Microscopy

Direct identification of bacteria

1st Practical part

Microbiological diagnostics

- Direct identification
  - Microscopic identification
  - Cultivation
  - Demonstration of bacterial components in clinical material
  - Closer determination of the pathogen
  - Determination of susceptibility to ATB
- Indirect identification
  - Serology

Direct identification

Microscopy

- Light microscopy
  - Magnification 10-100x
  - Bacteriology, mycology, parasitology
- Fluorescence microscopy
  - Magnification 10-100x
  - Mycobacteriology, mycology, virology
- Electron microscopy
  - Magnification 10-100 000x
  - Especially Virology

Direct identification

Microscopy

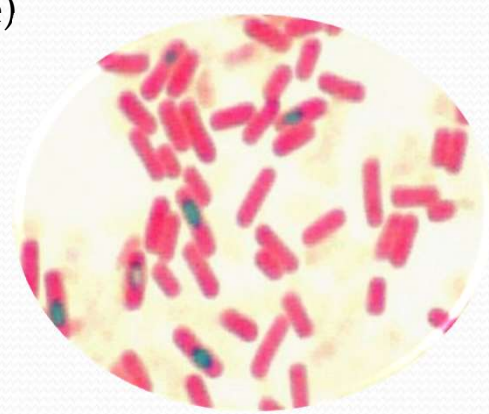
- Light microscopy
  - Native preparation
  - Observation of living microorganisms
  - Movement
- Parasitology
  - Observation of eggs and cysts of parasites
- Mycology
  - Lye (2% KOH) preparations
- Fixed specimen
  - Use for staining methods
  - Simple staining methods
  - according to Loeffler
- Diagnostic staining methods

Enterobius vermicularis – native microscopy

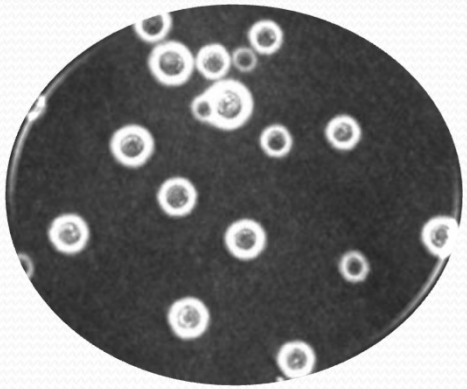
Direct identification

Microscopy

- Diagnostic staining techniques
  - Gram stain
  - Division into groups Gram positive and Gram negative (or unstable)
  - Based on the different structure of the bacterial wall
- Ziehl-Neelsen staining
  - For the diagnosis of acid-resistant microorganisms
  - Mycobacteria (mycolic acids)
- Burri's india ink staining
  - Proof of capsule – factor of virulence
  - capsules are represented by uncolored space
- Albert staining
  - Demonstration of metachromatic ganules
- Staining according to Wirtz - Conklin
  - For proof of spores
- Giemsa staining
  - It is performed in blood smears
  - Parasitology



Bacillus - Wirtz-Conklin staining



Cryptococcus neoformans – Burri's staining

Preparation of slides

- Fixed specimen made of clinical material
  - Spread the clinical material over the entire surface of the slide and allow to dry
  - Fix by stretching in flame (3x) or in methanol for 5 min
- Fixed preparation from pure culture
  - Drop a drop of physical solution on the glass
  - Resuspend a small amount of colony in a drop of saline and allow to dry
  - Fix by stretching in flame (3x) or in methanol for 5 min

