SAWA UNIVERSITY

بكتـريـا طبيـة 1

Differential staining of bacteria (Part 2)

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Second Stage — المرحلـــــــــة / الثانيـــة

تدريسي المادة : دكتــور معيــن حســن

محاضرة رقم 9

Lecture No. 9

Practical الجانب العملي

2- Acid fast stain (Ziehl-Neelsen stain)

- ***Mycobacterium tuberculosis*** **did not stain readily with primary stain but once stained, did not lose the stain even after washing with acid alcohol mixture. Hence, they are called acid fast bacteria. The technique is diagnostically important in identification of acid-fast *Mycobacterium* species and *Nocardia* species. Acid-fast organisms contain mycolic acid that renders the cell wall impermeable to most stains and detergents.**
- **Ziehl-Neelsen procedure is the most widely used acid-fast stains.**

1. **The smear is flooded with hot carbol fuchsin to facilitate stain penetration into bacteria.**
2. **Stained smears are washed with acid alcohol mixture that decolorized non acid-fast bacteria.**
3. **Methylene blue is used as counter stain for staining nonacid fast bacteria. Carbol fuchsin has more affinity for lipids than acid alcohol hence remains bound to cell wall of acid-fast bacteria when washed with acid alcohol.**

- **Requirements**

  A. Ziehl- Neelsen Carbol fuchsin stain

  b. Acid alcohol

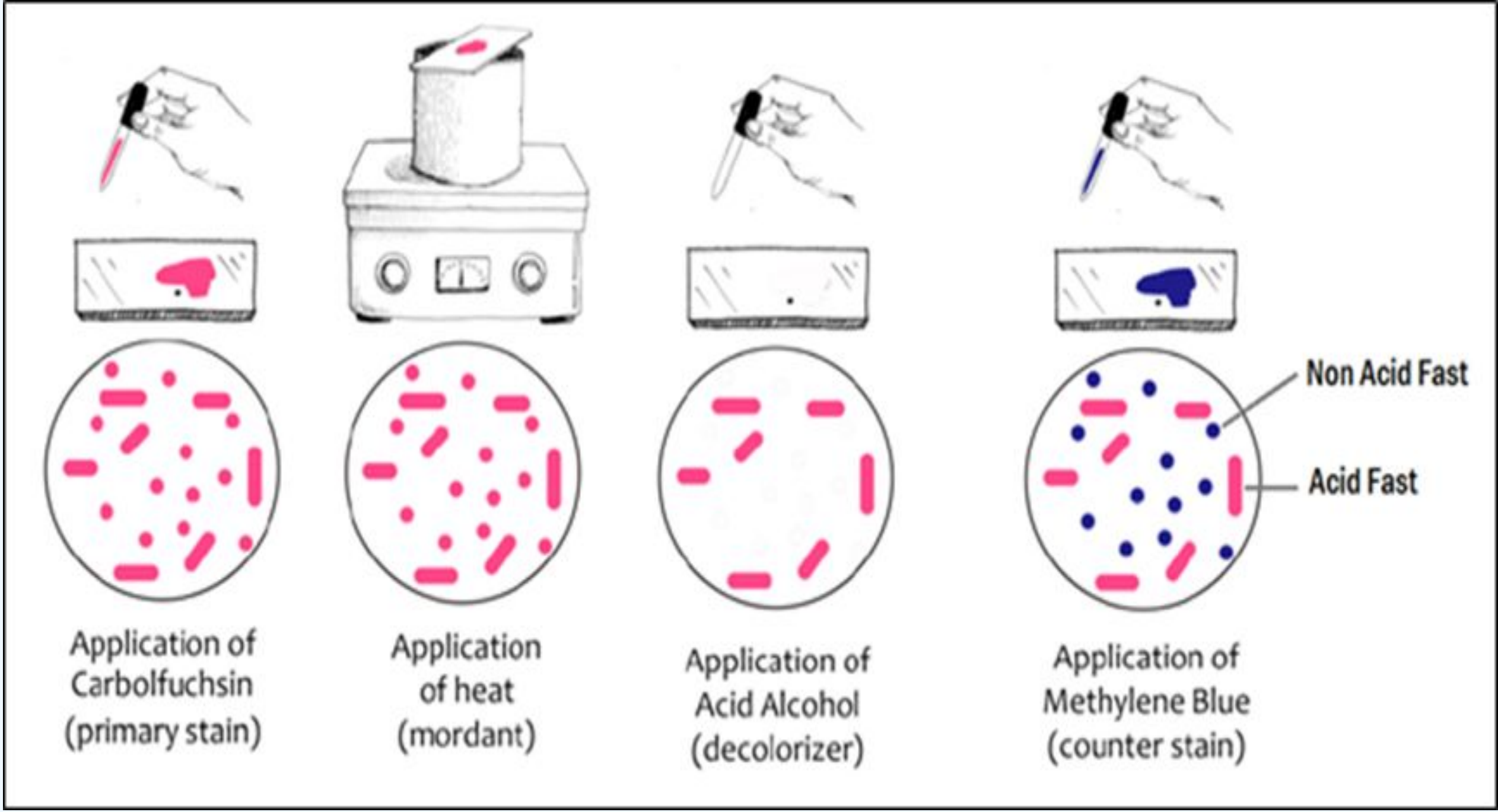
  c. Counter stain: Methylene blue or malachite green

