

SNS COLLEGE OF ALLIED HEALTH SCIENCE
Affiliated to The Tamil Nadu Dr M.G.R Medical University, Chennai



DEPARTMENT OF OPERATION THEATRE AND ANAESTHESIA

TECHNOLOGY

COURSE NAME: MICROBIOLOGY

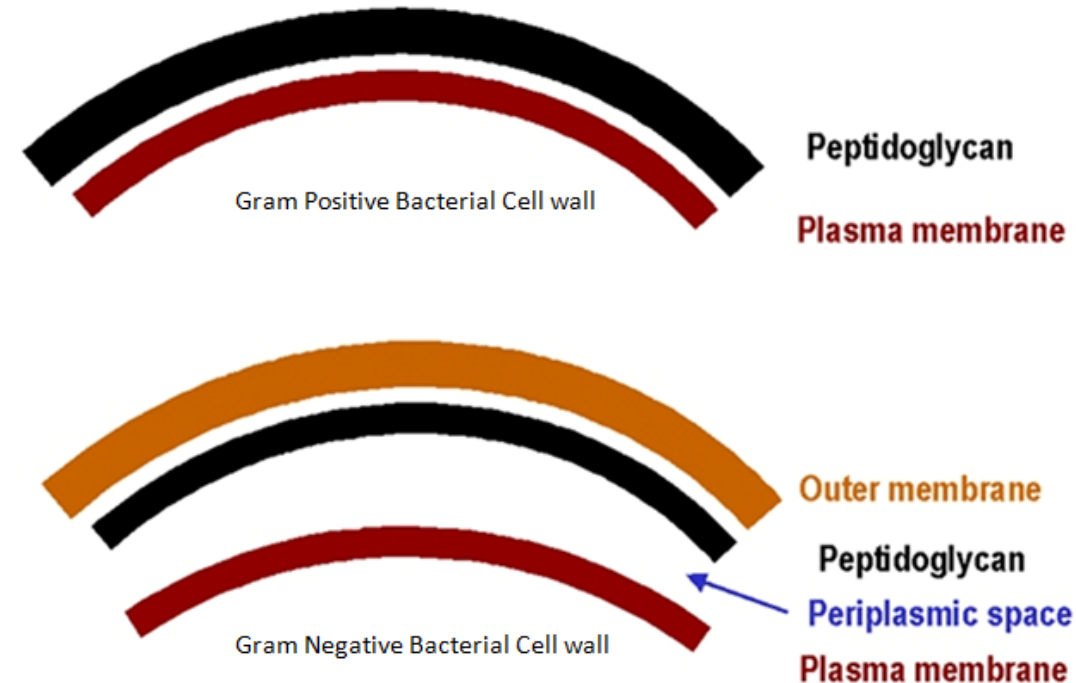
UNIT : 1

TOPIC : GRAM'S STAINING

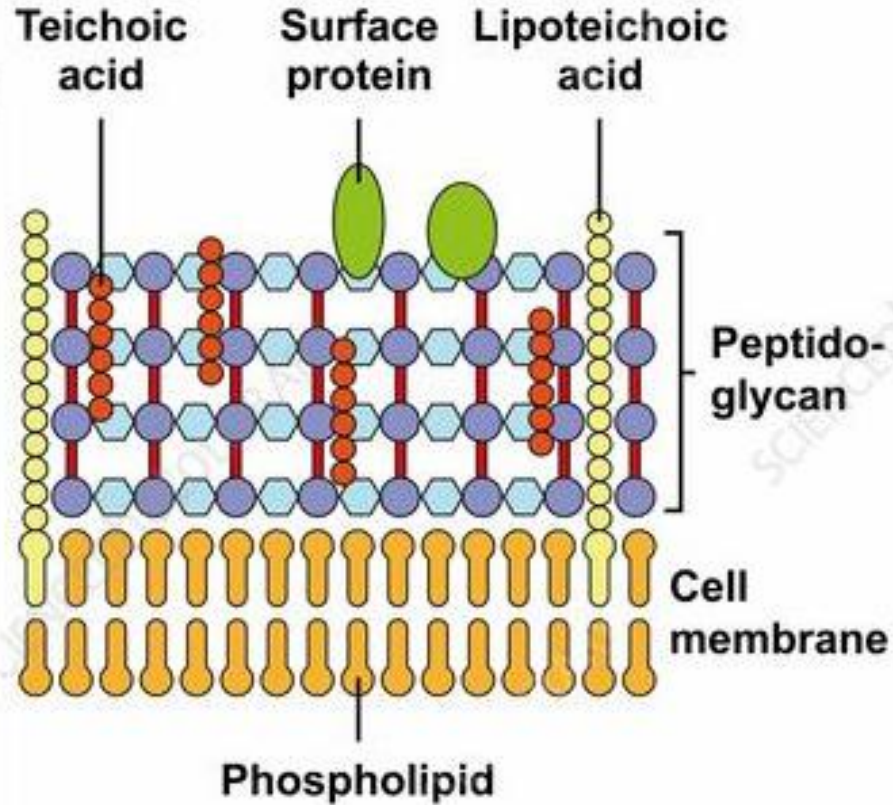
FACULTY NAME: MITHRA V

GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

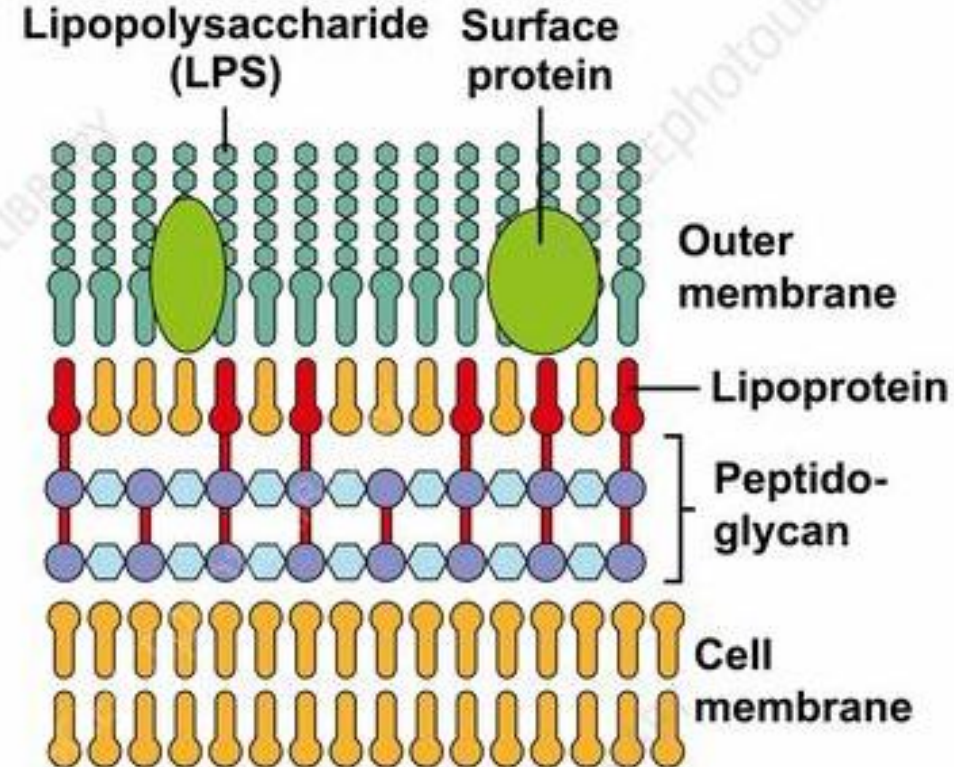
- Bacteria are a large group of minute, unicellular, microscopic organisms, which have been classified as prokaryotic cells, as they lack a true nucleus.
- The cell wall of **gram-positive** bacteria is composed of **thick** peptidoglycan layer.
- The cell wall of **gram-negative** bacteria is composed of **thin** peptidoglycan layer.



Gram Positive Bacteria



Gram Negative Bacteria



GRAM'S STAIN (DEFINE)

- Differential staining procedure used to categorize bacteria as Gram positive or Gram negative based on the chemical and physical properties of their cell's walls.
- Named after Hans Christian Gram, the Danish bacteriologist who originally devised it in 1844.
- First test performed for the identification of bacteria.
- The cell walls for gram-positive microorganisms have a higher lipid content than gram-negative cells

