3. Special stains (structural stains)

A. Capsule Staining (negative 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. The capsule stain employs an acidic stain and a basic stain to detect capsule production. Negative staining methods contrast a darker colored, background with stained cells but an unstained capsule. The background is formed with **India ink** or **nigrosine** or **Congo red**. A positive capsule stain requires a mordant that precipitates the capsule. By counterstaining with dyes like crystal violet or methylene blue, bacterial cell wall takes up the dye. Capsules appear colorless with stained cells against dark background.

► Procedure of Capsule Staining

1. Place a small drop of a negative stain (India Ink, Congo Red, Nigrosin) on the slide.

2. Using sterile technique, add a loop-full of bacterial culture to slide, smearing it in the dye.

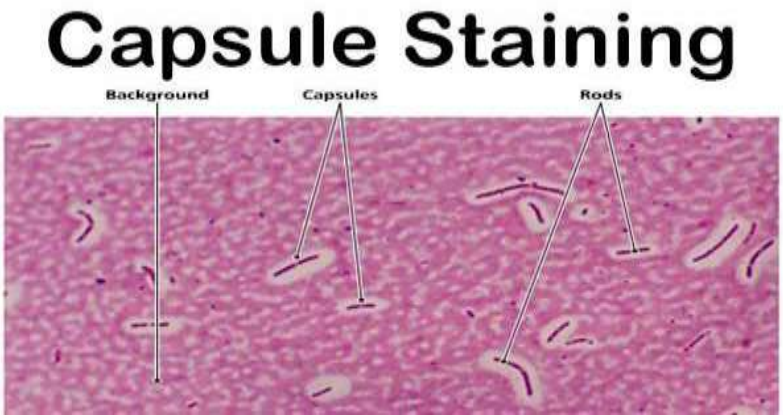
3. Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for 5-7 min.

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

5. Flood the smear with crystal violet stain (this will stain the cells but not the capsules) for about 1 min. Drain the crystal violet by tilting the slide at 45-degree angle and let stain run off until it air dries.

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

Note: negative staining is a mild technique that may not destroy the microorganisms, and is therefore unsuitable for studying pathogens.



Result of Capsule Staining
Capsule: Clear halos zone against dark background
No Capsule: No hales zone

B. **Endospore Staining** The Schaeffer-Fulton method is used to distinguish between the vegetative cells and the endospores. A primary stain (**Malachite green**) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green penetrate the spore wall) the endospore by heating. In this technique heating acts as a **mordant**. There is no need of using any decolorizer in this spore staining as the primary dye malachite green bind relatively weakly to the cell wall and spore wall. In fact, If washed well with water the dye come right out of cell wall however not from spore wall once the dye is locked in. Water is used to decolorize the vegetative cells.

Note: In Gram Staining and AFB Staining we use Alcohol or Acid Alcohol or Acid as decolorizer but in spore staining water is sufficient (to be used as decolorizer) because:

1. Malachite green dye is water- soluble and does not adhere well to the cell wall.

2. Vegetative cells have been disrupted by heat, because of these reasons, the malachite green rinses easily from the vegetative cells. As the endospores are resistant to staining, the endospore are equally resistant to de-staining and will retain the primary dye while the vegetative cells will lose the stain. The addition of a

secondary stain (**safranin**) is used to stain the decolorized vegetative cell.

► Procedure of endospore stain:

1. Prepare smears of organisms to be tested for presence of endospores on a clean microscope slide and air dry it.

2. Cover the smears with a piece of absorbent paper cut to fit the slide and place the slide on a wire gauze on a ring stand.

3. Saturate the paper with malachite green and holding the Bunsen burner in the hand heat slide until steam can be seen rising from the surface. Remove the heat and reheat the slide as needed to keep the slide steaming for about three min. As the paper being to dry add a drop or two malachite green to keep it moist, but do not add so much at one time that the temperature is reduced.