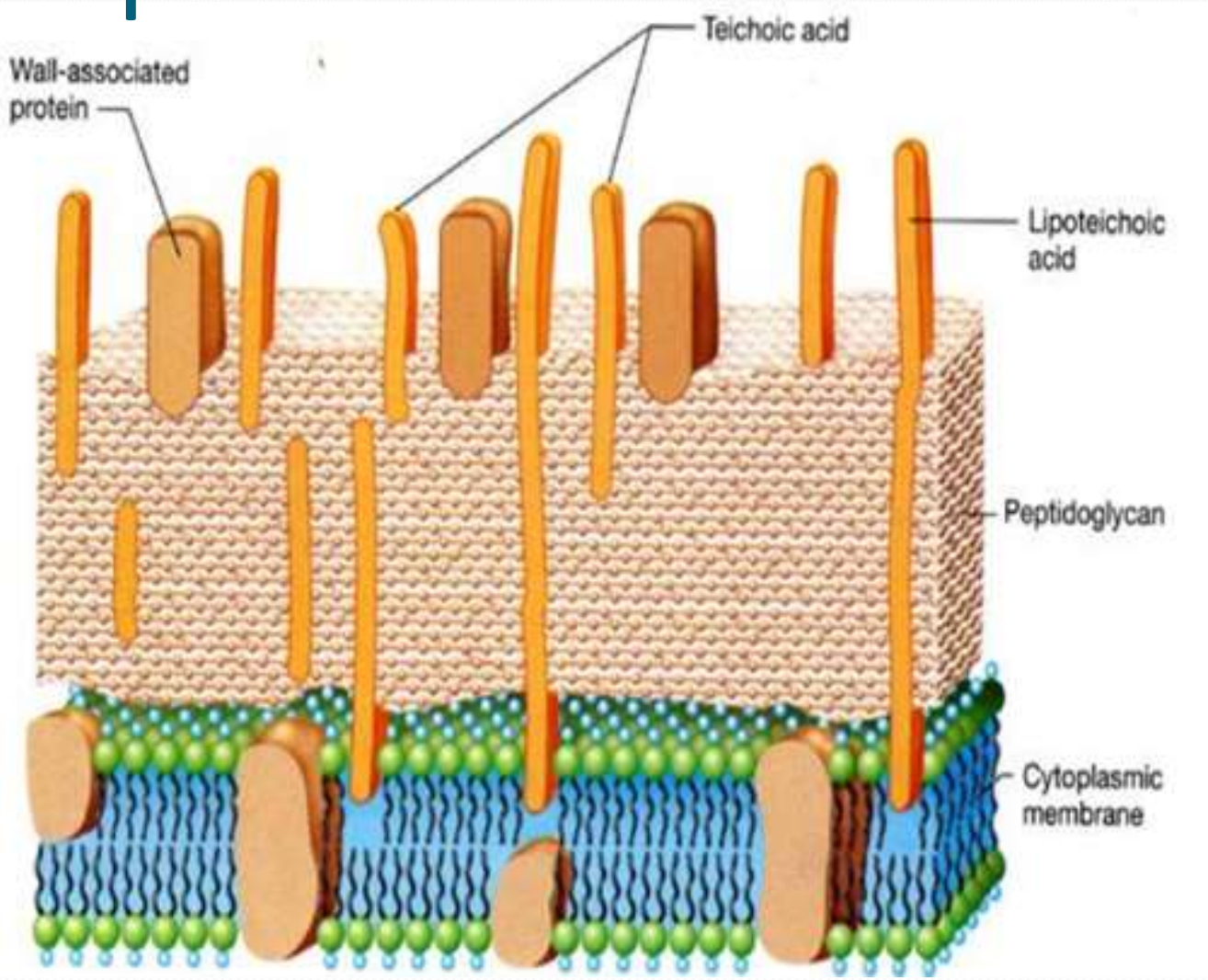
Gram staining

- Basic microbiological staining
  - Distinguishes bacteria into Gram positive (blue-violet), Gram negative (pink-red)
  - Based on the different structure of the cell wall
  - Formation of a color complex
  - Crystal violet + Lugol (iodine)
  - The complex is decolorized with acetone in gram-negative bacteria and the bacteria are further stained with safranin / carbolfuchsin

Gram positive bacteria

- The cell wall is formed by a massive layer of peptidoglycan
  - Composed of aminosaccharides
  - N-acetylmuramic acid
  - N-acetylglucosamine
- Acid-based transpeptides bind to each other - transpeptidation
- Teichoic acid chains run perpendicular
- The diameter of the cell wall is about 20 nm

