Practical Microbiology

Lab . (9)

Bacteria Staining (Part - 2)

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A number of other stains are used in microbiology to demonstrate spore, flagella, capsules and granules, and for staining bacteria in tissue sections used for identification and diagnosis .

Spore stain :(see figure 1 and 4)

Spores represent a chemically and physically resistant form of the vegetative bacteria cell. Spore staining is applied to bacterial spore formers.

Endospore production is a very important role and characteristic of some bacteria such as Bacillus and Clostridium, allowing them to resist different environmental factors that help the bacteria to increase the virulent factors , spored bacteria usually resistance to desiccation, chemical exposure, extreme heat, etc.

They bacteria spore , identified in the 1800s (by John Tyndall developed a process for destroying them with intermittent heat procedure), although the stain procedures to identify them did not develop until the early twentieth century. **Endospores are not for reproduction**

The stimulation for sporulation and forming spores can different and depending of some factors such as nutrient depletion, desiccation, PH, chemicals, temperature, and other factors.

After staining with simple or Gram stain .The vegetative portion of the cell takes up the stain but the spore does not, the spore appears as a white retractile body within the vegetative cells

The principal of spore stain can discusses by , a spore forms inside of the vegetative cell, the spore wall chemically changes and thicken. This sporulation process changes the spore's stain ability to penetrate and react , making it increasingly resistant to the staining dyes, and so a gimmick—steaming---enhances the primary dye's penetration .so The primary dye is malachite green is a relatively weakly binding dye to the cell wall and spore wall. In fact, if washed well with water, the dye comes right out of the cell wall, however not from the spore wall once the dye is locked in. That is why there does not need to be a decolorize.in this stain: it is based on the binding of the malachite green and the permeability of the spore vs. cell wall. . The steaming helps the malachite green to permeate the low-permeability spore wall.

The identification of spores is also very important for the clinical microbiologist who is analyzing a patient's body fluid or tissue since there are not that many spore forming **genera**.

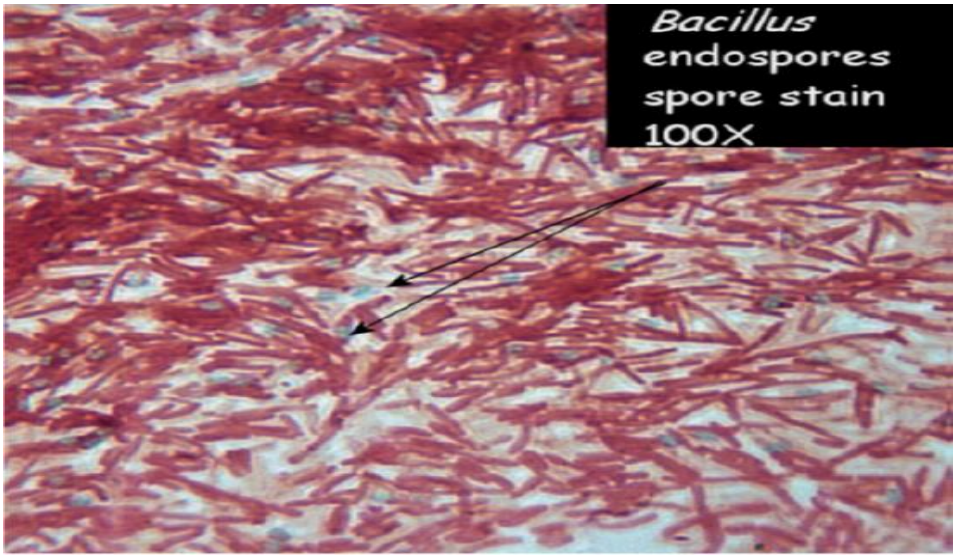


Figure (1) : Spore Stain

In fact, there are two major pathogenic spore-forming genera, Bacillus and Clostridium, together causing a number of lethal diseases---botulism, gangrene, tetanus, and anthrax,

Procedure of Spore Stain : see figure 2 and 3

1- Prepare a fixed smear of the *Bacillus* species---air-dry and heat-fix .

2- Flood the smear with 5% the primary dye, malachite green and let the stain react for 1 minute .Using your bunsen burner, heat the top of the stained slide until steam rises but do not let it boil. This step is performed by passing the burner back and over the slide for about 5 minutes.

3- Wash well with water.

4- Counter stain with the safranin for 1 minute.

