

Practical 4

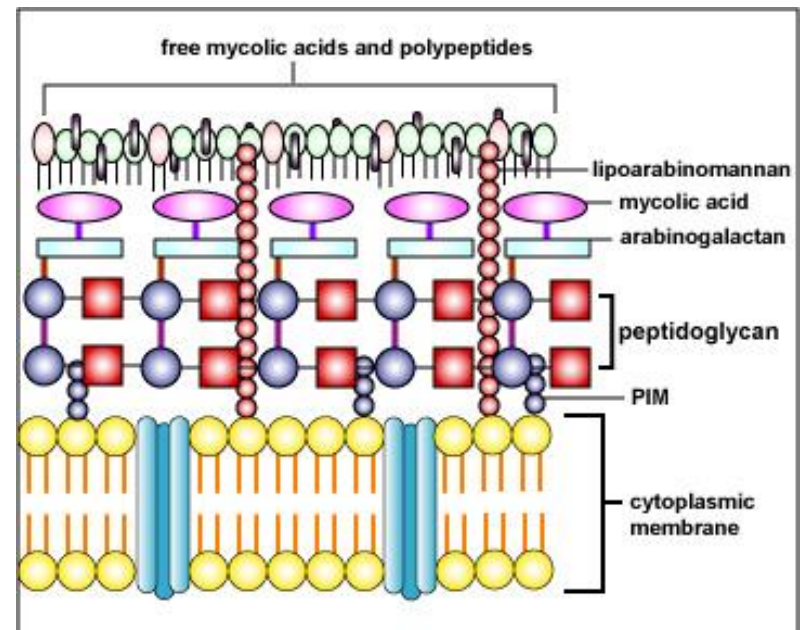
- Acid fast staining –
- *Mycobacterium tuberculosis*

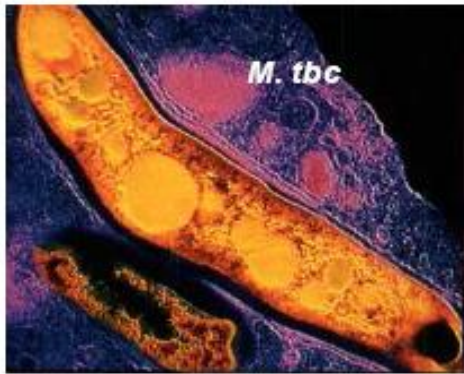
Acid fast bacteria

- **PRINCIPLE:**
- The lipoid capsule of the acid-fast organism takes up carbolfuchsin
- resists decolorization with an acid rinse.
- The lipoid capsule of the mycobacteria is of such high molecular weight that it is waxy at room temperature and successful penetration by the aqueous-based staining solutions (such as Gram's) is prevented.

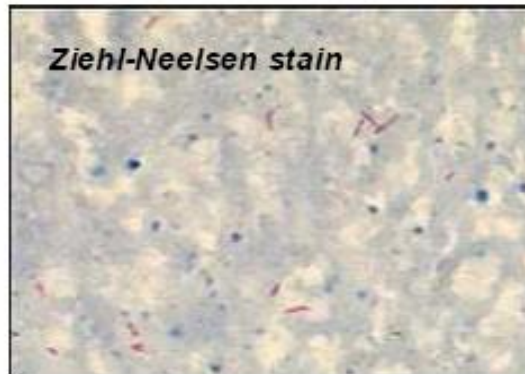
Acid fast bacteria

- Bacterial cell resistant to colourisation with some staining (Gram)
- Their cell wall is relatively not permeable, containing lipids, fatty acids...(*Mycobacterium tbc*, *Corynebacteria*...)
- If stained (with strong basic stains -basic fuchsin in 5% phenol)
they resist to decolorisation
with strong acids
(20% H_2SO_4)

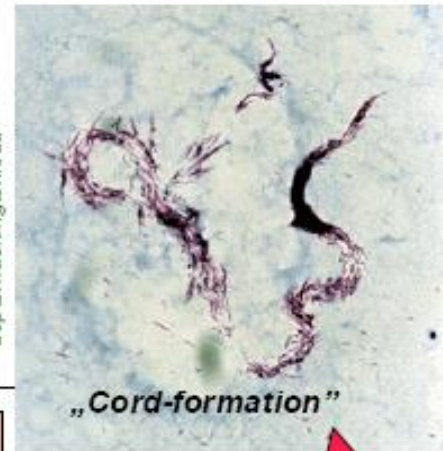




M. tbc



Ziehl-Neelsen stain



„Cord-formation”

