Endospore Staining by Schaeffer –Fulton Method.

Principle: Endospore staining is a differential staining technique where the spore is stained in a manner so that it can be distinguished from the vegetative part of the cell. Spores are structures remarkably resistant to heat, radiation, chemicals and other agents that are typically lethal to the organism. The heat-resistence of spores has been linked to their high content of calcium and dipicolinic acid. Although sporulation is genetically regulated, the event is initiated when the organism senses depletion of nutrients or during unfavorable environmental conditions. During sporulation, a vegetative cell gives rise to a new intracellular structure termed as endospore that is surrounded by an impermeable layer called sporecoat. Complete transformation of a vegetative cell into a sporangium and then into a spore requires 6-8 hours in most spore-forming species. An endospore develops in a characteristics position within a cell, i.e. either central ,subterminal or terminal .Once an endospore is formed in a cell, the cellwall disintegrates ,releases the endospore that becomes an independent spore. Endospores may remain dormant for long period of time. However, a free spore may return to its vegetative or growing state with the return of favourable conditions. The spores are differentially stained by using special procedures that help dyes penetrate the spore wall. An aqueous primary stain (Malachite Green) is applied and steamed to enhance penetration of the impermeable spore cores. Once stained, the endospores do not readily decolourize and appear green within red cells.

Suitable organism: *Bacillus subtilis*

Procedure: 1. Make a smear of the organisms on separate grease-free slides

2 Air dry and heat fix the smear.

3. Flood the smears with **malachite green** solution (stain for the spore).

3. Heat the slide to steaming and continue steaming for 5 minutes adding more stains to the smear from time to time. (a set of slides may be steamed for 10 and 15 minutes as well)

4. Wash under gentle stream of tap water.

5. Counter stain with **safranin** for 30 secs.

6. Wash smear with distilled water.

7. Air dry and examine under microscope.

<u>Dorner's Method</u>:

Procedure: 1) 1ml Carbol fuchsin + 1 ml culture

2) boiling for 5, 10 & 15 min.

3) one drop placed in a grease free slide with inoculating needle .

4) The drop mixed with nigrosin.

5) Smear made very quickly.

6) observed under microscope.

••• Staining Endospores •••

Due to the highly resistant nature of endospores, they are not easily penetrated by stains. Thus, it is necessary to steam the stain into an endospore. The Schaeffer-Fulton method is the most commonly used endospore staining technique, which uses Malachite green as the primary stain. Once the endospore has absorbed the stain, it is resistant to decolorization, but the vegetative cell is easily decolorized with water (leaving the vegetative cells colorless). Finally, the vegetative cells are counterstained with Safranin to aid in their visualization. When viewed under a microscope, the endospores appear green, while the vegetative cells are red or pink. The steps in the endospore staining technique are listed below.

1. Using aseptic technique, prepare a bacterial smear on a clean slide, air dry and gently heat fix.

2. Prepare a boiling water bath.

3. Cover the slide with a piece of paper towel, and place on a staining rack over the water bath.

4. Flood the paper towel on the slide with **Malachite green** (primary stain).

5. Steam the slide for five minutes.

6. Remove the slide from the water bath, and remove the paper towel from the slide.

7. Allow the slide to cool, and then rinse with deionized water until the water runs clear.

8. Pour off any excess water and apply **Safranin** (counterstain) for two minutes.

9. Rinse excess Safranin off with deionized water, and blot the slide dry with blotting paper.

10. Examine the slide with a light microscope under oil immersion objective