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Microbiology Lab

((Spore stain))

Lab/7

2 stage

By

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Spore stain:

Bacterial spores are bacterial mechanism that is intentionally set in an attempt to secure themselves to the adverse effects of the external environment.

Endospore staining is a technique used in bacteriology to identify the presence of endospores in a bacterial sample. Within bacteria, endospores are protective structures used to survive extreme conditions, including high temperatures making them highly resistant to chemicals.

At the bacterial spore staining, there are two methods commonly used, the method of **Schaeffer Fulton and Klein methods**. The difference of these two methods is the dye used. In the method of Schaeffer Fulton dyes used are Malachite Green and Safranin, while in the Klein method using dye Carbol Fuchsin and Methylene Blue.

Schaeffer Fulton method is a method often used by laboratory technicians, due to staining time faster than the method of Klein.

Principle of Endospore Staining

In the Schaeffer-Fulton`s method, a primary stain-malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolorized with water. Safranin is then applied to counterstain any cells which have been decolorized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green.



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Reagents used for Endospore Staining:

Primary Stain: Malachite green (0.5% (wt/vol) aqueous solution)
0.5gm of malachite green 100 ml of distilled water.

Decolorizing agent: Tap water or Distilled Water.

Procedure of Endospore Staining:

- 1- Take a clean grease free slide and make smear using sterile technique.
- 2- Air dry and heat fix the organism on a glass slide and cover with a square of blotting paper to fit the slide.
- 3- Saturate the blotting paper with malachite green stain solution and steam for 5 minutes, keeping the paper moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.
- 4- Wash the slide in tap water.
- 5- Counterstain with 0.5% safranin for 30 seconds. Wash with tap water; blot dry.
- 6- Examine the slide under microscope for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.

Result of Endospore Staining:

Endospores: Endospores are bright green.

Vegetative Cells: Vegetative cells are brownish red to pink.



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*Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spore shape may also be of diagnostic use. Spores may be spherical or elliptical.

