



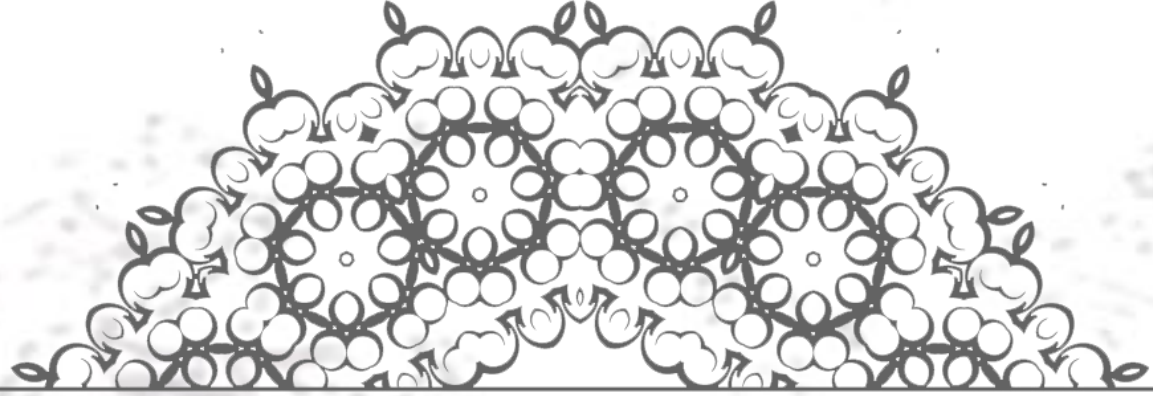
# 460 MBIO

# Medical Bacteriology

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2017

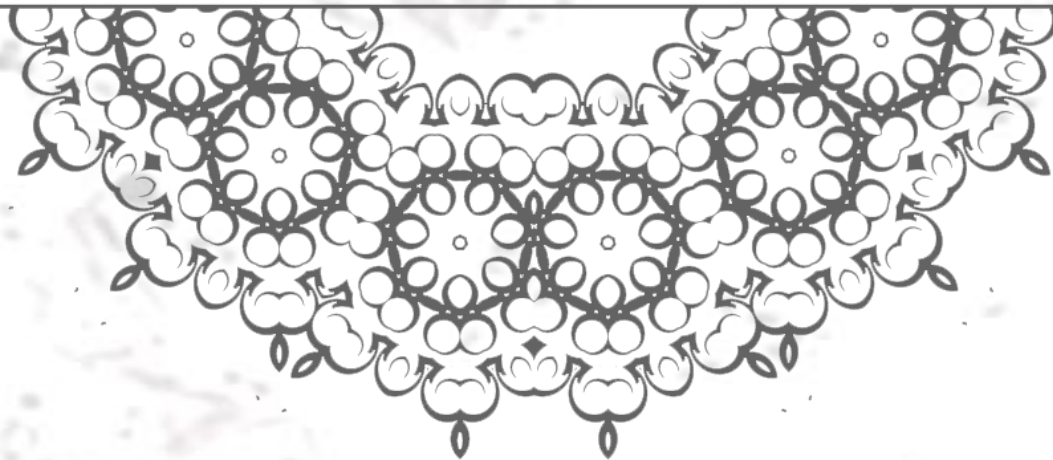




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**Lab 5**  
**Gram Positive Group**  
( *Acid-fast Bacteria* )

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## ❖ General Characters of Acid-fast Bacteria :

- Include the *Mycobacterium* and few species of *Nocardia*.
- Acid fastness is due to high lipid content of cell envelop, high lipid content is difficult to stain by ordinary dye but requires special dye as Carbofuchsin with heating, and once stained are difficult to decolorize with acid-alcohol mixture.

## ❖ General Characters of *Mycobacterium* :

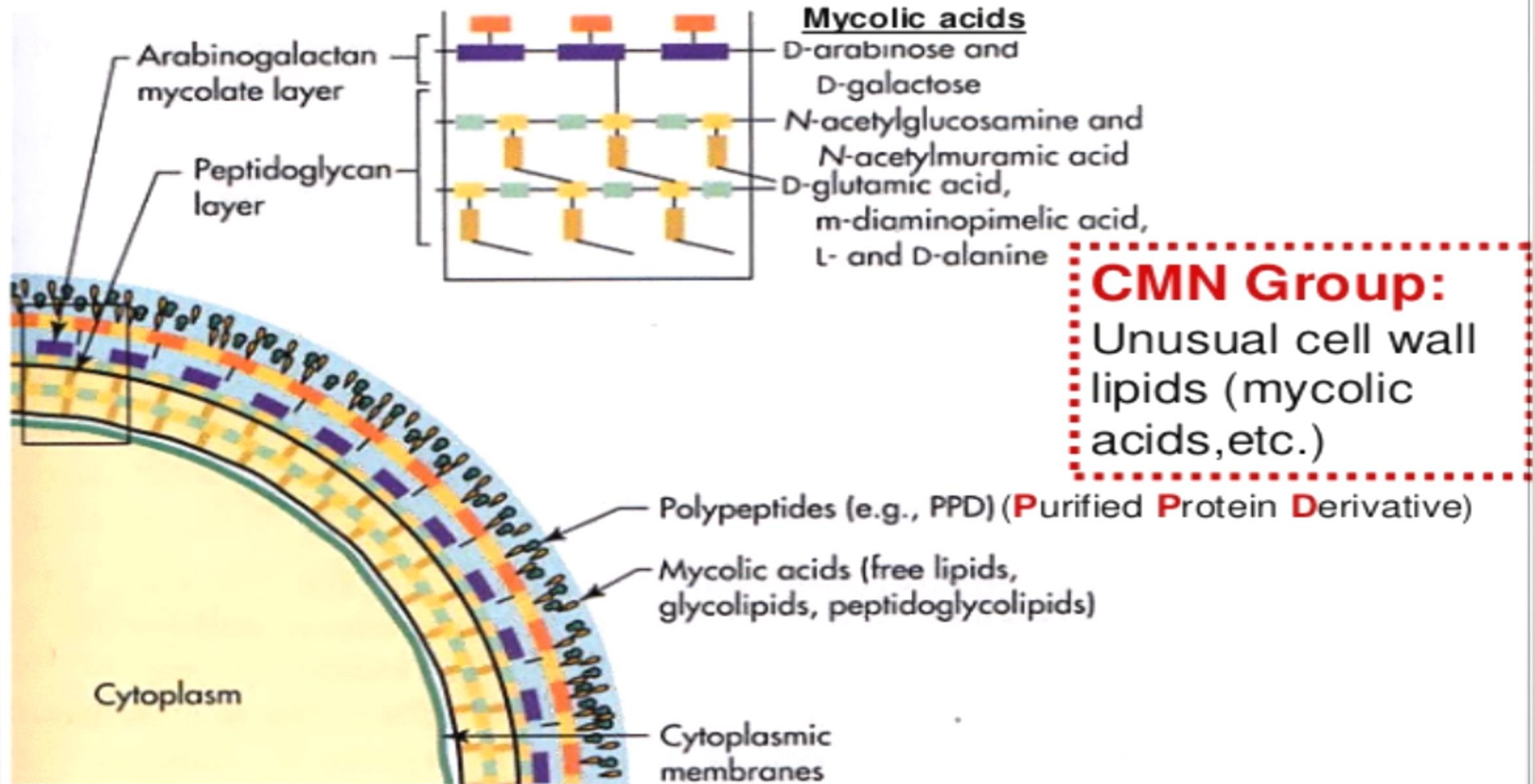
Gram positive pleomorphic rods, slender (thin) straight or slightly curved rods .

3 Non (motile – spore forming – capsulated)

Obligate aerobic, catalase positive.

*Mycobacterium* contains 40% lipid content in their cell envelop.