5- Examine under oil immersion .**The vegetative part of the cell appears red while the endospore is green.**

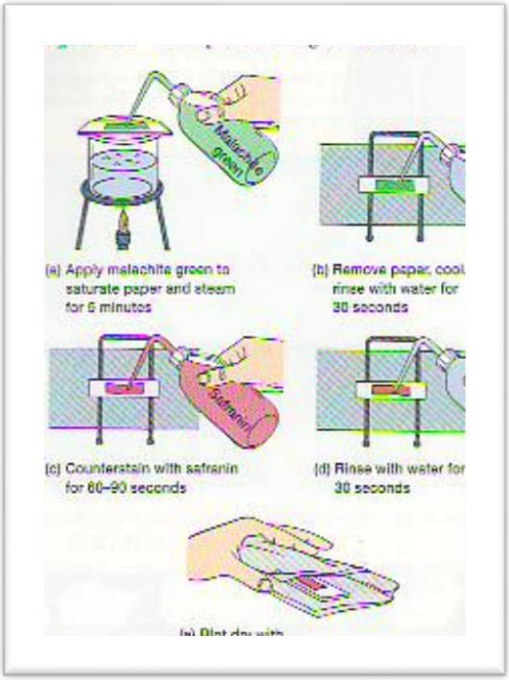


Figure (2) : Procedure of spore stain

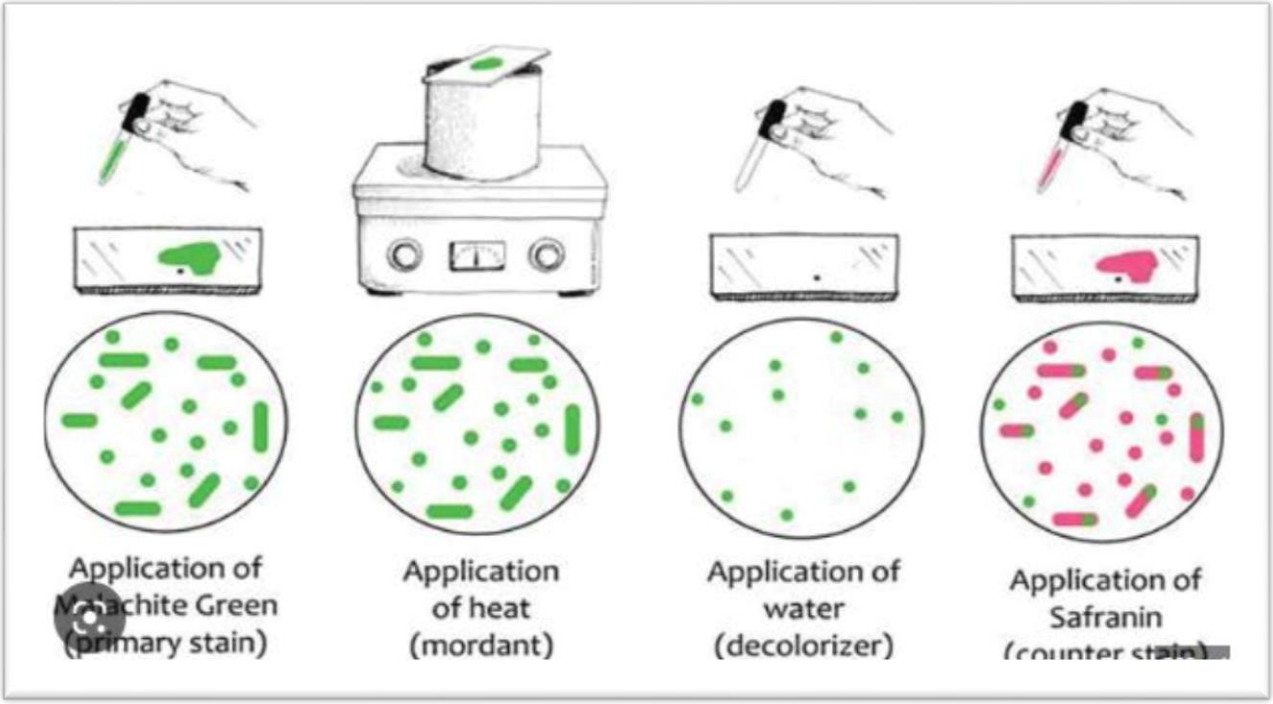


Figure (3) : Spore stain procedure

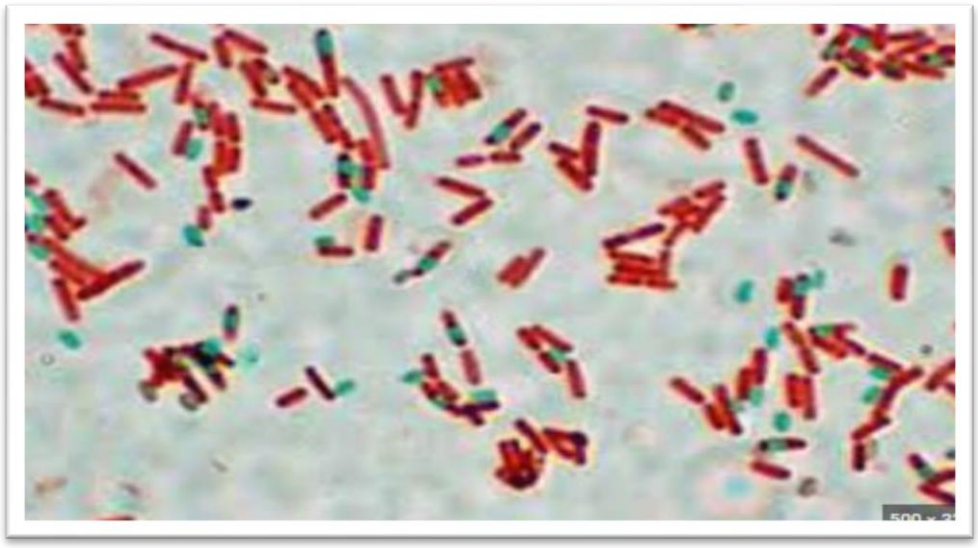


Figure (4) : Spore stain result (spore green color)

Negative Staining: It is used for capsule detection .

In negative staining the background observed darker than the bacteria .

Nigrosin or India ink is used. These stains have a negative charge that is repelled by the negative charge of the microorganism resulting negative or indirect staining of the microbial cell. (see Figure- 6)

Technique ;see figure 5

1- Place 2 to 3 drops of water on a side.

2- Take a small amount of growth from capsulated bacteria and mix .The bacteria with water.

3- Add a drop or two drops of nigrosin or India ink close to the bacteria suspension.

4- By inoculating loop, mix the stain with the bacteria suspension

5- Spread the bacteria suspension by another slide.

