Capsule stain
Principle, Procedure and Results

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Capsule stain

- It is a well organized gelatinous layer that is secreted by the cell, surrounds and adheres to the cell wall.
- Capsule is not common to all organisms and the organisms that have a heavy capsule are generally virulent and capable of producing disease.
- It protects bacteria against the phagocytic activities of host phagocytes.
- It also serves as a barrier against antimicrobials preventing them from entering the cell.

Capsule stain(cont..)

- Most capsules are polysaccharides (ex: *Streptococcus pneumoniae*) in nature but some are glycoproteins or polypeptides.
- Since capsule fails to retain standard dyes, it can't be stained by simple staining procedure or even gram stain, but it can be visualized indirectly using negative staining technique.
- A basic dye is used to stain the bacterial cell and acidic dye is used to colour the background where the capsule appears as a clear halo zone between the coloured background and the stained cell.


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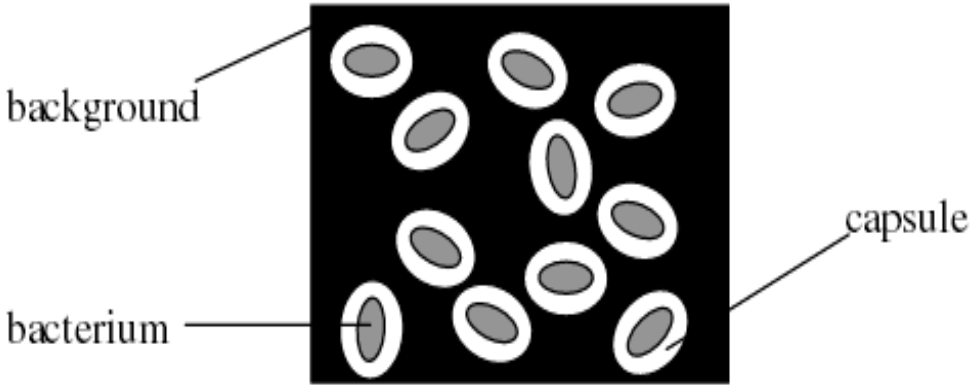
Principle of Capsule Stain

Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces. Therefore, the best way to visualize them is to **stain the background using an acidic stain** and to **stain the cell itself using a basic stain.** *(capsule does not take any stain).* A clear halo is the capsule.



Capsule Stain procedure

1. Place a single drop of India ink on a clean microscope slide, adjacent to the frosted edge.

2. Using a flamed loop and sterile technique, remove some *Klebsiella pneumoniae* from culture tube or plate and mix it into the drop of India ink. Be sure there are no large clumps of organism, but try to avoid spreading the drop.

3. Place the end of another clean microscope slide at an angle to the end of the slide containing the organism. Spread out the drop out into a film. This is done by contacting the drop of India ink with the clean microscope slide and using the capillary action of the dye/ slide to spread the India ink across the smear.

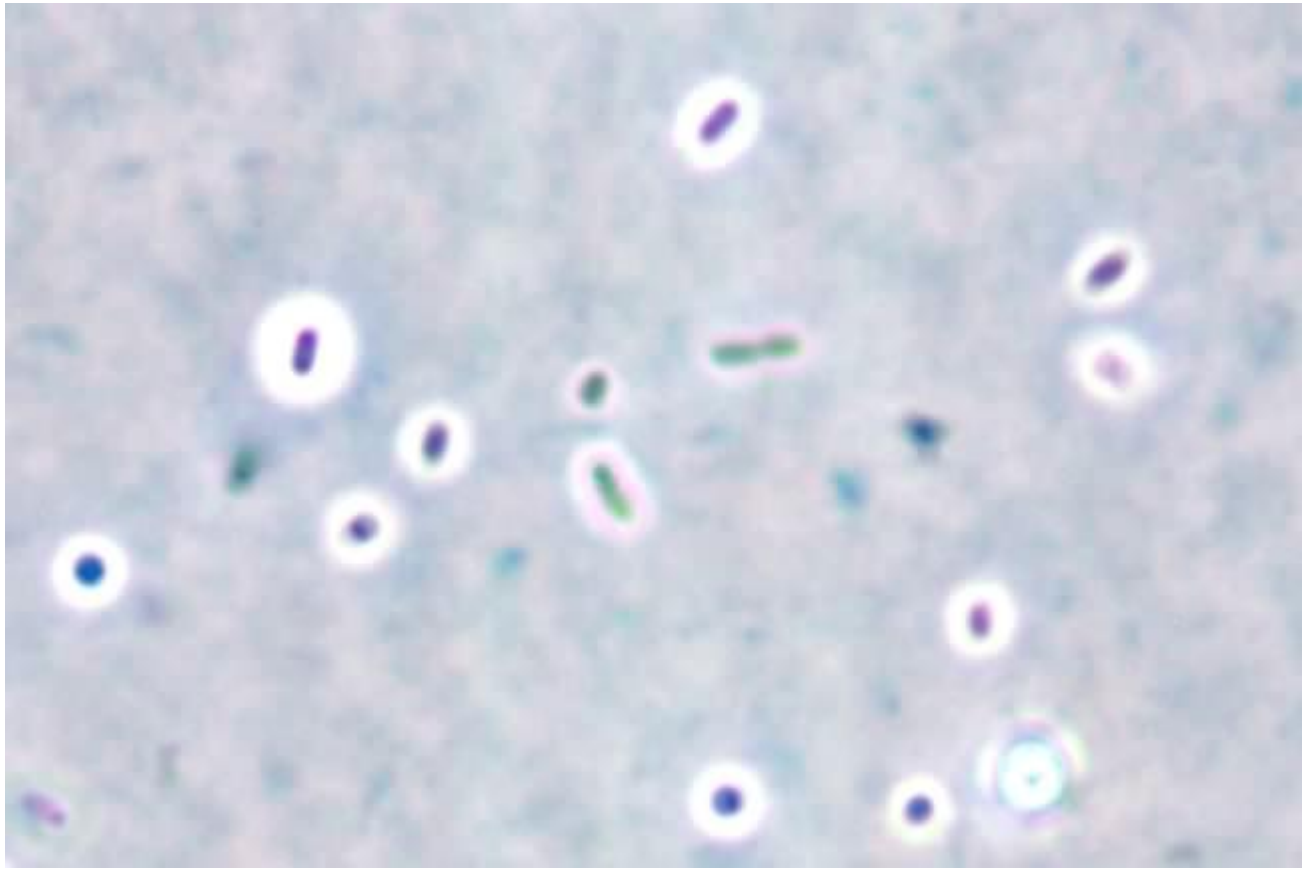
4. Allow the film to air dry (will take 5-7 minutes). DO NOT heat or blot dry! Heat will melt the capsule!

5. Saturate the slide with crystal violet for 1 minute and rinse slightly & very gently with water. Be cautious water may remove the capsule from the cell.

Let the slide air dry for a few minutes. DO NOT blot the slide! Blotting will remove the bacteria from the slide and/or distort the capsule.

6. Observe the slide under oil immersion.

Results: Look for purple cells surrounded by a clear halo on a dark background. The halo is the capsule.



Capsule staining by India ink method (at 1000x magnification)

THE END