***Gram staining***

**Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents.**

1. **Gram-positive bacteria *(thick layer of peptidoglycan-90% of cell wall*)- stains purple**
2. **Gram-negative bacteria *(thin layer of peptidoglycan-10% of cell wall and high lipid content)* –stains red/pink**

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

* **Application of mordant:** The iodine solution (mordant) is added to form a crystal violet-iodine, all cells continue to appear blue.
* **Decolorization step**: The decolorization step distinguishes gram-positive from gram-negative cells.
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.
* **Application of counterstain (safranin):** The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.



**Gram Staining Procedure\:**

1. Flood air-dried, heat-fixed smear of cells for **1 minute** with **crystal violet** staining reagent.
2. Wash slide in a gentle and indirect stream of tap water for **2 seconds.**
3. Flood slide with the mordant: **Gram’s iodine.** Wait **1 minute.**
4. Wash slide in a gentle and indirect stream of tap water for **2 seconds**.
5. Flood slide with**decolorizing agent (Acetone-alcohol decolorizer)**. **Wait 10-15 seconds** or add drop by drop to slide until decolorizing agent running from the slide runs clear.
6. Flood slide with a counterstain, **safranin**. Wait **30 seconds to** **1 minute**.
7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.
8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.



**Results:**

* Gram-negative bacteria will stain pink/red and
* Gram-positive bacteria will stain blue/purple.



