SNS COLLEGE OF ALLIED HEALTH SCIENCE

Affiliated to The Tamil Nadu Dr M.G.R Medical University, Chennai

DEPARTMENT OF CARDIAC TECHNOLOGY

COURSE NAME: MICROBIOLOGY

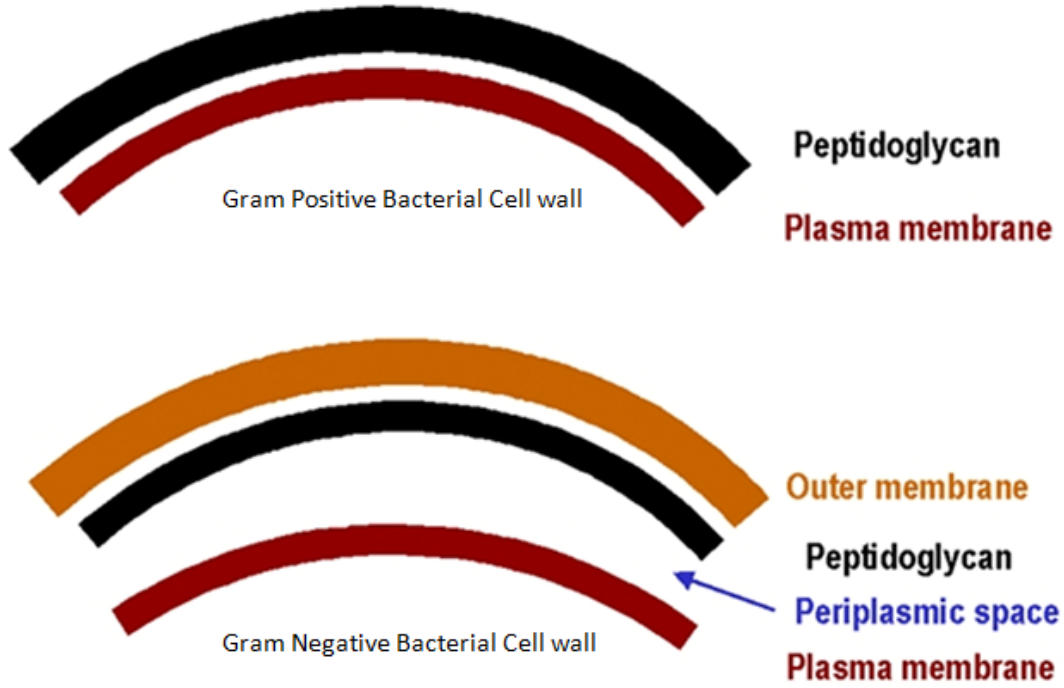
UNIT : 1

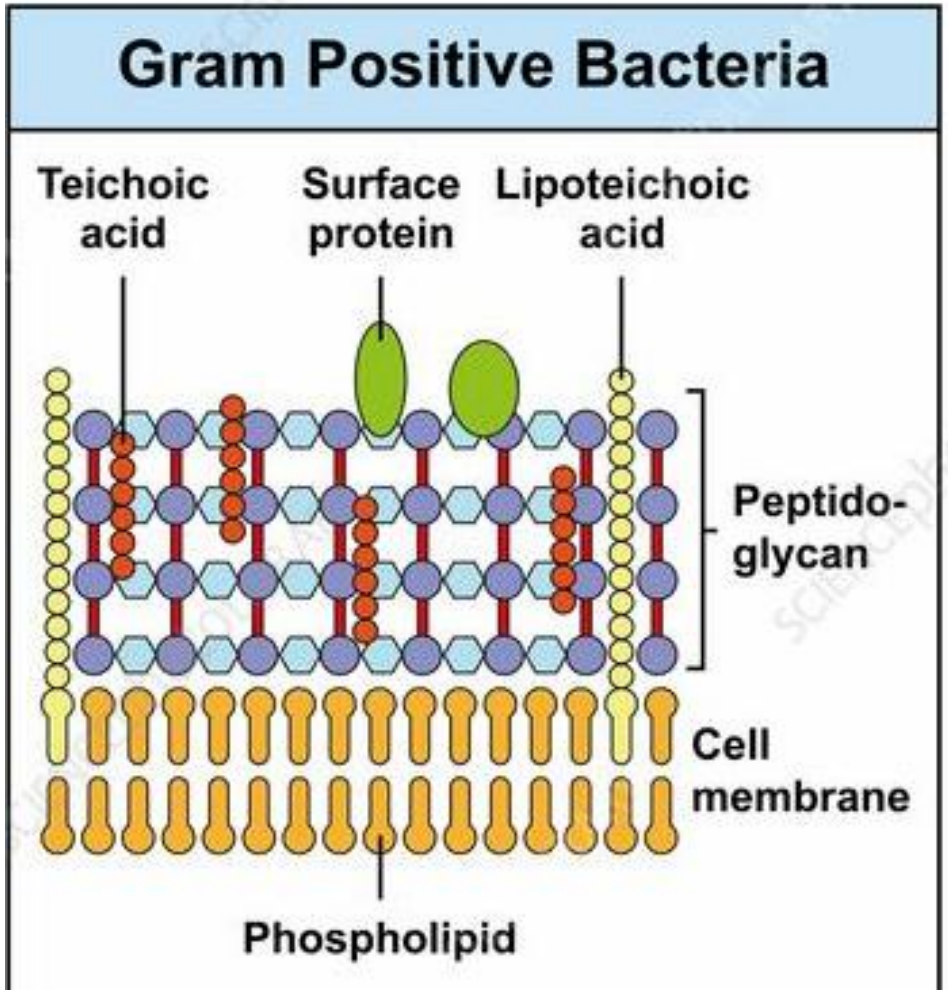
TOPIC : GRAM'S STAINING

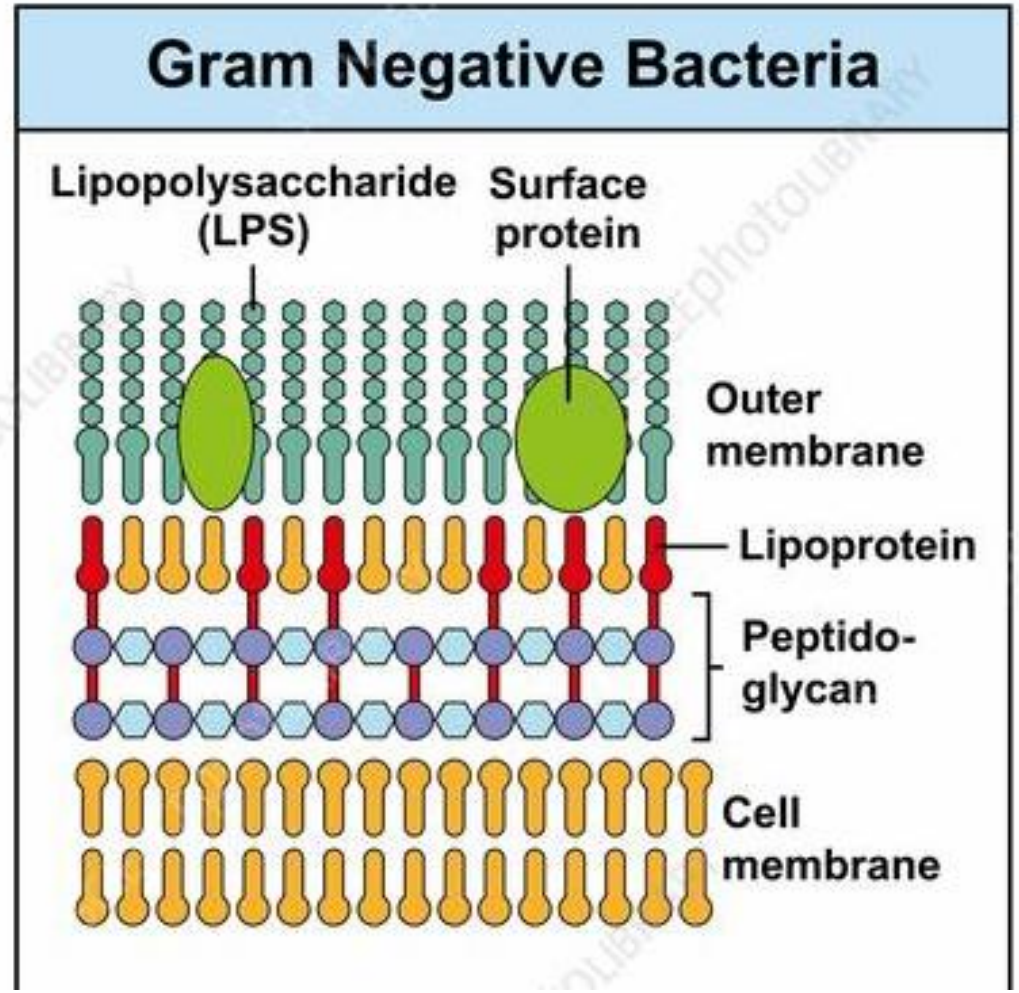
FACULTY NAME: MITHRA V

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
Teichoic acid
Surface protein
Lipoteichoic acid
Peptido-glycan
Cell membrane
Phospholipid


Gram Negative Bacteria
Lipopolysaccharide (LPS)
Surface protein
Outer membrane
Lipoprotein
Peptido-glycan
Cell membrane

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)

Prepare a smear of bacterial culture

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Stain with Crystal violet for 30 seconds

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Rinse in distilled water for 2 seconds

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Grams Iodine for 1 minute

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Rinse in distilled water

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Wash in 95% ethanol or acetone for 10 to 30 seconds

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Rinse in distilled water

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Safranin for 30 – 60 seconds

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Rinse in distilled water and blot

