SNS COLLEGE OF ALLIED HEALTH SCIENCES

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Affiliated to Dr MGR Medical University, Chennai

DEPARTMENT OF PHYSICIAN ASSISTANT

COURSE NAME: CLINICAL MICROBIOLOGY

TOPIC : ACID FAST BACILLI AND ITS STAINING

ACID FAST BACILLI

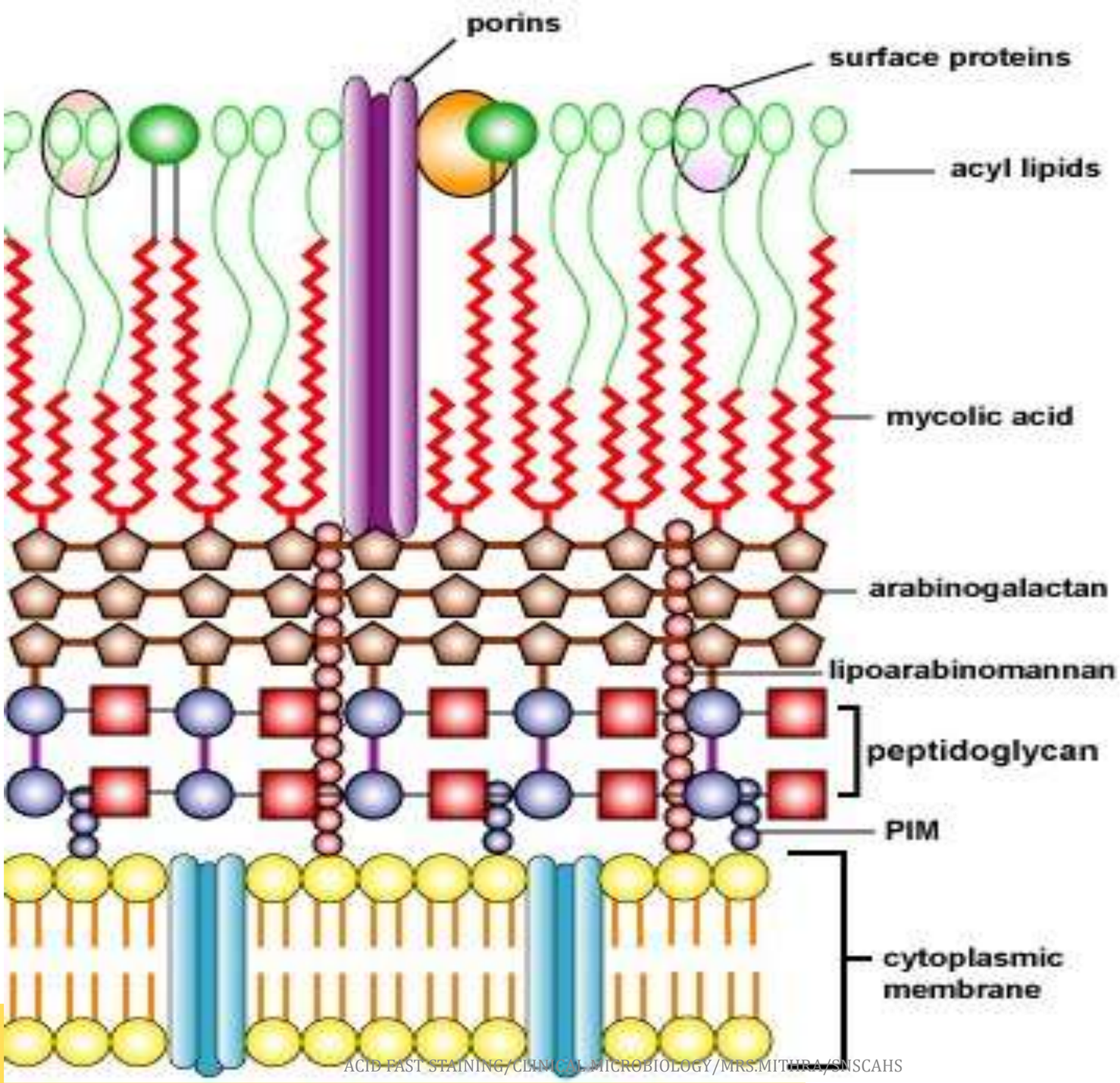
- Acid-fast organisms have cell wall similar to Gram-positive bacteria made up of a thick peptidoglycan layer.
- In addition, the cell wall of acid-fast organisms consists of large amounts of glycolipids especially mycolic acid and lipoarabinomannan (LAM), which are fatty acids.
- Acid-fast bacteria are those bacteria that are not easily decolorized using acid-alcohol once they have been stained using carbol fuchsin dye.
- As a result, they appear red after the staining technique.

