Microbiology Lab Experiment Changes

Experiment #: 3-9

Title: Endospore Stain

Live Organisms: *Bacillus subtilis* or *Bacillus cereus*, soil plates

Changes:

Modified procedure:

1. Make spore smears more cloudy than usual.
2. Put **heat fixed** smear onto "staining hot plate" in fume hood.
3. Place a filter paper over smear. Saturate filter paper with Malachite Green stain.
4. Allow stain to sit approximately 5 minutes.
5. Get a paper towel to carry slide to sink. Throw filter paper into trash. Rinse slide well.
6. Decolorize by rinsing well with water.
7. Counterstain with Safranin for 1 minute.
8. Rinse with water. Blot dry. You may wipe **BOTTOM** of slide with lens cleaning fluid and tissue.

Each student will make one slide of each culture: Bacillus and soil plate.

Note: Malachite green fades in the mounting medium of a prepared slide. Most commercially prepared spore stain slides are stained with carbol fuschin.

Take Home Lesson: You need to know the reagents and their function in the staining procedure. Why do we use the endospore stain? For what organisms do you expect to find spores? Be able to recognize that an organism has produced endospores based on its appearance under the microscope. Note that spores can be internal (endospores) or external (free spores) relative to the organism. Be careful not to mistake free spores for cocci.