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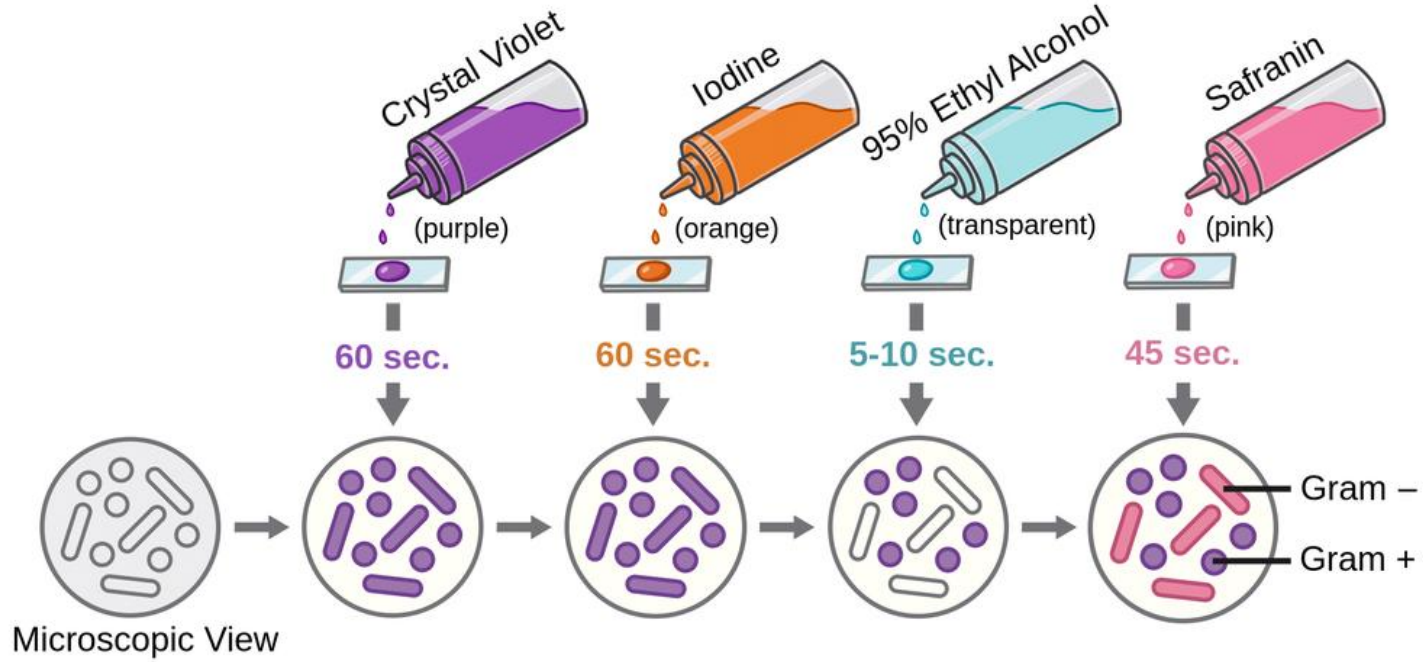
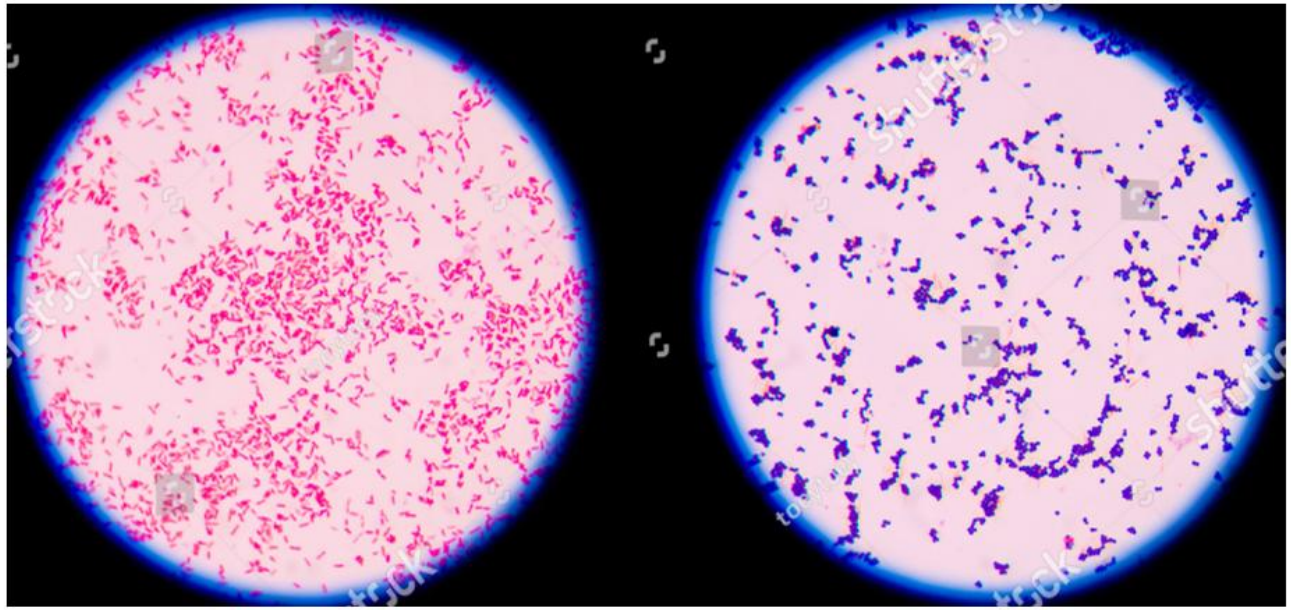
Observe under microscope



INTERPRETATION (TEST)

EXAMINING THE GRAM STAIN

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



GRAM NEGATIVE GRAM POSITIVE

Shape:

