

Spore Stain Procedure

I. Principle

The observation of spores is important for the identification of the spore-forming genera, *Bacillus* and *Clostridium*. These organisms may easily over-decolorize, making it difficult to distinguish them from gram-negative rods. The spore stain is a differential stain for the detection of spores that may otherwise be difficult to observe. Malachite green has a higher affinity for spores but washes out of vegetative cell material. Safranin is used as a counter-stain to stain vegetative bacteria but does not replace the malachite green adsorbed by endospores.

II. Specimen Information

Suspect isolates of *Bacillus* or *Clostridium* species.

III. Reagents & Equipment

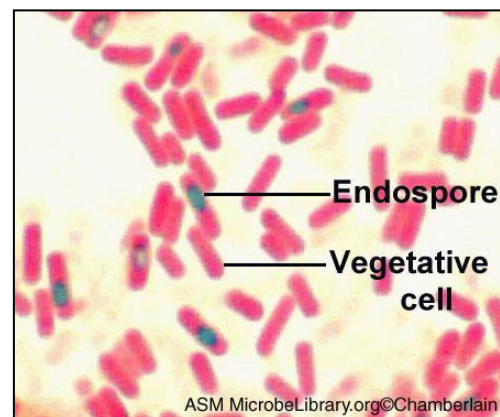
- A. Glass slides
- B. Saline
- C. Staining rack
- D. 5% Malachite green stain
 1. 5 g malachite green/100 mL distilled H₂O
 2. Outdate indefinite
- E. Safranin (observe manufacturer's expiration date)

IV. Procedure

- A. Emulsify organism obtained from the area of **heaviest** growth in the primary quadrant of plated medium into a drop of saline on a glass slide.
- B. Allow the slide to air dry.
- C. Flood the smear with Malachite green and stain for 10 min.
- D. Rinse slide over sink with tap water.
- E. Counterstain with safranin for 30 s.
- F. Rinse with tap water. Blot edge of slide to remove excess water and allow smear to dry.
- G. Examine smear under oil immersion (100X) for the presence and location of spores.

V. Interpretation

Spores are stained green and may appear at several locations within the cell (central, subterminal, terminal) or as free spores. Bacteria (vegetative cells) and cellular debris will appear pink from the safranin counterstain.



VI. Quality Control

- A. Quality control is performed each time the test is performed. Control organisms and their expected results are:
 - 1. *Bacillus* species (spores present)
 - 2. *E. coli* (no spores)
- B. If controls do not display expected results, the staining of patient specimens must be repeated using a new control slide. Notify the supervisor.

VII. References

- A. Washington, J. 1985. Laboratory Procedures in Clinical Microbiology. 2nd ed. p. 90, Springer-Verlag.

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Updates and Revisions: