*Capsule Stain*

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.

***Principle:***

**In capsule staining, a smear of bacteria is made at the center of a slide. It is not heat-fixed, as the cell shrinkage caused by heating may create a clear zone around the cell, which may be mistaken as capsule.**

**Moreover, as the capsular material is water-soluble and may be washed away with vigorous washing, only two reagents are used in staining without any in-between water-washing.**

 **One of the two reagents is the primary stain, crystal violet. It imparts dark purple-blue color to both, the cell as well as the capsule.**

**However, it is ‘absorbed’ into the cell due to the ionic nature of the cellular components, whereas it simply ‘adheres’ to the capsule, because of the non-ionic nature of the capsular material. To prevent of the dislodging of the capsular material, the smear is not water-washed.**

**The second reagent, copper sulphate acts both as a decolorizing agent and a counter-stain. It decolorizes by removing excess crystal violet from around the cells as well as the crystal violet adhering to the capsule. It also counter- stains the capsule and imparts a light blue color to it. The capsule now appears light blue, whereas the cell appears deep purple-blue.**

***Procedure:***

1. **Place a small drop of a negative stain (India Ink, Congo Red, Nigrosin, or Eosin) on the slide.**
2. **Using sterile technique, add a loopful 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 minutes.**
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 minutes. Drain the crystal violet by tilting the slide at a 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 zones surrounding the cells.**

1. Colour of the ar**ea surrounding the cells:**

Light blue: Capsule present (capsulated bacteria)

Dark purple-blue: Capsule absent (non-capsulated bacteria)

**2. Colour of the cells:**

Dark purple-blue

**3. If capsulated, size of the capsules:**

Small, moderate or large.

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**Capsule:** Clear halos zone against dark background