6- Observe under oil immersion

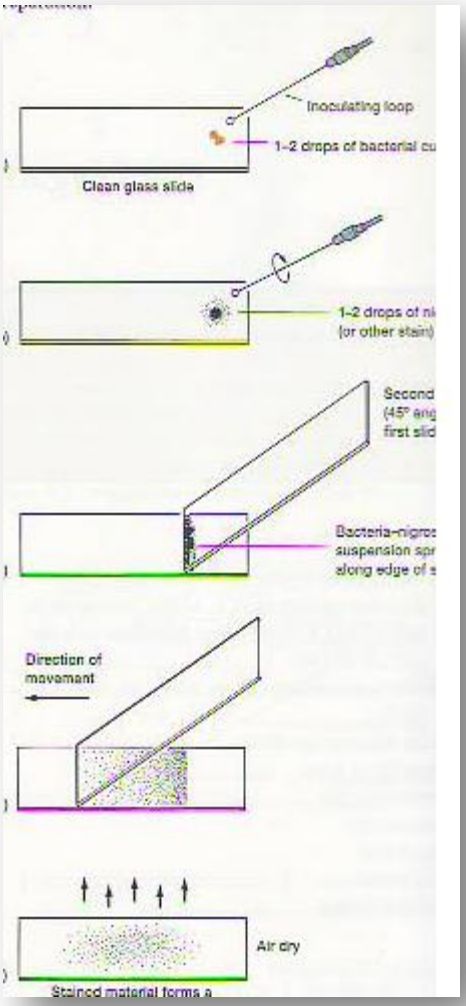


Figure (5) : Negative stain Procedure

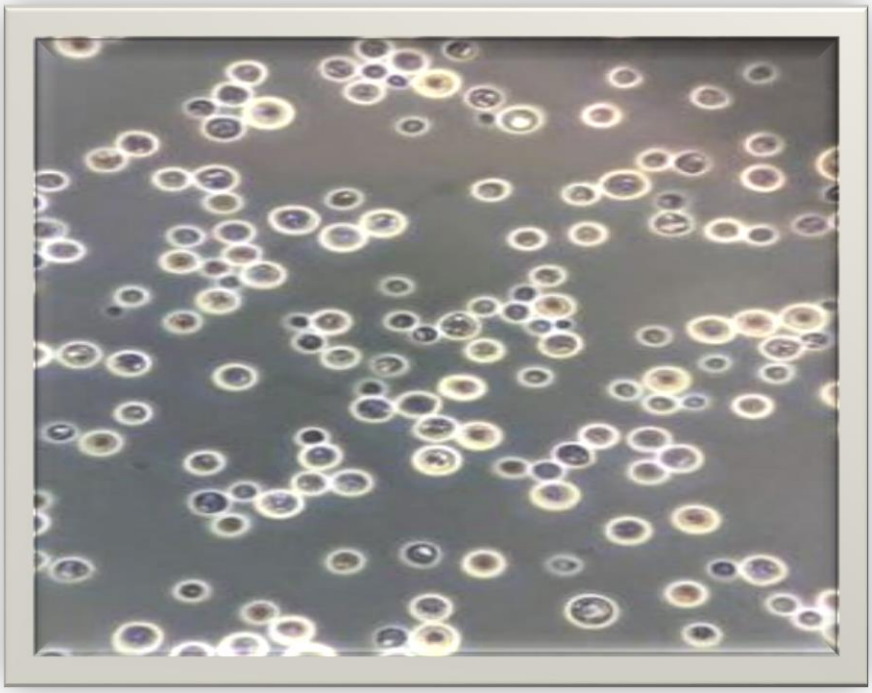


Figure (6): Negative Staining

Capsule staining

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A capsule is a gelatinous outer layer secreted by some 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.

Examples of capsulated bacteria.

- *Streptococcus pneumoniae.*
- *Klebsiella pneumoniae.*

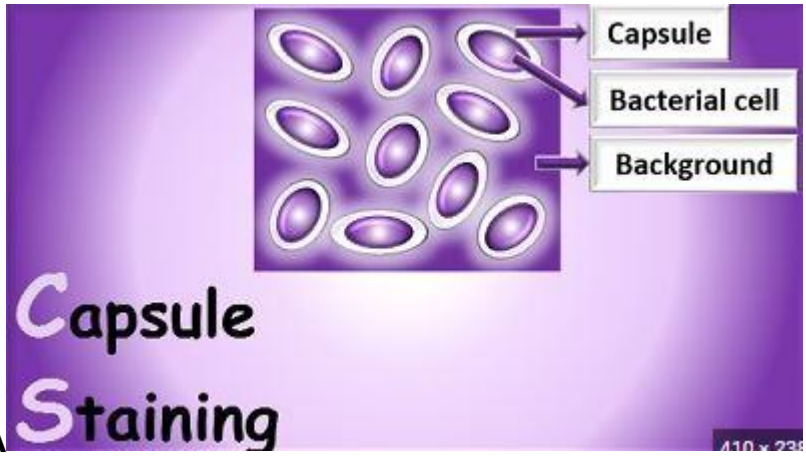
- *Haemophilus influenzae.*
- *Pseudomonas aeruginosa.*
- *Neisseria meningitidis.*

Principle of Capsule Staining

Capsules stain very poorly with reagents used in simple staining and a capsule stain can be, depending on the method, a misnomer because the capsule may or may not be stained.

Negative staining methods contrast a translucent, darker colored, background with stained cells but an unstained capsule.

The background is formed with india ink or Nigrosin or congo red. A positive capsule stain requires a mordant that precipitates the capsule. By counter staining with dyes like crystal violet or methylene blue, bacterial cell wall takes up the dye. Capsules appear colorless with stained cells against dark background.see fifure 7(a,b and c)

A 

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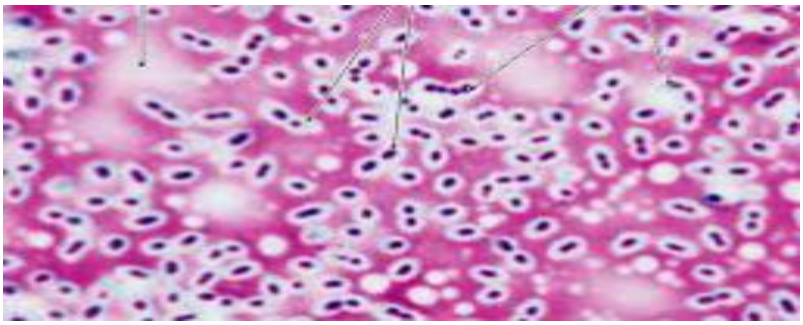

