Endospore staining

Microbiology I

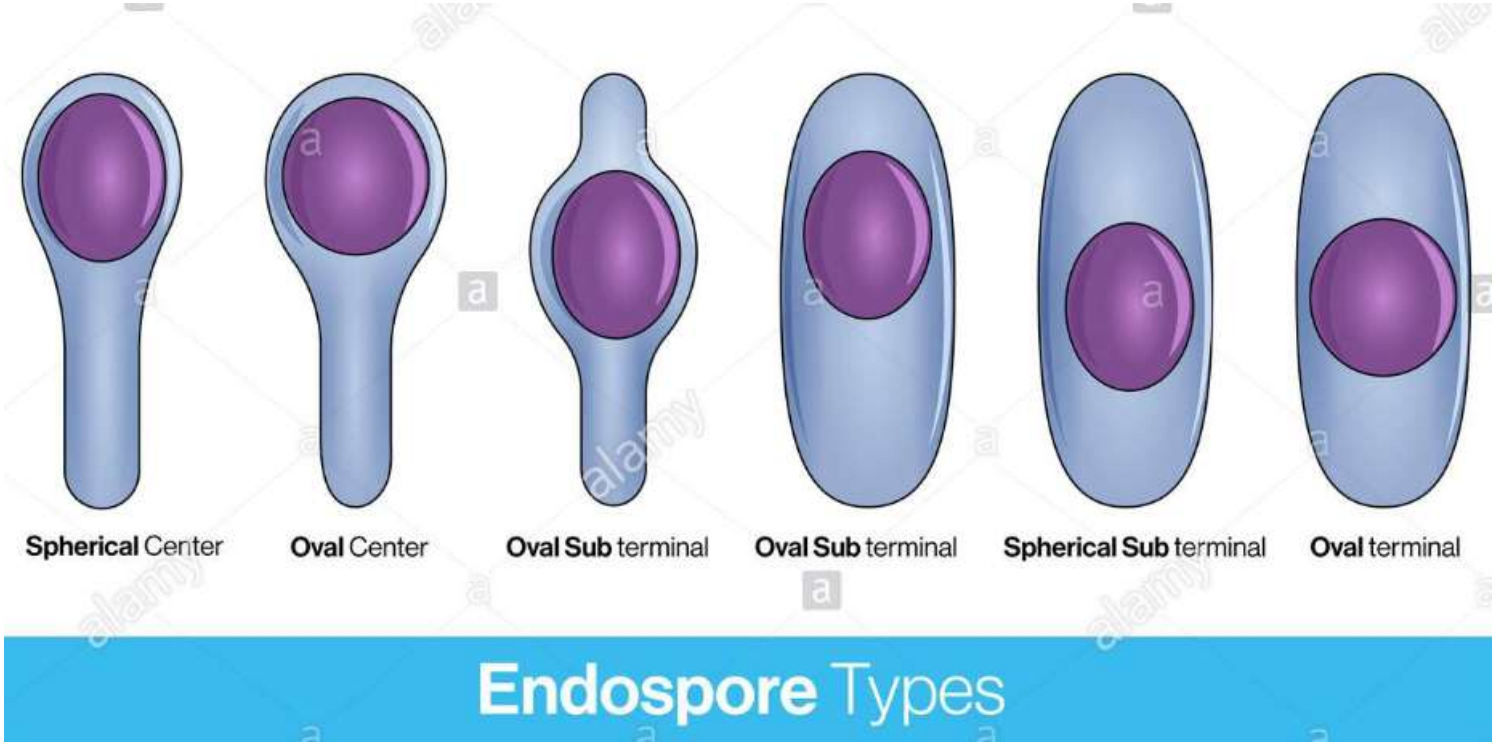
3.1.21

Introduction

- In 1922, **Dorner** published a method for staining endospores.
- **Shaeffer and Fulton** modified Dorner's method in 1933 to make the process faster
- The endospore stain is a differential stain which selectively stains bacterial **endospores**.
- The main **purpose** of endospore staining -
- differentiate **bacterial spores** from other **vegetative cells** and
- differentiate **spore formers** from non-spore formers.

Principle

- **Bacterial endospores** are metabolically inactive, highly **resistant** structures produced by some bacteria as a defensive strategy against unfavorable environmental conditions.
- The bacteria can remain in this suspended state until conditions become favorable and they can germinate and return to their vegetative state.
- Spores may be located in the **middle** of the cell, at the **end** of the cell or **between** the **end** and **middle** of the cell.
- Spore shape may also be of diagnostic use. Spores may be **spherical** or **elliptical**.


