STAINING TECHNIQUES

(GRAM STAIN)

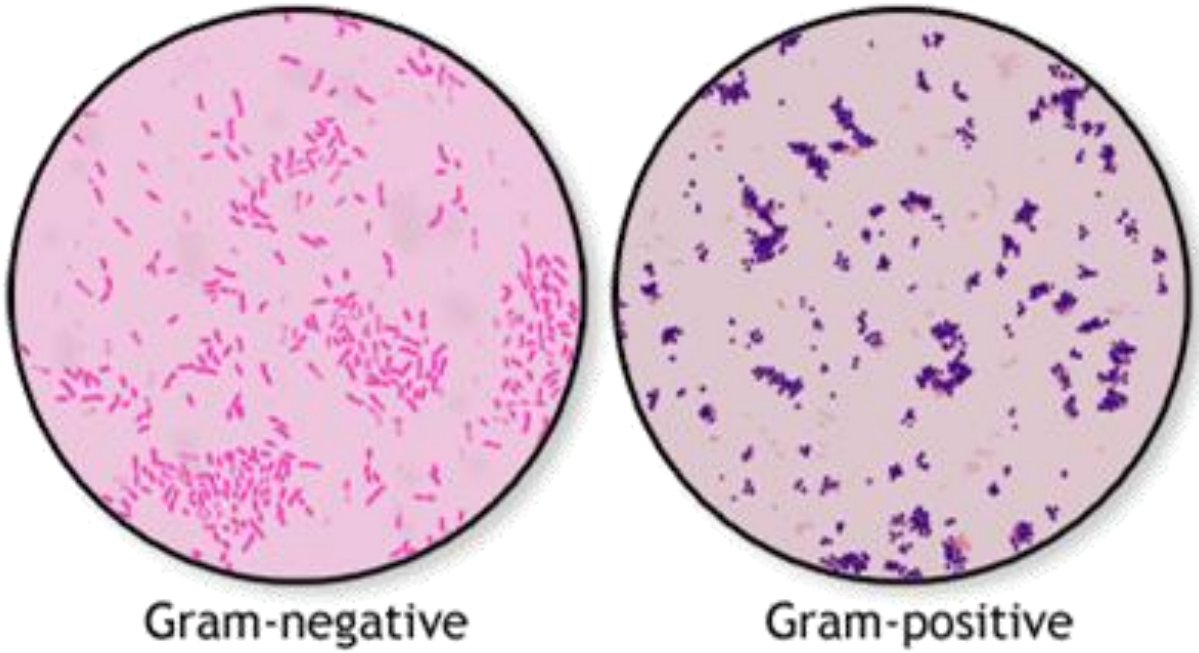
“ 240 MIC ”

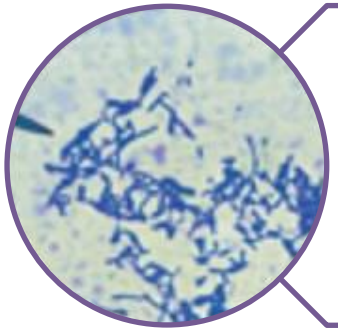
نورة الكـبيسي

Nalkubaisi@ksu.edu.sa

2021

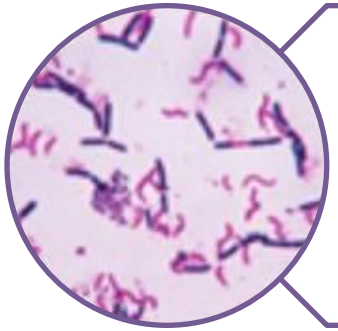
Since living bacteria are generally colorless and almost invisible because of their lack of contrast with the water in which they may reside, **STAINING** is necessary in order to make them **readily visible** for observation of intracellular structures as well as overall morphology.





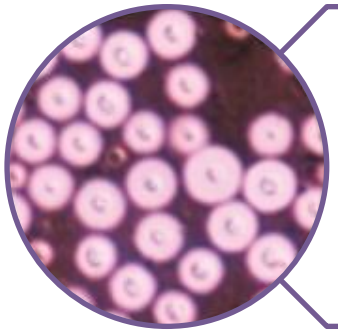
1. Simple staining :

* Only one dye is used.
* Differentiation among bacteria is impossible.
* eg. Simple staining.



2. Differential staining:

* More than one dye is used.
* Differentiation among bacteria is possible.
*eg. Gram's staining, Acid-fast staining.



