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FN M+H Fakultní nemocnice Motol a Homolka

Národní institut virologie a bakteriologie

Microscopy in bacteriology

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Microbiology I.
Practicals

WHAT IS IT?

- Diagnostic technique primarily intended for the detection of microorganisms

Resolution: 0.2 µm

Bacteria size: approx. 1-2 µm

Leukocyte size: approx. 10-20 µm

- Types

Type	Use
Light – native preparation	Motile bacteria
Light – stained preparation	Almost all bacteria
Fluorescent	Microfungi
Electron	Viruses

0.2 nm

Significance in microbiology

Advantages

- Easy processing
- **Quick results**
- Low cost

Disadvantages:

- Low sensitivity
- Low specificity
- Human factor

When to do it?

- Wherever possible (liquid materials) and where it provides useful information

If it can be convincingly identified microscopically, it is not necessary to verify the finding by other methods (typically mycology and parasitology)

What needs to be processed?

- Cerebrospinal fluid
- Sputum
- Primarily sterile (liquid) material
- **Positive blood cultures**

Laboratory response

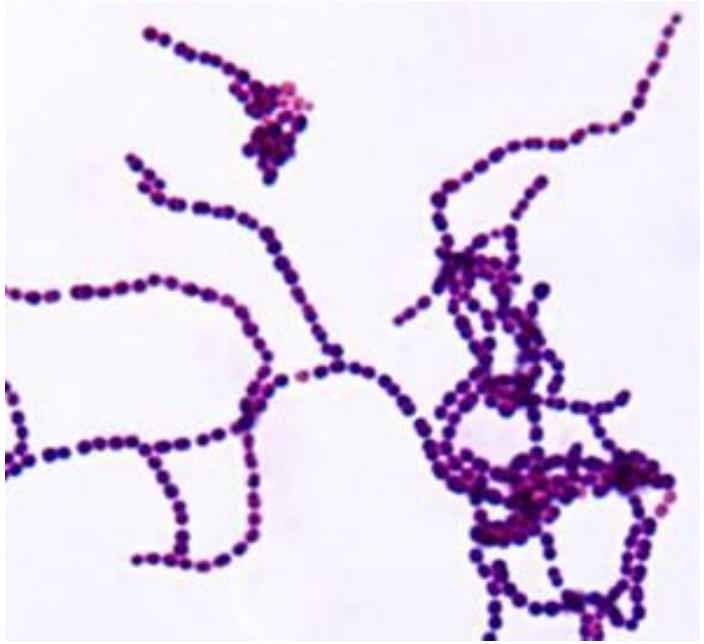
- It is **within hours** of delivery of the material to the laboratory

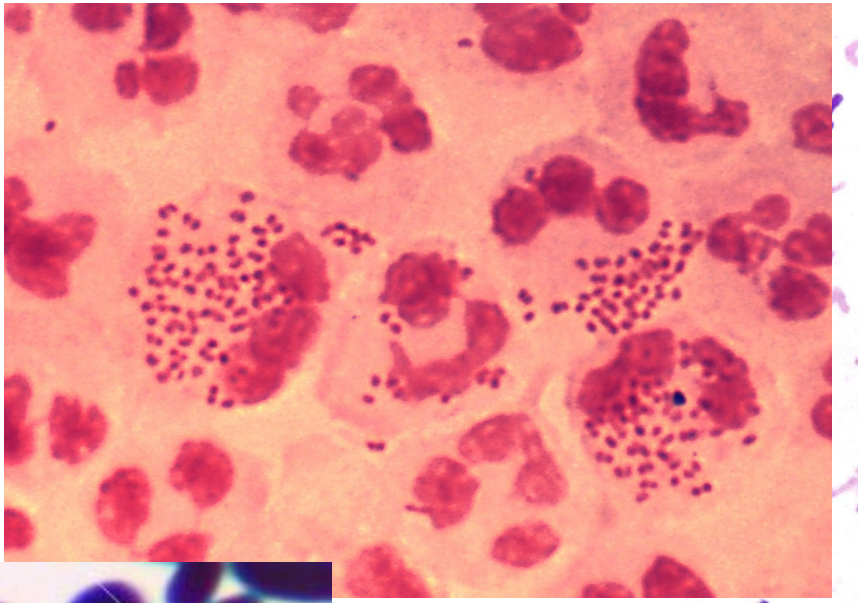
- If you want to know the result **as soon as possible**, call on the same day for the result

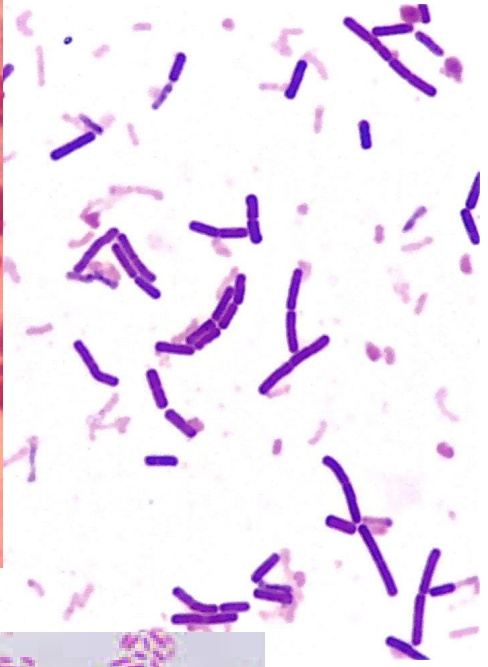
What do we need for this?