Spherical Center
Oval Center
Oval Sub terminal
Oval Sub terminal
Spherical Sub terminal
Oval terminal
Endospore Types

- **Schaeffer-Fulton`s method**:
- a primary stain-**malachite green** is forced into the spore by steaming the bacterial emulsion.
- **Malachite green** is water soluble and has a low affinity for cellular material
- so **vegetative cells** may be **decolourized** with **water**.
- **Safranin** is then applied to counterstain any cells which have been decolorized.
- At the end of the staining process, **vegetative cells** will be **pink,** and **endospores** will be **dark green**.

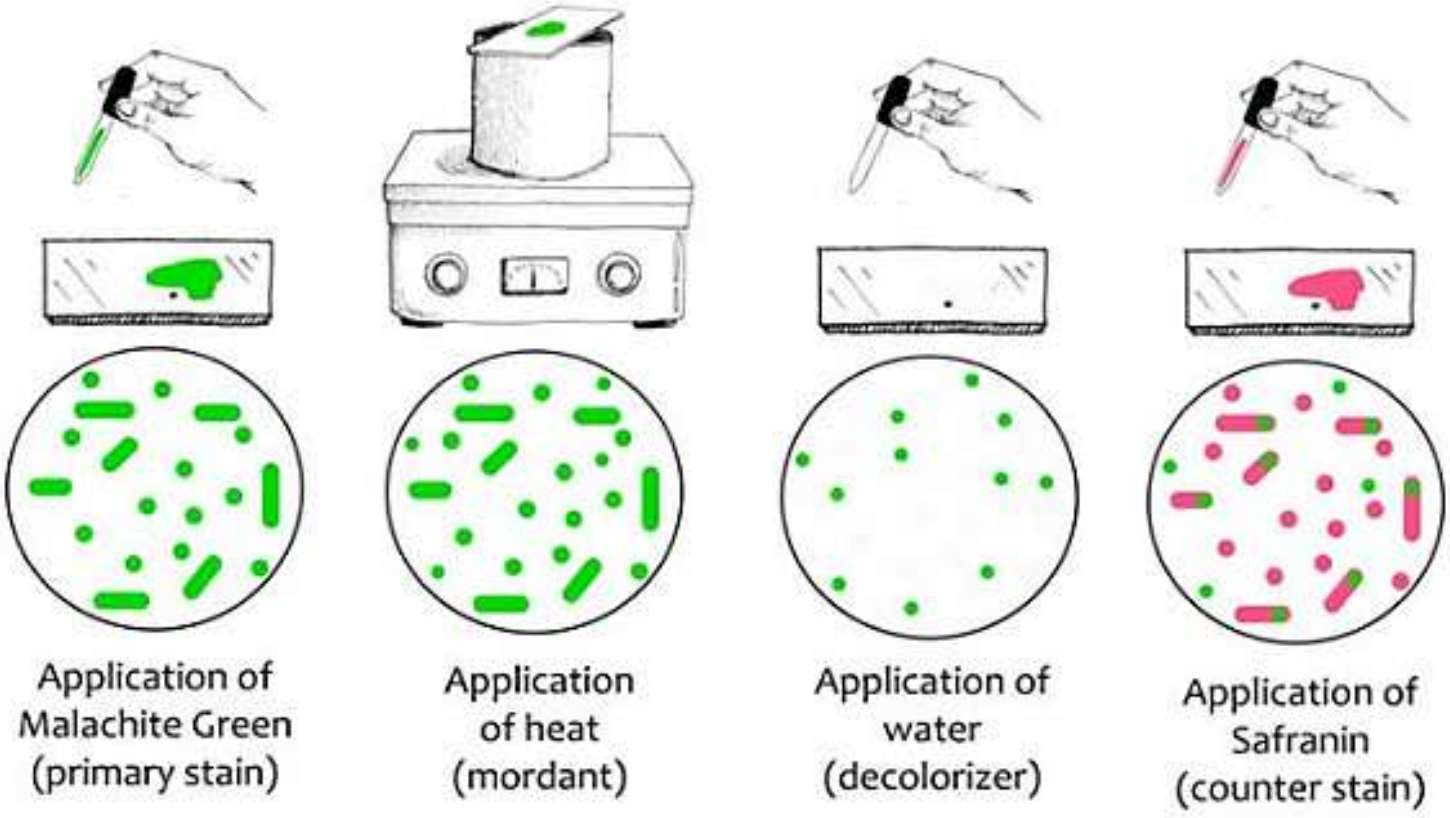
Reagents used for Endospore Staining

- **Primary Stain: Malachite green (0.5% (wt/vol) aqueous solution)**
  0.5 gm of malachite green
  100 ml of distilled water