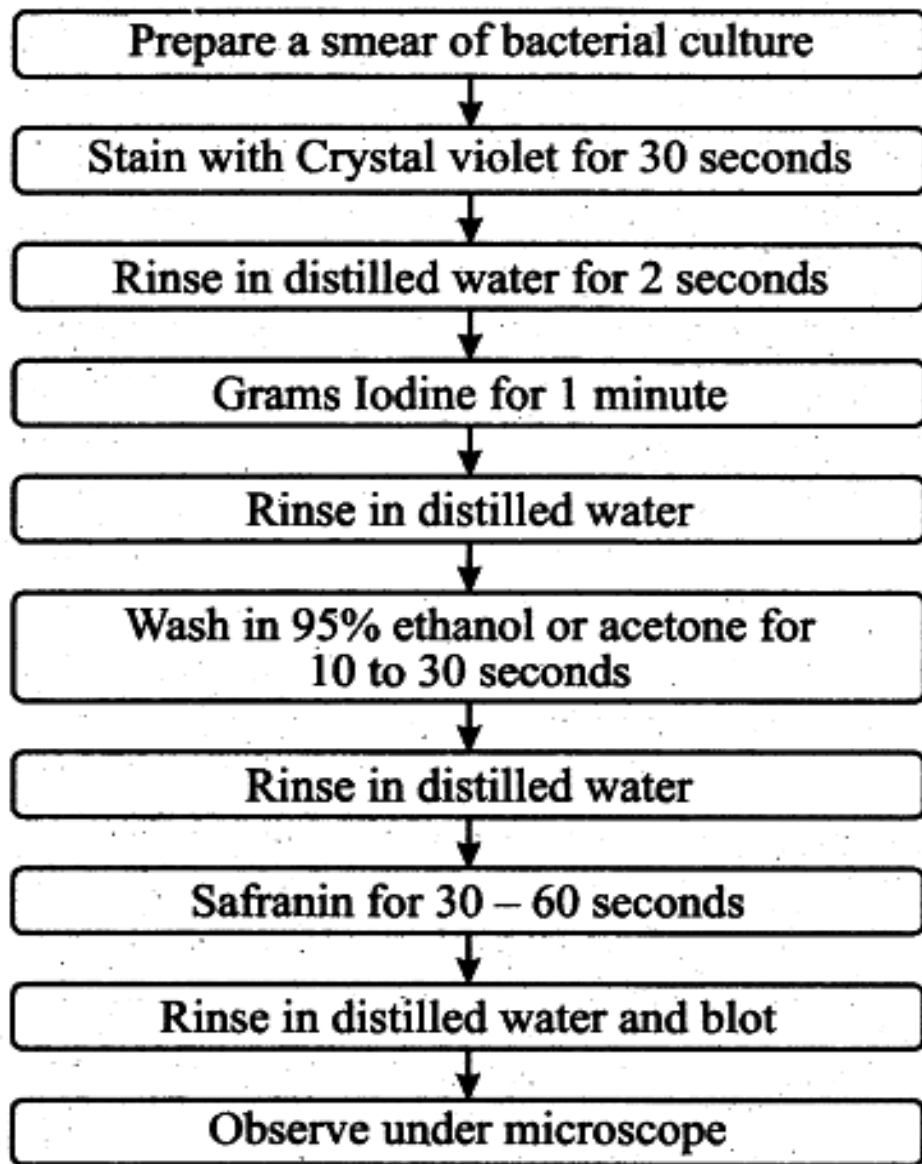
GRAM STAINING - COMPONENTS

- Primary stain (**Crystal violet**, methyl violet)
- Mordant (Gram's Iodine)
- Decolourizer (**ethyl alcohol**, acetone)
- Counterstain (Dilute carbol fuchsin, **safranin**)