# Lipid-Rich Cell Wall of Mycobacterium





## ❖ General Characters of *Mycobacterium* :

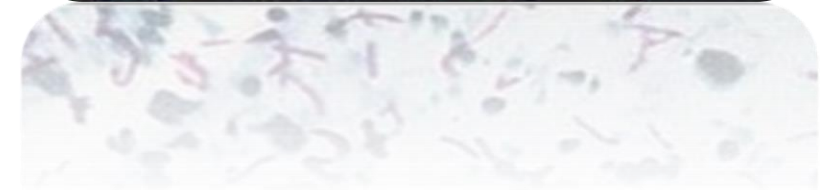
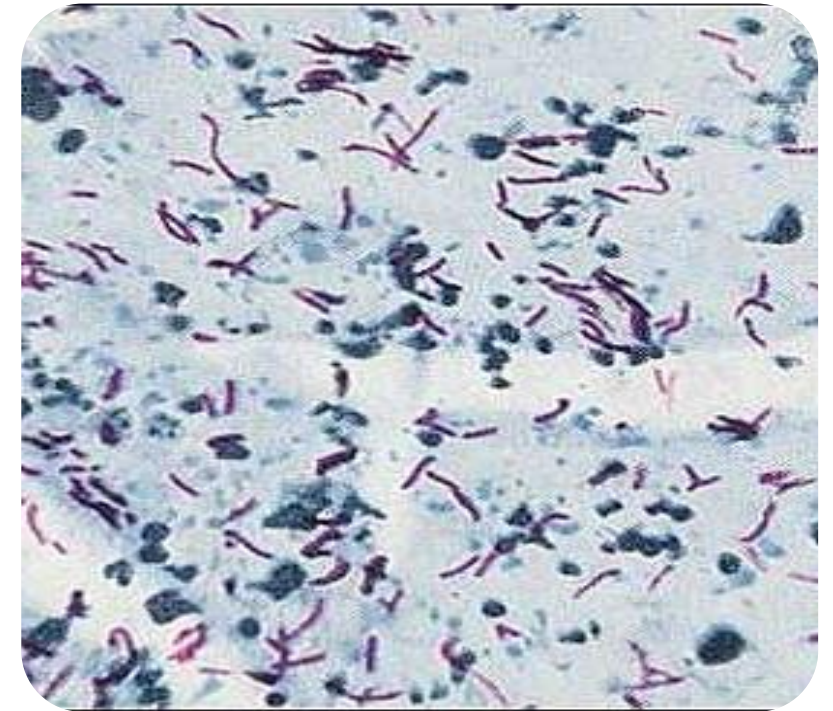
Most Mycobacteria are found in habitats such as water or soil. However, a few are intracellular pathogens of animals and humans.

The Mycobacterium include 2 species :

- *Mycobacterium tuberculosis*, which causes tuberculosis
- *Mycobacterium leprae*, which causes leprosy (uncommon)

## ❖ Laboratory diagnosis of *Mycobacterium tuberculosis*:

- **Specimen** : According to site of infection; sputum, CSF and EMU.
- **Morphology** :
  - Microscopical (Gram stain)
  - Macroscopical (Cultural characteristics)