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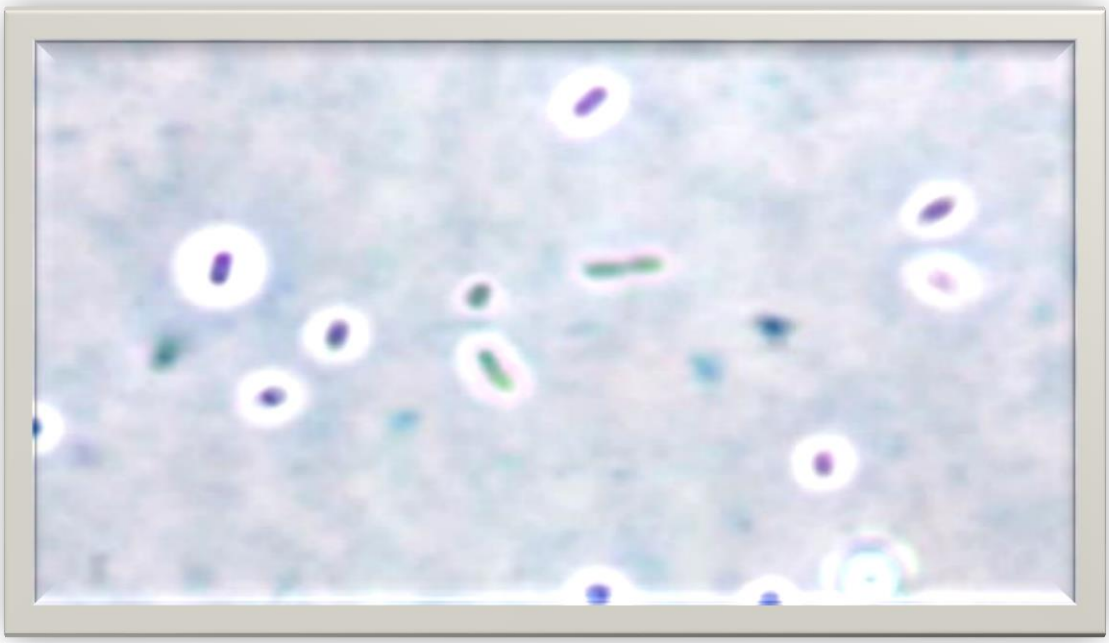
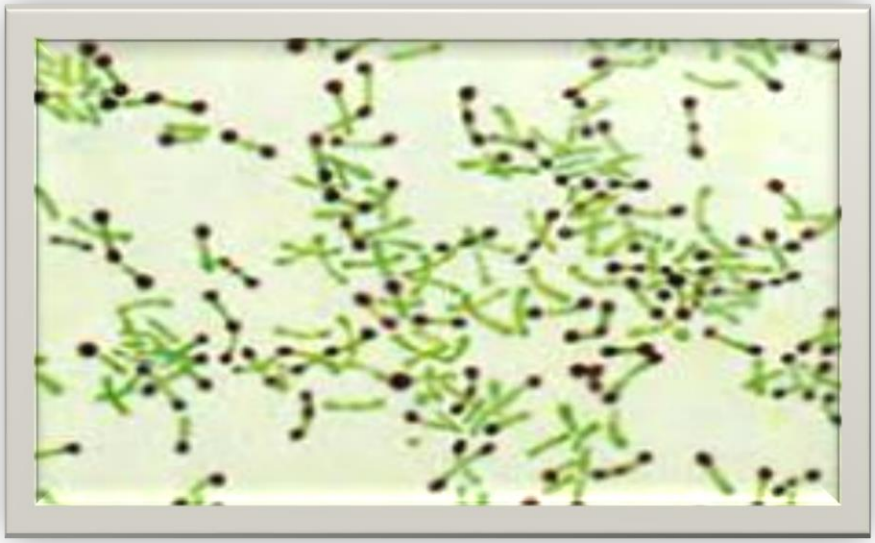


Figure (7) (a,b and c): Capsule Staining

Albert stain : is a kind of differential stain used for staining and identifying metachromatic granules found in Corynebacterium diphtheriae**.** The granules appear purple-black when exposed to Albert's stain and against the light green cytoplasm. Albert stain only acts to stain metachromatic granules and no other granules in the bacteria.

Albert stain is basically made up of two stains that are **toluidine blue' O' and malachite green** both of which are basic dyes with high affinity for acidic tissue components like cytoplasm .(figure 8 a and b)



A .

B

Figure. 8 (A and B) : Albert staining

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