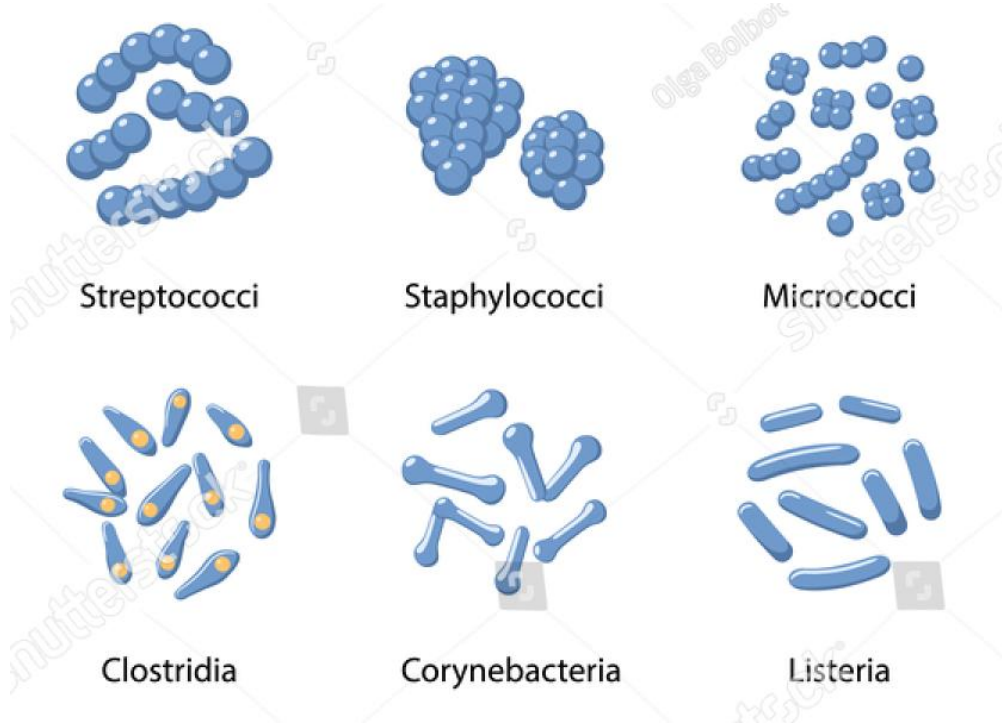
The most common shapes include round (*cocci*) or rod-shaped (*bacilli*).

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.

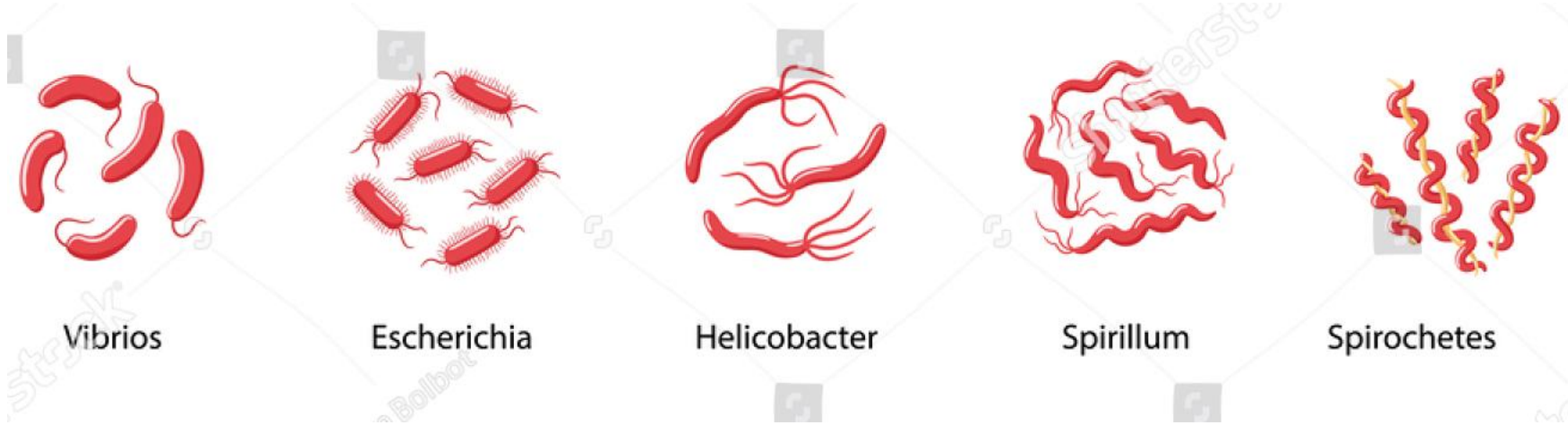


GRAM-NEGATIVE BACTERIA

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



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- 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