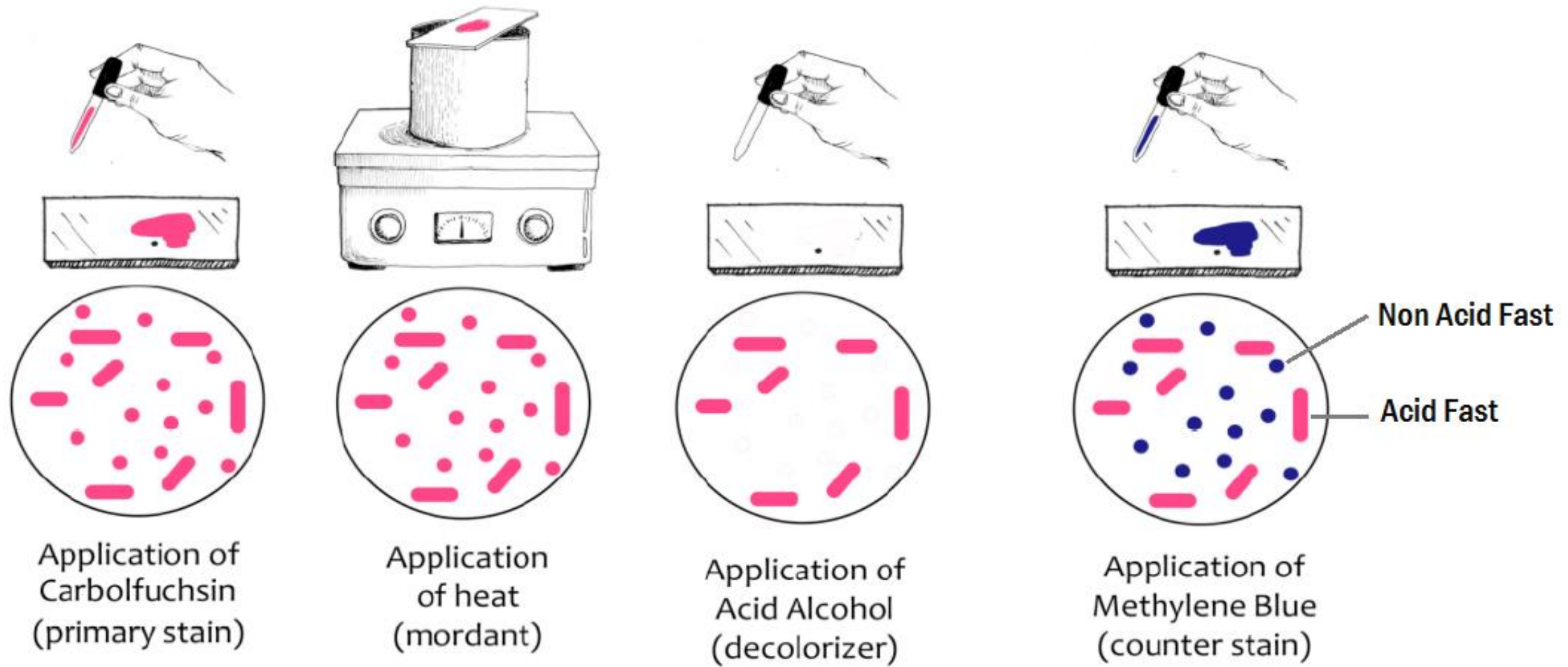


## ■ 2<sup>nd</sup>: Morphology, Microscopical :

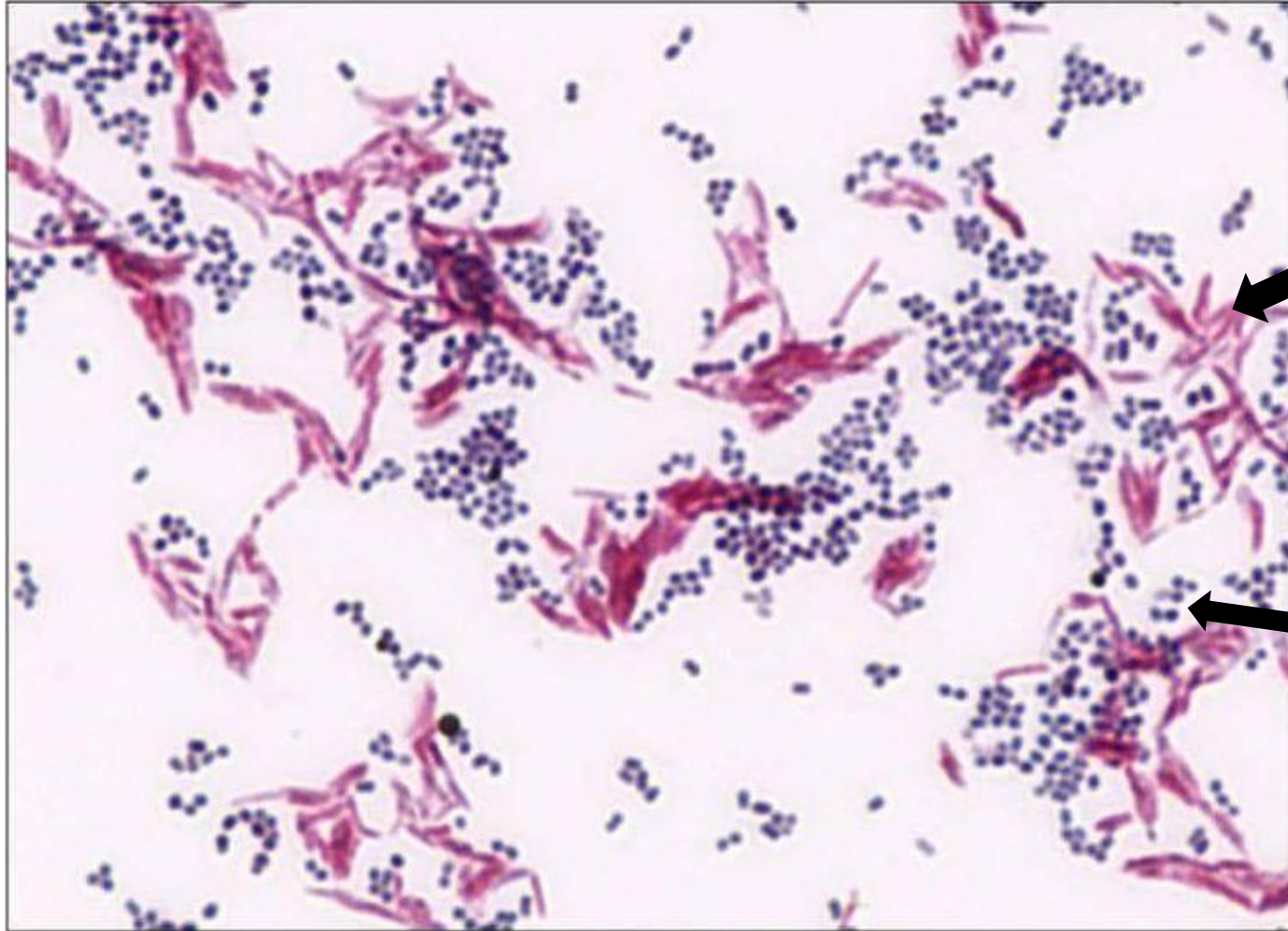
- Acid fast stain

- The acid-fast stain is a differential stain used to identify acid-fast organisms such as members of the genus *Mycobacterium* .
- The primary stain used in acid-fast staining, **carbolfuchsin**, is lipid-soluble and contains **phenol**, which helps the stain penetrate the cell wall. This is further assisted by the addition of **heat**.