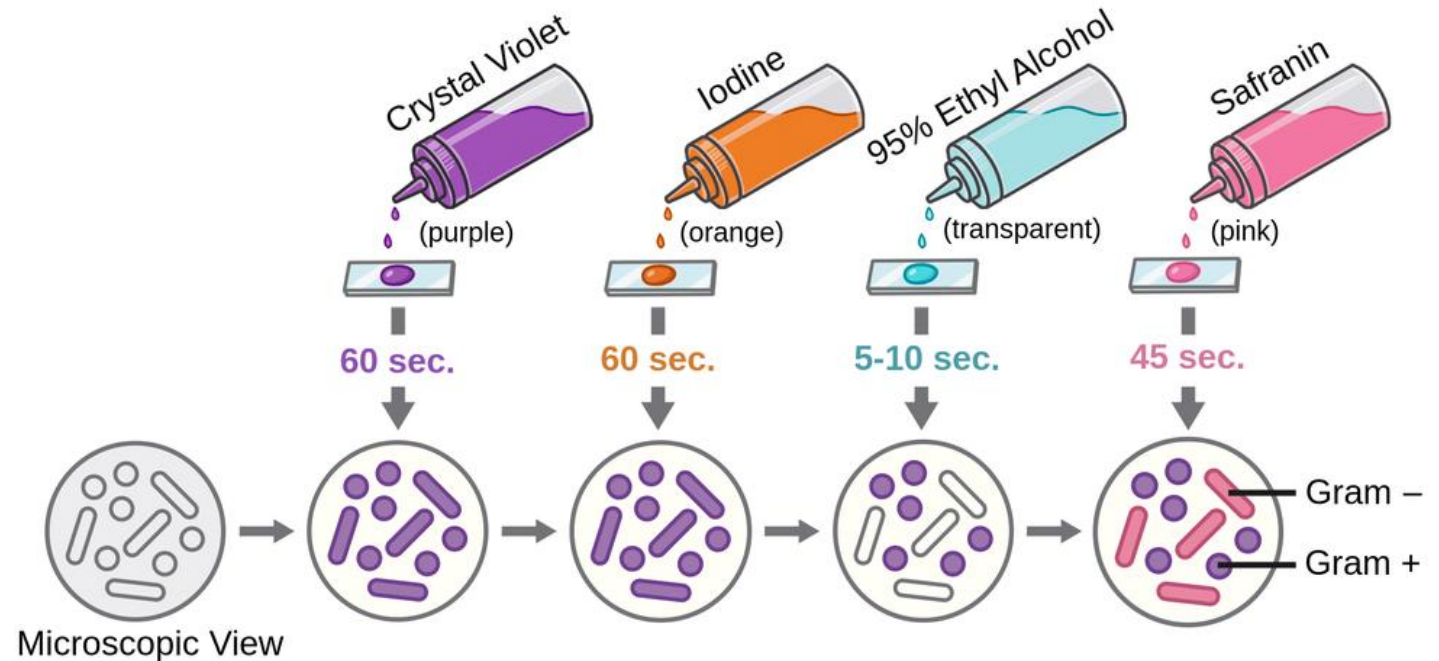


PRINCIPLE

- Crystal violet ions penetrate the cell wall of both types of cells.
- Iodine - forms a crystal violet iodine complex - “fixing” the dye.
- Following iodine, the cells are treated with decolorizer, a mixture of ethanol and acetone, which dissolves the lipid layer from the gram negative cells.
- The addition of a safranin that is counterstain to dye the gram negative cells with a pink color for easier observation under a microscope.
- Thus, **gram-positive** cells will be stained **purple/violet** and **gram-negative** cells will be stained **red/pink**.



PROCEDURE (PROTOTYPE)



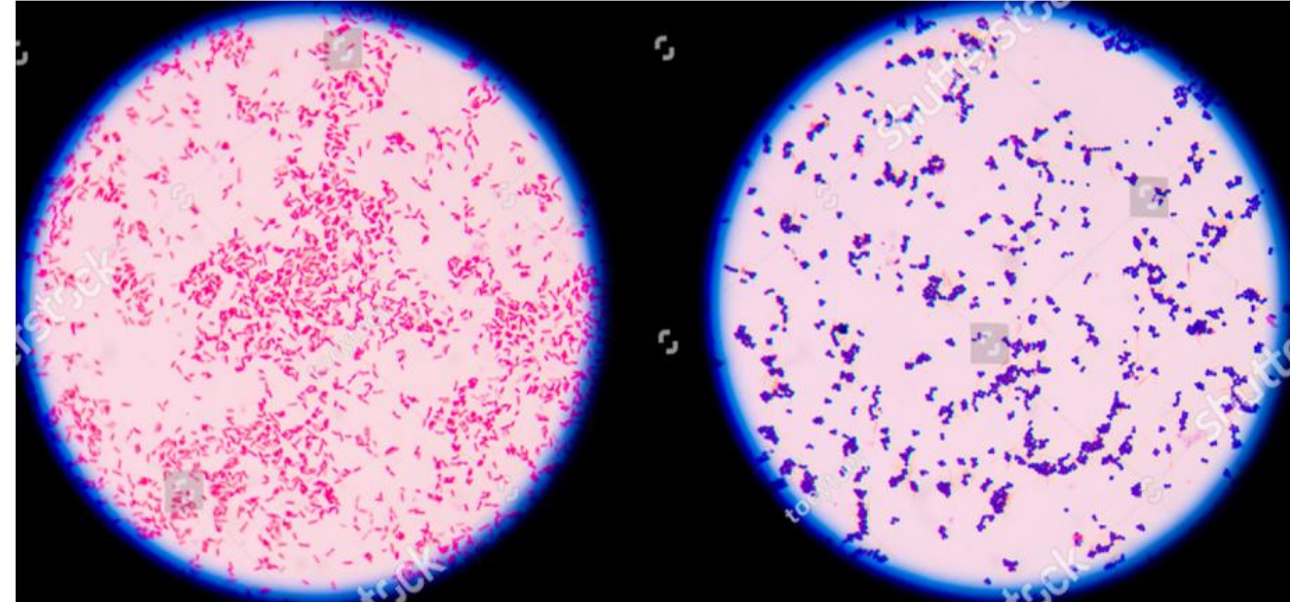
INTERPRETATION (TEST)

