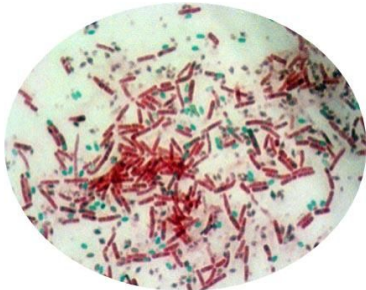


Endospore Stain

Results & Observations



Purpose:

- A differential stain used to detect the presence and location of spores in bacterial cells.
- The schaeffer-Fulton method.
- Endospores are colored green. Vegetative cells are colored red.
- Sporogenesis: the formation of endospores within a vegetative cell. Germination: Process that happens when an endospore is reverted or converted into a vegetative cell
- Sporogenesis of bacteria is not means of reproduction

Procedure

1. Place a piece of paper towel over the slide
2. Cover the paper with malachite green, 3-
3. Steam the slide for 5 minutes then rinse, 4-
4. Cover smear with safranin for 30 seconds and rinse

Interpretation & Questions:

1. **You forgot to heat-fix your slide. What should you expect to see?**
Heating or steaming the smear with the primary stain, malachite green, enhances the penetration of the stain into the endospore.

Heat-fixing the slide fixes the bacteria to the slide surface. If this step is not done, the bacteria in the smear would be washed off of the slide during the staining and decolorization steps. You would not see anything on the slide under the microscope.

2. **How would you describe the location of the endospores on your slides? (Name organism/culture sample)**
During unfavorable conditions (especially when carbon and nitrogen become unavailable) endospores can form within different areas of the vegetative cell. They can be central, subterminal, or terminal. Central endospores are located within the middle of the vegetative cell. Terminal endospores are located at the end of the vegetative cell. Subterminal endospores are located between the middle and the end of the cell.

3. **How would you describe the morphology and arrangement of the cells in your stained preparations?**
Endospores also may be

differentiated based on shape- either spherical, or elliptical oval-and size relative to or not.

4. List the reagents used in performing an endospore stain and describe their function.

- Malachite green - primary stain; malachite green dyes the endospore a blue-green or aqua color.
- Water - decolorizer; water removes stain from vegetative cells.
- Safranin - counterstain or secondary stain; safranin stains decolorized vegetative cells red.

5. What differences did you observe in the appearance of the endospore stained slides of the 24-hour-old culture and 1-week-old culture?

At 72 hours, endospores would have formed.