- **Decolorizing agent**
  Tap water or Distilled Water

- **Counter Stain: Safranin**
  Stock solution (2.5% (wt/vol) alcoholic solution)
  2.5 gm of safranin O
  100 ml of 95% ethanol

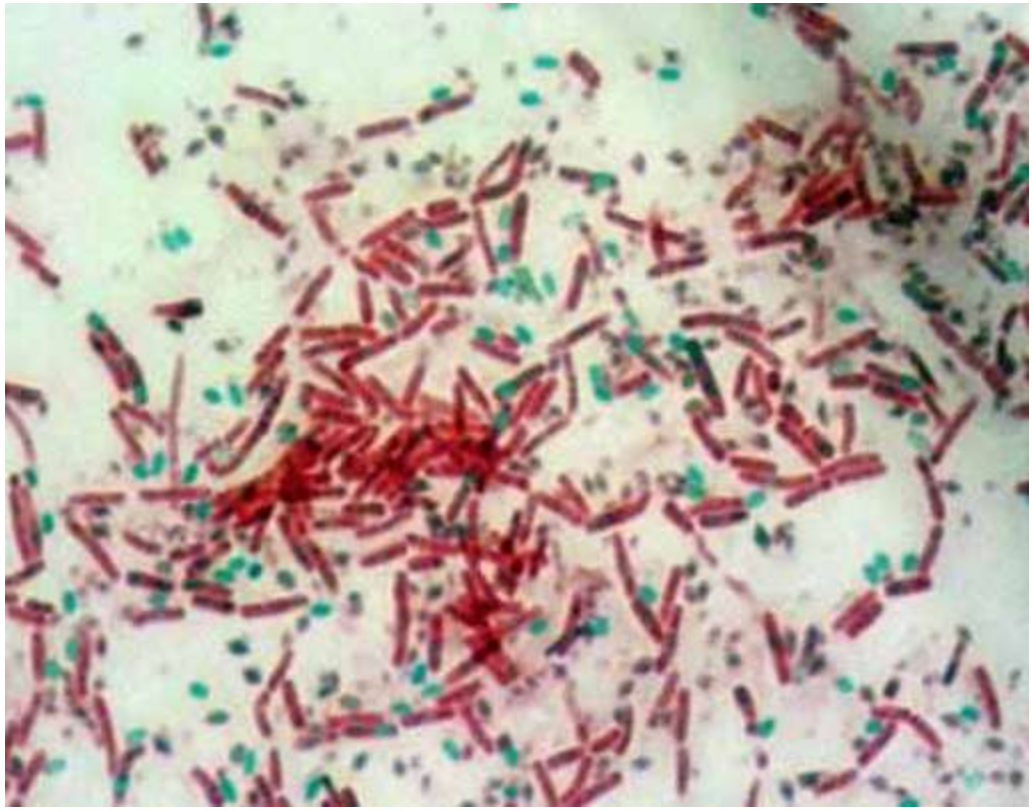
Procedure of Endospore Staining



Procedure

1. Take a clean grease free slide and make smear using sterile technique.
2. Air dry and heat fix the organism on a glass slide and cover with a square of blotting paper or toweling cut to fit the slide.
3. Saturate the blotting paper with **malachite green** stain solution and steam for **5 minutes**, keeping the paper moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.
4. Wash the slide in **tap water.**
5. Counterstain with **0.5% safranin** for **30 seconds**. Wash with tap water; blot dry.
6. Examine the slide under microscope for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.

Result of Endospore Staining



Endospores: Endospores are bright green.
Vegetative Cells: Vegetative cells are brownish red to pink.

Examples of Endospore Staining

Positive	Negative
Clostridium perfringens	*E. coli*
C. botulinum	*Salmonella spp*
C. tetani	
Bacillus cereus	
Sporolactobacillus spp	