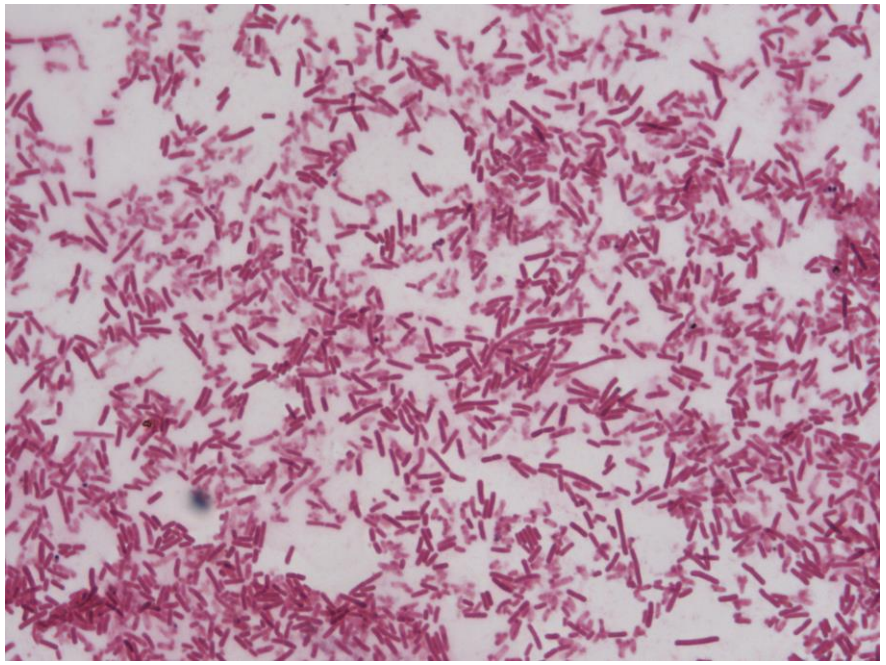
V – crystal violet
L – Iodine solution
A – alcohol (ethanol), acetone
S – safranin

V – crystal violet
L – Iodine solution
A – alcohol (ethanol), acetone
K - karbolfuchsin

+ Bacteria not stained by gram stain

https://link.springer.com/article/10.1007/s11051-019-4617-z

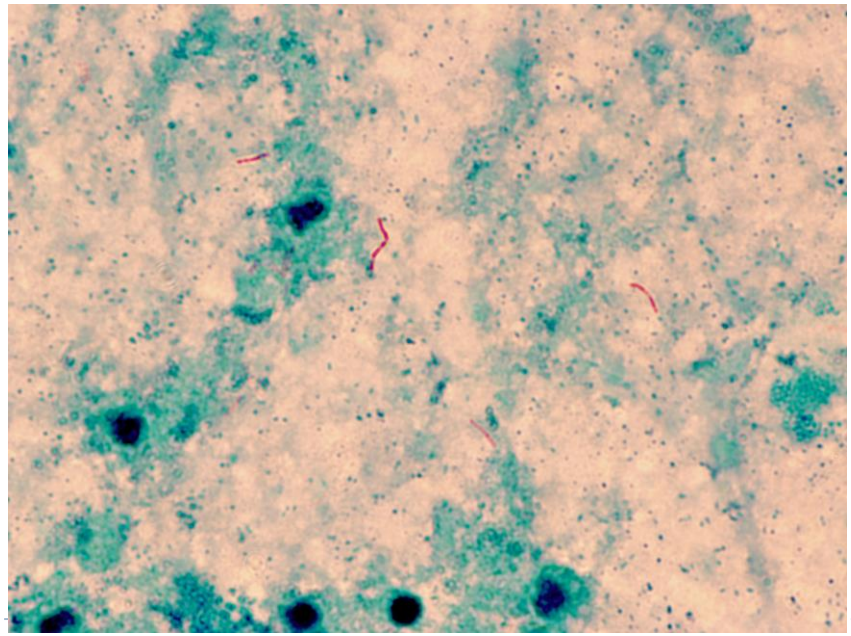




Ziehl-Neelsen stain (Acid fast)