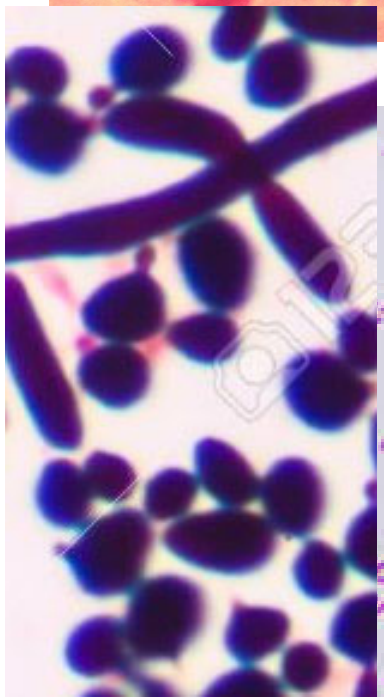
- For bacteriology: light microscope
- **Preparations**
  - Native with physiological solution (or KOH for fungi)
  - Stained
    - **Gram – bacteria, yeasts**
    - Ziehl-Neelsen – mycobacteria, nocardia
    - Ink (Burri) – for the presence of capsules (cryptococci)
    - Wirtz-Conklin – bacterial spores
    - Giemsa – pneumocysts, parasites
    - Lactophenol blue – fungi

Gram staining









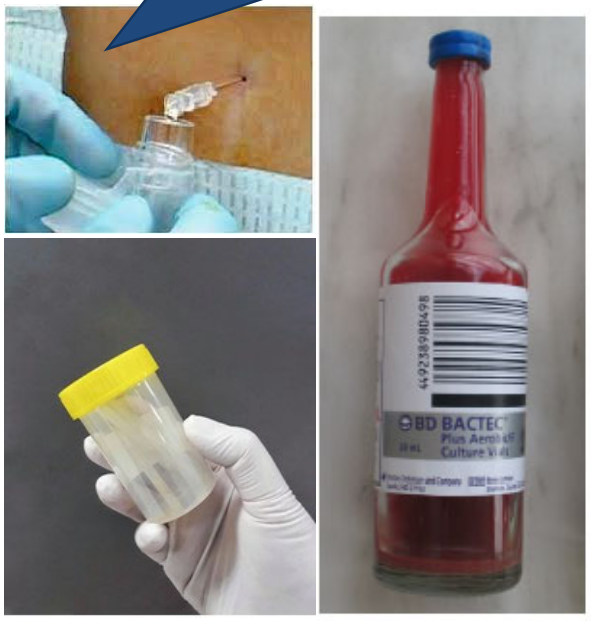
In practice – what **do we examine under the microscope**?

- **Clinical samples** (newly received)
  - Sputum – validity verification
  - CSF
  - Pathological secretions – liquid samples from sterile sites (pus, contents, punctures, etc.; possibly tissue after homogenisation)
  - Limited sensitivity (10^4 –10^5 CFU)

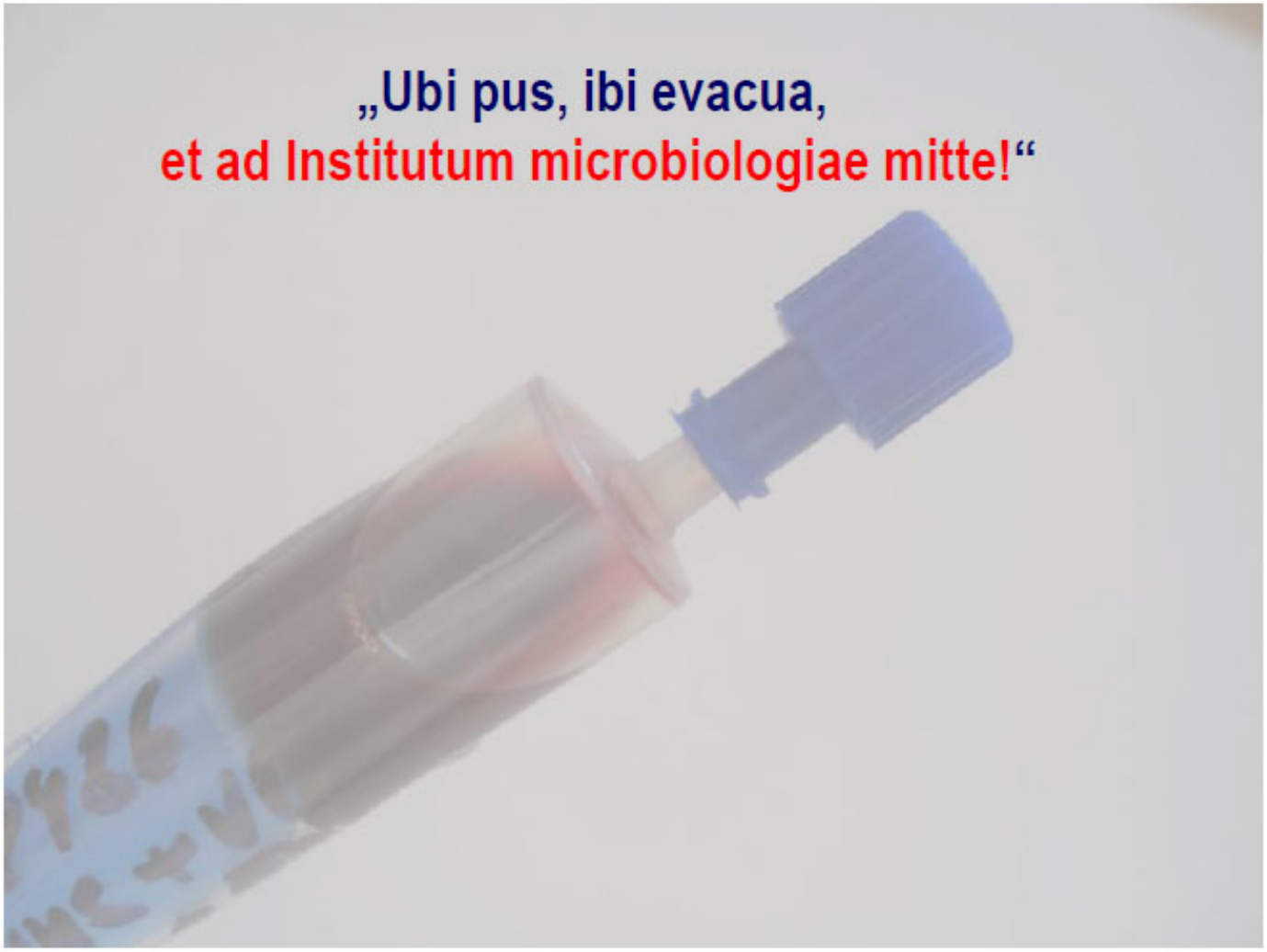
- **Positive blood cultures**
  - Patient with suspected bloodstream infection
  - First, inoculation of the blood culture bottle
  - The device detects bacterial growth in the medium (CO_2) and signals it (by beeping)

- **Microbial cultures** (bacteria, yeasts and moulds)
  - Unidentifiable strains
  - Confirmation of disputed identification

Fast and inexpensive method – tells you whether microbes are present → significance in initial differential diagnosis and patient treatment management.



Remember!



What do we **not** examine under a microscope?

- Swabs from the throat, rectum, superficial wounds, etc.

