

Capsule Staining

Principle: A capsule is a gelatinous outer layer that is secreted by the cell and that surrounds and adheres to the cell wall. It is not common to all organisms. Cells that have a heavy capsule are generally virulent and capable of producing disease since the structure protects bacteria against normal phagocytic activities of the host cell. Chemically capsular material is a polysaccharide, glycoprotein or a polypeptide. The ability to form capsule is genetically determined.

Suitable organism : *Klebsiella aerogens* of 36 - 48 hr milk culture by growing them on Milk medium.

Milk medium : composition (per litre) : -

Skim milk powder - 100.0g

Peptone - 5.0g

pH – 7.2

Procedure: 1. Put a drop of **nigrosin** or Indian ink (negative stain, live cells repel them) close to one end of the thin glass slide.
2. Add 2 loop-full of a broth culture into the drop of the stain and mix.
3. Prepare a smear of the suspended organism using the edge of a second slide.
4. Air dry the smear, do not heat fix. (The smear should not be heated because resulted cell shrinkage may create a clear zone around the organism that is an artifact that can be mistaken for the capsule)
5. Examine under microscope.

Capsule appears as a circumscribed layer around the cell which can be easily made visible against a dark background.

Procedure2 (Anthony Method)

1. Put 2 loopfulls of culture of *Klebsiella aerogens* on one end of the slide.
2. Prepare a heavy smear of the organism and allow to air dry.
3. Flood smear with **crystal violet** for 2 minutes(1% aq. sol). At this point the cell and the capsular material will take on dark colour.
4. Wash off the dye with **20% CuSO₄** soln. Because the capsule is non-ionic unlike the bacterial cell, the primary stain adheres to the capsule but does not bind to it. In the capsule staining method CuSO₄ soln is used as a decolorising agent rather than water. The CuSO₄ washes off the purple primary stain out of the capsular material without removing the stain bound to the cell wall. At the same time the decolorizing capsules absorbs the CuSO₄ and capsule will now appear as a contrasting blue hallow surrounding the deep purple colour of the cell.
5. Drain of CuSO₄.
6. Air dry and observe under microscope using oil-immersion objective.

*** Capsule is a part of the cell while slime is a secretion. When the slime gets hard and no longer exists as a secretion it becomes a capsule.