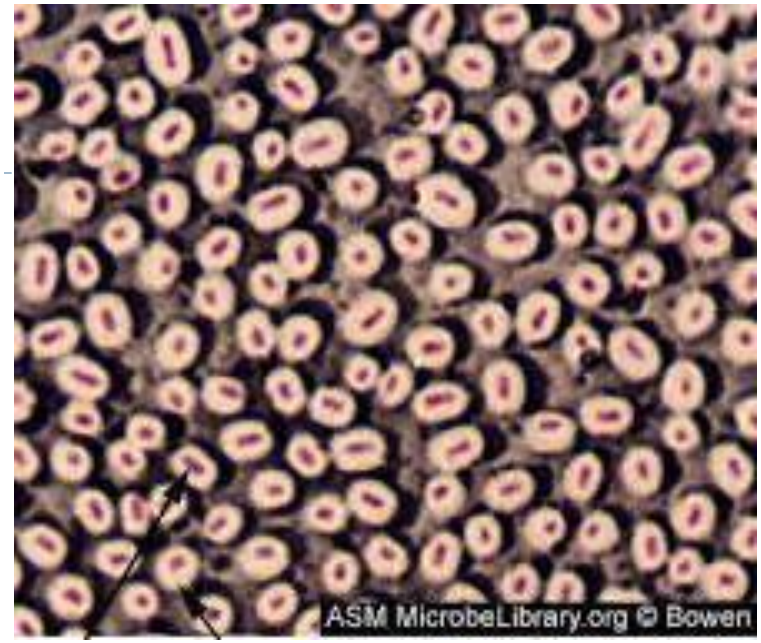
- Acid fast bacteria (*Mycobacterium tuberculosis*)

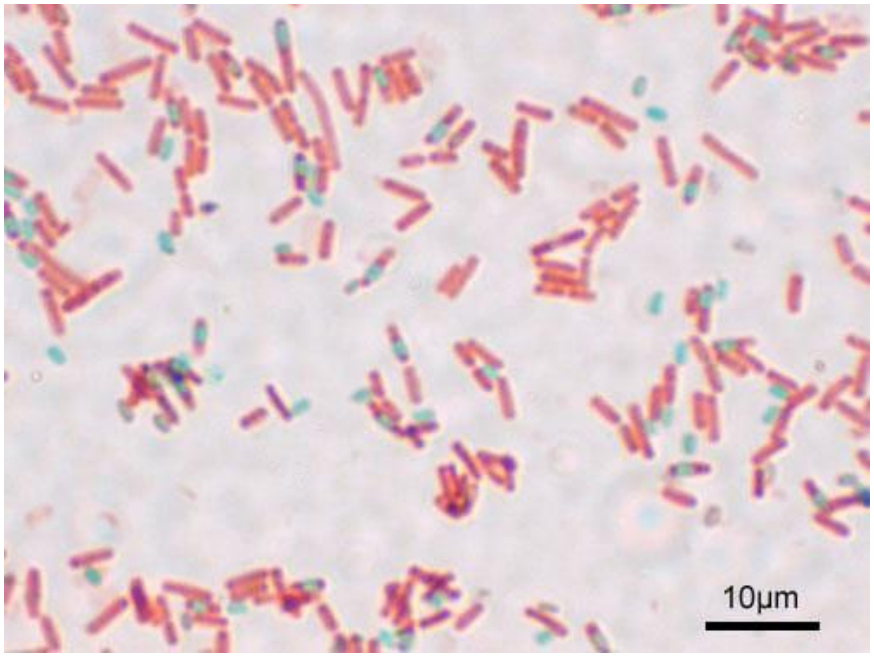
- Karbolfuchsin + heat
- Acid alcohol (ethanol + Θ HCl)
- Malachite green

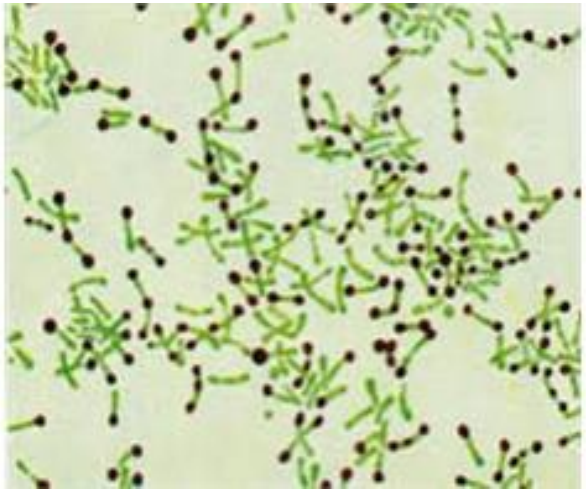


Other stain types

- Burri stain (capsules)
- Wirtze-Conklin stain (spores)
- Albert stain (metachromatic granules)