Gram negative	Gram positive	Complex (acid-fast)
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peptidoglycan



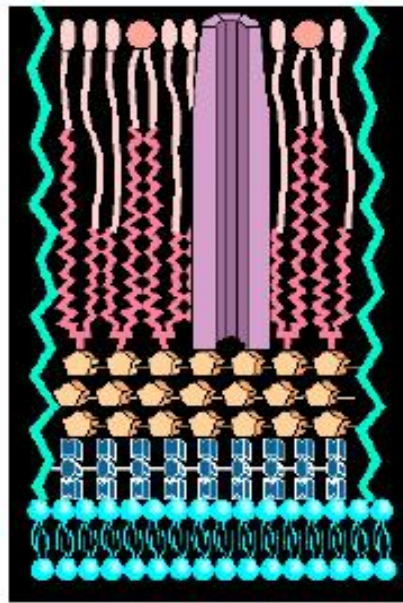
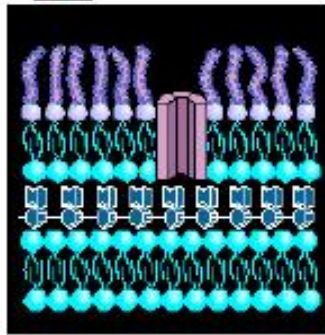
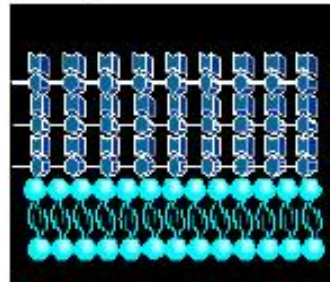
Lipid+LPS



lipid bilayer
plasma membrane



porins



acyl lipids



Mycolic acid
(mycolyltrehalose:
„cord-factor”)



D-arabinose
D-galactose

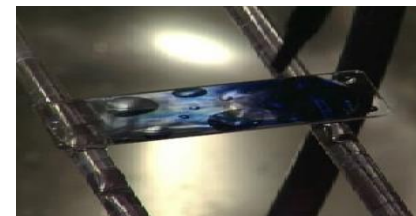


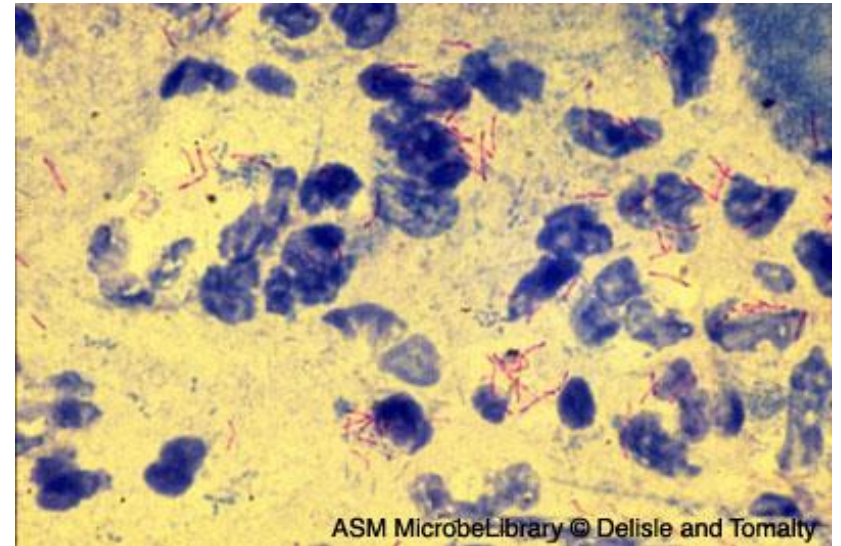
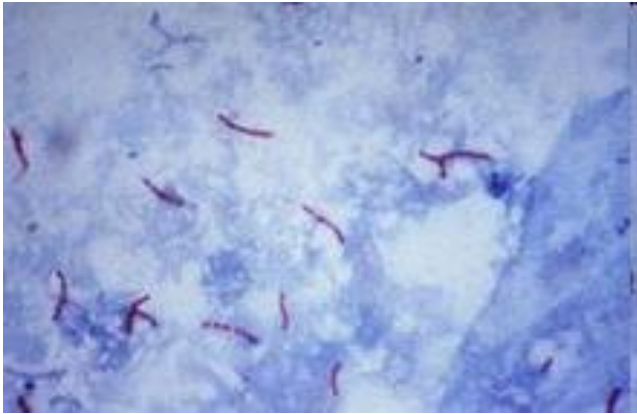
Ziehl Neelsen staining