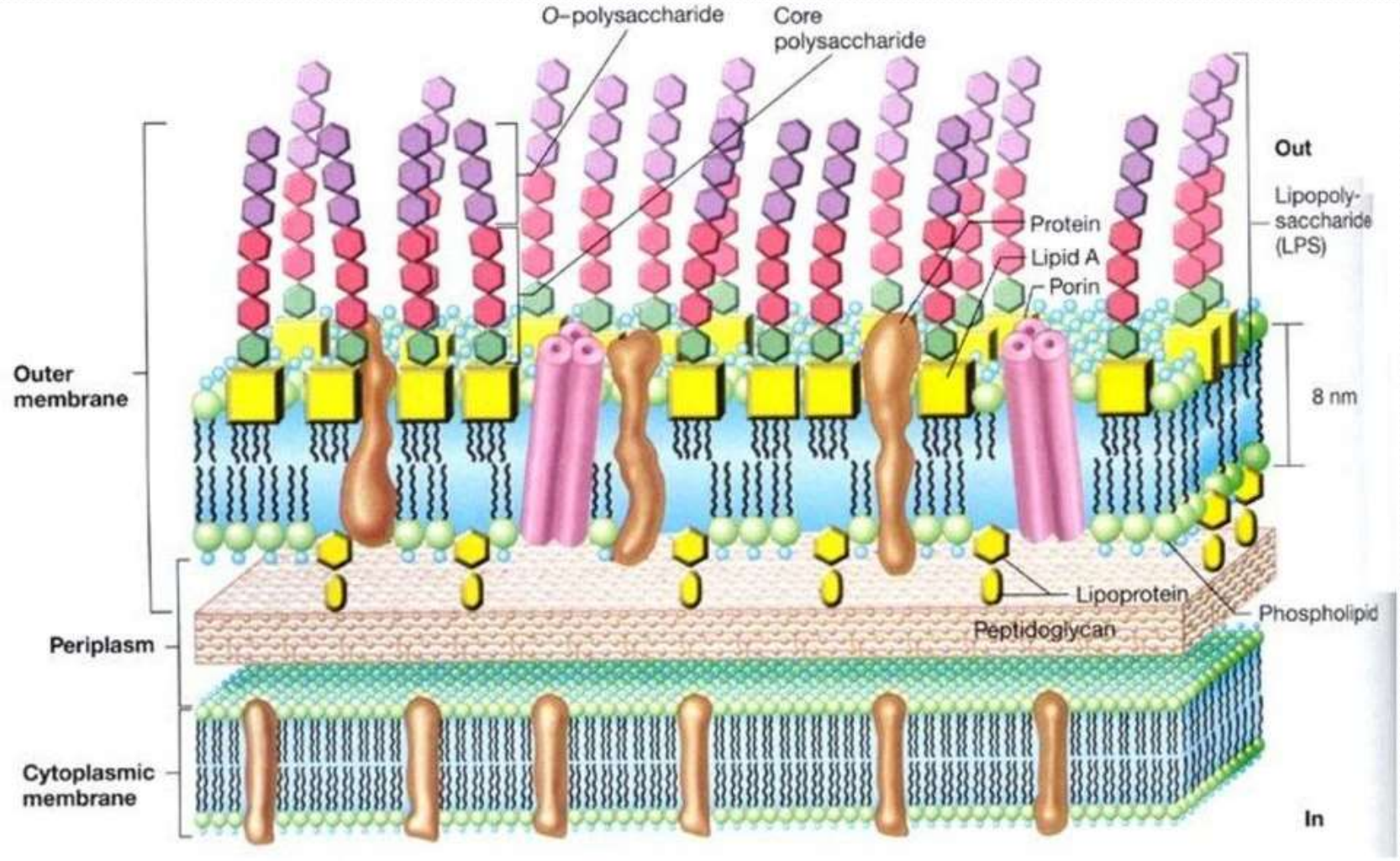
Gram positive bacteria



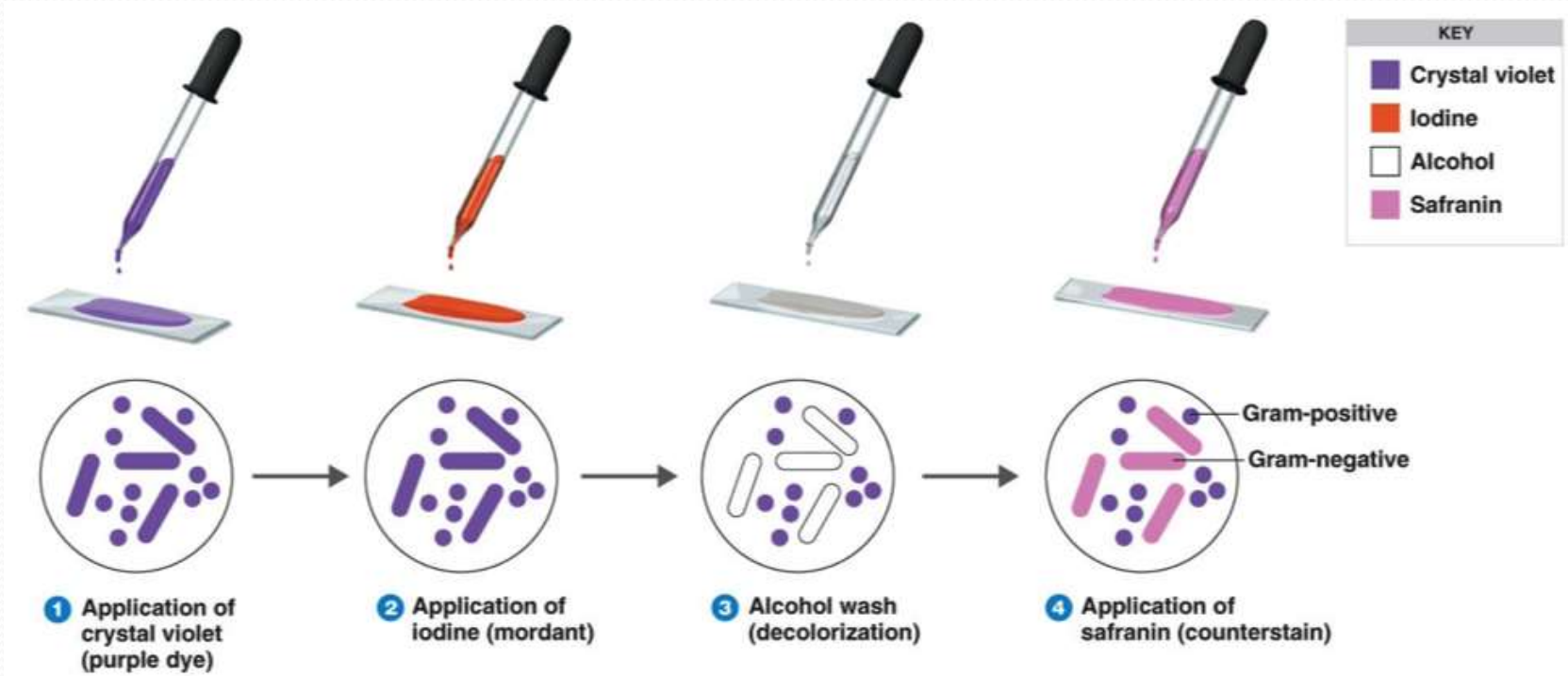
Gram negative bacteria

- The cell wall is composed of an outer membrane and a periplasmic space with peptidoglycan, which is thin, almost monolayer and contains diaminopimelic acid.
- The outer membrane consists of a phospholipid bilayer that contains proteins and lipopolysaccharides
  - Proteins - some of them form porines
  - Lipoproteins provide tight binding of the outer membrane to the peptidoglycan
- Lipopolysaccharides (endotoxin)
  - Lipid part immersed in the membrane - lipid A
  - Core (carbohydrate residues) connecting lipid A and antigen O
  - The polysaccharide part protrudes from the membrane - antigen O
- The outer membrane prevents the penetration of some substances
  - Crystal violet
  - Some ATBs, eg erythromycin
  - Bile acids
- The periplasmic space contains enzymes
  - Inactivation of some ATBs (beta-lactamases)