- Stool samples for bacteriology, can only be examined under a microscope for
  - parasitology (+tapes!)
  - virology (electron microscope)

Microscopy of clinical materials

1) Sputum microscopy

- Gram staining

- **We are interested in**:
  - Squamous epithelium
  - Microbes
  - (culture appearance)

	Criteria	*Score*
Neutrophils (pus cells) count (Score A)	< 10 neutrophil/10x field	0
	10-25 neutrophils/10x field	+1
	>25 neutrophils/10x field	+2
Macroscopy (Score B)	Mucoid, Mucopurelent, Purelent, or Blood stained	+1
Squamous epithelial cell count (Score C)	< 10 Squamous epithelial cell/10x field	0
	10-25 Squamous epithelial cell /10x field	-1
	>25 Squamous epithelial cell /10x field	-2

- **Sputum validity** – Bartlett score (values -2 to +3)
  - Score 0 and below: no inflammation or contamination
  - The more leukocytes and fewer squamous cells, the more valid
  - Abscessing pneumonia may have different sputum than mild pneumonia

Samples from DCD

* **Sputum** – expectorated sputum. Valid sputum – high number of leukocytes, few large epithelial cells, no contaminating URT microbiota

Invalid sputum

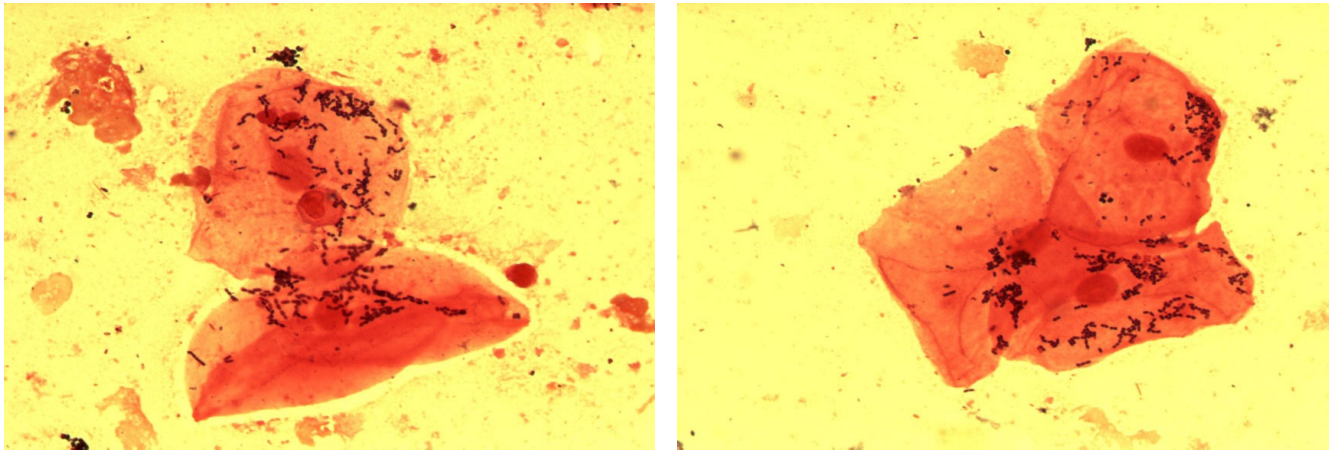


Fig. Large epithelial cells from HCD (saliva in the sample) covered with commensal Gram-positive flora (usually viridans streptococci)

Valid sputum

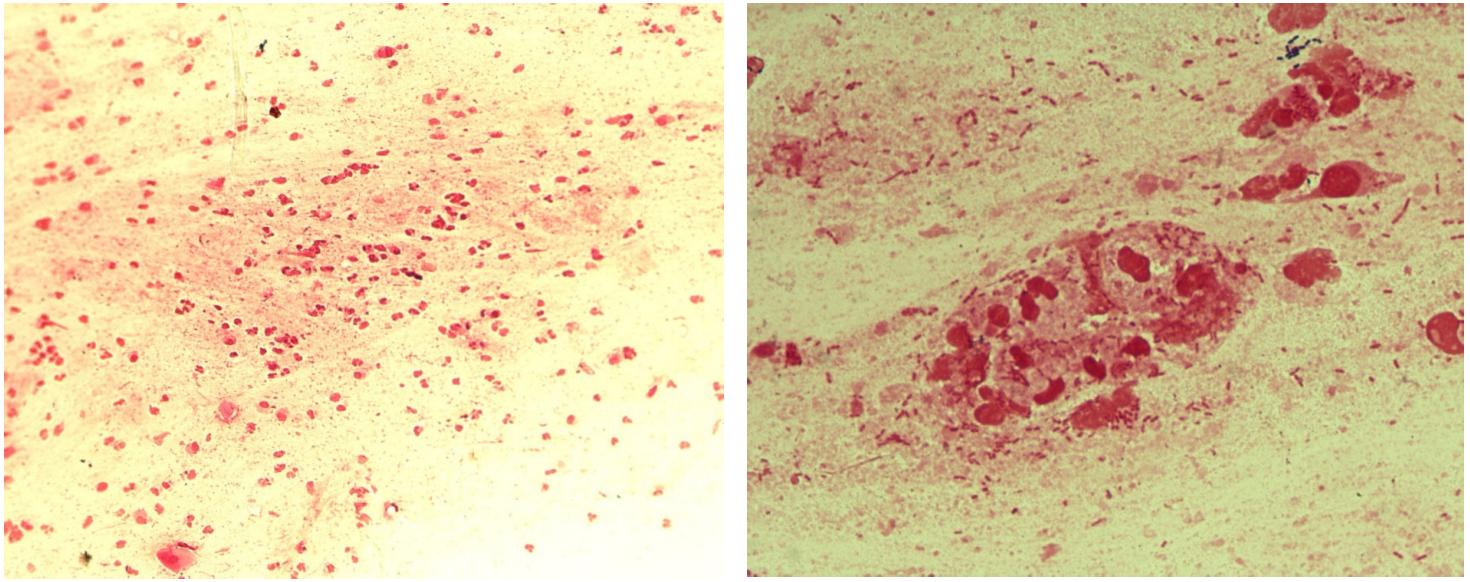
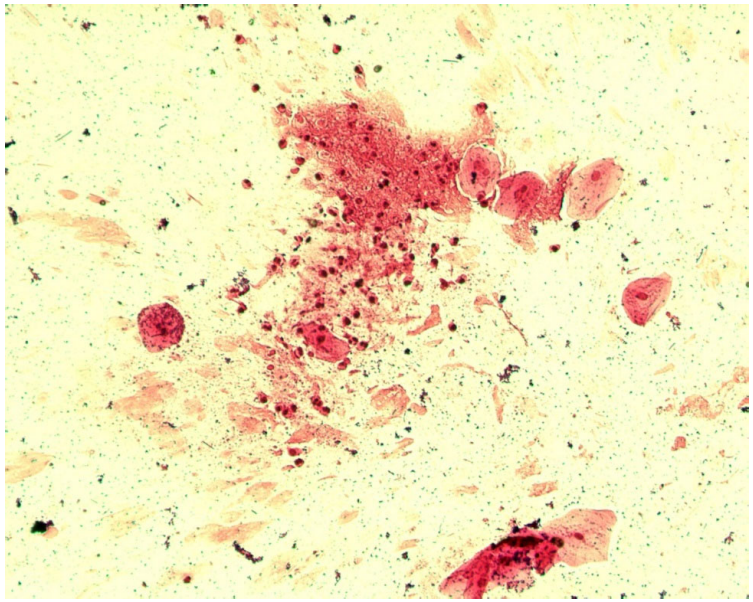
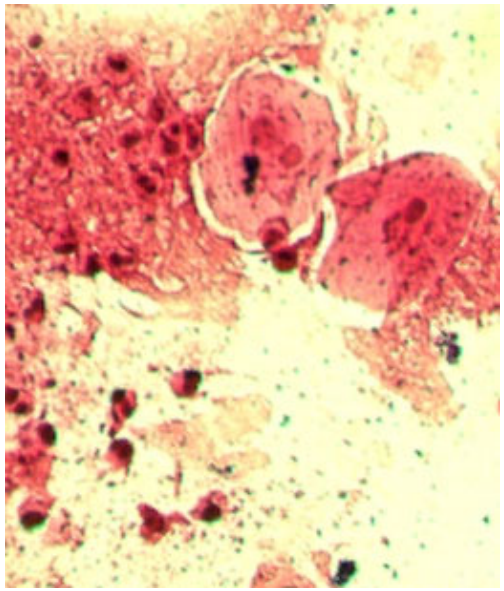