  d. Bacterial cultures: *Mycobacterium spp*

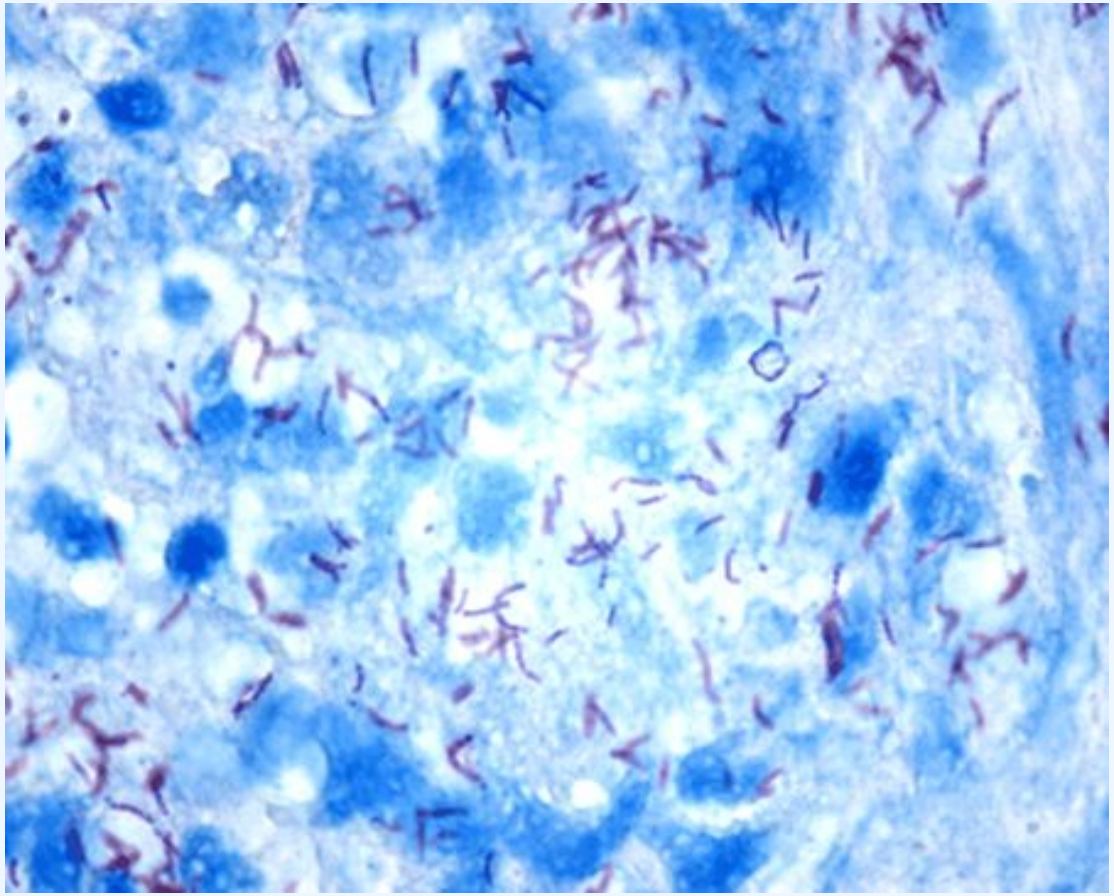
- **Procedure**

- **Ziehl- Neelsen Carbol fuchsin method**

1. **Prepare and heat fix the smears of the cultures on clean grease free slides.**
2. **Cover the smears with boiled carbol fuchsin and leave it for 5-8 min.**
3. **Gently wash with water and then with decolorizer (acid alcohol) for 15-20 sec. or until no more color comes out. Wash the slides with water.**
4. **Counter stain for 30-60 sec. with methylene blue or malachite green.**