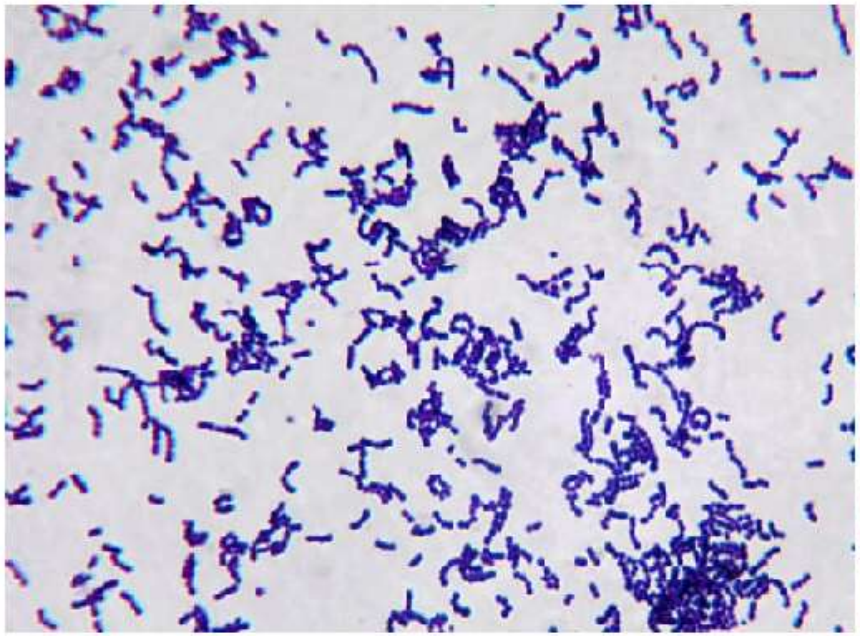
Gram negative bacteria



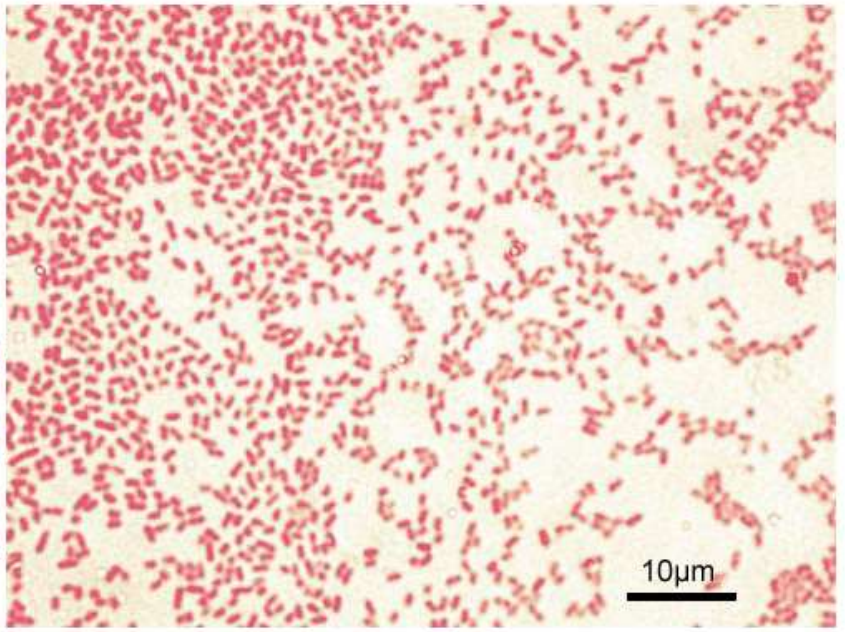
Gram staining



Gram staining



Gram Positive Bacteria



Gram Negative Bacteria

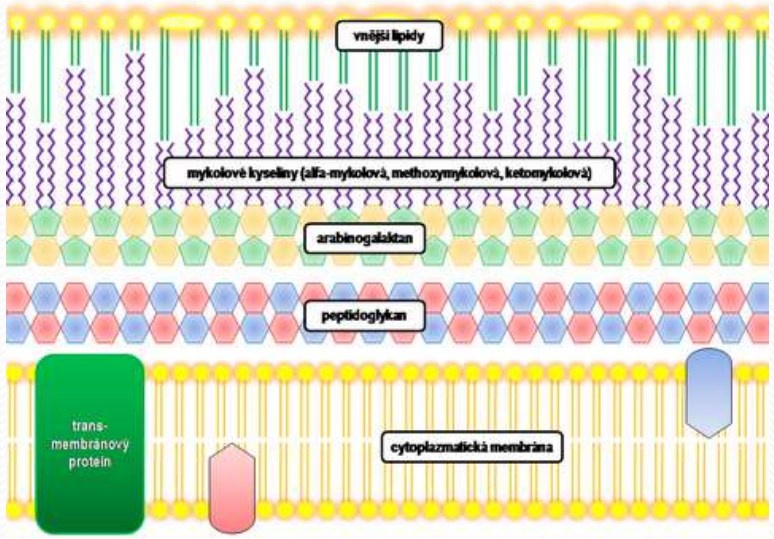
- **Gram Positive** : Dark purple
- **Gram Negative** : Pale to dark red
- **Yeasts** : Dark purple
- **Epithelial cells** : Pale red

Ziehl-Neelsen (acid-fast) staining

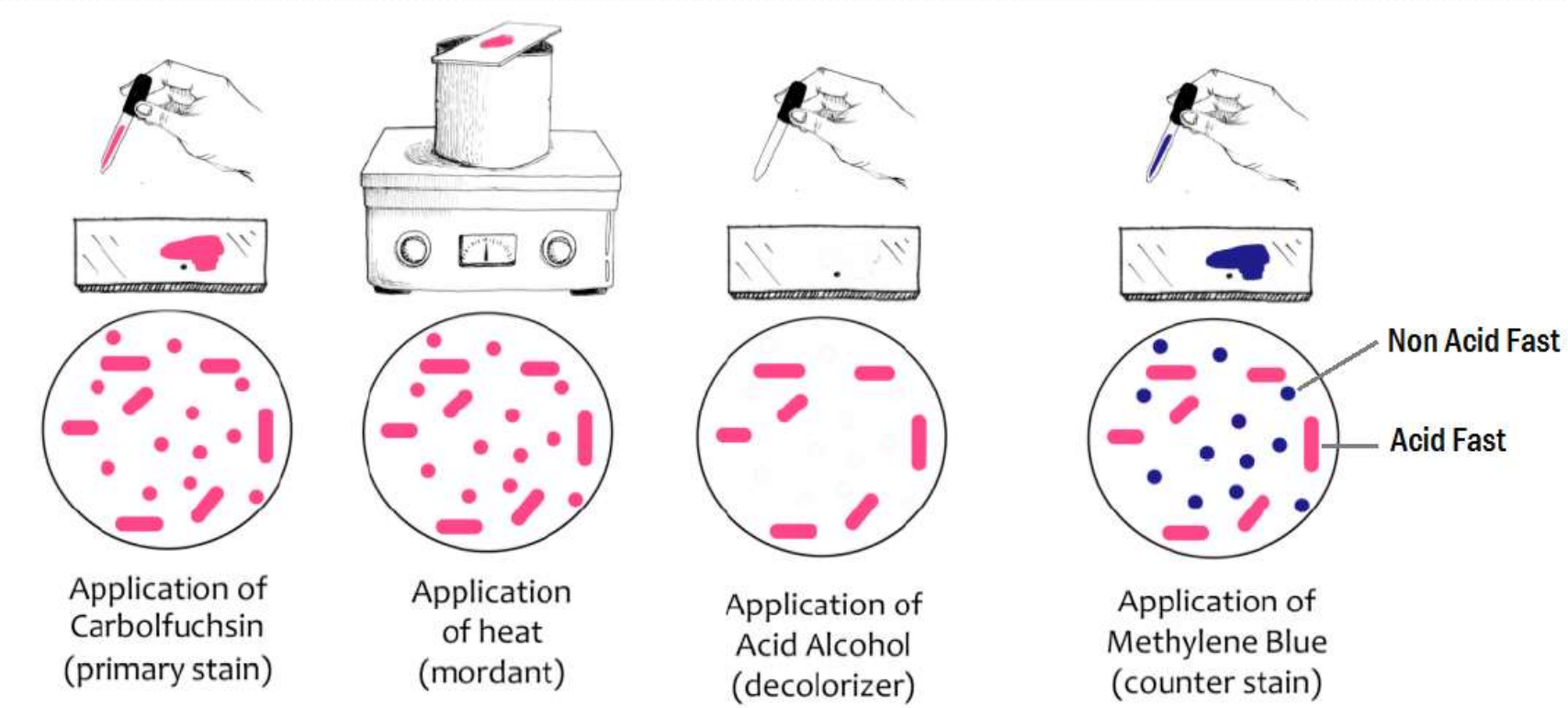
- For the diagnosis of acid-resistant microorganisms
  - Mycobacteria, nocardia
  - Acid resistance is due to the high content of lipids in the cell wall - mycolic acid
- Principle of dyeing
  - Formation of cell wall complexes with fuchsin
  - Dye penetration facilitated by elevated temperature
- The complex is not discolored by acids, bases or alcohol
- The background of the preparation is stained with a contrast agent
- Acid-resistant microorganisms are pink on a blue / green background

