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### Microbiology Lab

**((Capsule stain))**

**Lab/6**

**2 stage**

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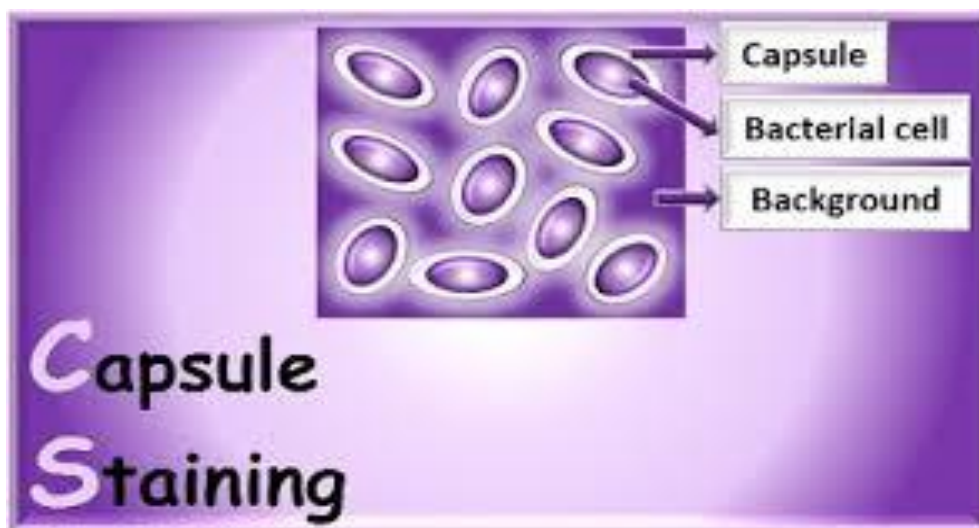


### Capsule Stain:

One key bacterial adaptation is the capsule, an outer layer of polysaccharides that covers the cells of many different bacterial species. Capsules act as a sort of magic cloak, protecting bacteria from toxic compounds and desiccation and allowing them to adhere to surfaces and to escape the immune system of the host.

**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.





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### 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.