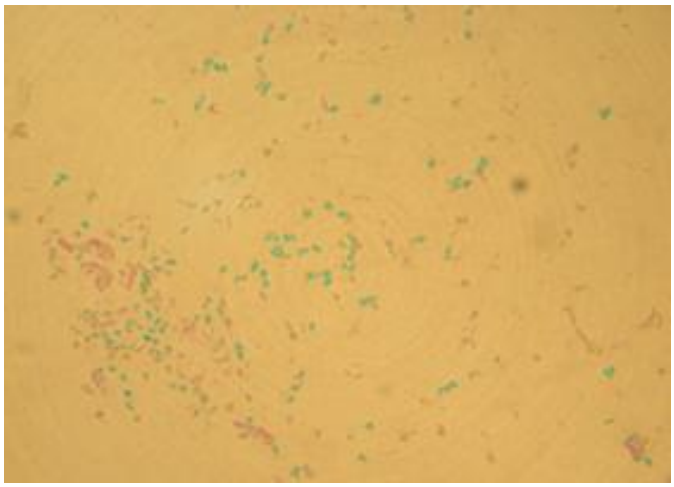
Wirtz's Endospore Stain

Endospore formation is characteristic of *Clostridum* and *Bacillus* spp. The ability to concentrate and coat their protoplasm allows them to survive the adverse environmental conditions they experience in their soil habitat. This also allows the spores to resist staining. The "live" organisms are easily visualized with simple stains and Gram's stains.



Endospores are typically highly refractile, light striking them is deflected. Many *Bacillus* species have inclusion bodies that are highly refractile. These inclusion bodies may look like endospores with regular staining. The presence of endospores must be confirmed with endospore specific stains. The presence, and characteristic shape and position of endospores require special procedures to permeate the endospore coat. Most endospore stains involve heating the slides while keeping them continually moist with the dye. While quicker, it produces volatile chemicals and is just a big mess. The same results can be obtained by letting the dye sit on the slide for 30 minutes. It is a good idea to start this slide first and work on another stain while you are waiting for the dye to permeate the endospore.

General Considerations

You will be using a *Bacillus* species for the endospore stain. The shape and position of *B. cereus* spores are very similar to those of *B. anthracis*. *Bacillus* does not start forming spores until it runs out of food. If the cultures are too young, you will mostly see just the pink rods of the bacteria. If the cultures are too old, you will mostly see just the small green ovals of the endospores. Ideally, you should see the green oval bodies of the endospore surrounded by the pink vegetative bacterial cell. Select a sample from the middle of a colony with the straight inoculating needle for the best results. The edge of the colony is still actively growing and will have few endospores.

Materials

- Malachite Green Stain (5%)
- Safranin (0.25%) - counter stain
- *Bacillus* spp. plate

Wirtz's Endospore Stain Procedure

1. Make a smear of *Bacillus* and methanol fix.
2. Flood the smear with malachite green stain.
3. Allow the stain to sit for **at least 30 minutes**.
    - Add more stain if it starts to dry out.
4. Rinse the slide with distilled water.
5. Flood the slide with safranin (0.25%) for 1-5 minutes.
6. Rinse the slide with distilled water and let air dry.
7. Observe under oil immersion.
    - The endospores are aqua and the bacterial cells are pink.