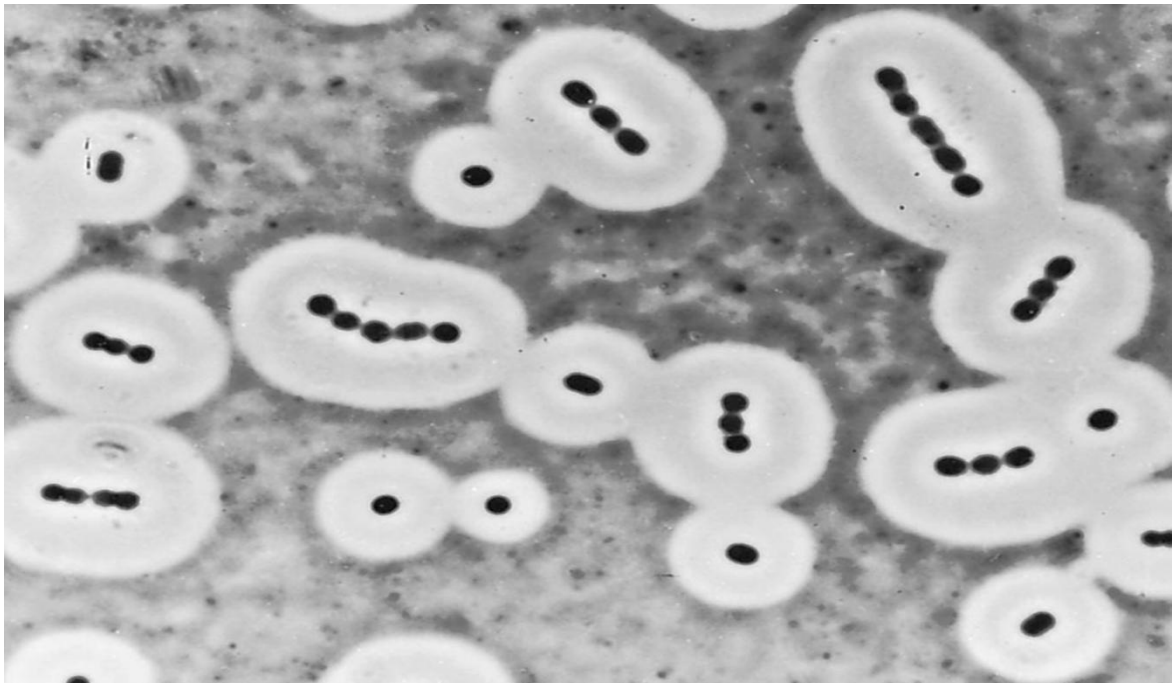
Capsule staining

Microbiology I



Introduction

- Capsule stain is a type of differential stain that uses acidic and basic dyes to stain background & bacterial cells respectively so that presence of capsule is easily visualized.

- Capsule is synthesized in the cytoplasm and secreted to the outside of the cell where it surrounds the bacterium.

- Most bacteria have capsules made of polysaccharide layer, some are made up of polypeptide, or glycoprotein.

- Capsules are associated with virulence in several microorganisms, it is essential to identify them.
- *Streptococcus pneumoniae* and *Neisseria meningitides*

Principle

- Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces.
- Capsulse are visualized by staining the background using an acidic stain
- Ex. Nigrosine, congo red
- Cell is stained using a basic stain
- Ex. crystal violet, safranin, basic fuchsin, & methylene blue.
- Two commonly used methods for capsule staining are:
- 1. **India ink method**
- 2. **Anthony's stain method**

India ink method

- In this method, two dyes: crystal violet, and India ink are used.
- The capsule is seen as a clear halo around the microorganism against the black background.
- Ex. *Cryptococcus.*
- The background will be dark (color of India ink).
- The bacterial cells will be stained purple (crystal violet – basic dyes as they are positively charged).
- The capsule (if present) will appear clear against the dark background.

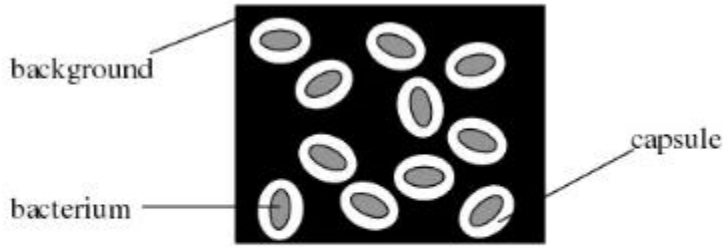


Image 1: Capsule Staining *(source-microbugz)*

Anthony's stain method

- Primary stain is **crystal violet** and the cells are stained purple.
- 20% copper sulfate solution used as both the decolorizing agent and counterstain.
- It decolorizes the capsule by washing out the crystal violet, but will not decolorize the cell.
- As the copper sulfate decolorizes the capsule, it also counterstains the capsule.
- Thus, the capsule appears as a faint blue halo around a purple cell.

Materials and reagents required

- **Test bacteria:** 36-48 hour culture of capsulated bacteria e.g. ***Klebsiella pneumoniae*** growing on a slant of **EMB Agar**
- Stain solutions:

1. crystal violet & indian ink (Nigrosin)
2. crystal violet & copper sulphate solution – Anthony's method.