3. Special staining:

* More than one dye used.
* Special structures are seen.
* eg. Capsule staining, Spore staining.

PREPARING A SMEAR

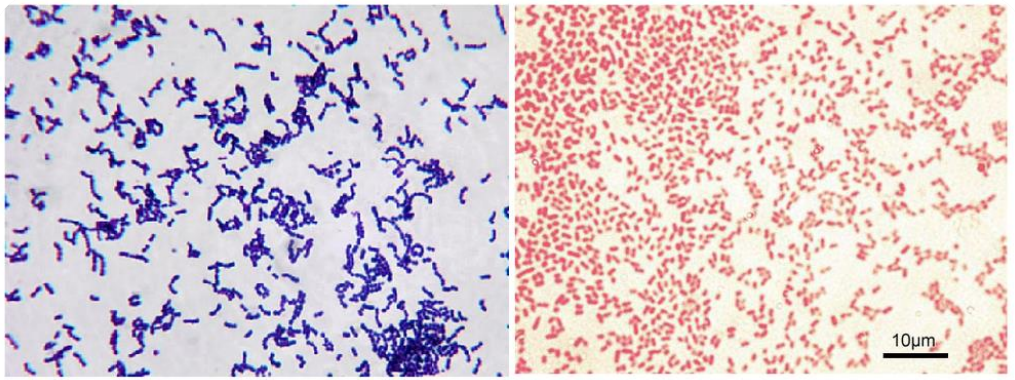
A properly prepared smear accomplishes two things:

A) It causes bacteria to adhere to a slide so that they can be stained and observed.

B) It also kills them, rendering pathogenic bacteria safe to handle.

THE GRAM STAINING

- Gram staining method, the most important procedure in Microbiology, was developed by Danish physician **Hans Christian Gram** in 1884.
- Gram staining is still the cornerstone of bacterial identification and taxonomic division.
- The differences in cell wall composition of Gram positive and Gram negative bacteria accounts for the Gram staining differences.
- Gram positive cell wall contain **thick layer** of **peptidoglycan** with numerous teichoic acid cross linking which resists the decolorization.



The primary stain is **Crystal violet**, and all cells take up the purple crystal violet stain.

Following the primary stain, **Gram's Iodine** is applied to the bacterial smears.

The iodine acts as a mordant:

- Enhancing the ability of the stain to enter and bind to the bacteria.
- Specifically, the iodine binds with crystal violet and locks it into peptidoglycan of bacteria.
- Intensifies the purple color.

The decolorizing agent used in the Gram staining procedure is **95% ethanol**:

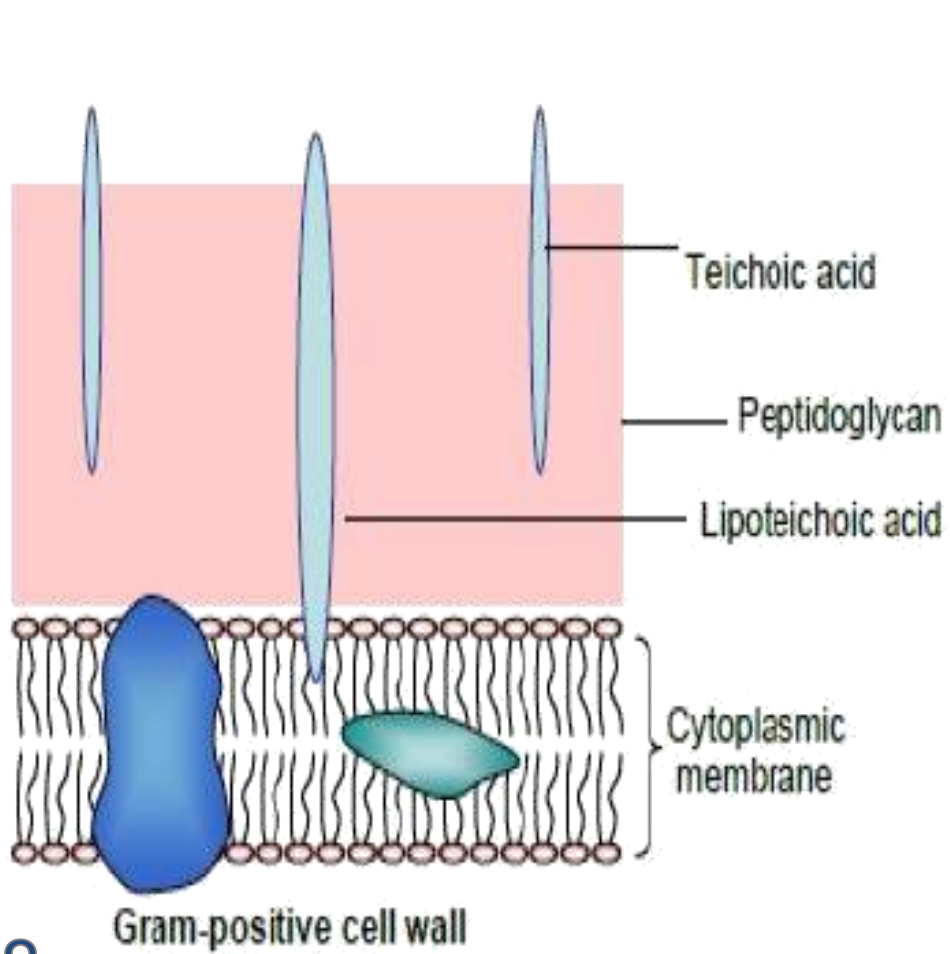
- a lipid solvent that melts the Gram negative outer membrane and leads to decolorization of Gram negative cells.
- It also dehydrates proteins, helping the primary stain to remain in Gram positive cell walls.

