Endospore Stain

- 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 perfringes, and Clostridium difficile*.
- 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, waste lack of nutrients, and dessication.
- Spores are resistant to heat, dessication, chemicals, and radiation.
- Bacteria can form endospores in approximately 6 to 8 hours after being exposed to adverse conditions.
- The normally growing cell that forms the endospore is called a vegetative cell.
- Spores are metabolically inactive and dehydrated.
- They can remain viable for thousands of years.
- When spores are exposed to favorable conditions, they can germinate into vegetative cell within 90 minutes.
- Endospores can form within different areas of the vegetative cell.
- They can be central, subterminal, or terminal.
- Central endospores are located within the middle of the vegetative cell.
- Terminal endospores are located at the end of the vegetative cell.
- Subterminal endospores are located between the middle and the end of the cell.
- Endospores can also be larger or smaller in diameter than the vegetative cell.
- Those that are larger in diameter will produce an area of "swelling" in the vegetative cell.
- These endospore characteristics are consistent within the spore-forming species and can be used to identify the organism.
- Because of their tough protein coats made of keratin, spores are highly resistant to normal staining procedures.
- There are three components in the spore stain procedure. They are:
    - Malachite green (primary stain)
    - Water (decolorizer)
    - Saffranin (counterstain)

Acid-Fast Staining

- The acid-fast stain is a differential stain used to identify cells capable of retaining a primary stain when treated with acid alcohol.
- Very useful for identifying bacteria in the genus *Mycobacterium*, some of which are pathogens (i.e. *Mycobacterium leprae, Mycobacterium tuberculosis*).
- Also useful for identifying other organisms which could be pathogenic such as members of the *Nocardia* genus and parasites in the genus *Cryptosporidium* and the genus *Isospora*.
- Few organisms are acid-fast, so this stain is run only when there is suspicion of an infection by an acid-fast organism.
- Acid-fast positive cells contain mycolic acids in their cell wall.
- Mycolic acid is a waxy substance which does not allow the cells to be stained by simple stains, but when stained by carbolfuchsin can retain this stain even acid alcohol decolorizer is used.
- There are two methods:
    1. Ziehl-Neelsen (ZN), which uses heat to drive the carbolfuchsin in the cells.
    2. Kinyoun (K), which uses a more concentrated, more lipid soluble form of carbolfuchsin.
- There are three components in the acid-fast procedure. They are:
    1. Carbolfuchsin= A primary stain that is a phenolic compound that is lipid soluble. Stains cells reddish purple.
    2. Acid-alcohol= A decolorizer that decolorizes non acid-fast cells.
    3. Methylene blue= A secondary stain that stains non acid-fast cells blue.

Capsule Stain

- There are two common ways that the capsule stain is performed
- It can be prepared as a negative stain or a background stain.
- As mentioned before, the stain is designed to stain everything but the organism (the background).
- The polysaccharide or polypeptide composition of the capsules may make staining difficult.
- The smear is prepared in the same manner as the negative stain, and the slide is not heat-fixed, since application of heat may destroy or distort the capsule.
- The negative stain is air-dried before a basic stain is applied, which in many cases is crystal violet.
- The slide is then carefully rinsed and blotted, then viewed.
- In another technique, two loopfuls of organism are spread as a thin film on a slide and allowed to air dry.
- The smear is flooded with crystal violet for 2 minutes then washed off with 20% copper sulfate.
- The copper sulfate is not allowed to go down the sink!
- The slide is then blotted and viewed.