BACTERIAL STAINING

Disclaimer

All the content material provided here is only for teaching purpose.

Microscopy helps to Measure and Observe the Bacteria

- **Measurement**
  - **Microorganisms are very small**
  - **Use metric system**
  - **Metre (m) : standard unit**
  - **Micrometre (μm) = 1 x10^{-6} m**
  - **Nanometre (nm) = 1 x10^{-9} m**
  - **Angstrom (Å) = 1 x10^{-10} m**

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Why we should be Stain *Bacteria*

Bacteria have nearly the same refractive index as water, therefore, when they are observed under a microscope they are opaque or nearly invisible to the naked eye. Different types of staining methods are used to make the cells and their internal structures more visible under the light microscope.

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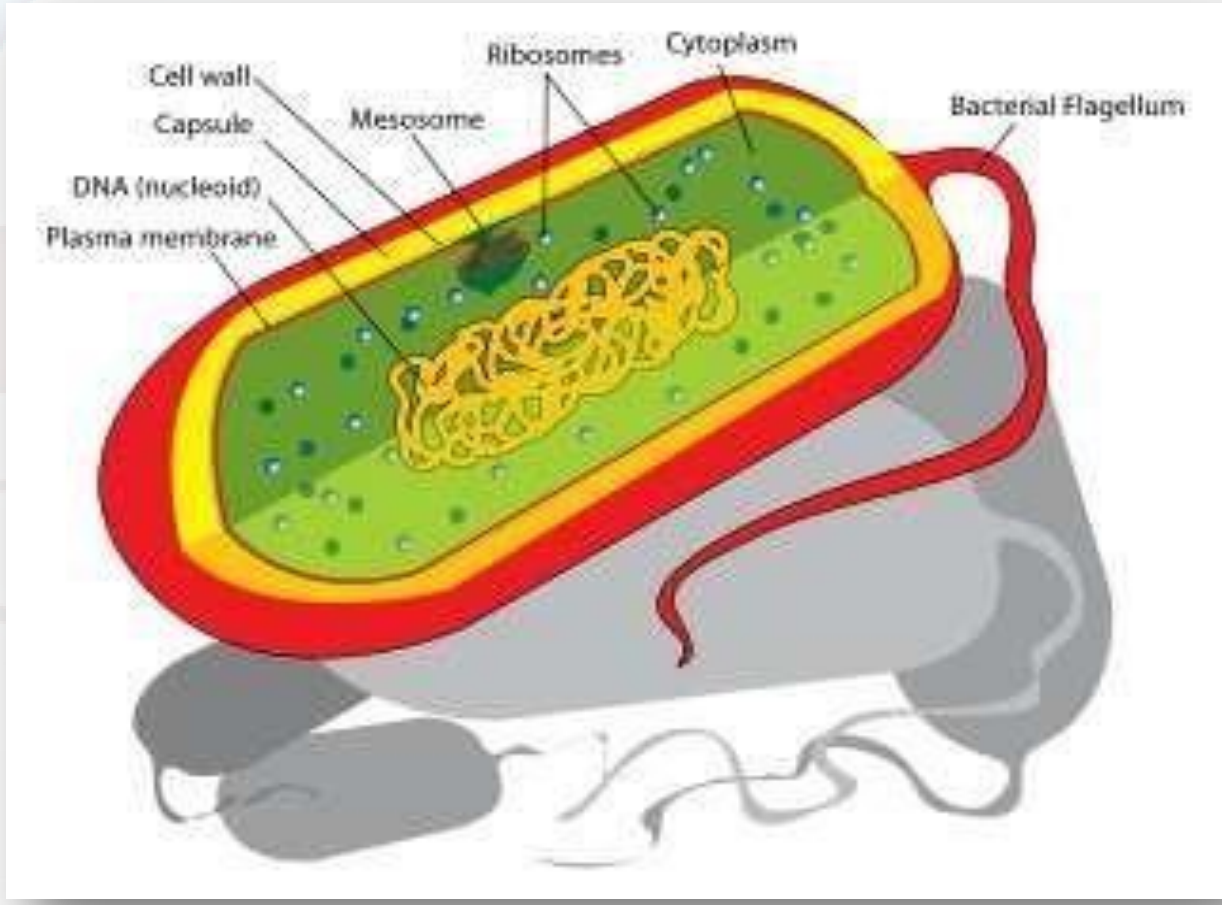
Staining helps in observation of Bacteria

