Direct detection of infectious agent in clinical specimen

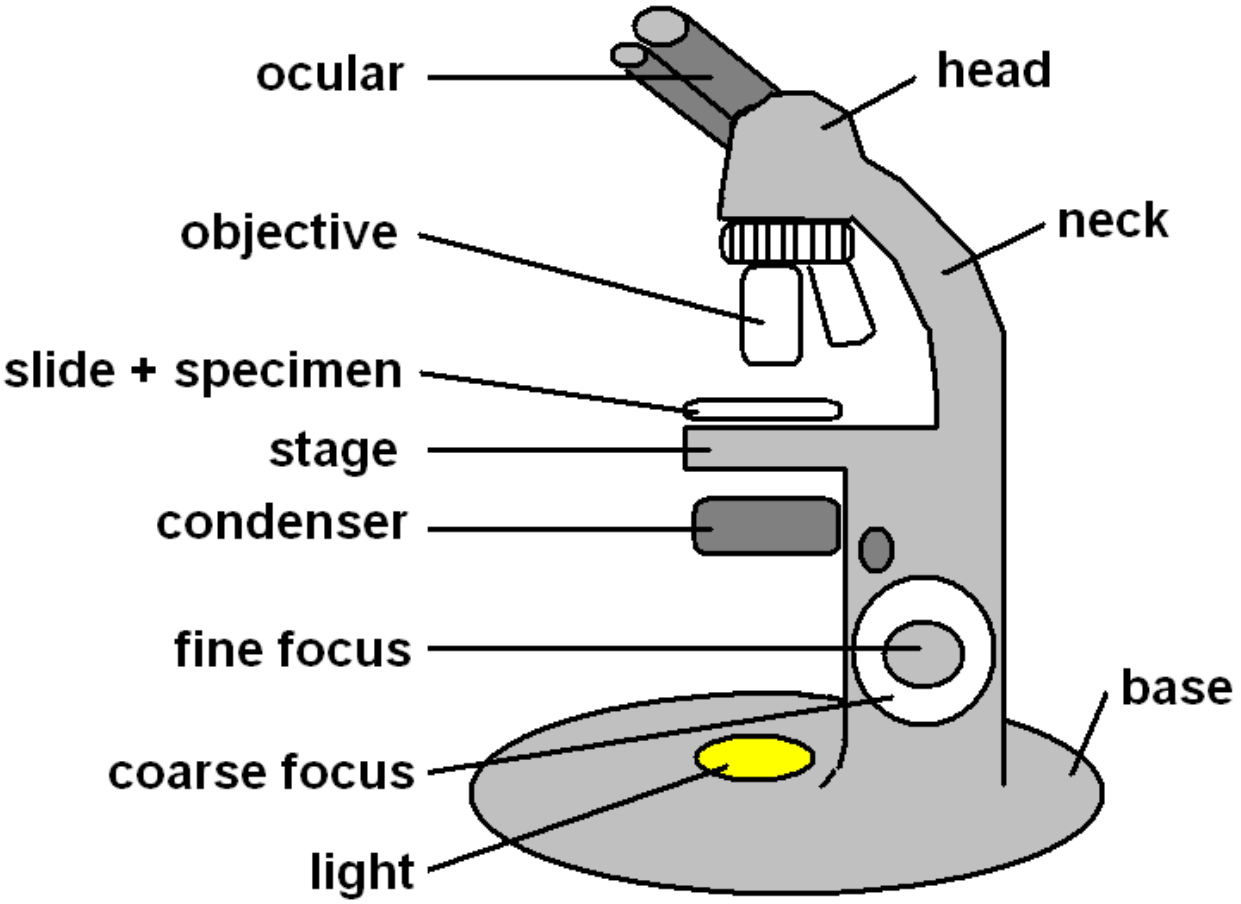
Microscopy

- **is the technical field of using microscopes to view objects or their details which could not be seen with unaided eye (advantage – quick, cheap, but low sensitivity)**
- **branches of microscopy and their principles**
- - **light** (optical): visible light transmitted through or reflected from the sample through a single or multiple lenses to allow a magnified view of the sample. Image is detected directly by the eye, imaged on a photographic plate or captured digitally.
- **techniques:**
- bright field microscopy
- dark field microscopy (oblique ligt illumination, unstained samples, only scattered light with object create image, higher contrast)
- phase contrast microscopy (unstained samples, phase shifts in the light passing through a transparent specimen are converted into contrast changes in the image)
