Lecture 6

- Bacterial Staining / Gram Staining

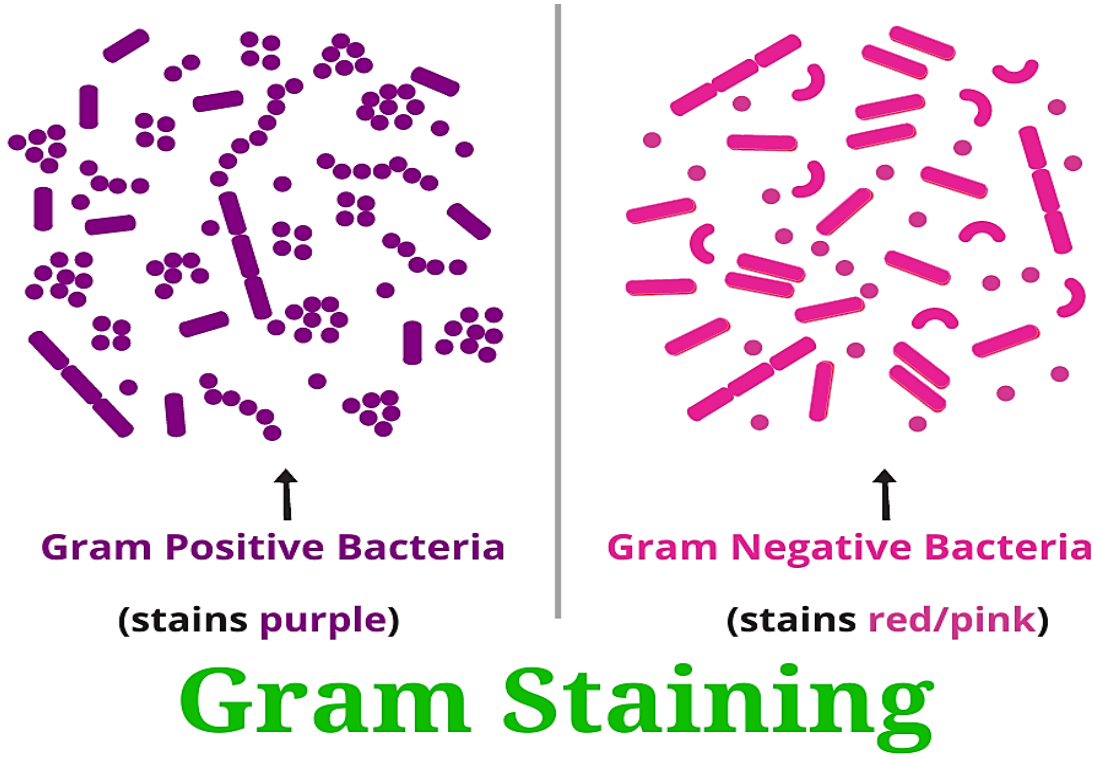
• Gram stain

• The most important differential stain used for diagnostic identification of bacteria, its developed by Hans Christian Gram in 1884.

Gram staining divides bacteria into two categories

1- gram-positive bacteria

2- gram-negative bacteria.

