

SNS COLLEGE OF ALLIED HEALTH SCIENCE
Affiliated to The Tamil Nadu Dr M.G.R Medical University, Chennai



DEPARTMENT OF CARDIO PULMONARY PERFUSION CARE

TECHNOLOGY

COURSE NAME: CLINICAL MICROBIOLOGY

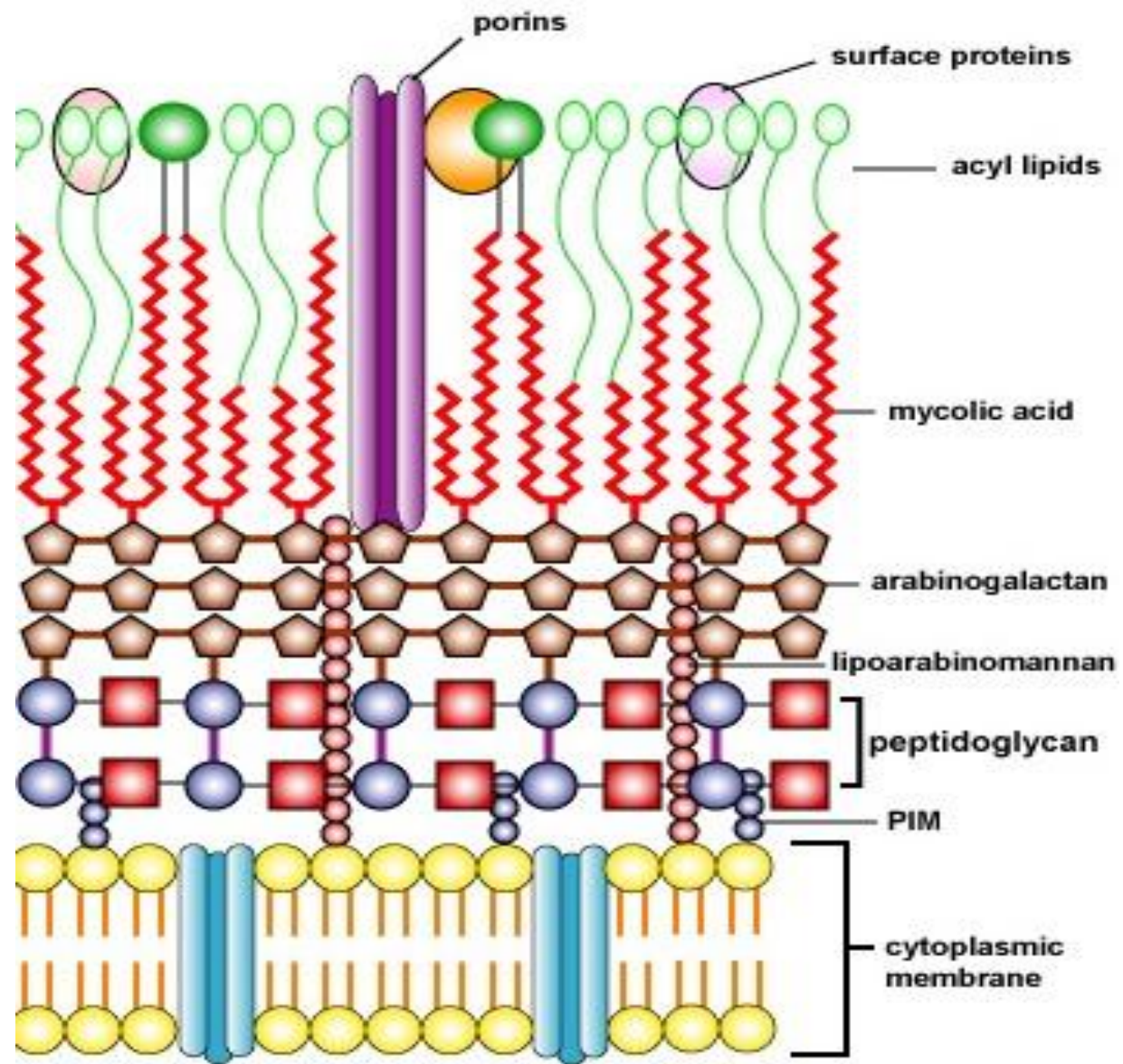
UNIT : 1

TOPIC : ACID FAST STAINING

FACULTY NAME: MITHRA V

ACID FAST BACILLI (DEFINE)