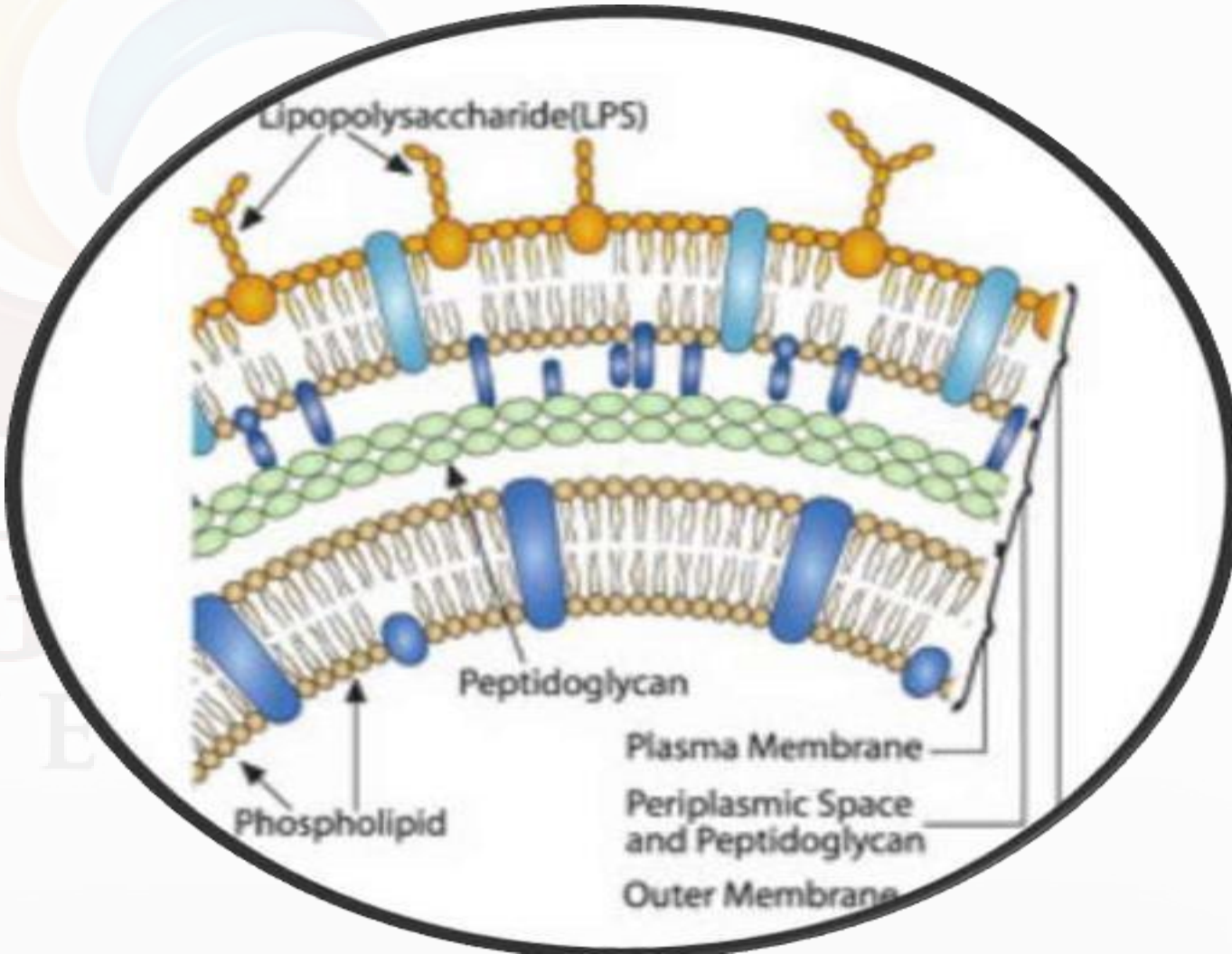
- **Microscopes are of little use unless the specimens for viewing are prepared properly. Microorganisms must be fixed & stained to increase visibility, accentuate specific morphological features, and preserve them for future use.**