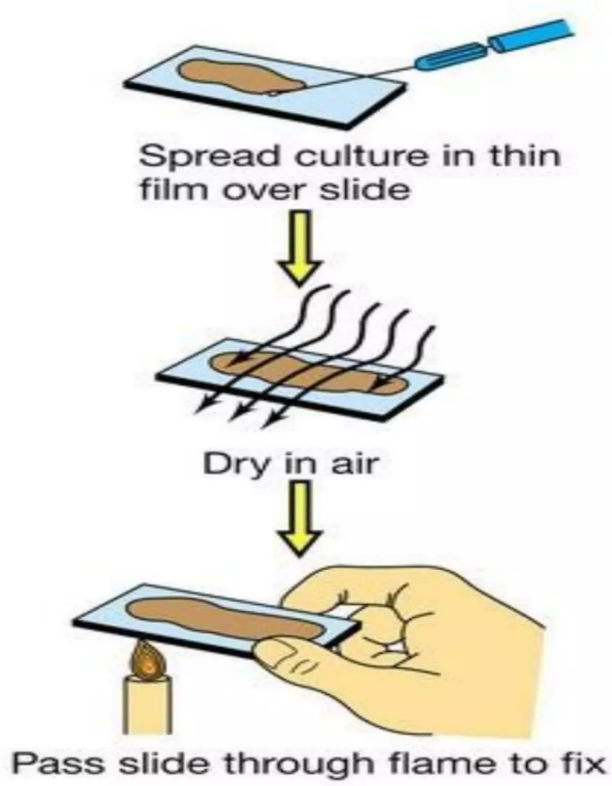


- Steps in gram staining

Step 1 : Preparation of smear on a glass slide

1. Take a loop of bacterial culture sample on a clean and grease-free slide.

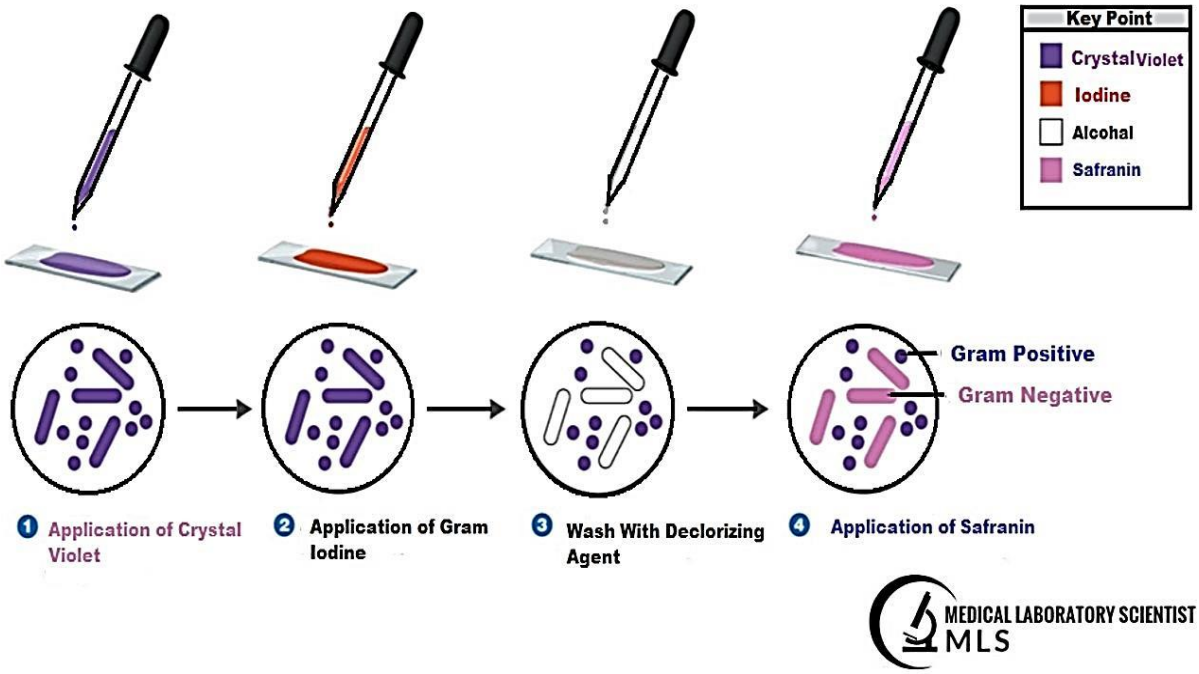
2- Make a smear on the slide, Air dry, and heat fix the smear. (Gently pass the slide through the flame 2-3 times)



Step 2 : Gram Staining

1. Add Crystal Violet on smear and stand for **1 min**
2. Gently wash the slide using tap water
3. Add gram's Iodine solution for **1 min**
4. Gently wash the slide using tap water
5. Add Ethyl alcohol (95 %) stand for **15 -30** seconds
6. Gently Wash the slide using tap water
7. Add counterstain safranin stand for **1 min**

8. Observe the slide under the microscope with a 100 X objective oil immersion lens.



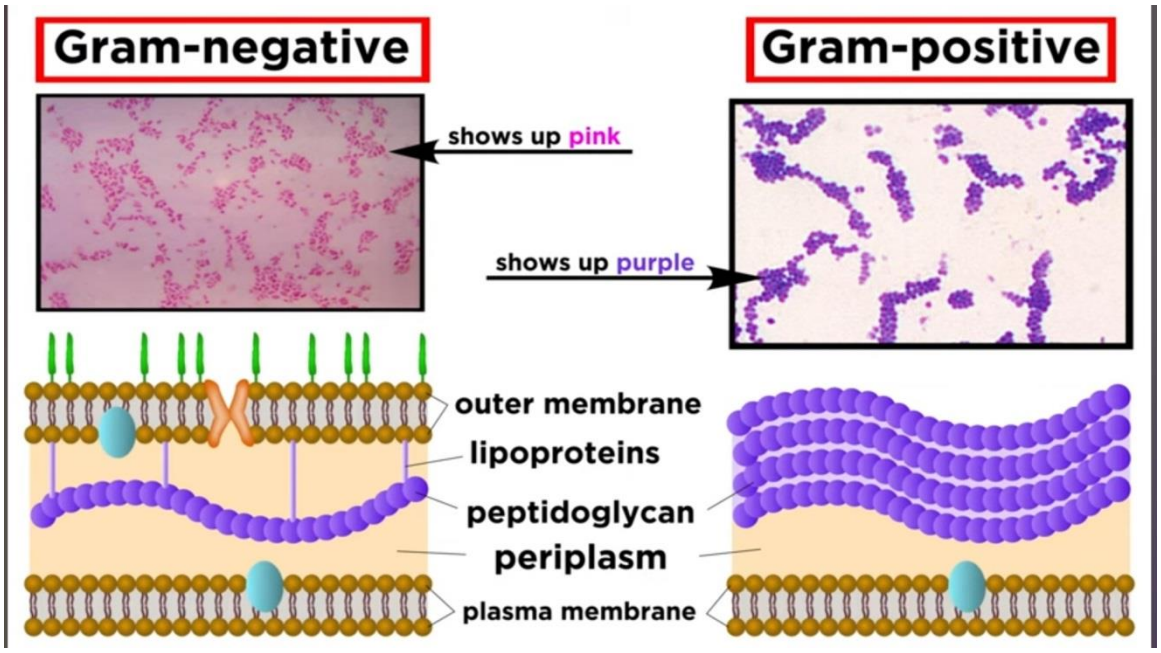
Results:

Bacteria retain the crystal violet and gram iodine mixtures complexes after the wash in 95% ethanol solution in purple color and are named as **Gram positive Bacteria**, those which miss these complexes of solution are red in color by counter stain the safranin or 10% fuchsine are named as **Gram negative Bacteria**.

- The theory of Gram stain differentiation is based on cell wall structure and lipid component; therefore, it comes out with two theories below:

1. Cell wall theory: Gram-positive bacteria have heavy peptidoglycan, which helps decolorizer (alcohol) to dehydrate the Gram-positive cell wall and traps the crystal violet complex inside the cell wall and maintains the purple color of crystal violet.

2. Lipids theory: Gram-negative bacteria have high lipid amount (10–15%) in the cell wall, which makes decolorizer (alcohol) easily to remove the crystal violet complex, and then colorless cell wall is stained with safranin and appears pink/red color.



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