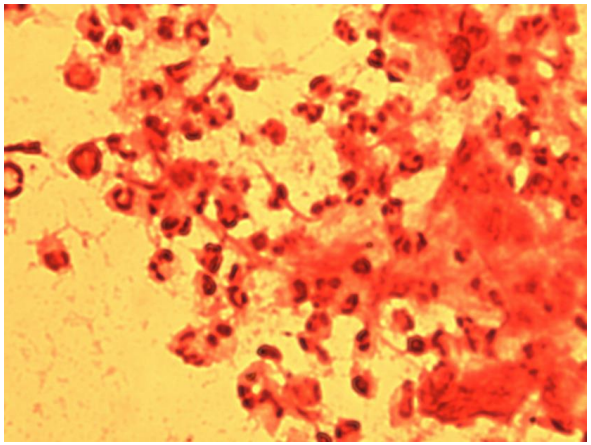
https://www.lobachemie.com/Staining-Solutions-00823/ALBERT-STAIN-A-SOLUTION-CASNO-NA.aspx

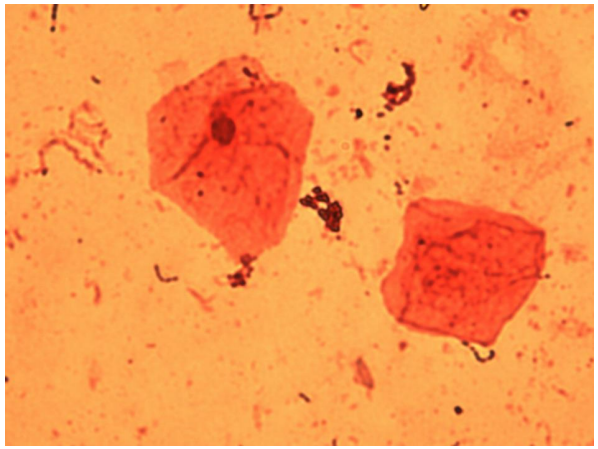
https://microbiologia.net/microbiologia/tinciones-colorantes/

