

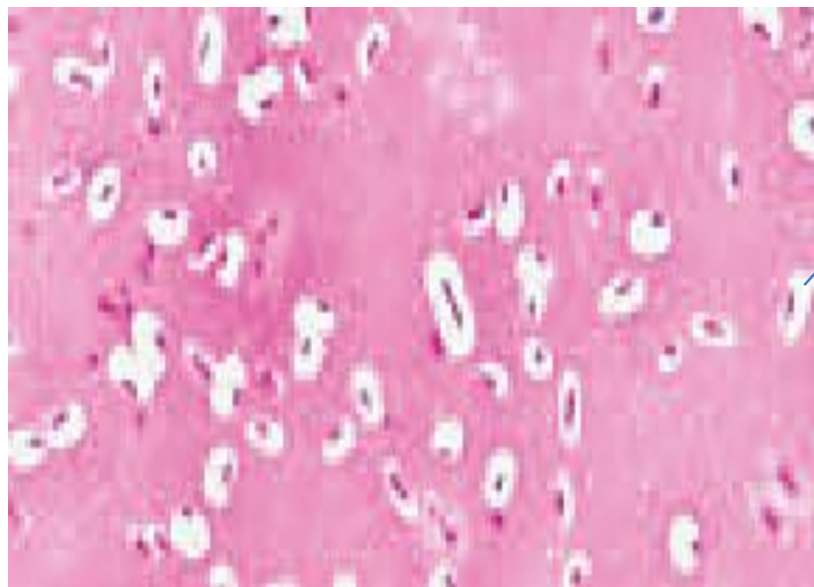
Capsule Stain

Purpose

The capsule stain is a differential stain used to detect cells capable of producing an extracellular capsule. Capsule production increases virulence in some microbes (such as the anthrax bacillus *Bacillus anthracis* and the pneumococcus *Streptococcus pneumoniae*) by making them less vulnerable to phagocytosis.

Principle

Capsules are composed of mucoid polysaccharides or poly peptides that repel most stains because of capsules are non-ionic . The capsule stain technique takes advantage of this phenomenon by staining *around* the cells. Typically, an acidic stain, such as Congo red or nigrosin that stains the background , and a basic stain that colorizes the cell proper, are used . The capsule remains unstained and appears as a white halo between the cells and the colored background .



capsule

Capsules are not easily stained, because they are very fragile and are easily disrupted by water and heat. Capsular staining does not require heat-fixing a smear. The use of heat during fixation causes shrinkage, so the procedure is performed without heat.

Procedure:

A. Positive staining method.

1. A smear is prepared on a clean slide.
2. The smear should not be heat fix it should be just air dried.
3. After air drying the smear is flooded with 1 % Crystal violet for about 5 – 7 minutes.
4. After 5 minutes the smear is rinse with 20 % copper sulfate solution .
5. The slide is air dried and observed under oil immersion objective.

B. Negative staining method .

1. Place a drop of India ink on a clean slide.
2. Using aseptic technique, add a loop full of bacteria to the drop of India ink and mix.
3. Spread out the drop using a second slide in the same way one prepares a thin smear.
4. Allow the smear to air dry. Do not heats fix the smear .
5. Observe the slide under oil immersion .

spore stain

Purpose

The spore stain is a differential stain used to detect the presence and location of spores in bacterial cells. Only a few genera produce spores. Among them are the genera *Bacillus* and *Clostridium*. Most members of *Bacillus* are soil, freshwater, but a few are pathogens, such as *B. anthracis*, the causative agent of anthrax, but four pathogens are fairly well known: *C. tetani*, *C. botulinum*, *C. perfringens*, and *C. difficile*, which produce tetanus, botulism, gas gangrene, and pseudomembranous colitis, respectively.

Principle

An endospore is a dormant form of the bacterium that allows it to survive poor environmental conditions. Spores are resistant to heat and chemicals because of a tough outer covering made of the protein keratin. The keratin also resists staining, so extreme measures must be taken to stain the spore. In the Schaeffer-Fulton method. Spores may be located in the middle of the cell (central), at the end of the cell (terminal), or between the end and middle of the cell (sub terminal). Spores also may be differentiated based on shape—either spherical or elliptical (oval)—and size relative to the cell (*i.e.*, whether they cause the cell to look swollen or not).

Procedure:

- 1) Smear the organism and heat fix to a slide.
- 2) Place the slide over a steam bath and cover with Malachite Green.
- 3) Keep the stain over the bath for 3 - 5 minutes, recovering the slide with Malachite Green if some evaporates.
- 4) Dump the Malachite Green off and allow to cool.
- 5) Rinse the slide with water to remove excess stain.
- 6) Cover the smear with Safranin for two minutes.

- 7) Rinse the slide with water to remove excess stain .
- 8) Blot dry the stain and view under a microscope .

Endospore
producing
bacterium



Endospore
nonproducing
bacterium



1. Bacteria and endospores are transparent prior to staining
2. Steam is used to drive/force the primary stain **MALACHITE GREEN** into **all** bacteria as well as **endospores**.
3. Decolorization with water removes the **Malachite green** stain from the bacteria but **NOT** from the endospores.
4. Sample is counterstained with **SAFRANIN**, which is taken up by all bacterial cells but without steam the stain **CANNOT** be taken up by endospores.