Ziehl-Neelsen (acid-fast) staining

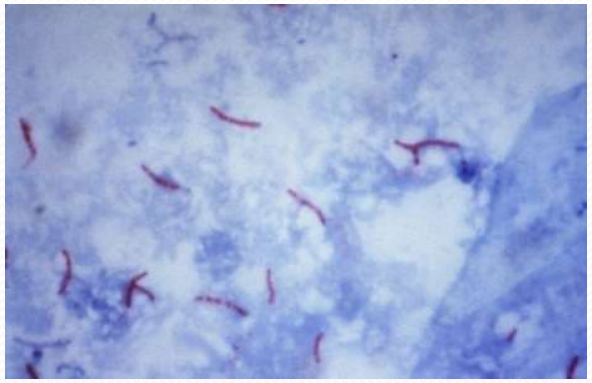
- The cell wall of mycobacteria consists of a thick layer of mycolic acids, a polysaccharide layer and a peptidoglycan
  - Mycolic acids (prevention of phagocytosis)
  - Long fatty acids with a cyclopropane core or methoxy or keto groups
- Polysaccharide layer
- Arabinogalactan

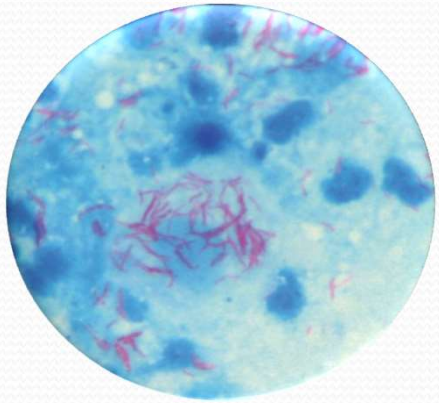


Ziehl-Neelsen (acid-fast) staining



Ziehl-Neelsen (acid-fast) staining



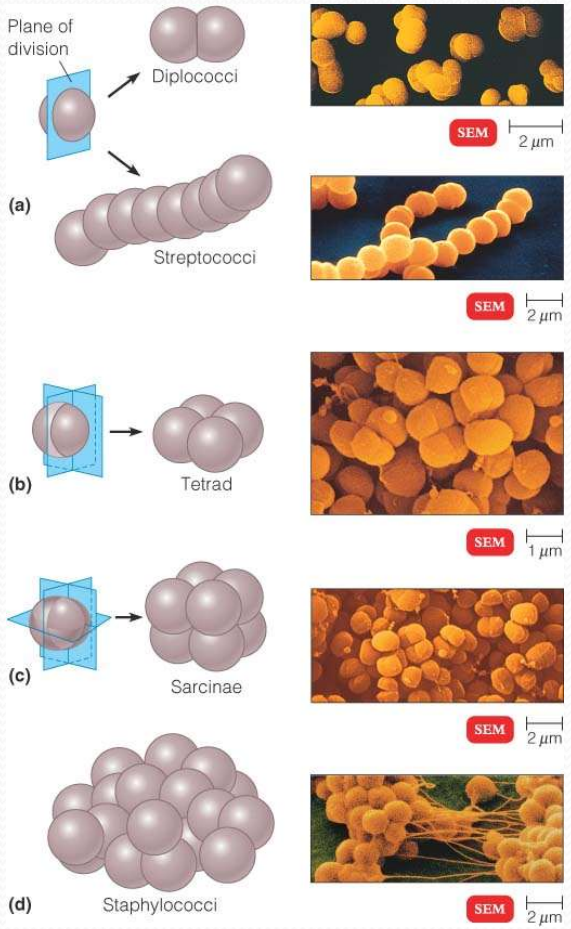


•**Acid Fast Bacilli** : Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded
•**Cells** : Green (malachite green) or Blue (methylene blue)
•**Background material** : Green (malachite green) or Blue (methylene blue)

Microscopic morphology

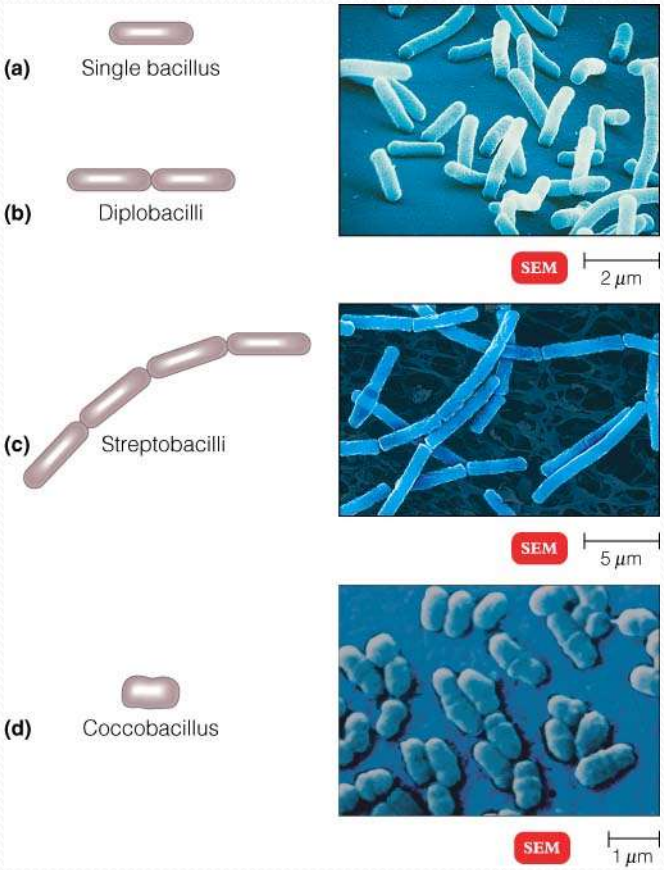