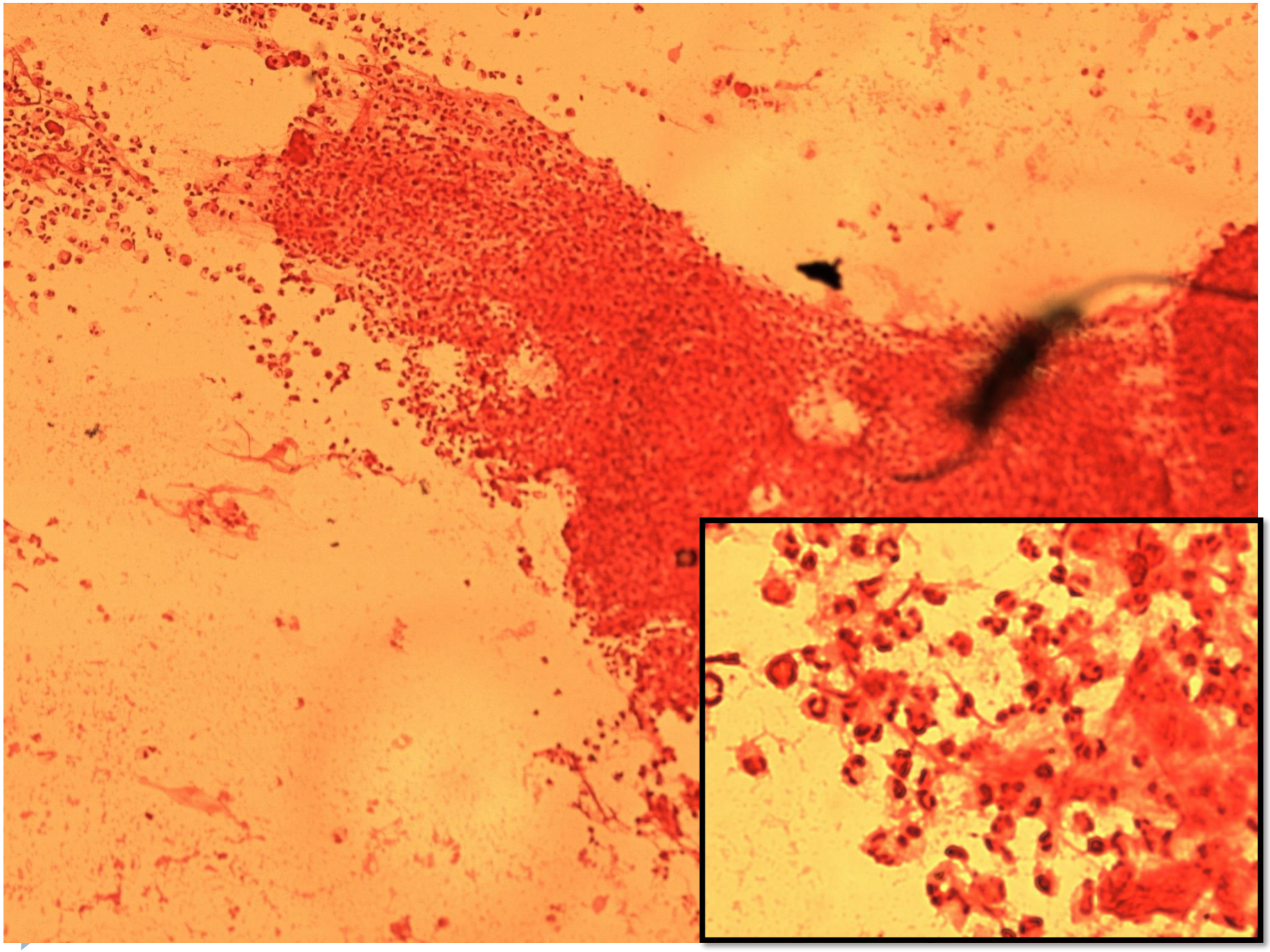
Practical lesson

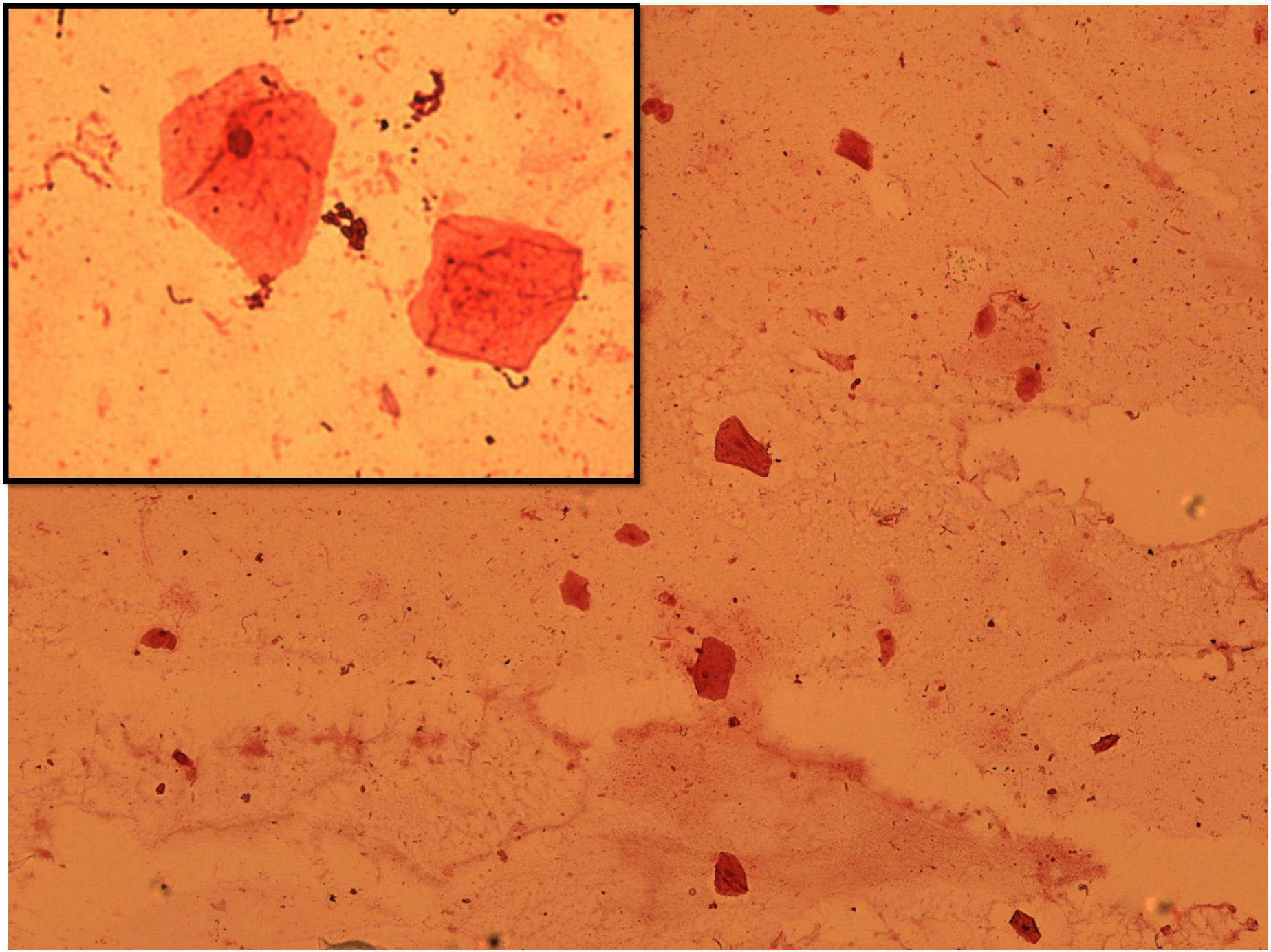
Part 1 – sputum validity assessment

- 10x10x magnification (without immersion oil)
- Valid sample:
  - Leukocytes >25 /ZP
  - Squamous epitelial cells <10 /ZP









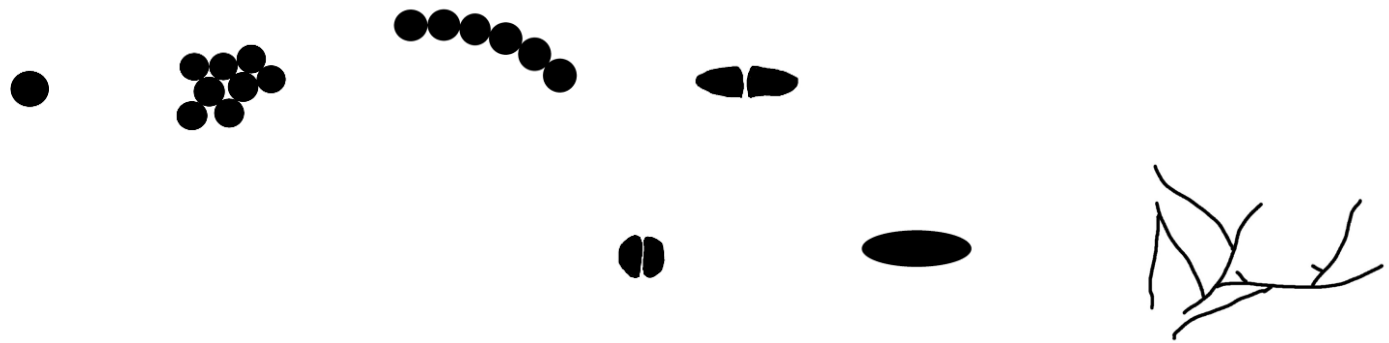
Part 2 – native microscopic slide

- Native vs. stained slides

- Put one drop of ph.solution on the slide
- Take a part of a grown colony by 1 µl (green) loop and stirr in the drop of ph.solution
- Cover with coverslip
- Use 40x-60x obj.

Part 3 – Gram stain (from grown culture)

1) Color (G+, G-)
2) Morphology
   - Cocci
     - In pairs (diplococci), in clusters, in chains
   - Rods
   - …



Part 3 – Gram stain (from grown culture)

- Mark the slide with pencil
- Put one drop of ph.solution on the slide
- Take part of a grown colony by 1 µl (green) loop and stirr in the drop of ph.solution
- Wait until the mixture is dry

- Fixation – 3x above the flame
- Crystal violet → 1 min, → rinse by water
- Iodine solution → 30s → rinse
- Ethanol/acetone → 30s → rinse
- Safranin → 30s → rinse

Use objective lens 100x magn. → total magn. 1000x. Use immersion oil
While using other objectives, don´t use immersion oil!
When your work with a micriscope is finished, please clean the objective with a bottle labeled „lihobenzín“ and turn off the light.

Conclusion - micriscopy

- Fast method
- In bacteriology we use gram stain mostly
- Not possible to use for species identification (shows only morphology)
- Indications:
  primary sterile liquid materials
  tissue
  sputum validity
  positive blood cultures
  (grown cultures)