- Acid-fast bacteria have higher amount of glycolipids, such as **mycolic acid** and **lipoarabinomannan (LAM)** – fatty acids.
- **Layer 1:** Thin layer of **peptidoglycan**.
- **Layer 2:** **Arabinogalactan** (D-arabinose and D-galactose).
- **Layer 3:** Mycolic acids consisting of **free lipids, glycolipids, and peptidoglycolipids** include **lipoarabinomannan** and **phosphatidyinositol mannosides (PIM)**.
- **Layer 4:** **Surface proteins**
- **Layer 5:** **Periplasm**



- **Examples of Acid-fast bacteria**

- *Mycobacterium tuberculosis* (causative agent of human tuberculosis)
- *Mycobacterium leprae* (the causative agent of human leprosy).
- *Mycobacterium Bovis*, *Rhodococcus equi*, and *Nocardia* species

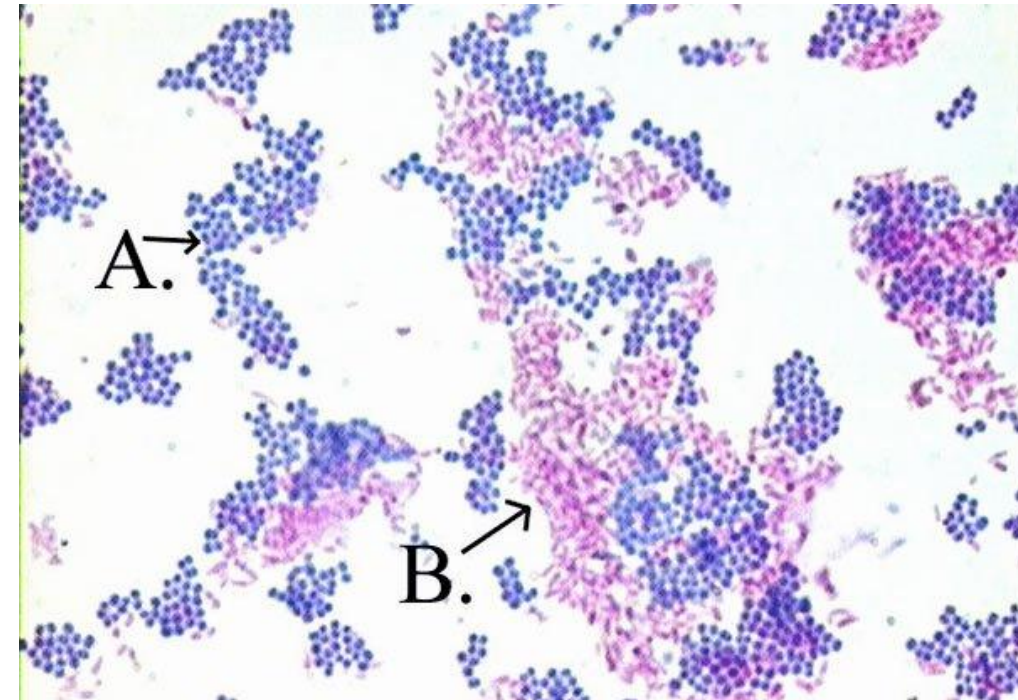
Examples of non-acid fast bacteria

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



ACID FAST STAINING (DEFINE)

- Originally pioneered by a scientist named **Paul Ehrlich** in the year 1882.
- Later, it was modified by **Ziehl and Neelson** in 1883.
- Also called as Ziehl Neelson staining.
- **Differential staining** - Used to distinguish between Non-acid fast (A) and Acid fast bacteria (B).