- fluorescence microscopy (structures of interest stained with fluorochromes emitt fluorescence)
- - transmission **electron microscopy**: except visible light using electron beams, higher resolution 0,05 nm
- - scanning electron microscopy: visualizes details on the surfaces of cells and particles, nice 3D view
- reference: basic microscopy course - ttp://www.epa.gov/ogwdw000/lt2/training/

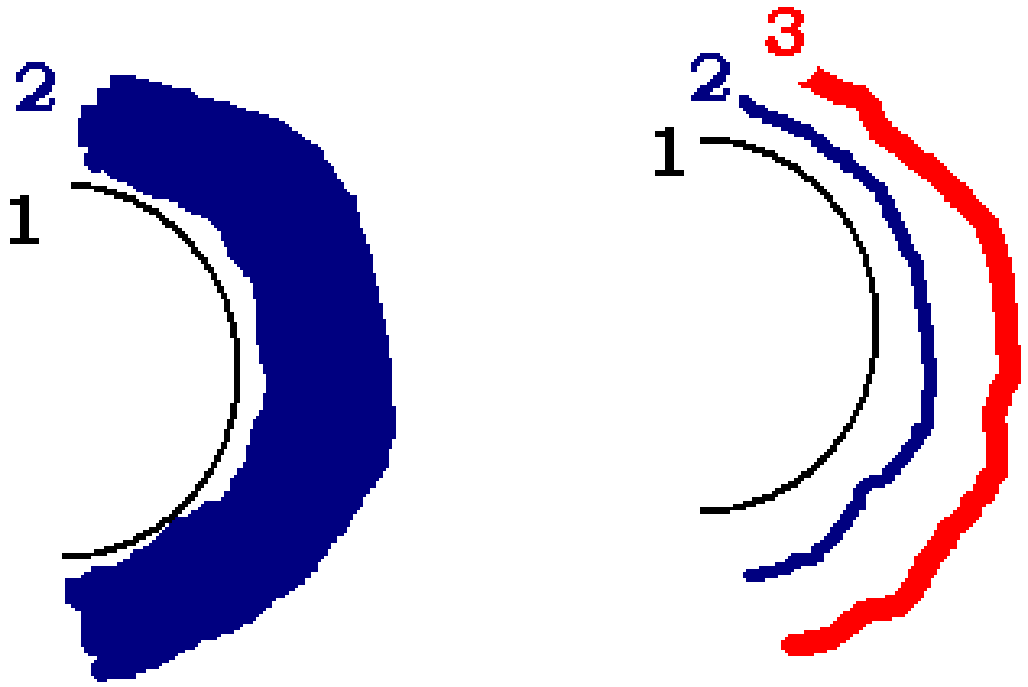
Suitable samples

- **Must be from primary sterile sites**
- **Positive hemocultures** (to detect agents of septicemia)
- **Cerebrospinal fluid** (to detect bacterial agent of CNS infection)
- Aspirates from any primary sterile sites (e.g. joint aspirate, peroperative material from internal tissue and fluids – incision/bioptate of endocard – bacterial endokarditis, abscess especially located in abdominal cavity…)
- fluorescent microscopy (structures of interest stained with fluorochromes emitt fluorescence)
- Dont observe stool to diagnostic bacterial GIT agent (polymicrobic sample)!!!