Fig. Leukocytes originating from DCD (left, low magnification) and uniform morphology of bacterial cells (right, high magnification, Gram-negative rods, left)

Sputum of poorer quality, still suitable for culture



A – low magnification



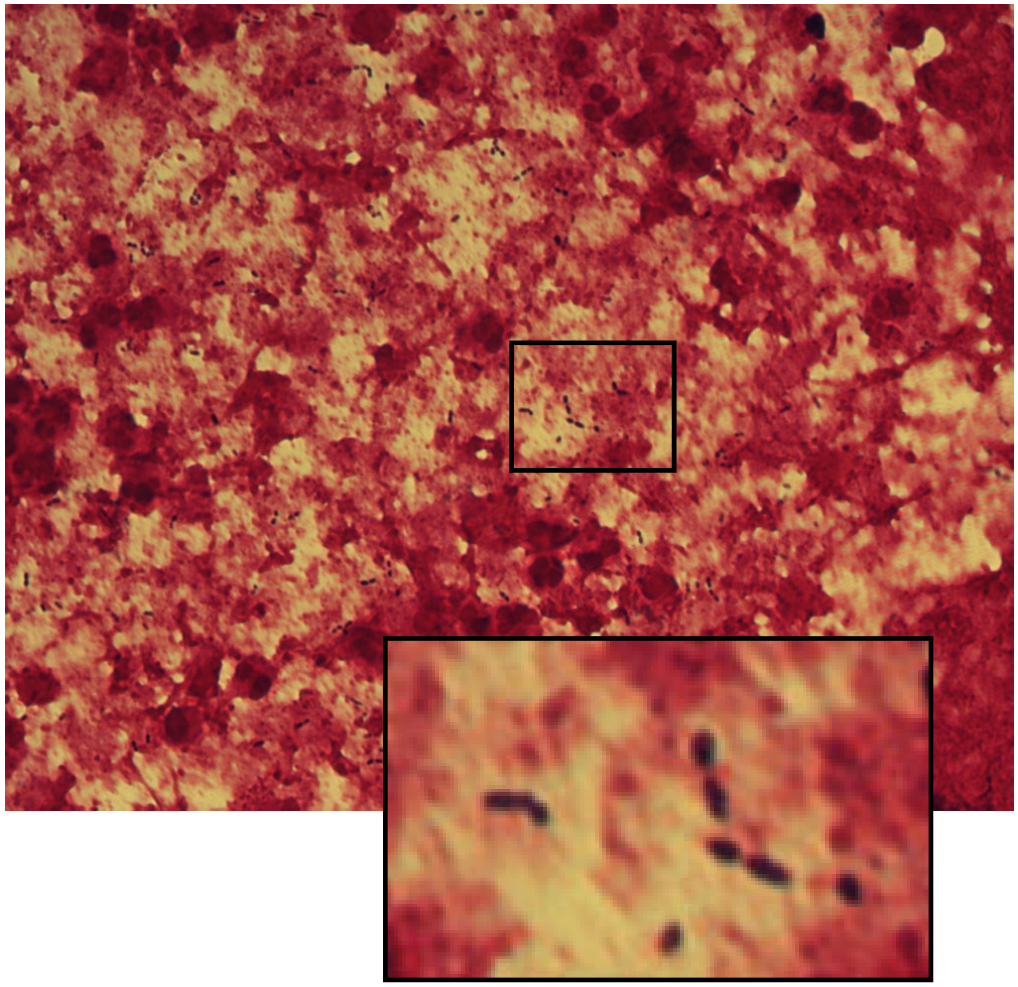
B – higher magnification

Fig. Large number of leukocytes from DCD and large epithelium from HCD

Significance

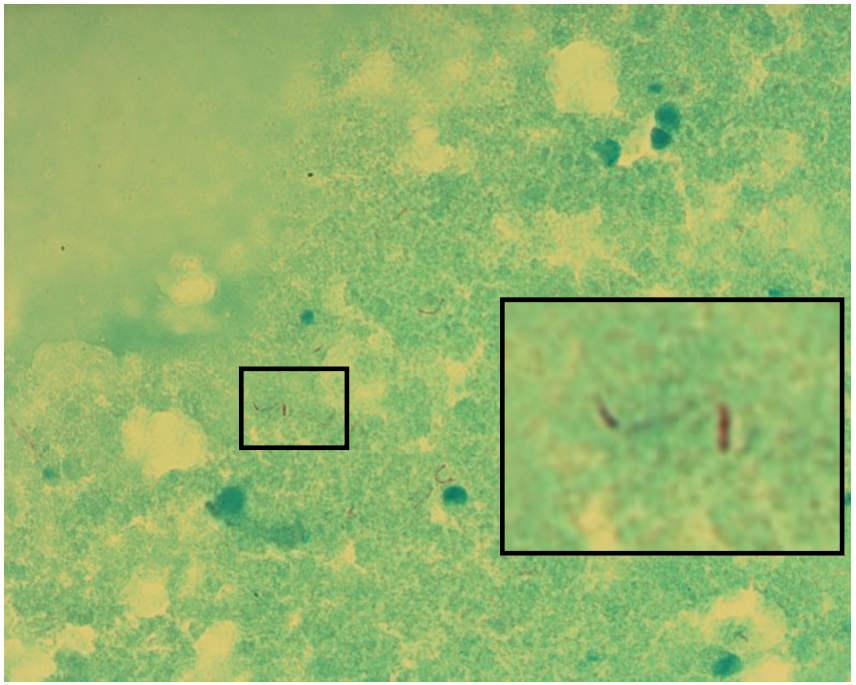
- Using microscopy, we can identify the agent and consult on treatment
  - G+ diplococci (see fig.)
  - G- rods

Atlas of Clinical Microbiology
Dept of Medical Microbiology, 2nd Faculty of Medicine, Charles University
Permanent preparations and e-versions available



Sputum microscopy for TB

- May be useful (see fig.), but must be targeted