REQUIREMENTS

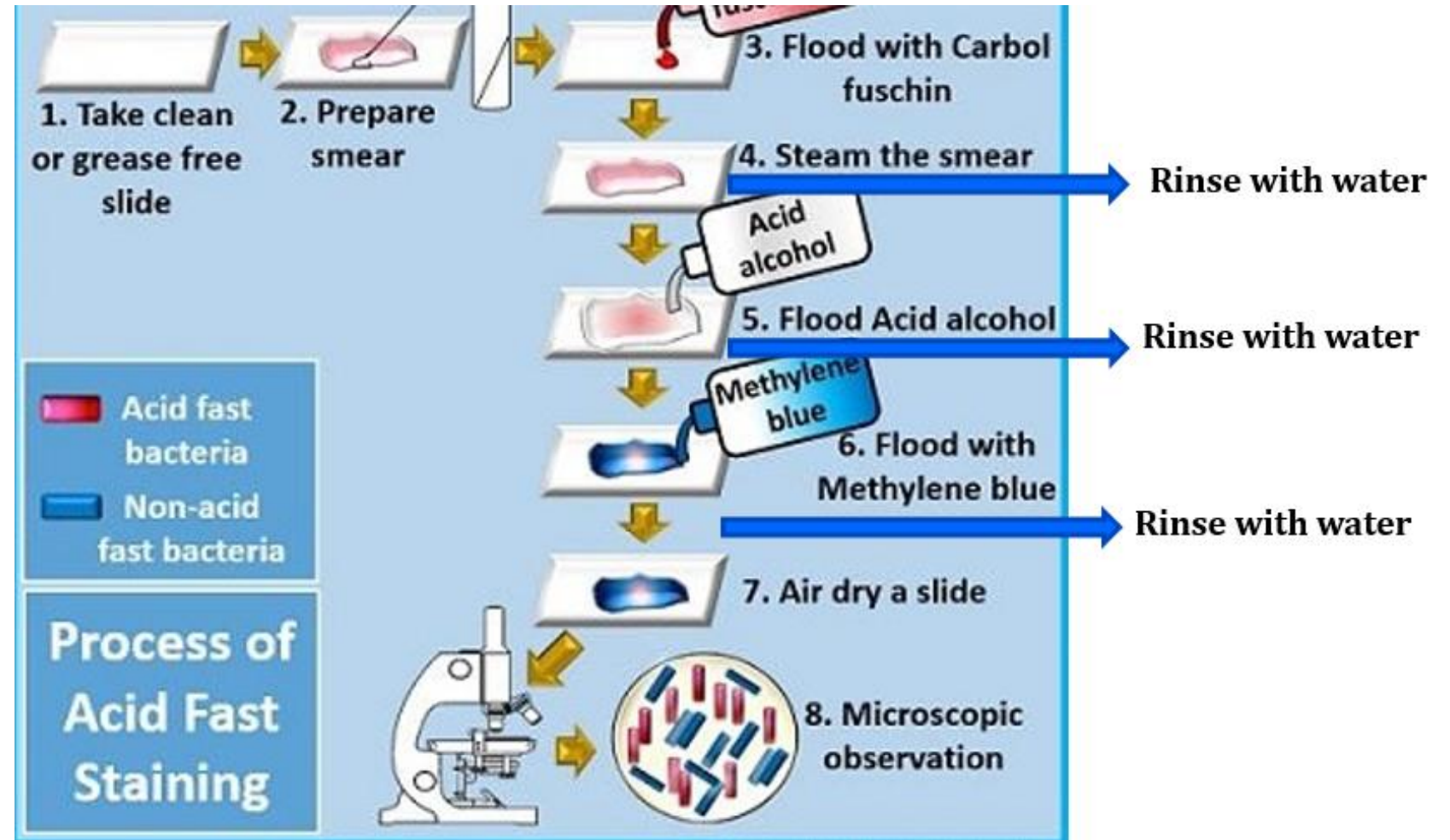
- **Ziehl Neelson Carbol Fuchsin (ZNCF)** - functions as a **Primary stain**.
- **Acid Alcohol** - serves as a **decolourizing agent**, which contains **3% of HCL** along with **95% ethanol**.
- **Methylene Blue** - functions as a **counterstain** and contains **3% of methylene blue**.