Examples of acid-fast bacteria:

- Mycobacterium tuberculosis (causative agent of human tuberculosis),
- Mycobacterium leprae (the causative agent of human leprosy).
- In addition, Mycobacterium Bovis, Rhodococcus equi, and Nocardia species fall under this category.

Structure and Composition of the Acid-Fast Cell Wall

- **Layer 1:** The acid-fast cell wall of *Mycobacterium* has a thin, inner layer of peptidoglycan.
- **Layer 2:** The peptidoglycan layer is, in turn, linked to arabinogalactan (D-arabinose and D-galactose).
- **Layer 3:** The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids.
- Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM).
- **Layer 4:** The outer surface of the acid-fast cell wall is studded with surface proteins that differ with the strain and species of the bacterium.
- **Layer 5:**The periplasm is the gelatinous material between the peptidoglycan and the cytoplasmic membrane.


porins
surface proteins
acyl lipids
mycolic acid
arabinogalactan
lipoarabinomannan
peptidoglycan
PIM
cytoplasmic membrane

Functions of the Acid-Fast Cell Wall Components

- **Layer 1:** The peptidoglycan prevents osmotic lysis.
- **Layer 2:** The arabinogalactan layer provides additional strength to the cell wall.
- **Layer 3:** The mycolic acids and other glycolipids also impede the entry of chemicals causing the organisms to grow slowly and be more resistant to chemical agents.
- **Layer 4:** The surface proteins in the acid-fast cell wall, including functioning as enzymes and serving as *adhesins.*
- **Layer 5:** The periplasm contains enzymes for nutrient breakdown.

ACID FAST STAINING

- Acid-fast staining was originally pioneered by a scientist named **Paul Ehrlich** in the year 1882.
- Later, it was modified by **Ziehl** and **Neelson** in 1883.
- Thus, acid-fast staining is also called ZiehlNeelson staining.
- It is a type of **differential staining** method used to distinguish between the acid-fast and non-acid fast bacteria.
- Mycobacterium is an acid-fast bacterium, which retains the colour of carbolfuschin even after the treatment with decolourizer.
- The mycobacteria species retain the primary stain's colour because they contain mycolic acid in their cell wall.

- **Mycolic acid** is a waxy substance that does not allow the decolourizer to enter the cell wall due to its waxy nature.
- Therefore, acid-fast staining discriminates the mycobacterium species from the other groups of bacteria.
- Non-acid fast bacteria quickly lose the primary stain's colour due to the absence of mycolic acid and appears blue.

- Acid-fast staining refers to one of the staining methods, which differentiates the *Mycobacteria species* from the other bacterial groups based on the **staining properties** and **cell wall differences**.
- Red-coloured acid-fast bacteria indicates the positive result of acid-fast staining.
- Oppositely, blue-coloured non-acid fast bacteria indicates the negative result of acid-fast staining.

Examples of **acid-fast bacteria**:

- *Mycobacterium tuberculosis*, *M. leprae*, *M. smegmatis*, *M. phlei* etc.

Examples of **non-acid fast bacteria**:

- *Escherichia coli*, *Staphylococcus aureus* etc.

