**Practical Biology Dr. Mayada S.H.**

**Observation of bacteria using staining procedures**

**1.Simple staining. 2.Gram staining.**

**1.Simple Staining**

**Smear preparation:-**

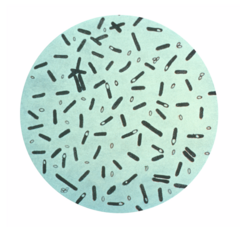
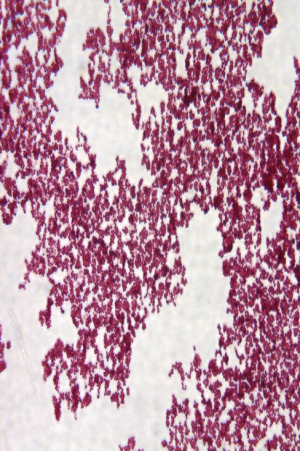
-A drop of water is placed in the centre of a slide.

-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**

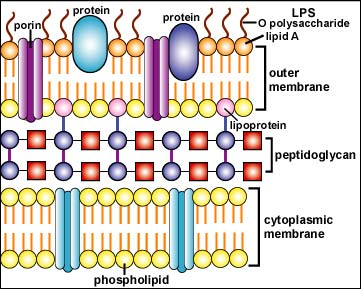
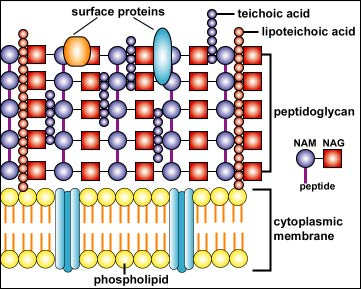
In 1884 Christian Gram Staining technique that separates bacteria into two groups:-

**1-Gram-positive bacteria**

**2-Gram-negative bacteria**

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

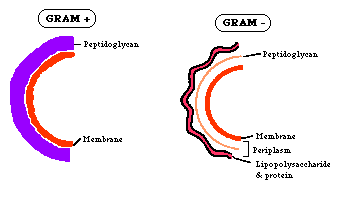
**Gram-positive cell wall Gram-negative cell wall**



**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 petidoglycan and teichoic acid.
* Bacteria stained pink are **Gram –** their cell walls have 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**

* ***Steptococcus***   **. *Staphylococcus***
* ***Lactobacillus*** **. *Bacillus***
* ***Clostridium***

**Gram-negative bacteria**

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

**Shapes of Bacteria**

bacteriashape