  - Other staining (Ziehl-Neelsen)
  - Lower sensitivity
    - A negative smear does not mean the absence of TB



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2) Cerebrospinal fluid microscopy

- Use:
  - Routine
  - **Suspected neuroinfection**

The same applies to other primarily sterile materials

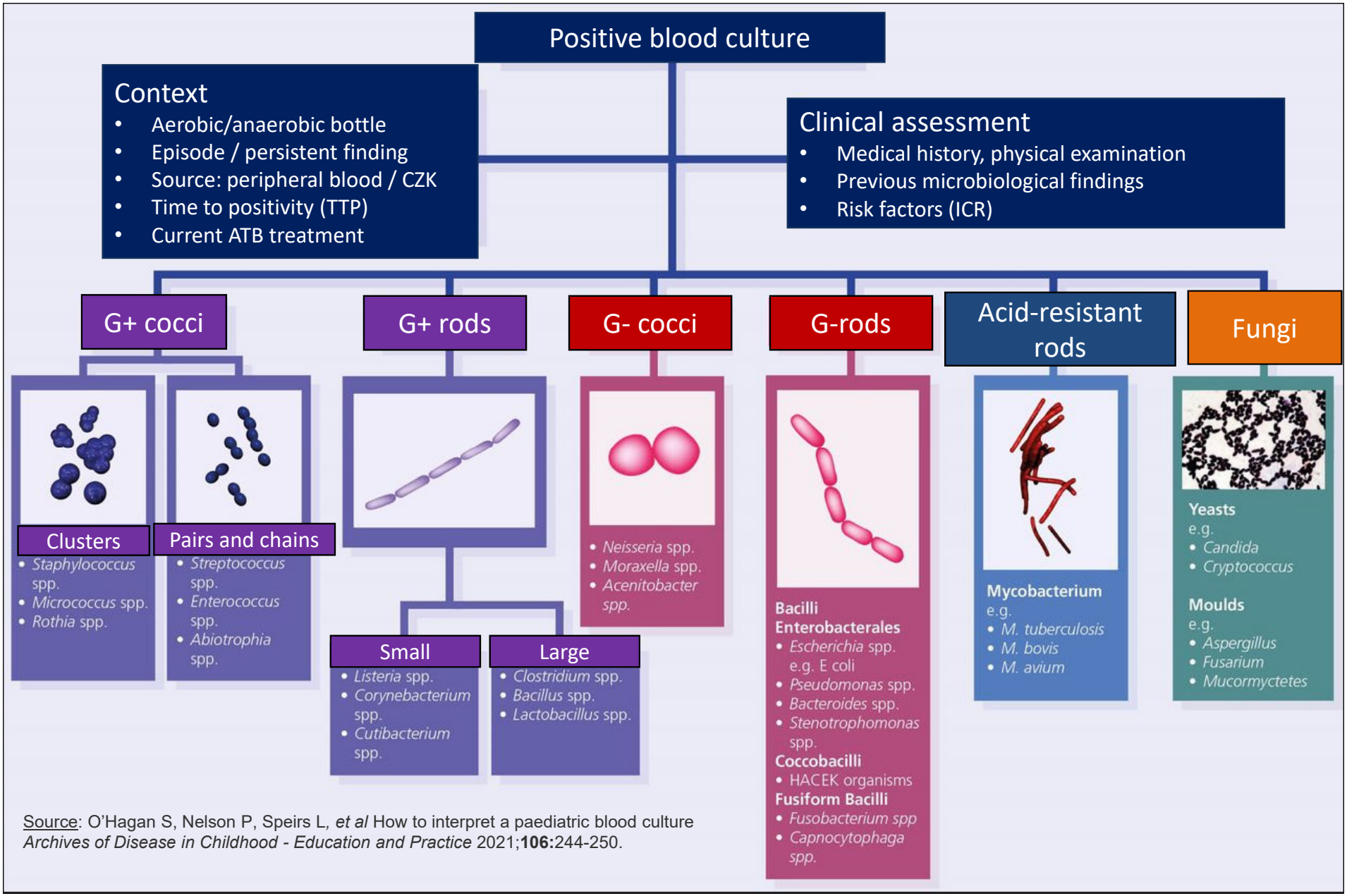
- In cases of neuroinfection:
  - Degree of inflammation
  - Microbes – we communicate the morphology to **the attending physician during the critical interval**
- When there are no microbes, we rely on PCR and serology
- Properly performed cerebrospinal fluid microscopy can be a life-saving procedure!

3) Blood cultures

Why examine positive blood cultures under a microscope?