Requirements of Acid-Fast Stain

- Acid-fast staining is a differential staining procedure, which uses the combination of three reagents:
- Ziehl Neelson Carbol fuschin
- Acid alcohol
- Loeffler's Methylene blue
- ***Ziehl Neelson Carbol Fuschin (ZNCF)***
- It functions as a **Primary stain**. ZNCF stain has a phenolic base and a carbolfuschin dye. A **phenolic base** of ZNCF shows a high affinity towards the lipid content.
- Thus, ZNCF dye can **solubilize** the lipoidal material in the cell wall and stain the cell red. Due to high lipid content, a mycobacterial cell wall is less permeable. So, a phenol base increases the **cell permeability**, by which a cell allows the stain to **penetrate**.

- ***Acid Alcohol***
- It serves as a **decolourizing agent,** which contains **3% of HCL** along with **95% ethanol**. Acid-fast bacteria contains a high lipid content, which prevents the cell from binding with stains and decolourizers.
- Thus, acid-fast bacteria will retain the colour of the primary stain and appear red.
- In contrast to this, non-acid fast bacteria lack a large amount of lipid content, as a result of which the cells lose the colour of primary stain and decolourizes.
- ***Methylene Blue***
- It functions as a **counterstain** and contains 3% of methylene blue.
- Methylene blue stains the **decolourized cells** of non-acid fast bacteria and make them appear blue.
- Unlike non-acid fast, an acid-fast bacteria will not take up the colour of methylene blue and appear red.