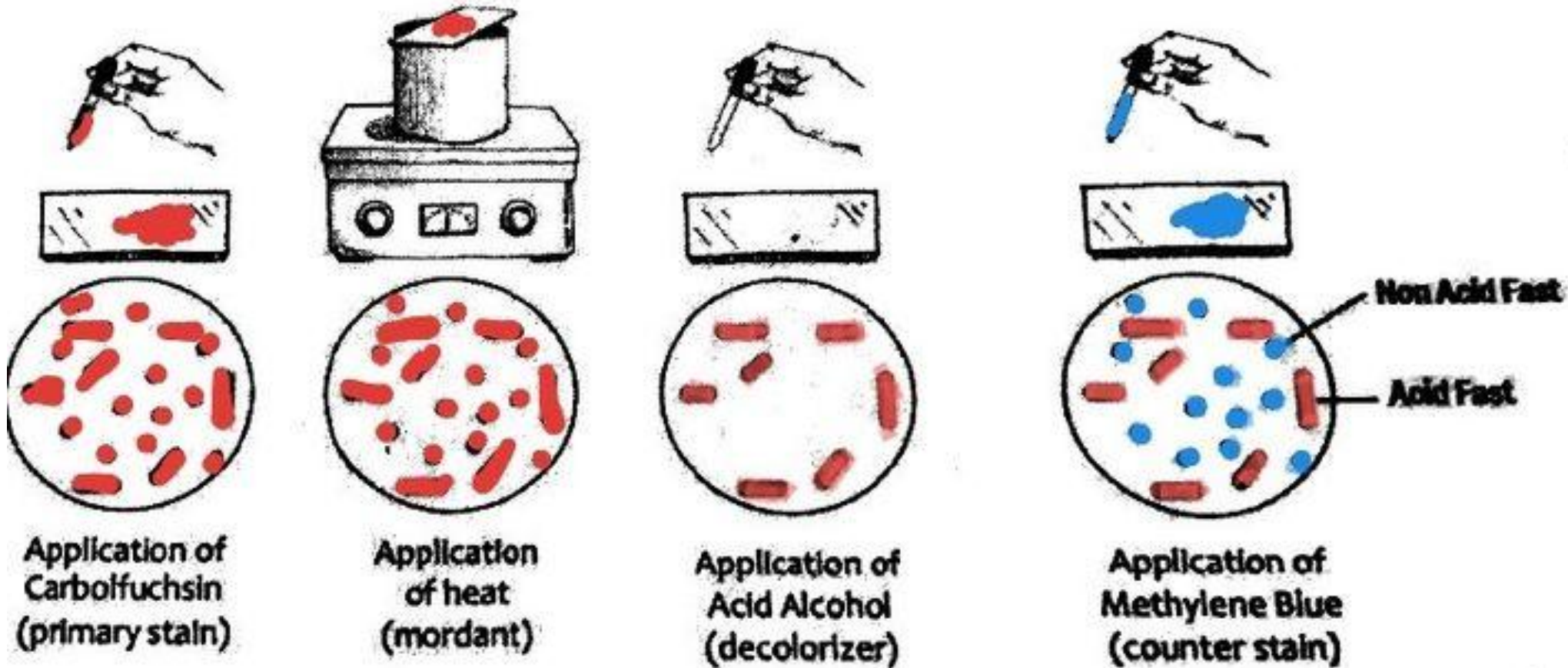
PRINCIPLE

- **Acid-fast bacteria** - high lipid content, retain the **red colour** of the primary stain.
- Mycobacterium - acid-fast bacterium, retains the colour of **carbolfuchsin** even after the treatment with decolourizer because of **mycolic acid** in their cell wall.
- 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 stains the **decolourized cells** of **non-acid fast bacteria** and make them appear **blue**.