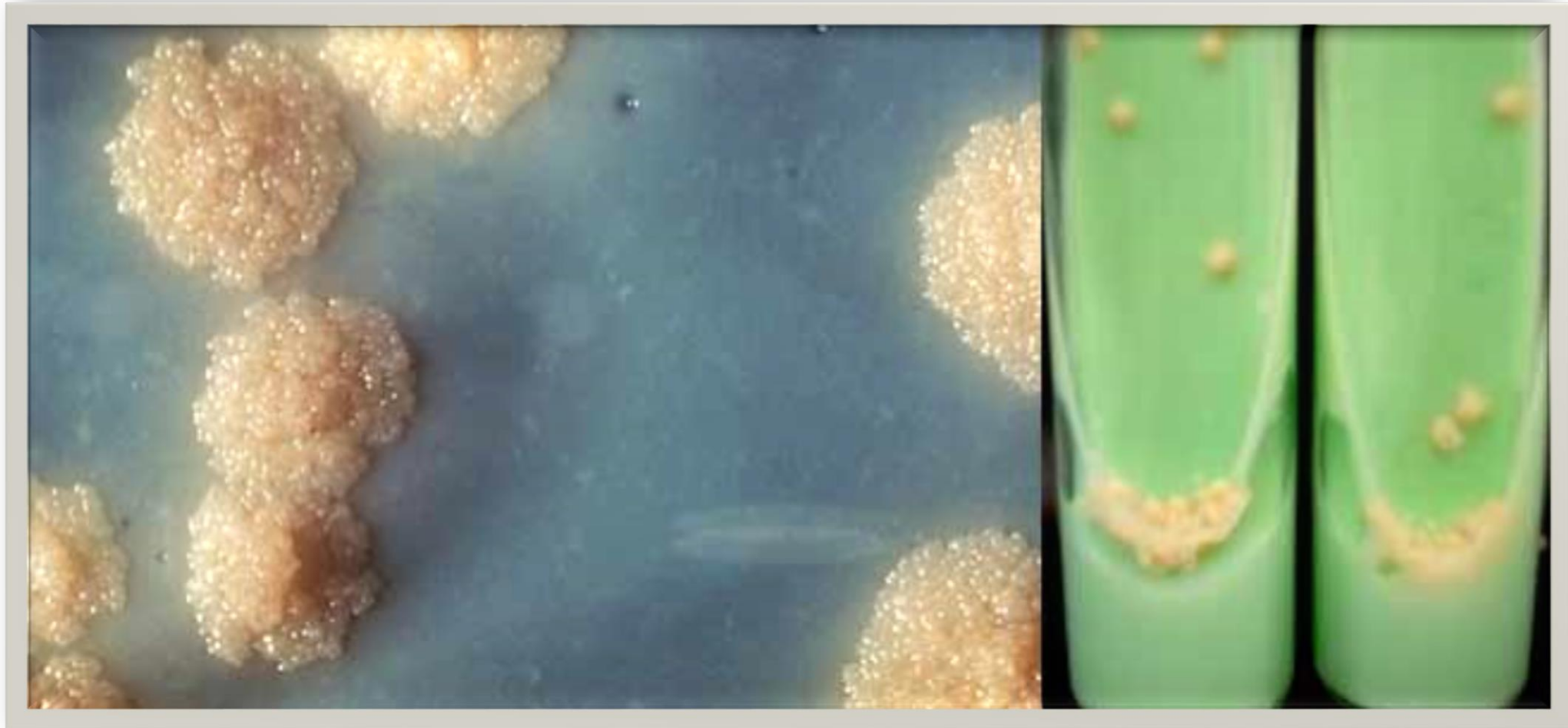


## Acid fast staining ziehl-neelsen method



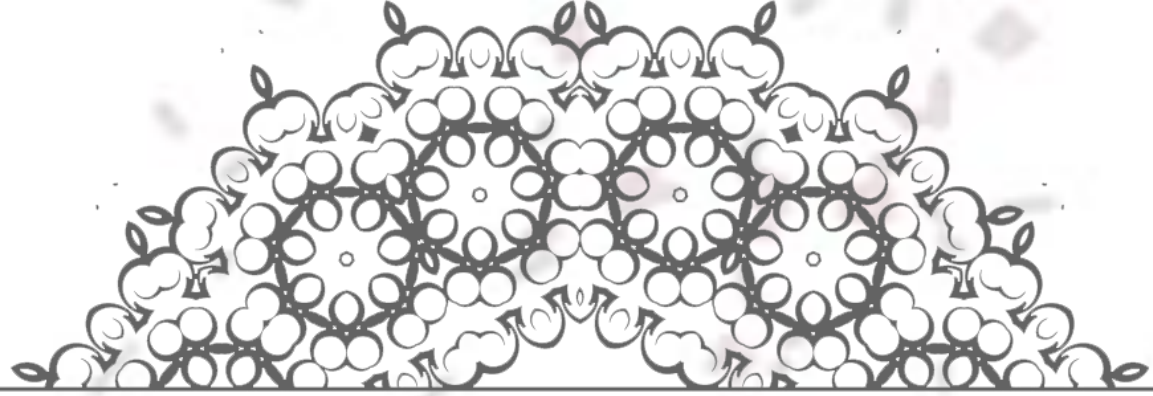
**Acid-fast bacteria  
appear red or pink**

**Non acid-fast  
bacteria or tissue  
appear blue**



Eight Week Growth of *Mycobacterium tuberculosis* on  
Lowenstein-Jensen Agar





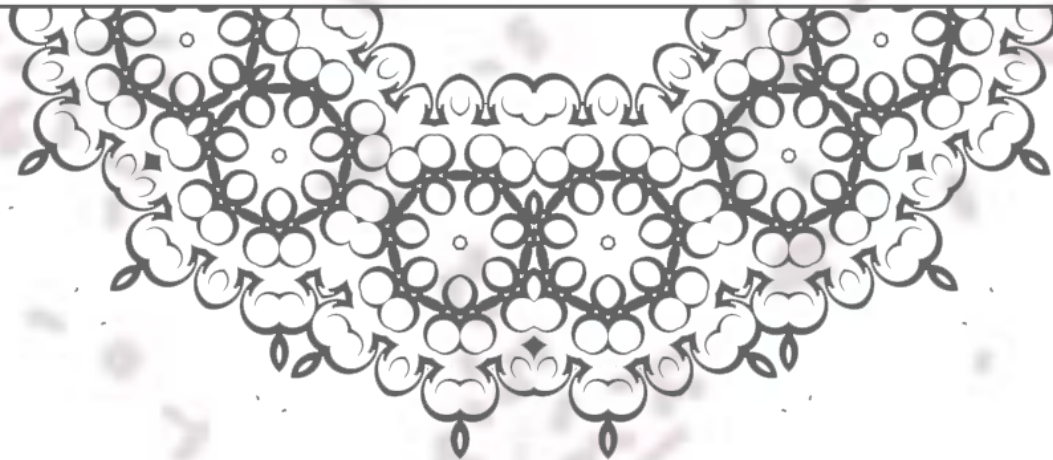
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# Lab 5

## Gram Negative Group

( *Haemophilus* )

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## ❖ General Characters of *Haemophilus* :

Gram negative coccobacilli, usually capsulated.