Stains and Staining

- Bacteria are slightly negatively charged at pH 7.0
  - Basic dye stains bacteria
  - Acidic dye stains background

- Simple stain
  - Aqueous or alcohol solution of single basic dye

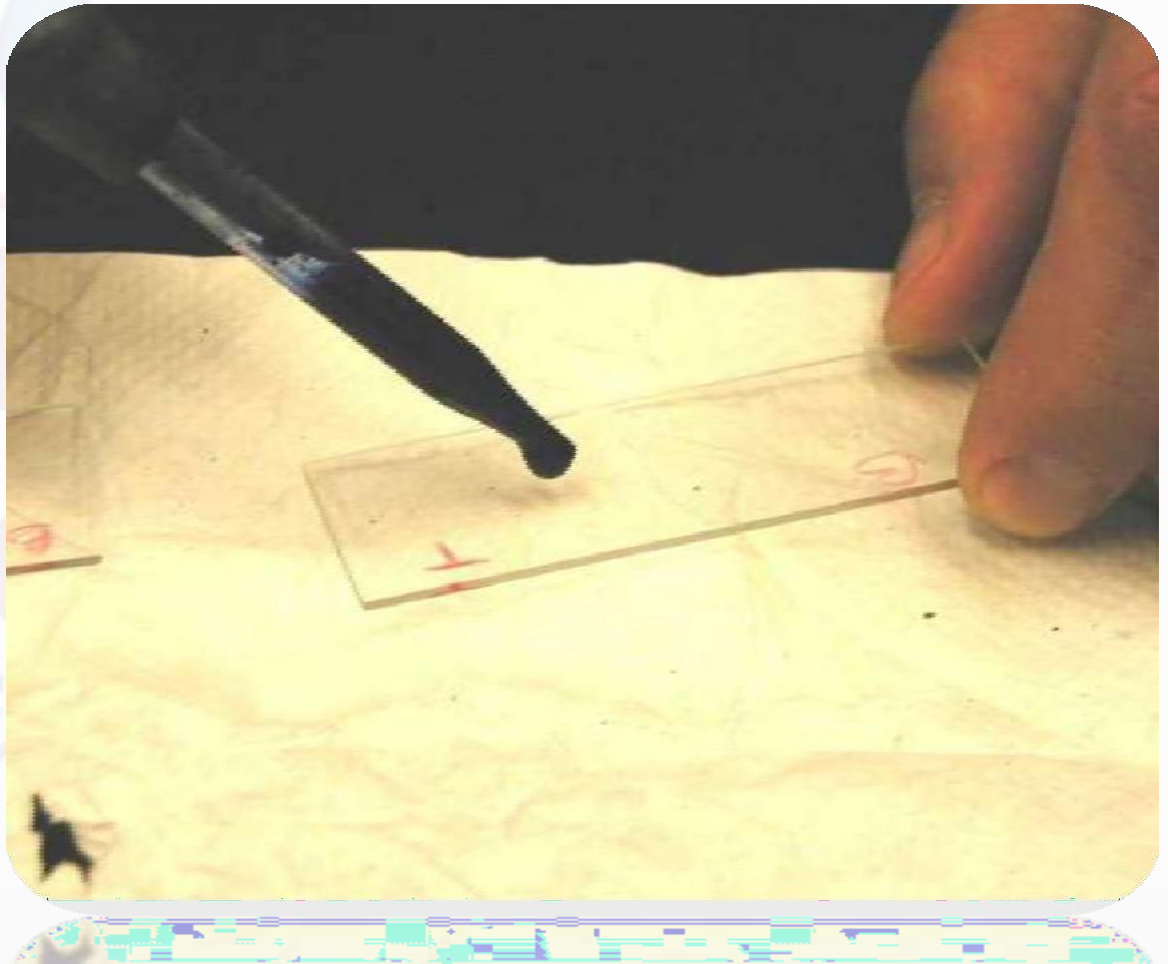


What is a Stain

- A stain is a substance that adheres to a cell, giving the cell color.
- The presence of color gives the cells significant contrast so are much more visible.
- Different stains have different affinities for different organisms, or different parts of organisms
- They are used to differentiate different types of organisms or to view specific parts of organisms