- To verify the **actual positivity**
  - If we do not see bacteria, something is wrong

- **Basic information about the morphology of microbes in blood cultures**
  - Possibility of immediate change of treatment to **targeted antibiotic therapy**
    - Empirical antibiotic therapy is much less effective
  - Impulse for further **identification methods**
    - Bacteria – indicative sensitivity within 2 hours
    - Key hyphae in yeasts – basic identification within 2 hours
  - Correctly performed **indicative sensitivity**
    - Correct antibiotic combinations
    - We know the next day at the latest



Source: O'Hagan S, Nelson P, Speirs L, *et al* How to interpret a paediatric blood culture *Archives of Disease in Childhood - Education and Practice* 2021;**106:**244-250.

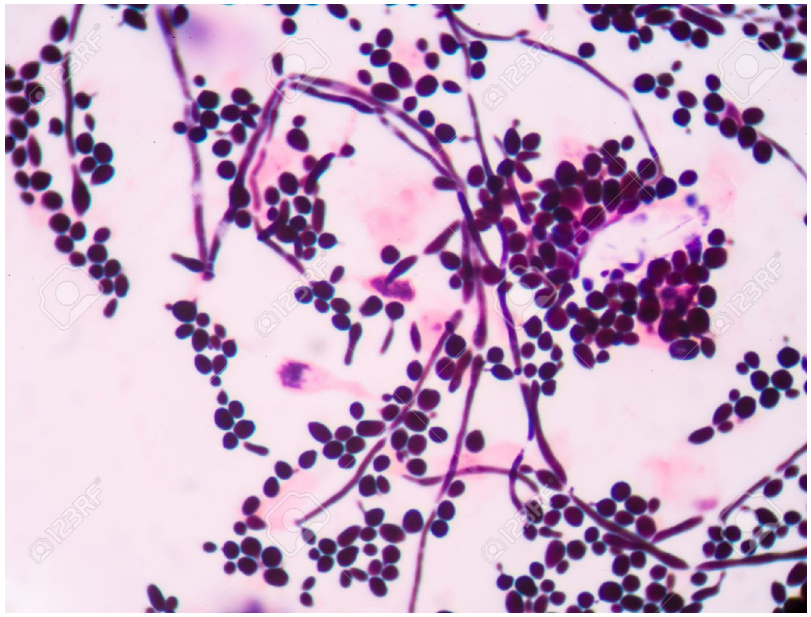
4) Overgrown cultures

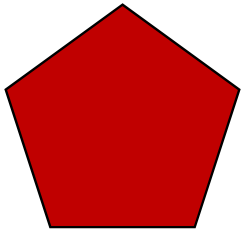
NOT STANDARD for bacteria.

IDENTIFICATION FROM CULTURE, but it helps:

- For bacteria and yeasts
  - If MALDI TOF fails (i.e. the gold standard for identification)

- **For moulds,** microscopy is indispensable for identification



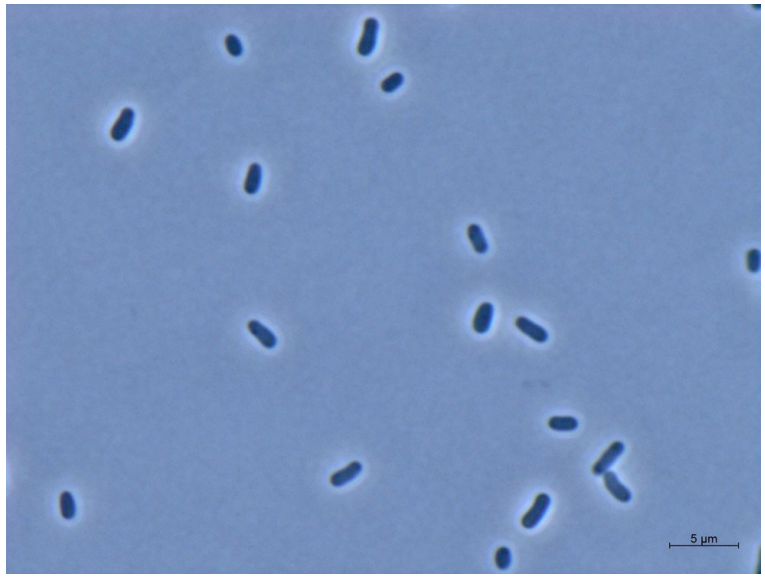


Practicals

Native slides

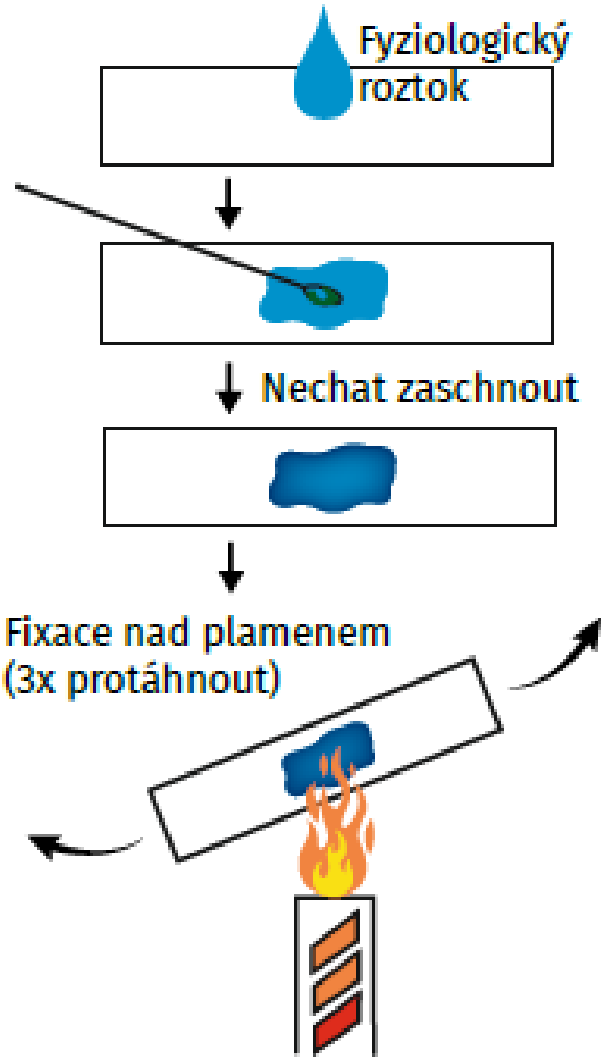
- Scorching the slide
- Drip saline
- Apply culture with a loop
- Place a cover glass and observe