EXAMINING THE GRAM STAIN

- **Color:**
- Gram-positive appear purple to blue
- Gram-negative appear pink to red.

Shape:

The most common shapes include round (*cocci*) or rod-shaped (*bacilli*).



GRAM NEGATIVE

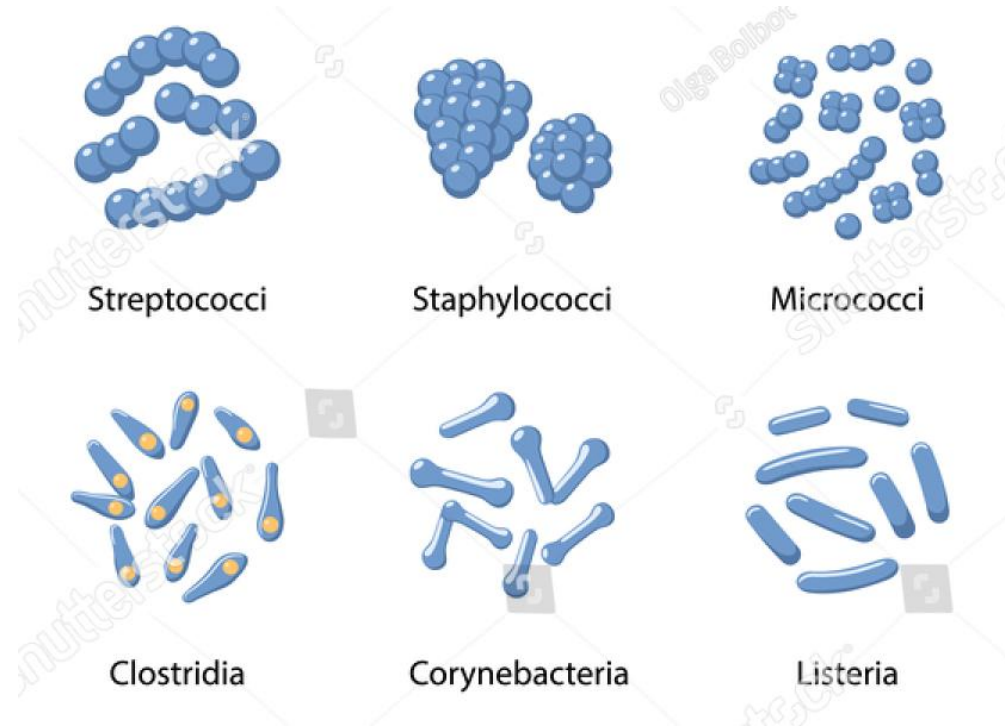
GRAM POSITIVE

GRAM-POSITIVE BACTERIA

- Cell walls that contain thick layers of peptidoglycan (90%)
- This causes them to appear blue to purple under a Gram stain.

Gram-positive organisms include:

- *Staphylococcus* species.
- *Streptococcus* species.
- *Corynebacterium* species.
- *Clostridium* species.
- *Listeria* species.



GRAM-NEGATIVE BACTERIA

- Cell walls with thin layers of peptidoglycan (10%) and high lipid (fatty acid) content.
- This causes them to appear red to pink under a Gram stain.

Gram-negative organisms include:

- *Neisseria gonorrhoeae*.
- *Escherichia coli* (E. coli).
- *Pseudomonas* species.
- *Proteus* species.
- *Klebsiella* species.



REFERENCES

- Topley & Wilson's Microbiology and Microbial Infections, 10th Edition, 2018
- Jawetz, Melnick, & Adelberg's Medical Microbiology, 28th Edition, 2021
- [Gram Stain Protocols - American Society for Microbiology \(PDF\)3](#)
- [Gram Staining: Principle, Procedure & Result Interpretation - Microbe Notes5](#)
- [Gram Stain: MedlinePlus Medical Test](#)

THANK YOU