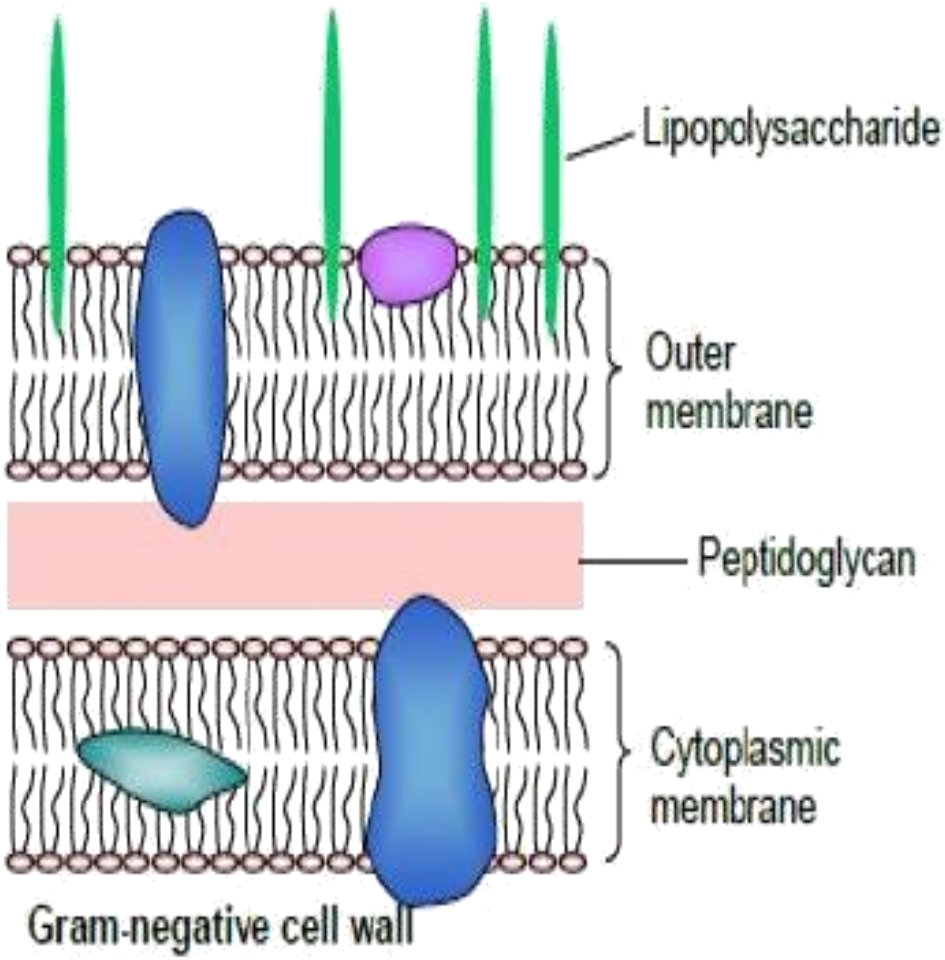
The counter stain then used is **Safranin:**

which stains the decolorized Gram negative cells pink.

At the end of the staining procedure

Gram positive cells are purple and Gram negative cells are pink.





The End