You see movement
– no fixation



1) Fixation by flame

- Add saline
- Spread the culture
- Leave to dry
- Fix

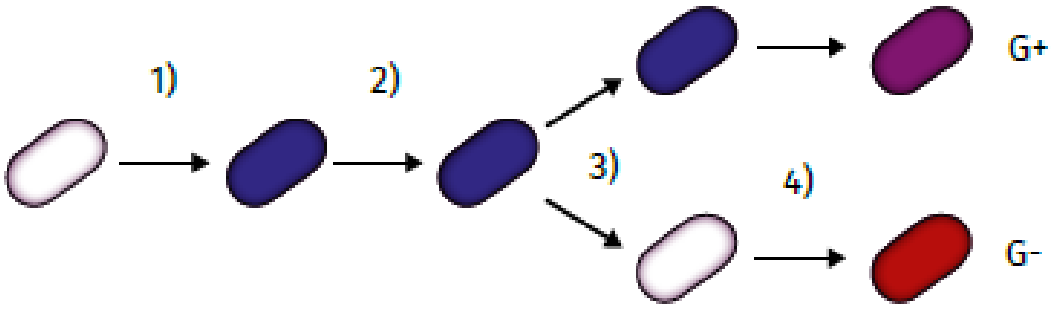


OBR. 185 PŘÍPRAVA FIXOVANÉHO PREPARÁTU

2) Gram staining

V-L-A-K

- **1.** Crystalline violet
- **2.** Lugol's solution (G+)
- **3.** Acetone (ethanol)
- **4.** Carbol fuchsin (G-)



3) Further staining in bacteriology

- **Ziehl-Nelsen** (TB)
  - Carbol fuchsin
  - Alcohol
  - Malachite
  - Rinsing

  TBC red on green background

- **Buri** (capsules)
  - Ink + culture and coating
  - Fixation
  - Violet

  Sheaths are white (not stained) on an ink background

- **Wirtz-Conklin** (spores)
  - Malachite
  - Carbol fuchsin

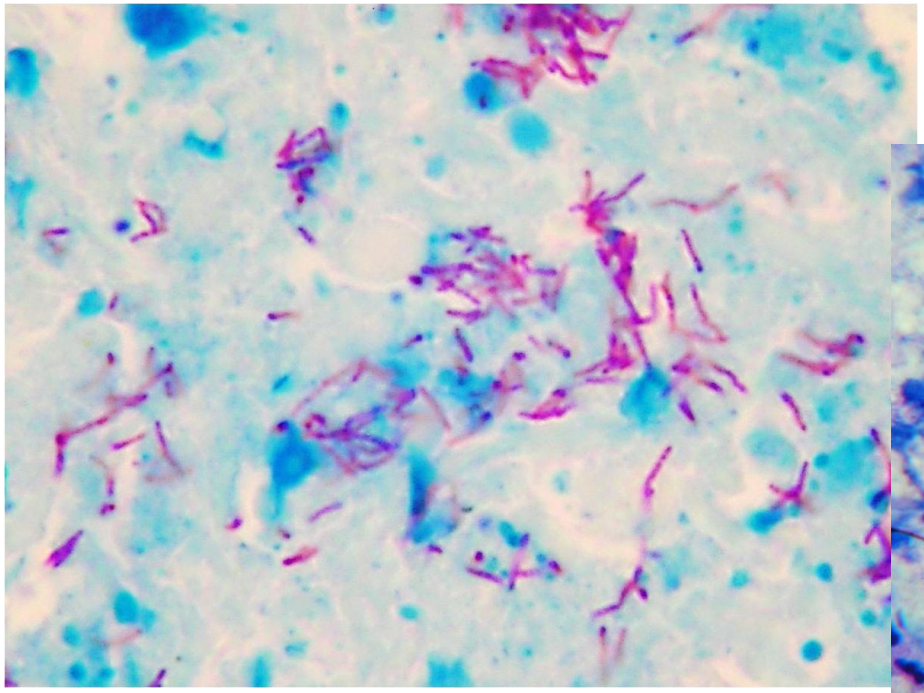
  Bacteria pink, spores green

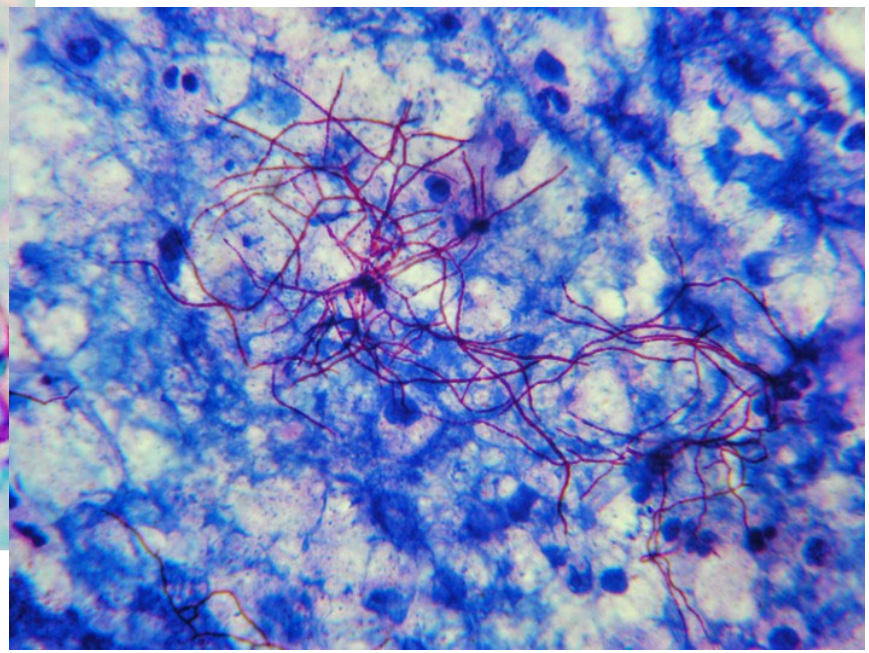
- **Albert** (granules)
  - Albert
  - Lugol