(Fig : Ziehl Neelsen staining procedure



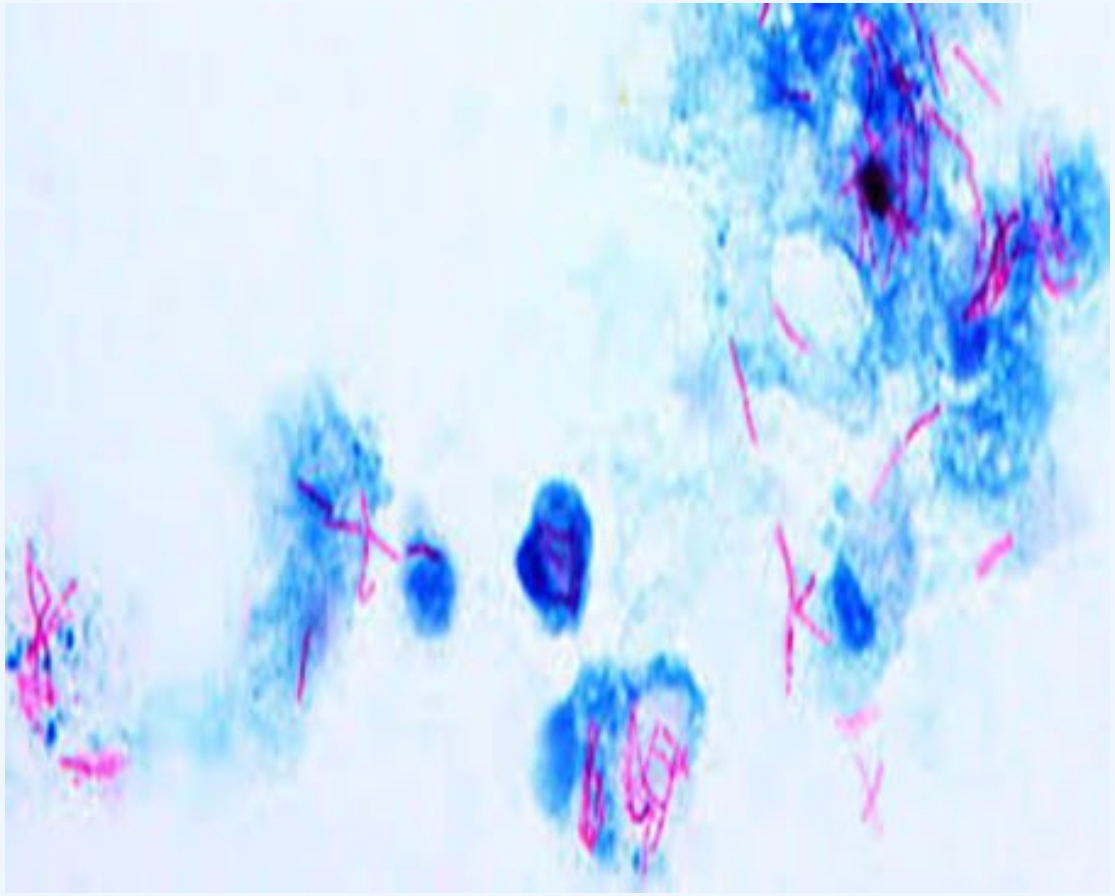


Fig : Acid fast stain (*Mycobacterium tuberculosis*)

Capsule Staining

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A capsule is a gelatinous outer layer secreted by bacterial cell and that surrounds and adheres to the cell wall. Most capsules are composed of polysaccharides, but some are composed of polypeptides. The capsule which can be found in both gram negative and gram-positive bacteria.