Staining Techniques

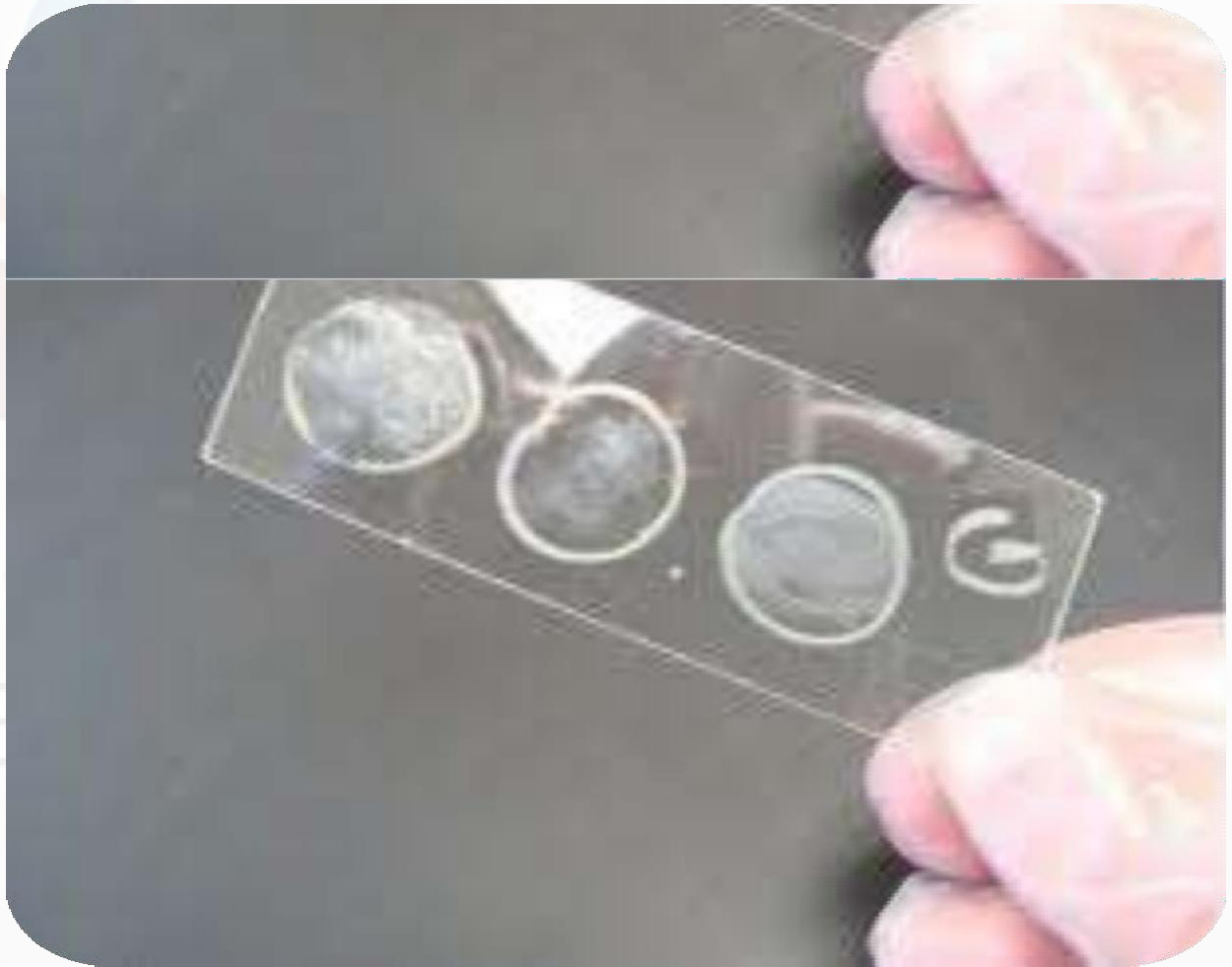
- **Staining** is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes.