  Bacteria green, granules blue

Ziehl-Neelsen

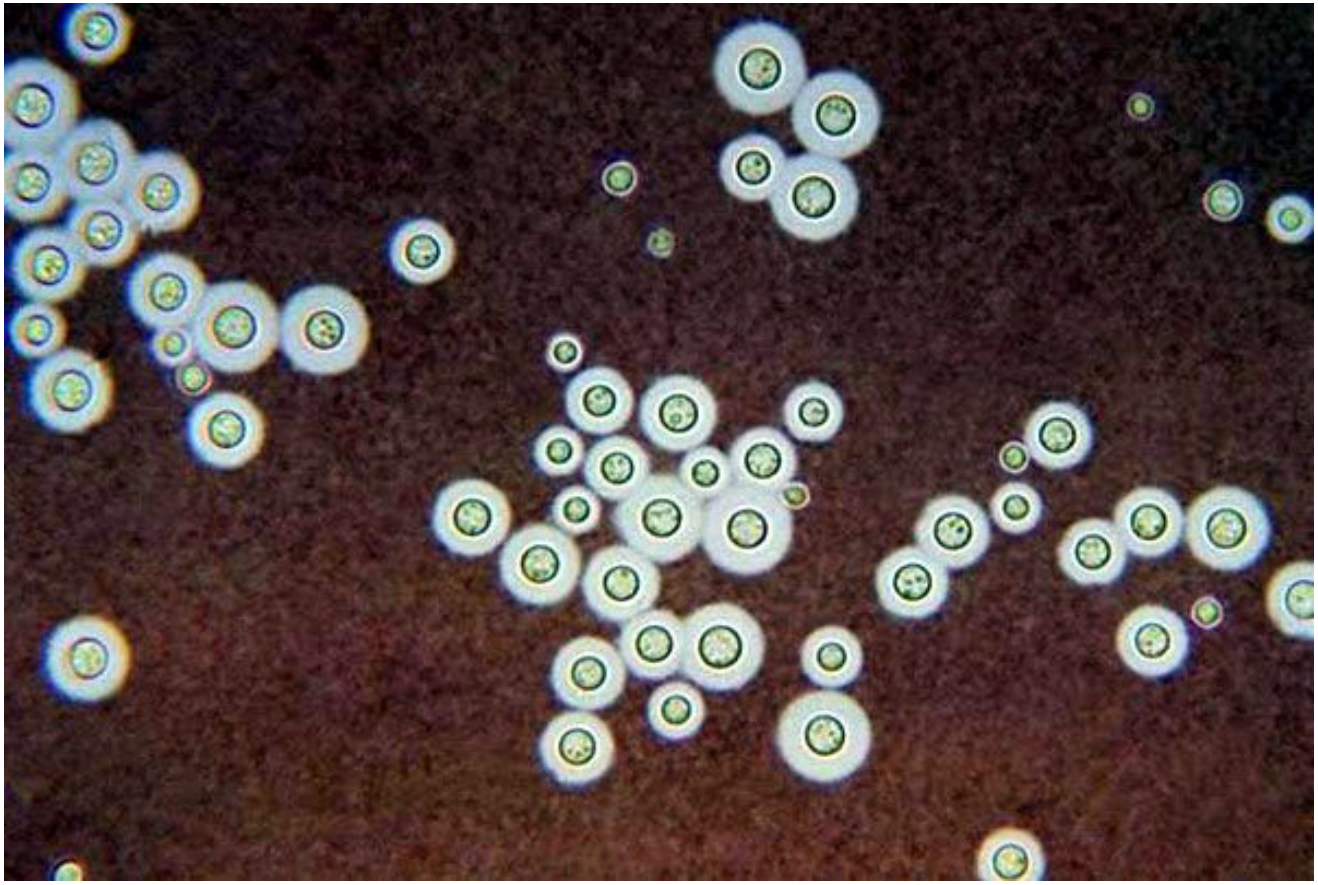
- What? Acid-resistant rods
- For what? Mycobacteria, nocardia



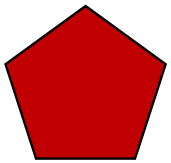


Buri (ink)

- What? Cases
- For what?
  - ***Cryptococcus*** (yeast),
  - **encapsulated bacteria** (e.g. *S. pneumoniae*)



Slides with basic morphologies



- g- rod
- g- diplococcus
- g+ rod
- g+ cocci in clusters
- g+ cocci in chains

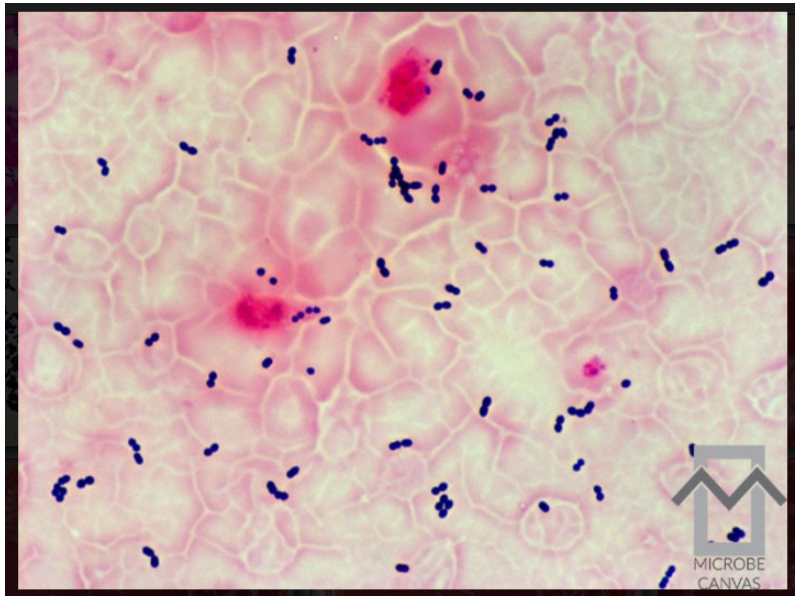
Interpretation

- **Cells**
  - Leukocytes
  - Erythrocytes
  - Epithelia

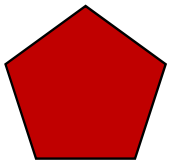
Is this the right material?

- **Microbes**
  - Yes/no
  - Staining and shape
  - Number (approximate)

Are the microbes there?



Conclusion: What do you think?



Let's get started!

Learn at home

Microbiology I (DA1104339)

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General Collapse all

- Oznámení
- Sylabus — Completion
- Introductory email — Completion
  All the necessary stuff before you start the course (credit, practical training and exam)
- Bacteria Overview — Completion
  Figures from the czech textbook (Hurych et al, 2021)
- Microscopy atlas — Completion
  Melter et al. (2025) - new edition