Mostly are fastidious, facultative anaerobic

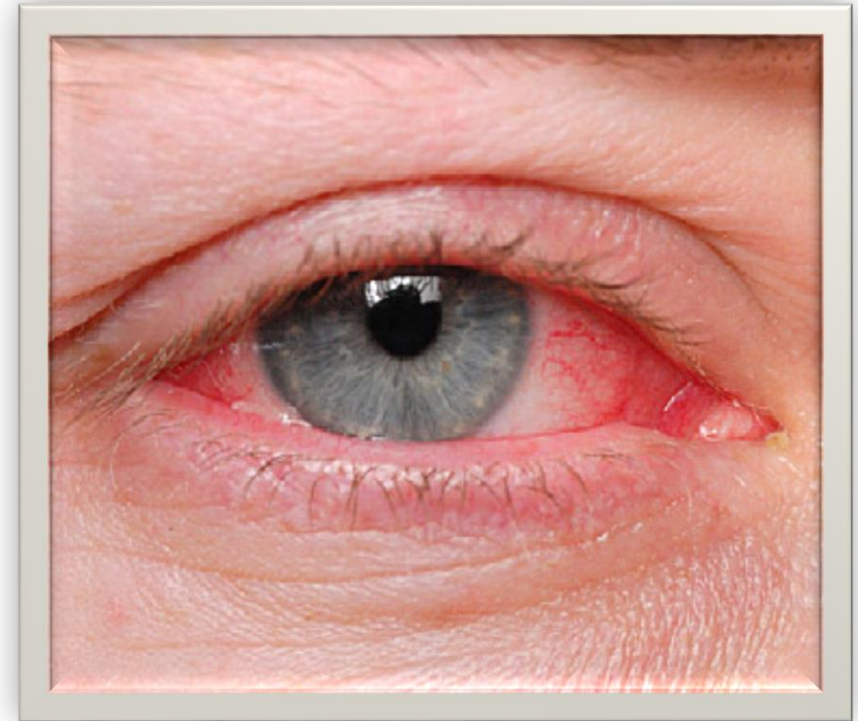
Non motile and non spore forming.

Oxidase and catalase positive.



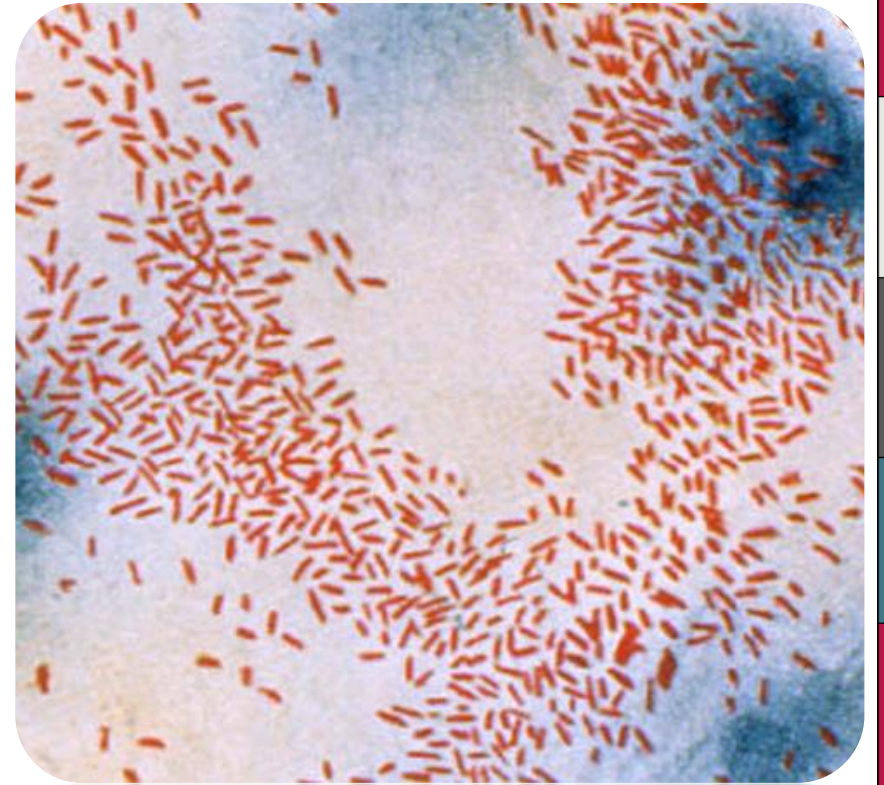
- Pathogenic as well as part of upper respiratory tract flora, such as :
    1. *Heamophilus influenza*, which causing each of (pneumonia, otitis media, epiglottitis, meningitis, bacteremia).
- Heamophilus influenzae* is the most important pathogen and have been subdivided according to Serotypes (capsular antigens), to six types from a through f, the most important type is type b.

2. *Haemophilus aegypticus*, the cause of conjunctivitis.
3. *Haemophilus parainfluenzae*.
4. *Haemophilus ducreyi*.



