

Observation of bacteria using staining procedures

1.Simple staining.

2.Gram staining.

1.Simple Staining

Smear preparation:-

- A drop of water is placed in the centre of a slide.
- One loopfuls of organisms is transferred to the centre of slide .
- Spread the organisms over the slide.
- The smear is allowed to dry.
- Slide is passed through flame several times to heat-kill and fix organisms

- A bacterial stain is stained with crystal violet (fuchsin, methylene blue) 1 min.
- Stain is briefly washed off slide with water , allow the slide to air-dry and examine with an oil immersion objective.

2.Gram Staining

In 1884 Christian Gram Staining technique that separates bacteria into two groups:-

1-Gram-positive bacteria

2-Gram-negative bacteria

Based on the ability to retain crystal violet during decolorization with alcohol.

The Gram stain, which divides most clinically significant bacteria into two main groups, is the first step in bacterial identification.

- Bacteria stained purple are **Gram +** their cell walls have thick peptidoglycan and teichoic acid.
- Bacteria stained pink are **Gram –** their cell walls have thin peptidoglycan and lipopolysaccharides with no teichoic acid.

In **Gram-positive bacteria**, the **purple crystal violet stain** is trapped by the layer of **peptidoglycan** which forms the outer layer of the cell.

In **Gram-negative bacteria**, the **outer membrane of lipopolysaccharides prevents the stain from reaching the peptidoglycan layer**. The outer membrane is then permeabilized by acetone treatment, and the **pink safranin counter stain** is trapped by the peptidoglycan layer.

The Gram stain has four steps:-

- 1. **Crystal violet**, the *primary stain*: followed by
- 2. **Iodine**, which acts as a *mordant* by forming a crystal violet-iodine complex, then
- 3. **Alcohol**, which *decolorizes*, followed by
- 4. **Safranin**, the *counterstain*.

Gram staining tests the bacterial cell wall's ability to retain *crystal violet* dye during solvent treatment.

- Safranin is added as a mordant to form the *crystal violet/safranin* complex in order to render the dye impossible to remove.

- Ethyl-alcohol solvent acts as a decolorizer and dissolves the lipid layer from gram-negative cells. This enhances leaching of the primary stain from the cells into the surrounding solvent.
- Ethyl-alcohol will dehydrate the thicker gram-positive cell walls, closing the pores as the cell wall shrinks.
- For this reason, the diffusion of the crystal violet-safranin staining is inhibited, so the bacteria remain stained.

Grampositive bacteria

- *Streptococcus* . *Staphylococcus*
- *Lactobacillus* . *Bacillus*
- *Clostridium*

Gram-negative bacteria

- *Escherichia* . *Salmonella*
- *Vibrio* . *Treponema*