The Function of bacterial capsule :

1-It is considered a virulence factor because it enhances the ability of bacteria to cause disease (e.g. prevents phagocytosis). The capsule can protect cells from engulfment by eukaryotic cells, such as macrophages.

2- Capsules also contain water which protects the bacteria against desiccation.

3-They also exclude bacterial viruses and most hydrophobic toxic materials such as detergents.

4-Immunity to one capsule type does not result in immunity to the other types.

5-Capsules also help cells adhere to surfaces.

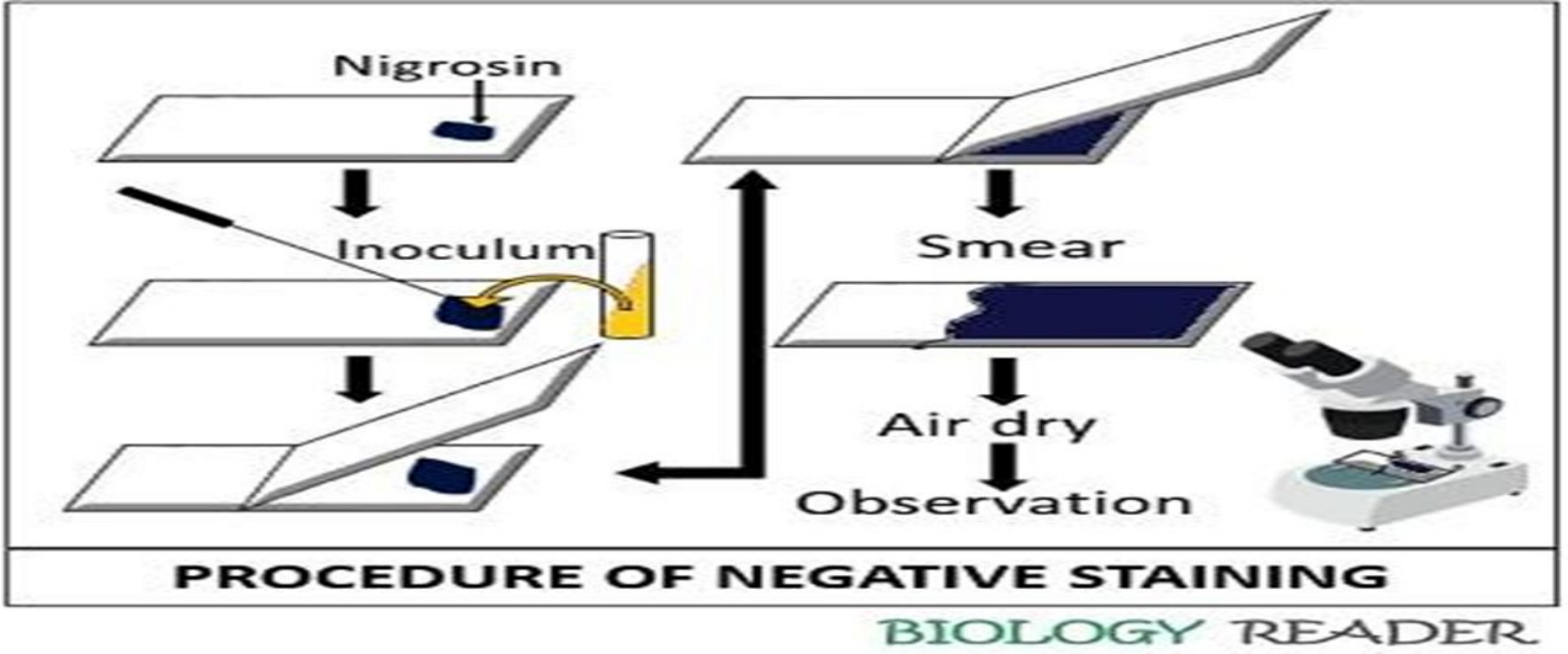
Procedure of Capsule Staining:

1. Place a small drop of a negative stain (India Ink, Nigrosin) on the slide. Using sterile technique, add a loop full of bacterial culture to slide, smearing it in the dye.

