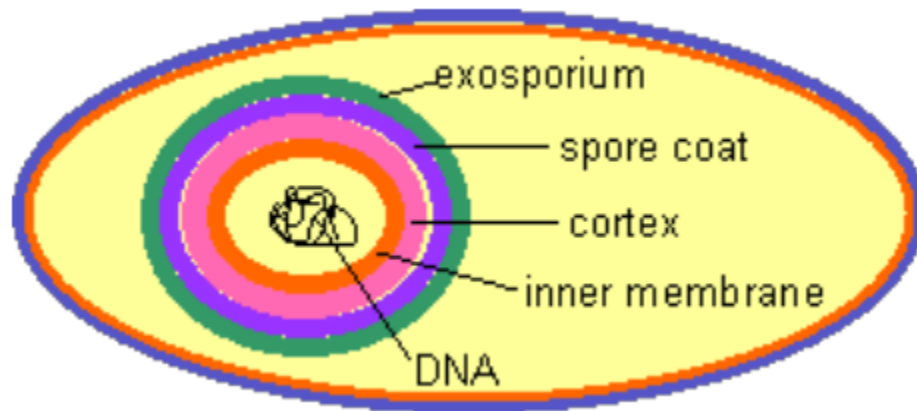


PW 03 : ENDOSPORE STAIN

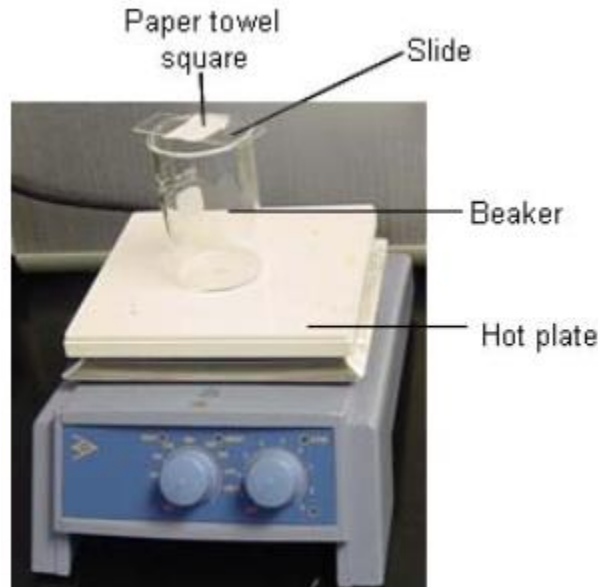
1. Introduction

The **endospore stain** is a differential stain which stains bacterial endospores or spores. Some bacteria, including those belonging to the genera *Clostridium* and *Bacillus*, have the capacity to produce metabolically inactive cells called spores. Spores are highly resistant to hostile chemical and physical conditions and are produced by a process called **sporulation** if environmental conditions become unfavorable for normal vegetative growth. The spore structures have a tough outer covering composed primarily of keratin which makes them resistant to heat, radiation, disinfectants, and desiccation. The bacteria will remain in this suspended state until conditions become favorable again and they can germinate and return to their **vegetative** state. The term **endospore** refers to the spore structure contained within a mother cell. The term **spore** refers to the spore structure that exists free of the mother cell. Endospores may be located in the middle of the cells (central), at the end (terminal), or between the end and the middle of the cells (subterminal). The endospores themselves may be round or oval.



The **primary stain** in the endospore staining procedure is **malachite green**, which stains both vegetative cells and endospores. As with the acid-fast stain, **heat** is required to penetrate the endospore coat. Once the cells have cooled, the cells are **decolorized** with **water**, which selectively removes the malachite green from all vegetative cells but not from endospores. The **counterstain** then applied is **safranin**, which stains the decolorized vegetative cells pink. Thus, at the end of the staining procedure, the endospores are dark green, and vegetative cells are pink.

Note: Sometimes the endospores don't take up the malachite green very well. In those cases, the endospores will appear as clear ovals or circles within a pink vegetative cell.



2. Cultures needed

Bacillus megaterium

3. Procedure

1. Fill a beaker about 1/4 to 1/3 full of **tap water** and place on a hot plate. Turn hot plate on “high” and allow water to boil. Once the water comes to a boil, reduce the heat.
2. Obtain a clean glass slide and prepare a bacterial smear. Air dry and heat fix the slide. Be sure to label your slide!
3. Place the slide on top of the beaker of water and let steam for 5 minutes.
4. Place a paper towel square on top of the smear area. This will hold the stain in place and keep it from running off the slide.
5. Apply enough Malachite Green to soak the paper towel square and allow to sit for 2-3 minutes, keeping the paper towel moist with stain. Do not let it dry!
6. Carefully remove the slide with a clothes pin (slide will be hot!) and place on the staining tray to cool. Remove paper towel square.
7. Tilt the slide and rinse with distilled water.
8. Cover the slide with Safranin and allow to sit for 30 seconds.

9. Tilt the slide and rinse with distilled water.

10. Blot your slide dry with Bibulous paper and observe your slide under oil immersion using proper microscope techniques. Endospores will appear as dark green or clear circles or ovals, whereas vegetative cells will be pink rods.

11. Clean your microscope with lens paper and lens cleaner.

4. Results

- Using colored pencils, draw the results of the endospore stain.
- Label an endospore and a free spore in the drawing. Are the endospores central, terminal, or subterminal?

5. Study questions

1. Why must heat be used with the application of the primary stain during endospore staining?
2. What advantage does the ability to sporulate give to pathogenic bacteria such as *Clostridium tetani*, *Clostridium botulinum* and *Bacillus anthracis*?