- Bacterial morphology (size, shape and arrangement of bacterial cells) is one of the mostly used feature for the differentiation of various bacterial species.
- However pleomorphic bacteria can assume several shapes, following are the three basic bacterial shapes:
  - Coccus (plural-cocci) : spherical
  - Bacillus (plural-bacilli) : rod-shaped
  - Spiral : twisted

Arrangements of Cocci



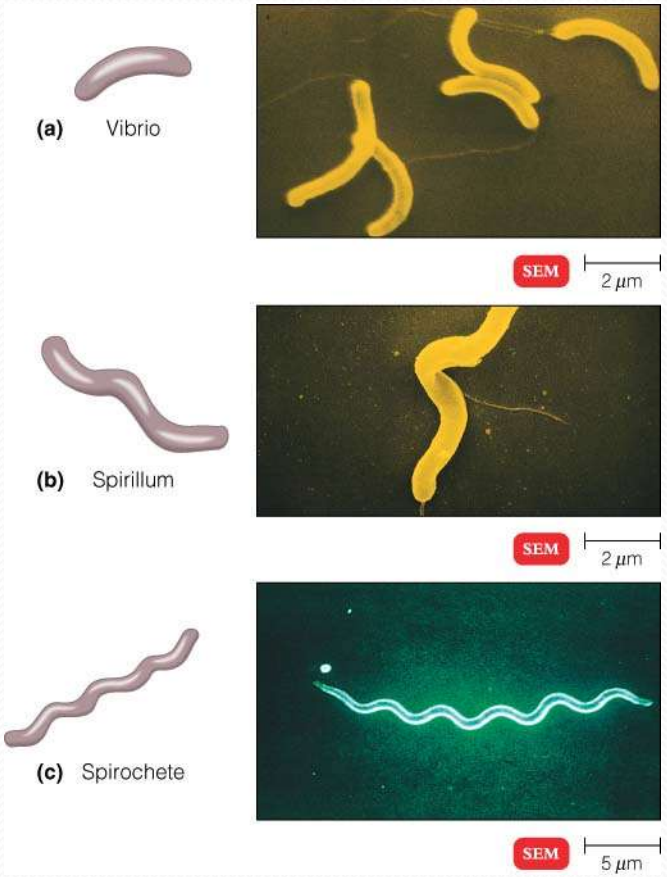
- **Diplococci** : Cocci that remain in pairs after dividing.
- **Streptococci** : Cocci that remain in chains, like beads on a string.
- **Tetrads** : The cocci that are arranged in packets of four cells, as the cells divide in two plains.
- **Sarcinae** : Cocci that divide in three planes and remain in groups cube like groups of eight.
- **Staphylococci** : Cocci that divide in multiple planes and form grape like clusters or sheets.