4. Remove the paper with tweezers and rinse the slide thoroughly with tap water.

5. After 5 min carefully remove the slide from the rack using a clothspin.

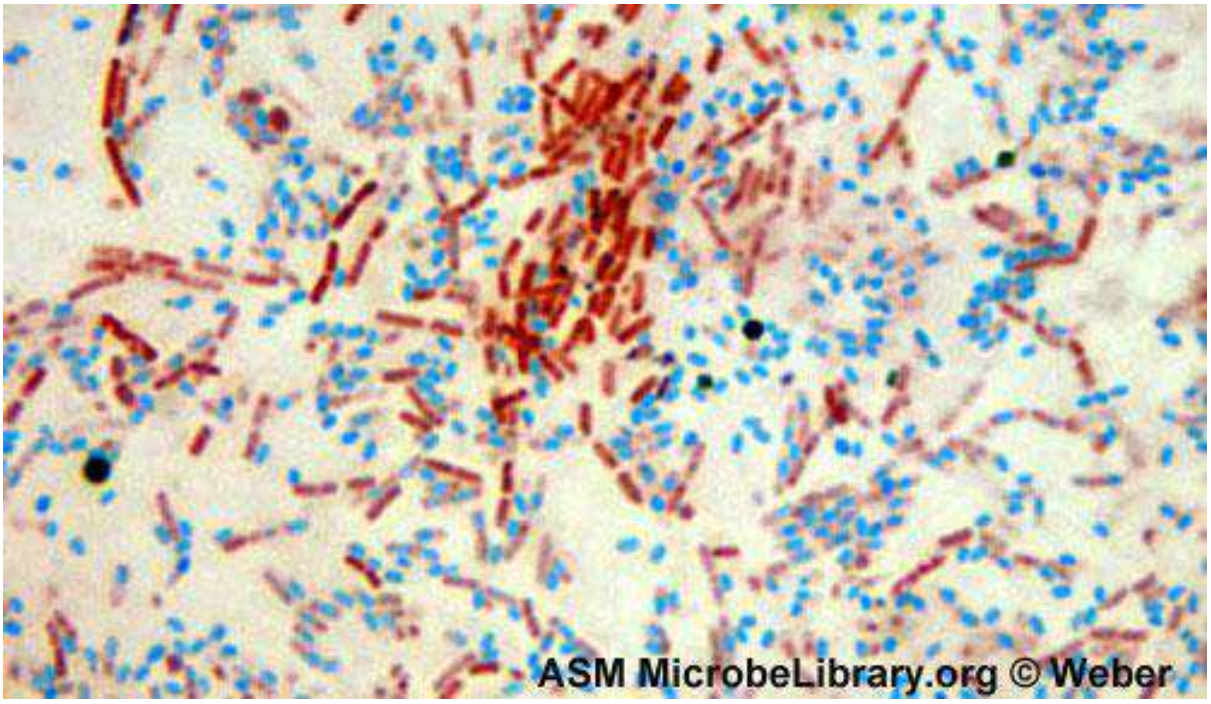
6. Remove the blotting paper and allow the slide to cool to room temperature for 2 min.

7. Rinse the slide thoroughly with tap water (to wash malachite green from both sides of the microscope slide).

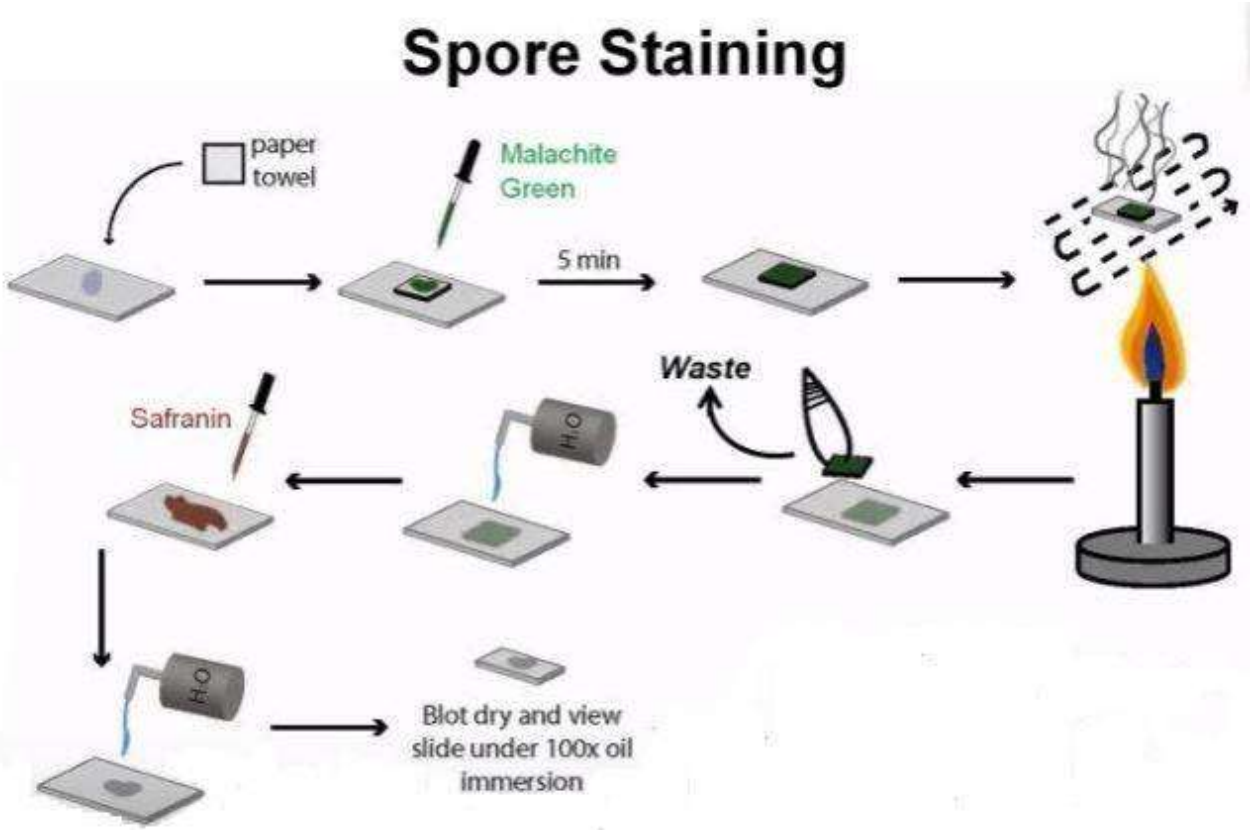
8. Stain the smear with **safranin** for 2 min.

9. Rinse both side of the slide to remove the secondary stain and blot the slide/air dry and exam.

10. The vegetative cells will appear red and the spores will appear green.



Results of endospores staining



Spore staining procedure