Light microscope – essentila tool in clinical microbiology lab

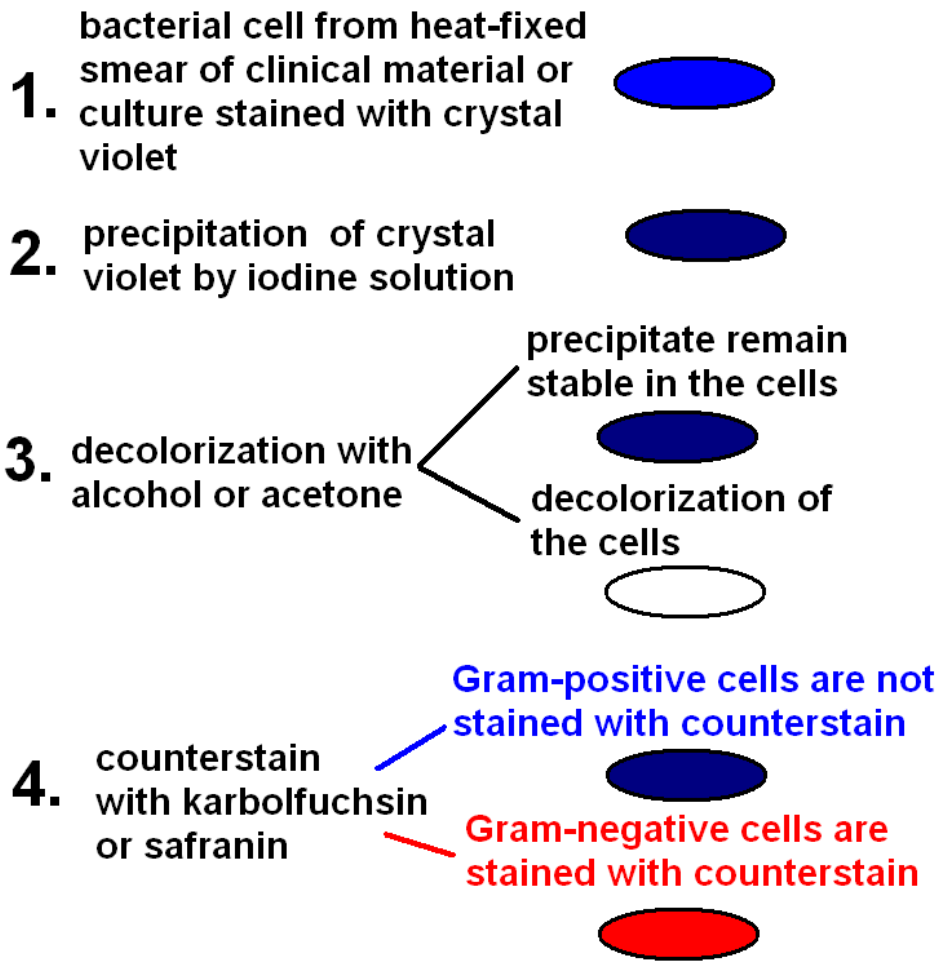


Main differencies in structure of Gram-positive and Gram-negative cell envelope

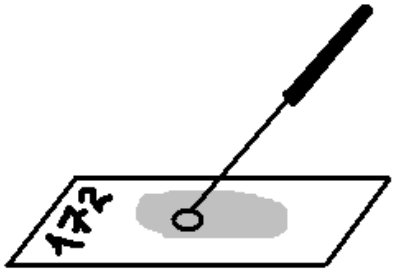


Note: 1. cytoplasmatic memrane, 2. cell wall (peptidoglycan), 3. outer membrane

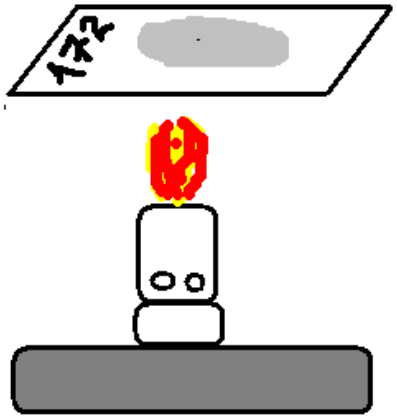
Gram stain


1.
bacterial cell from heat-fixed smear of clinical material or culture stained with crystal violet
2.
precipitation of crystal violet by iodine solution