- Used in the demonstration of acid-fast bacteria belonging to
- the genus 'mycobacterium', which include the causative agent for tuberculosis.

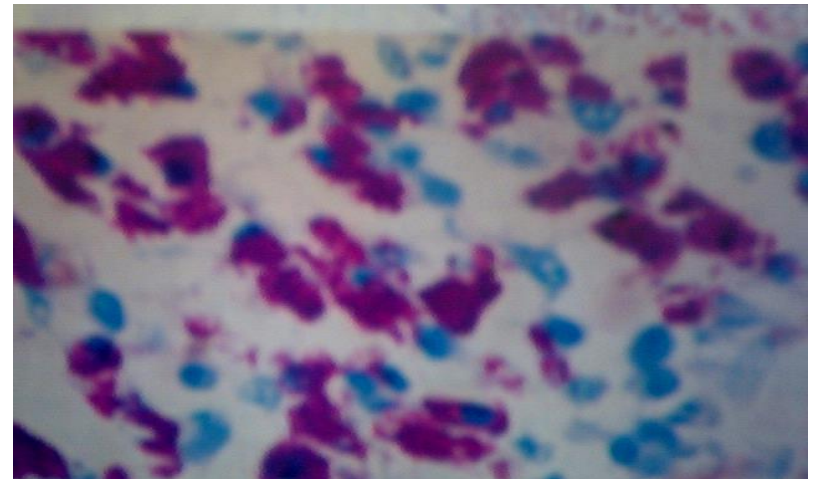
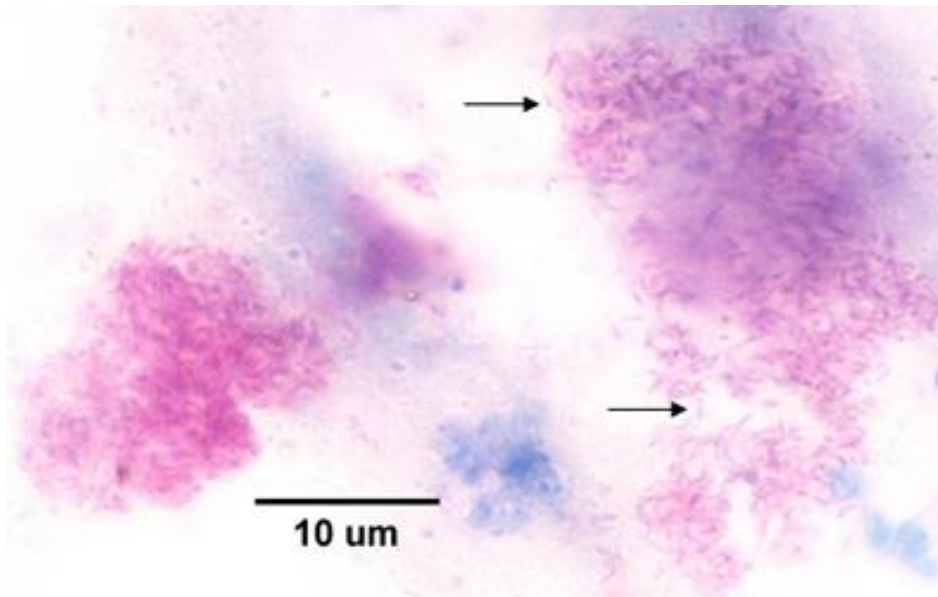
Ziehl Neelsen staining

