***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** differs from the **slime layer** that most bacterial cells produce in that it is a thick, detectable, discrete layer outside the cell wall. The capsule stain employs an acidic stain and a basic stain to detect capsule production.

Capsules stain very poorly with reagents used in simple staining and a capsule stain can be, depending on the method, a misnomer because the capsule may or may not be stained.

Negative staining methods contrast a translucent, darker colored, background with stained cells but an unstained capsule. The background is formed with **india ink or nigrosin or congo red**. India ink is difﬁcult to obtain nowadays; however, nigrosin is easily acquired.

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 colourless with stained cells against dark background.

Capsules are fragile and can be diminished, desiccated, distorted, or destroyed by heating. A drop of serum can be used during smearing to enhance the size of the capsule and make it more easily observed with a typical compound light microscope.

Reagents used for Capsule Staining

**Crystal Violet (1%)**
**Nigrosin**



***Procedure of Capsule Staining***

1. Place a small drop of a **negative stain** (India Ink, Congo Red, Nigrosin, or Eosin) on the slide.
Using sterile technique, add a loopful 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. Flood the smear with **crystal violet stain** (this will stain the cells but not the capsules) for about **1 minutes**. Drain the crystal violet by tilting the slide at a 45 degree angle and let stain run off until it air dries .
5. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.

***Result of Capsule Staining***



**Capsule:** Clear halos zone against dark background
**No Capsule:** No Clear halos zone

Examples of Capsule Positive and Negative