Smearing out of the *sample*

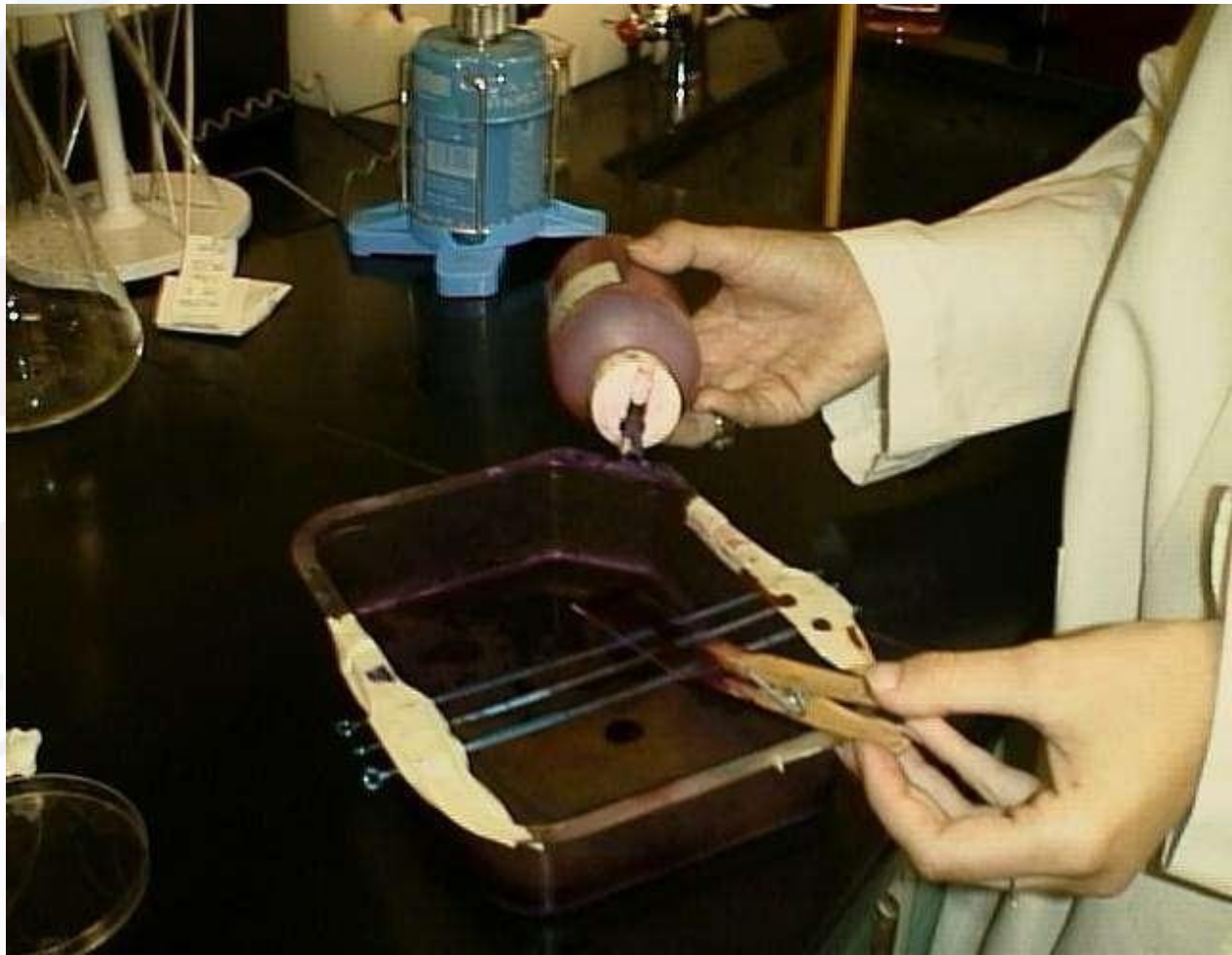
Fixation

- **Fixation**–which may itself consist of several steps–aims to preserve the shape of the cells or tissue involved as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the specimen so it will accept stains