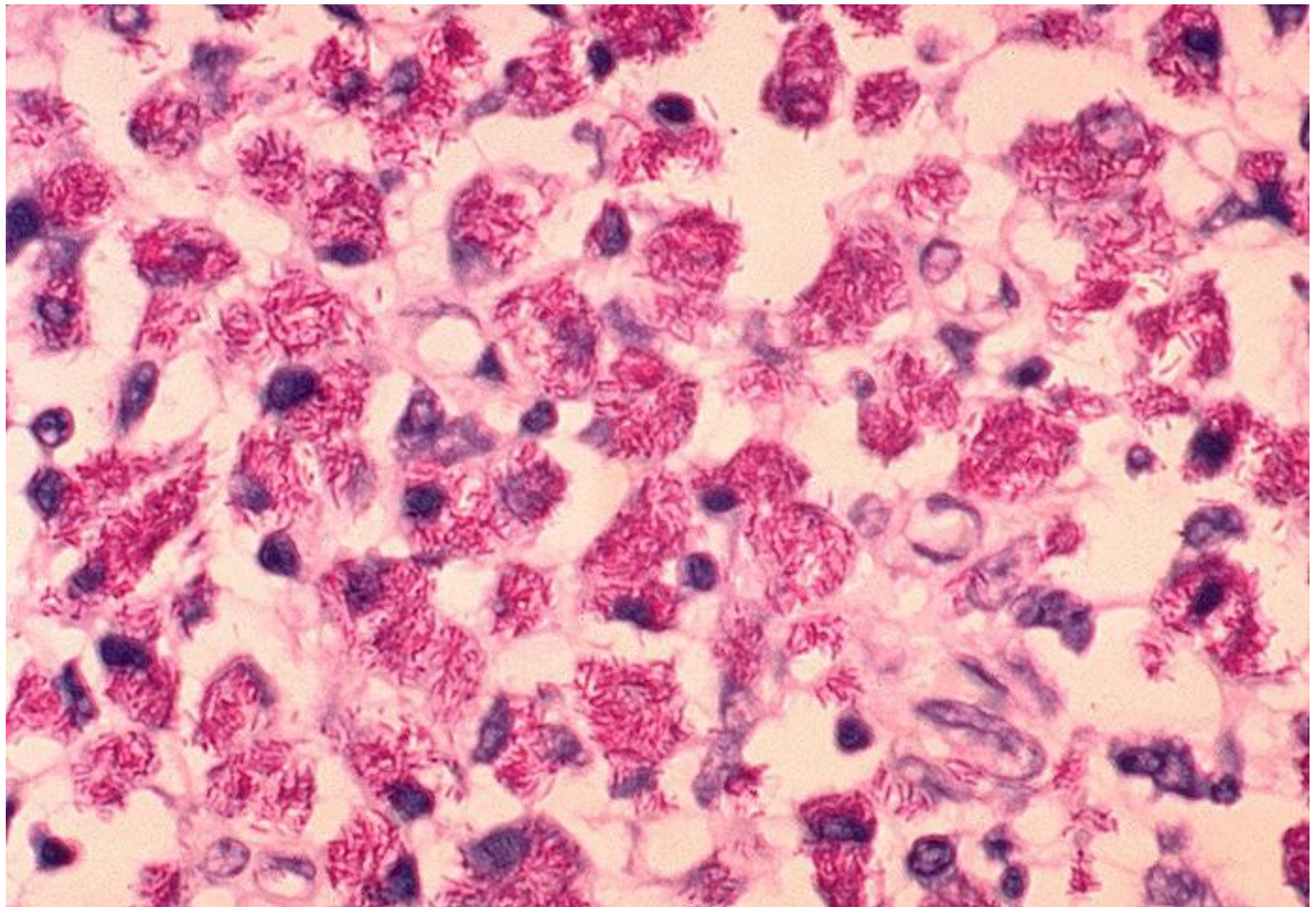
- Heat fixed smear
- Apply carbol fuchsin (basic fuchsin + phenol)
- Heating until evaporation (3x)
- Rinse with water
- Apply 3% HCl (or 95% alcohol)
- Counterstain with malachit green





Mycobacterium tuberculosis – Ziehl Neelsen





Fluorescent microscopy