Arrangements of Bacilli (rod shaped bacteria)



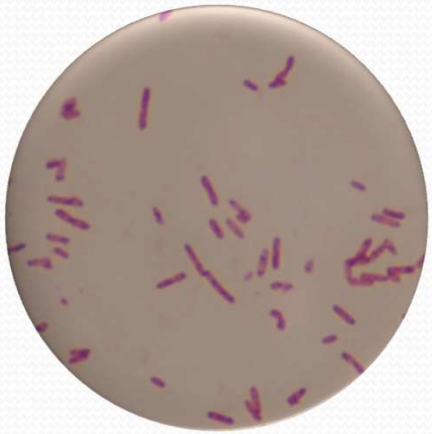
- **Diplobacilli** : Bacilli that remain in pairs after dividing.
- **Streptobacilli** : Bacilli that remain arranged in end-to-end chains.
- **Coccobacilli** : Bacilli that are so short and fat which look like cocci.

Spiral Bacteria

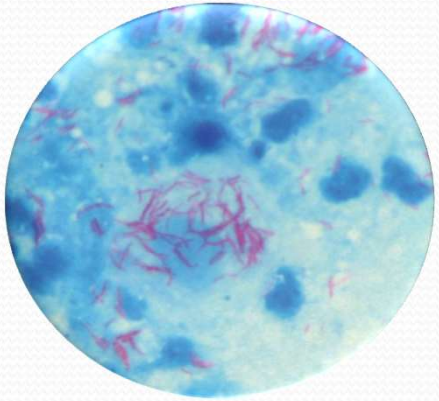


- **Vibrios** : Bacteria that are curved or comma-shaped.
- **Spirilla** : Bacteria that have a helical shape and fairly rigid bodies.
- **Spirochetes** : Bacteria that have a helical shape and flexible bodies.

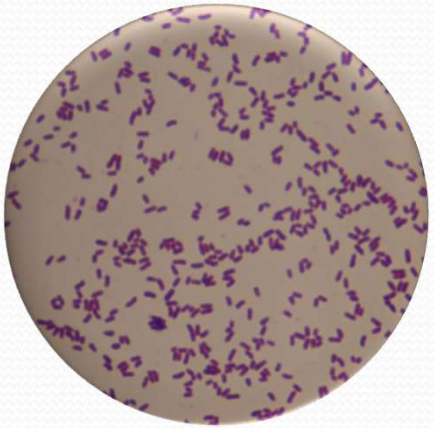
Microscopic morphology



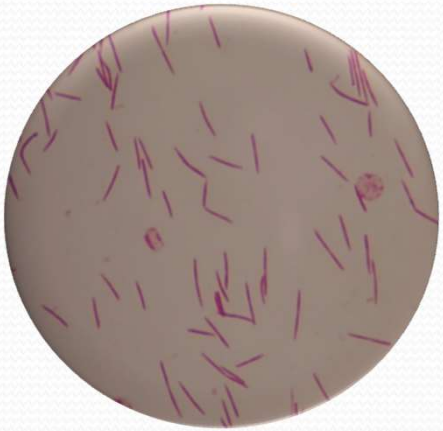
Pseudomonas aeruginosa – Gramovo barvení G-



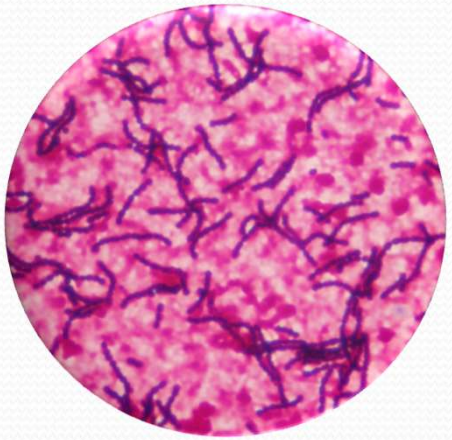
Mycobacterium tuberculosis – Ziehl-Neelsenovo barvení



Corynebacterium sp. – Gramovo barvení G+



Fusobacterium sp. – Gramovo barvení G-



Streptococcus pyogenes v HK – Gramovo barvení G+