

Endospore Staining

- A differential stain used to detect the presence and location of spores.
- Few genera produce spores, including *Bacillus* and *Clostridium*. Pathogenic strains include *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium difficile*, *Bacillus cereus* and *Bacillus anthracis*.
- What is an endospore, you ask? Well,,,
- An endospore is a dormant form of the bacterium that allows it to withstand harsh environmental conditions. Examples of harsh conditions include heat, UV radiation, disinfectants, toxins, lack of nutrients, and dessication.
- Once favorable conditions return, the endospore germinates to give rise to a new vegetative cell.
- Endospores have a tough outer covering made of keratin (a protein) that makes them resistant to these harsh conditions as well as to staining.
- Malachite green is used to stain the spore because it has a low affinity for cellular material. Therefore, vegetative cells and endospore mother cells can be decolorized with water and counterstained with saffranin.
- Heat is needed to drive the malachite green stain into the endospore.
- There are three components in the endospore stain procedure. They are:
 - Malachite green (primary stain)
 - Water (decolorizer)
 - Saffranin (counterstain)
- Endospore locations include central, terminal, and subterminal.

- PROCEDURE
 1. Prepare a smear. Air dry and heat fix.
 2. Place a small piece of paper towel over the smear – not hanging over the edge of the slide
 3. Saturate the paper towel with 5% solution of **malachite green** – set for 1 minute
 4. Heat slide until steam rises from stain (do not boil!) – add more stain as it evaporates and continue applying just enough heat to continue steaming for 5 minutes
 5. Allow slide to cool (prevents breaking) before washing (decolorize) with water – discard piece of towel in the trash (not in the sink!)
 6. Apply 0.5% safranin counterstain and allow it to react for 30-60 seconds

7. Wash with water and blot dry (do not rub)
8. Examine by brightfield microscopy using the 100X oil-immersion objective to discern colors.