3.
decolorization with alcohol or acetone
precipitate remain stable in the cells
decolorization of the cells
4.
counterstain with karbolfuchsin or safranin
Gram-positive cells are not stained with counterstain
Gram-negative cells are stained with counterstain

Preparing smear of clinical material or bacterial culture for differential staining

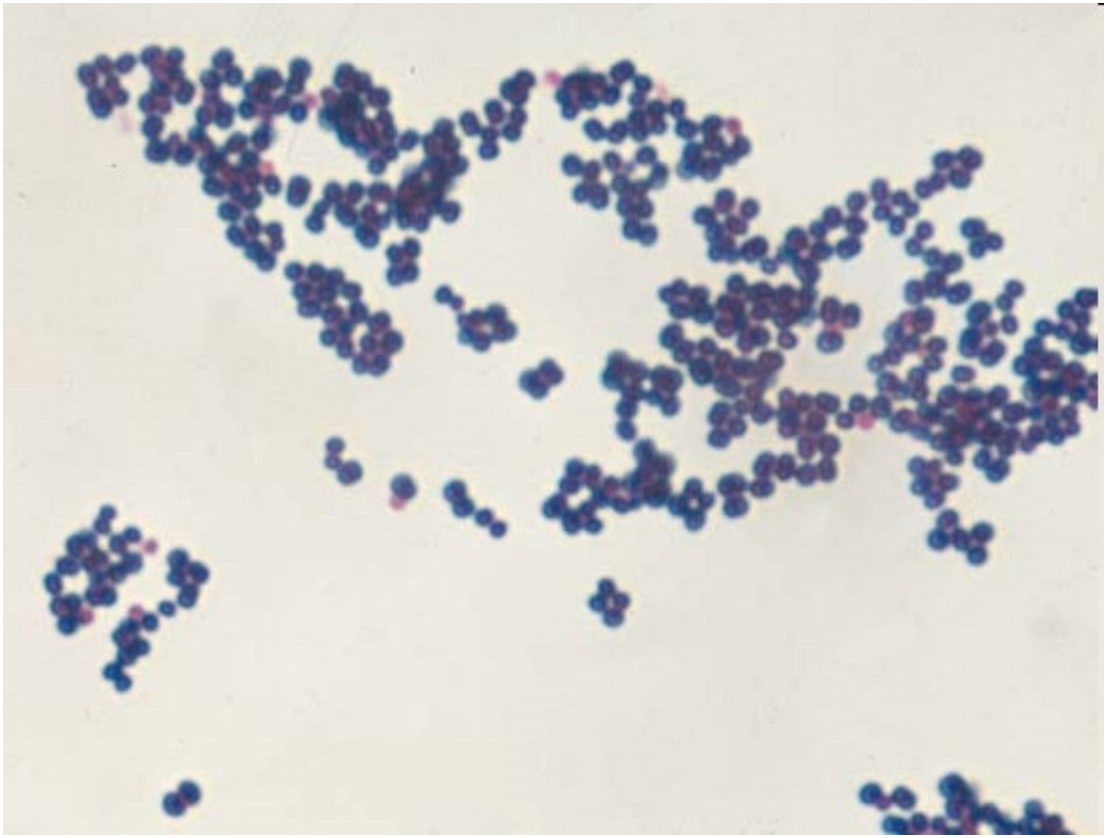


1. label slide
2. spread culture or material in thin film over slide
3. dry in air

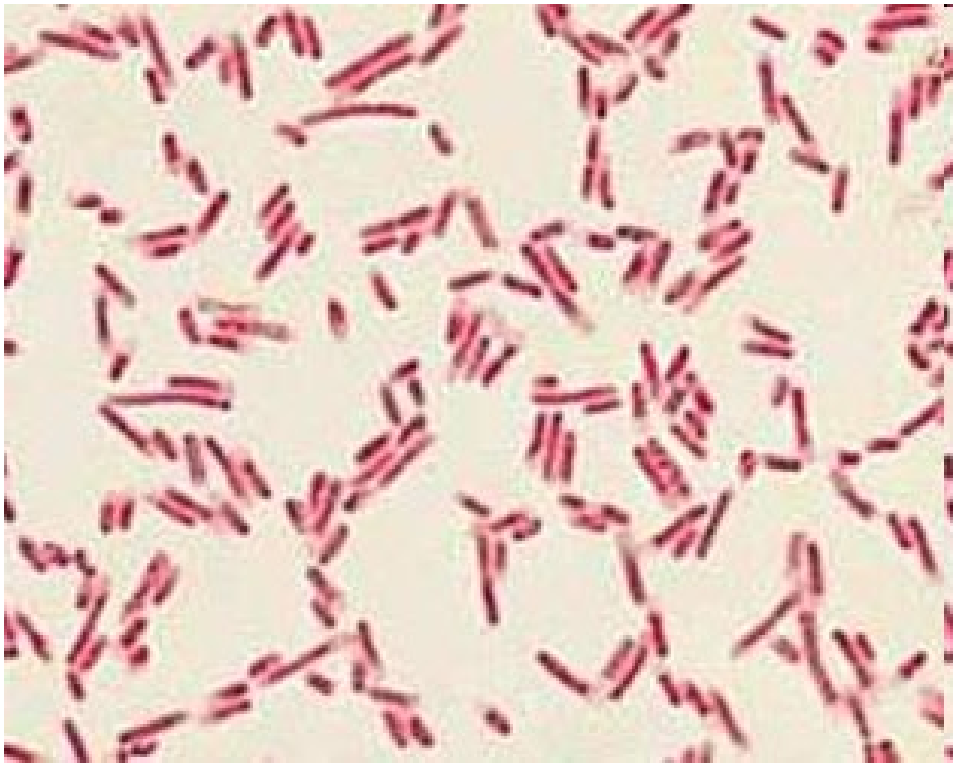


4. pass slide through flame to fix

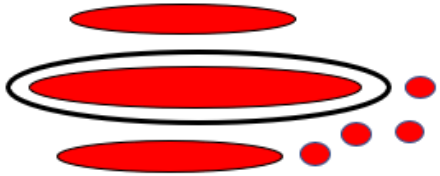
Gram-positive bacteria



Gram-negative bacteria



Ziehl-Neelsen staining

1. 

Presence of lipids in the outer layer – carbol fuchsin by heated disruption and staining of mycobacteria but also common microbiota in red (or special TB fuchsin is used, probably with an organic difference)

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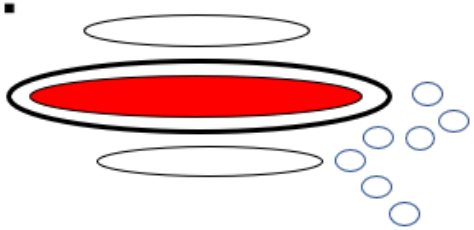
2. 

Acidic alcohol decolorizes the normal microbiota, leaving only the red-colored mycobacteria

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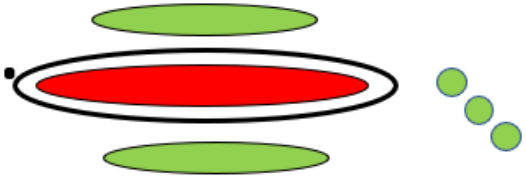
3. 

Mycobacteria will remain red in color and microbiota will stain green with malachite green.