Gram Staining

Principle of Gram staining

The structure of the organism's **cell wall** determines whether the organism is gram-positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria can retain the primary stain by resisting decolorization while others get decolorized by a decolorizer.

Those bacteria which retain the primary stain are called Gram-positive and those bacteria which get decolorized and then get counterstained are called Gram-negative.

Crystal violet (CV) dissociates into CV^+ and Cl^- ions in aqueous solutions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells purple.

Iodine (I), used as mordant interacts with CV^+ and forms large complexes of crystal violet and iodine ($CV–I$) within the inner and outer layers of the cell.

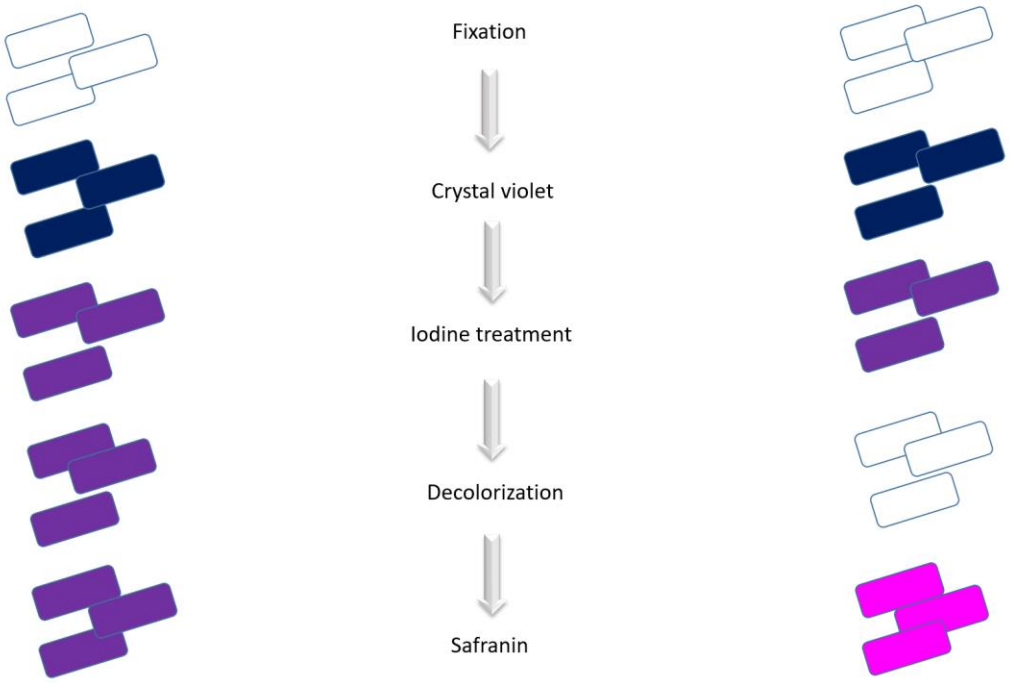
When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. Since Gram-negative organisms have a thin peptidoglycan layer (1-2 layers) and have an additional lipopolysaccharide layer that gets dissolved due to the addition of alcohol, so gram-negative organism fails to retain the complex and gets decolorized as the complex is washed away.

In contrast, a Gram-positive cell becomes dehydrated from an ethanol treatment. This closes the pores in the cell wall and prevents the stain from exiting the cell. The large $CV–I$ complexes become trapped within the Gram-positive cell also due to the thick and multilayered (40 layers) nature of its peptidoglycan.

After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged **safranin or basic fuchsin**, is applied last to give decolorized Gram-negative bacteria a pink or red color.

Steps in Gram Staining

Gram-Positive **Gram-Negative**



1. **Fixation of clinical materials** to the surface of the microscope slide either by heating or by using methanol. (methanol fixation preserves the morphology of host cells, as well as bacteria, and is especially useful for examining bloody specimen material).

2. **Application of the primary stain (crystal violet).** Crystal violet stains all cells blue/purple

3. **Application of mordant:** The iodine solution (mordant) is added to form a crystal violet-iodine (CV-I) complex; all cells continue to appear blue.

4. **Decolorization step**: The decolorization step distinguishes gram-positive from gram-negative cells.

5. The organic solvent such as acetone or ethanol extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-

walled, gram-positive bacteria. The gram-negative bacteria appear colorless and gram-positive bacteria remain blue.

6. **Application of counterstain (safranin):** The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.