## ❖ Laboratory diagnosis of *Haemophilus* :

- Specimen.
- Morphology :
  - Microscopical (Gram stain)
  - Macroscopical (Cultural characteristics)



- **Specimen :**
  - According to site of infection; swap, sputum, CSF.
- **Morphology :First Microscopical (Gram stain)**
  - Gram negative coccobacilli.



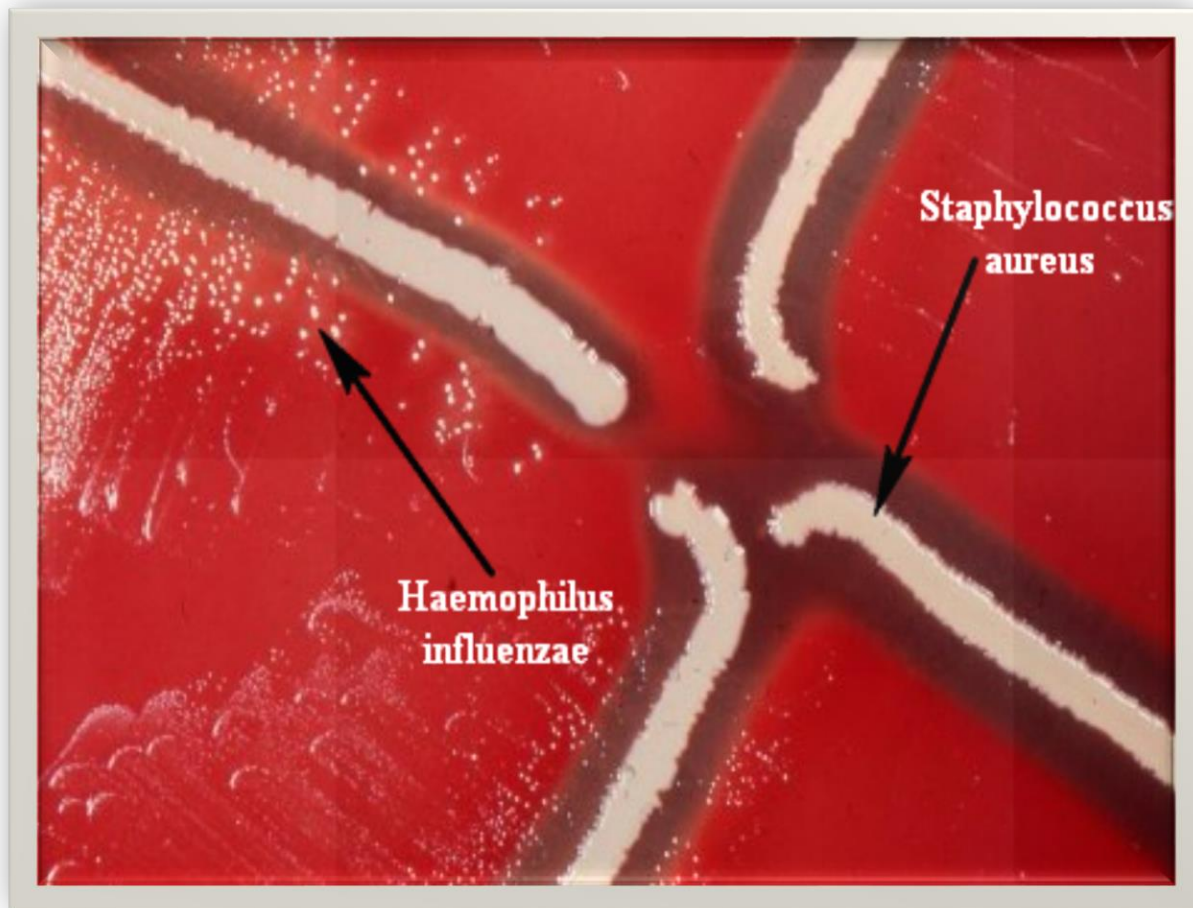
## 2<sup>nd</sup> : Morphology, Macroscopical

*H. influenzae* grow on blood agar or chocolate agar as it requires X factor and V factor that found on blood.

- **V factor** : Nicotinamide adenine dinucleotide NAD
- **X factor** : Hematein

<b>Blood Agar</b>	<b>Chocolate Agar</b>
A 24 h colony of <i>H. influenzae</i> on blood agar is very small usually non hemolytic	A 24 h colony of <i>H. influenzae</i> on chocolate agar is larger than that observed on blood agar





*H. influenzae* growing will on blood agar, because of presence of both X and V factors.

# Any Questions

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