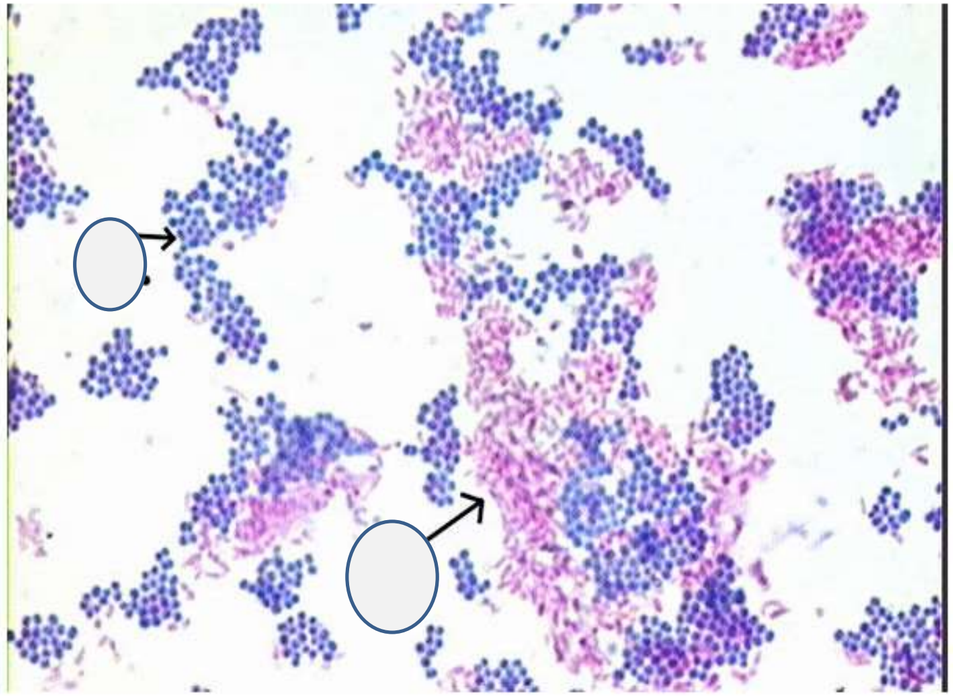
Principle

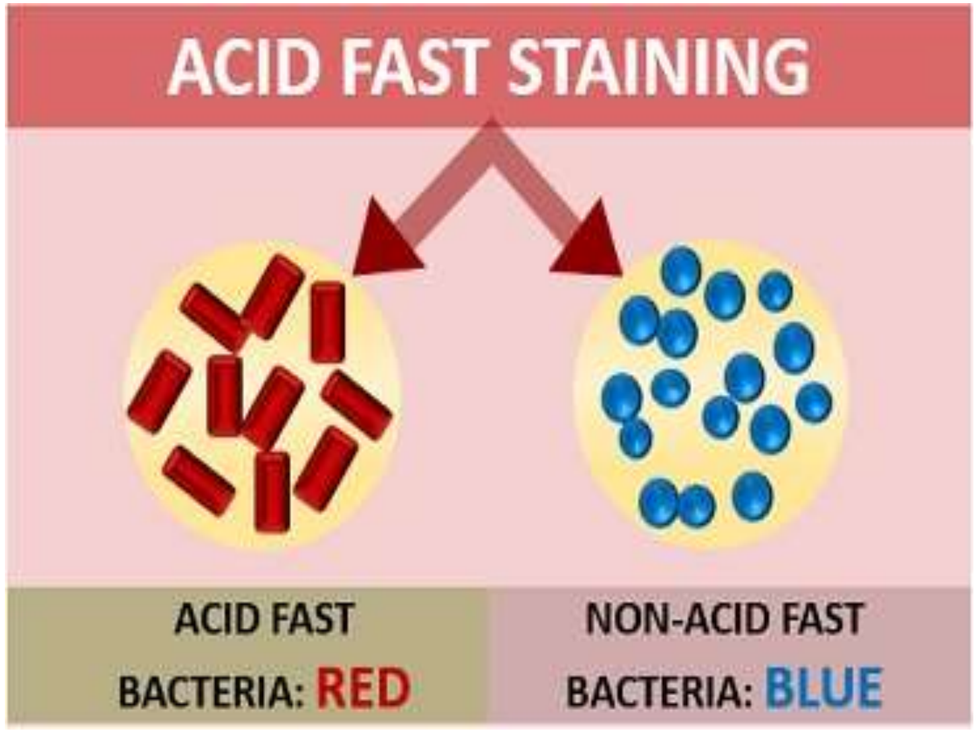
- A smear is subjected to **heat** after staining with Zeihl Neelson Carbol fuschin.
- All the cells appear red after 2-3 minutes because of primary stain (carbolfuschin)
- After this, a **decolourizer** (3% HCl) is added to the smear.
- The mycobacteria resist the effect of decolourizer because of the substantial lipoidal material or mycolic acid in the cell wall.
- They don’t allow the penetration of the acid decolourizer into the cell, and remains appear red.
- On the contrary, other bacteria will not resist the effect of decolourizer due to little or no lipoidal content, as the decolourizer causes leakage of primary stain by creating pores in the cell wall. Thus, a non-acid fast cell appears colourless.
- Only the decolourized or non-acid fast cells will take the blue colour of methylene blue stain on counterstaining. Conversely, the acid-fast cells will remain red in colour.

Procedure of Acid-Fast Stain

- Prepare bacterial smear on clean and grease free slide, using sterile technique.
- Air dry and heat fix a thin film of microorganisms. Allow the slide to cool.
- Flood the slide with Carbolfuchsin.
- Steam the slide with a Bunsen burner over the sink.
- Let the slide set for 5 minutes. Rinse with water.
- Flood slide with Acid Alcohol for 30 seconds. Rinse with water.
- Counterstain by flooding the slide with Methylene Blue for 30 seconds. Rinse with water.
- Air dry the slide
- View organisms using the oil immersion objective of your microscope.

Interpretation of Acid-Fast Stain





Acid fast: Bright red to intensive purple **-** Red, straight or slightly curved rods, occurring singly or in small groups, may appear beaded.
Non-acid fast: Blue color

Acid-fast vs Non acid-fast bacteria

- Acid-fast bacteria retain the red color of carbol fuchsin after discoloration using acid-alcohol,
- While the non-acid fast bacteria lose the red carbol fuchsin color after discoloration using acid-alcohol.
- In addition, acid-fast bacteria take a long time to grow (3 days-months)
- While non-acid fast bacteria grow very fast (within 24 hours).

Assessment

1. What is Acid fast Bacilli?
2. 5 layers of acid fast bacilli?
3. What is Acid fast staining?
4. Components involved in acid fast staining?
5. Principle and Procedure?
6. Interpretation of acid fast staining?

THANK YOU