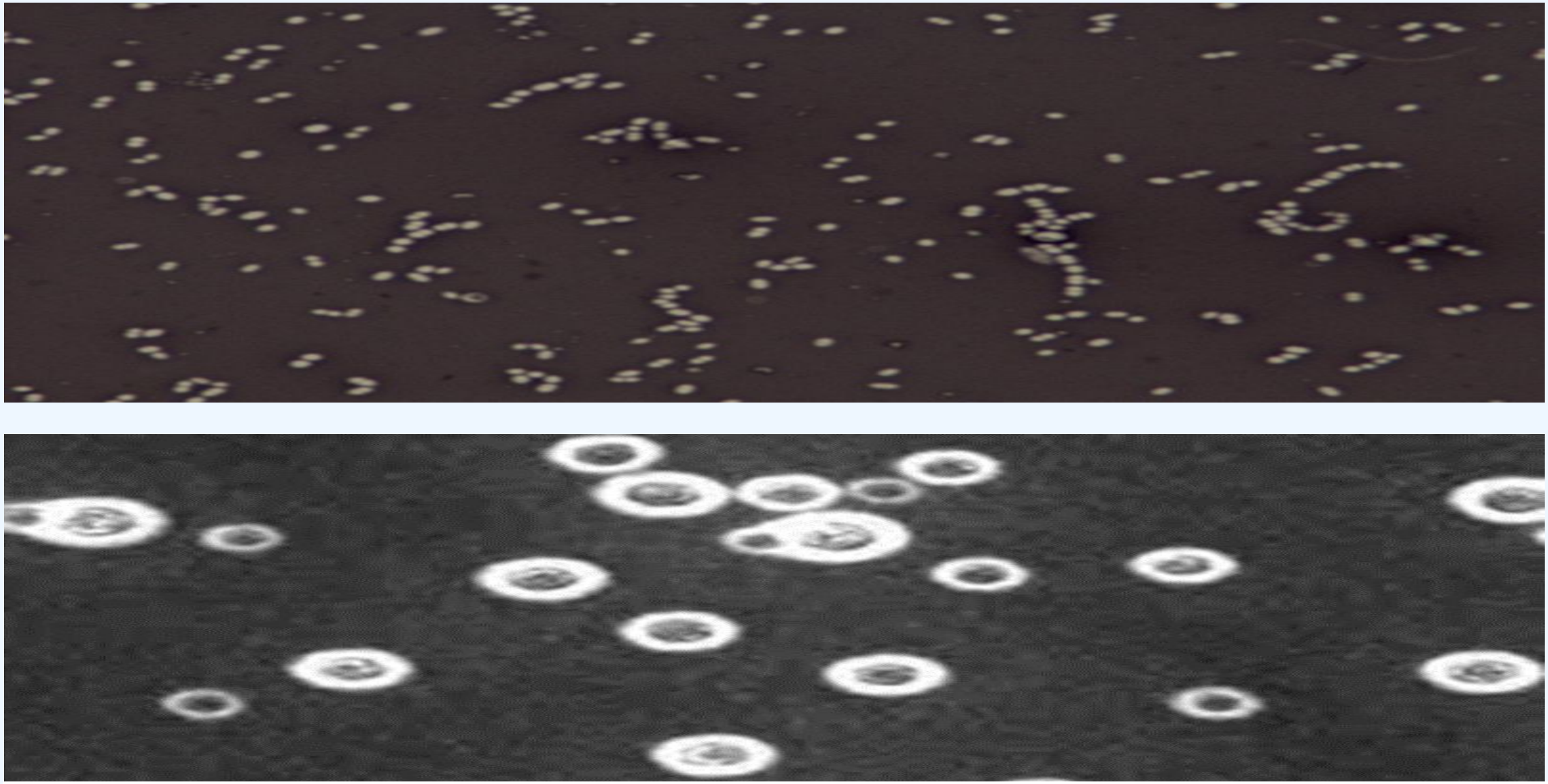
2. Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for 5-7 minutes.

3. Allow to air dry (do not heat fix).

4. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.



(Fig) Negative stain procedure



(Fig) Negative stain

QUIZ

Q1/ WHAT IS THE ACID FAST STAIN (ZIEHL-NEELSEN STAIN)?

Q2/ PROCEDURE OF CAPSULE STAINING:

Q3/ MENTION THE PROCEDURE OF ACID FAST STAIN (ZIEHL-NEELSEN STAIN)?

Any questions?