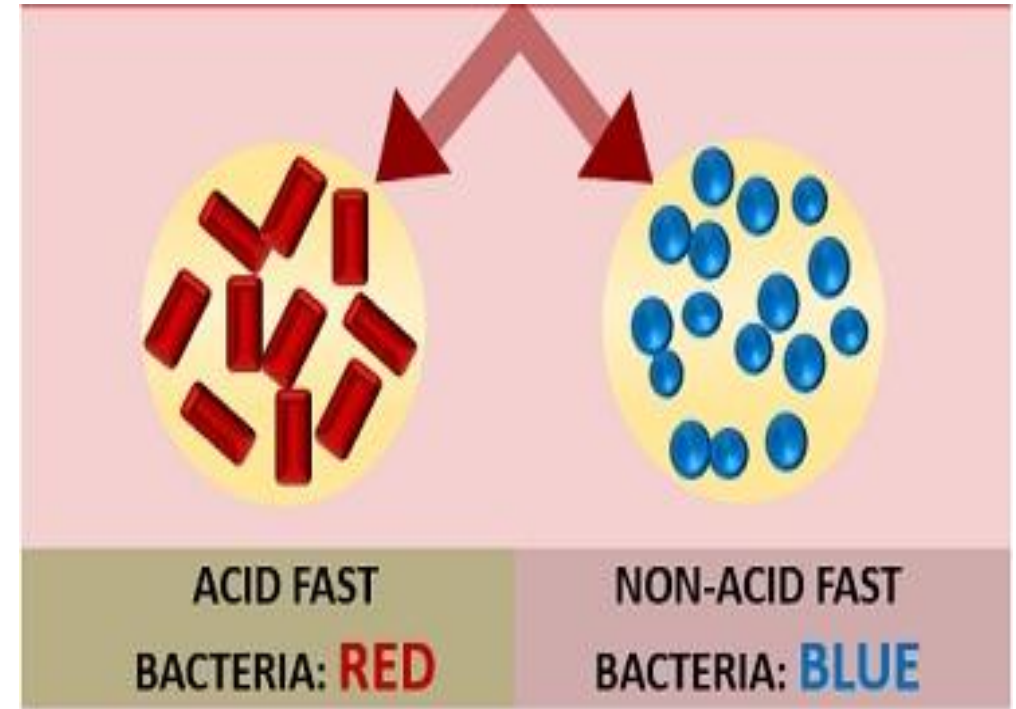
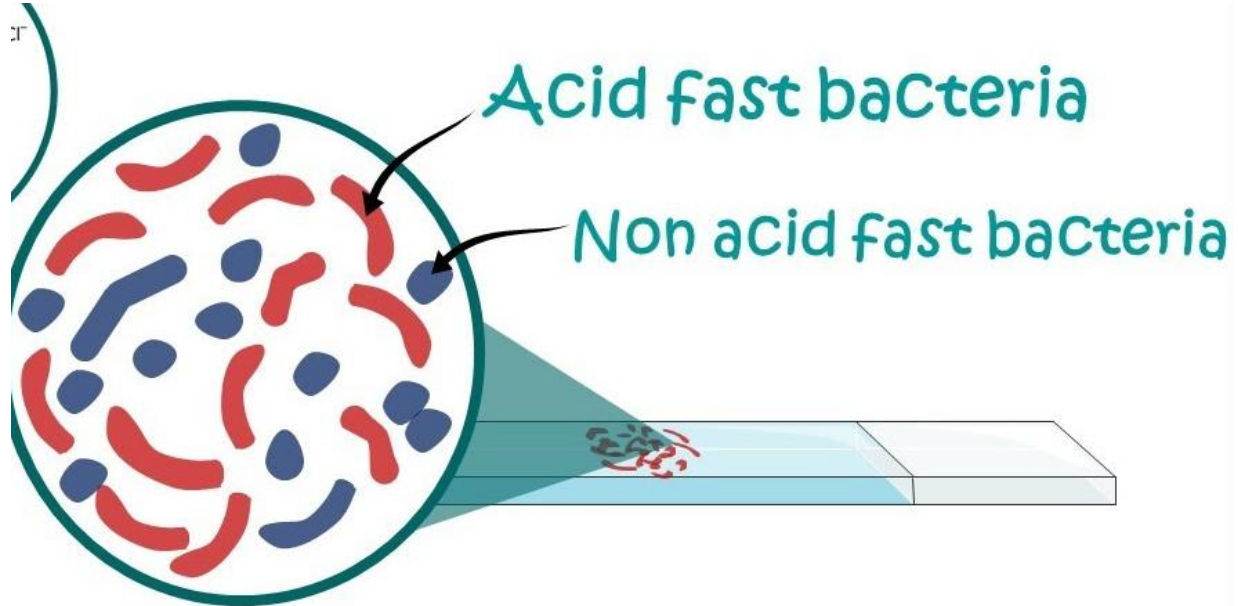
PROCEDURE (PROTOTYPE)



PROCEDURE (PROTOTYPE)



INTERPRETATION (TEST)



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 adding acid-alcohol and retain the **blue colour of methylene blue**.
- 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).

REFERENCES

- Ananthanarayan, R., & Paniker, C. K. J. (2022). *Textbook of Microbiology* (11th ed., 315–319).
- Jawetz, Melnick, & Adelberg's (2020). *Medical Microbiology* (28th ed., Chapter 11).
- CDC Laboratory Manual <https://www.cdc.gov/tb/lab/manual/chapter3-acid-fast-bacilli.html> Title: “Acid-Fast Bacilli (AFB) Smear and Culture”
- https://microbiologysociety.org/resource_library/knowledge-search/acid-fast-staining.html
Title: “Acid-Fast Staining: Principle, Procedure & Interpretation”

THANK YOU