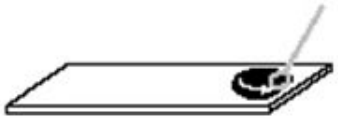
- Microscopic slides
- Inoculating loop
- Microscope with 100x objective lens (oil immersion)
- Immersion oil
- Gas burner
- Tissue paper

Capsule Stain procedure

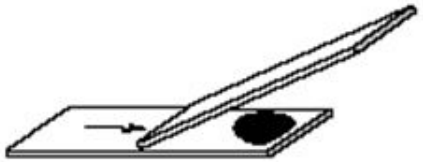
- **India Ink Method:**
- Place a single drop of **India ink** at the edge on a clean microscope slide
- Add a loopful of the bacteria culture and mix, prepare smear using another glass slide
- Allow the film to air dry (5-7 mins). DO NOT HEAT.
- Saturate the slide with crystal violet for 1 minute and rinse slightly & very gently with water.
- Let the slide air dry for a few minutes. DO NOT BLOT DRY.
- Observe the slide under oil immersion.

Procedural Diagram
Negative Stain

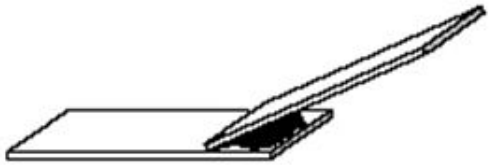
1. Begin with a drop of acidic stain at one end of a clean slide.



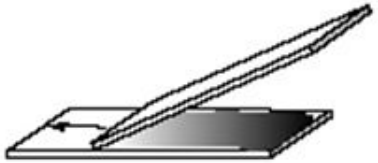
2. Aseptically add organisms and emulsify with a loop. Do not over-inoculate and avoid spattering the mixture. Sterilize the loop after emulsifying.



3. Take a second clean slide, place it on the surface of the first slide, and draw it back into the drop.



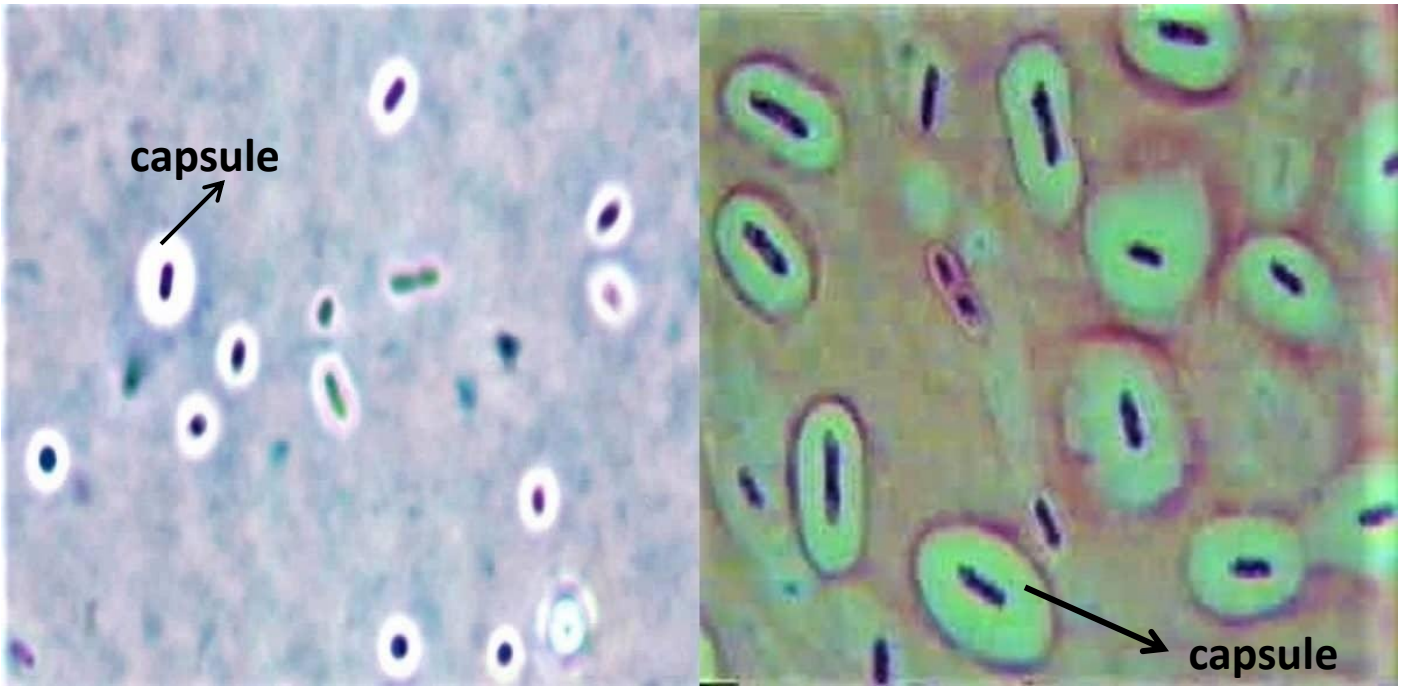
4. When the drop flows across the width of the spreader slide...



5. ...push the spreader slide to the other end. Dispose of the spreader slide in a jar of disinfectant or Sharps container.

6. Air dry and observe under the microscope. Do NOT heat fix.

Microscopic observation



Indian ink method

Anthony's method