Simple staining

- *simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation.*
- *The stain can be poured drop by drop on the slide*



Simple staining

- Methylene blue, Basic fuchsin
- Provide the color contrast but impart the same color to all the organisms in a smear
- Loffler's ethylene blue: Sat. solution of M. blue in alcohol - 30mlKoH, 0.01% in water - 100mlDissolve the dye in water, filter. For smear: stain for 3'. For section: stain

Simple staining (cont..)

- Dilute Carbol fuchsin:- Made by diluting Z-N stain with 10- 15 times its volume of water- Stain for 20-25 seconds, wash with water

Use: To demonstrate the morphology of Vibrio cholera

Polychrome methylene blue:

Use: M'Fadyean's reaction - B. anthracis

Simple Stains

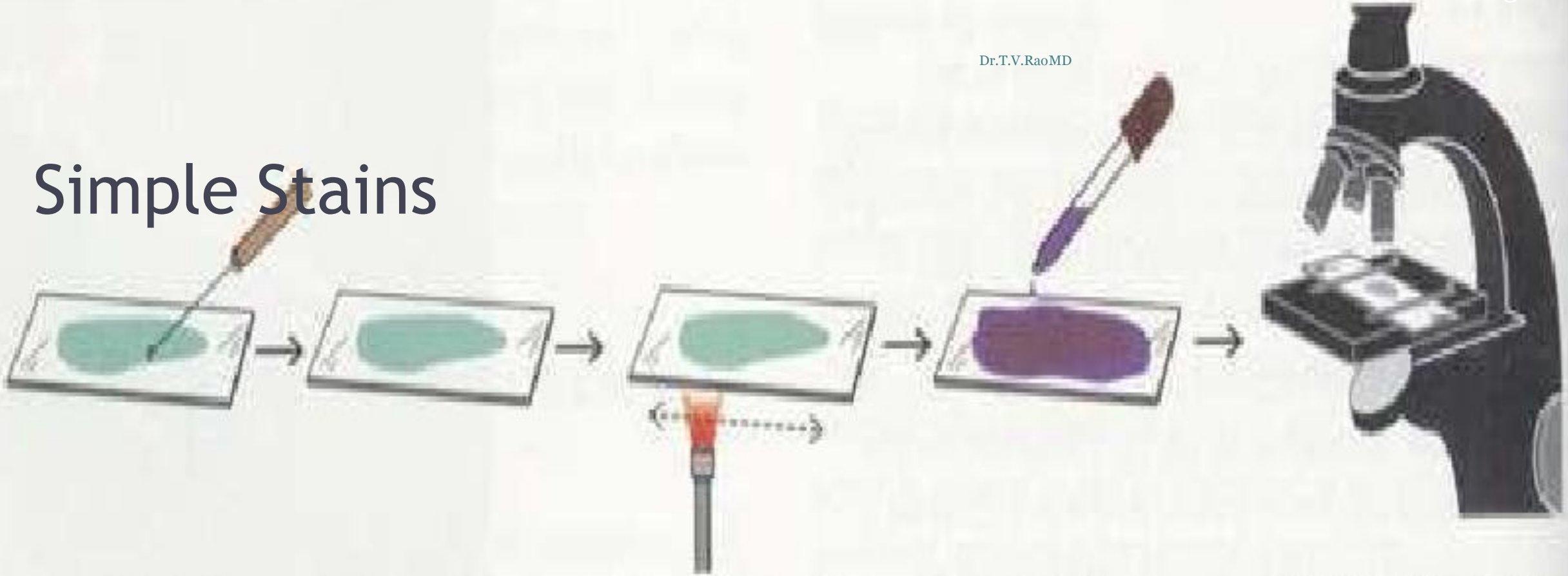
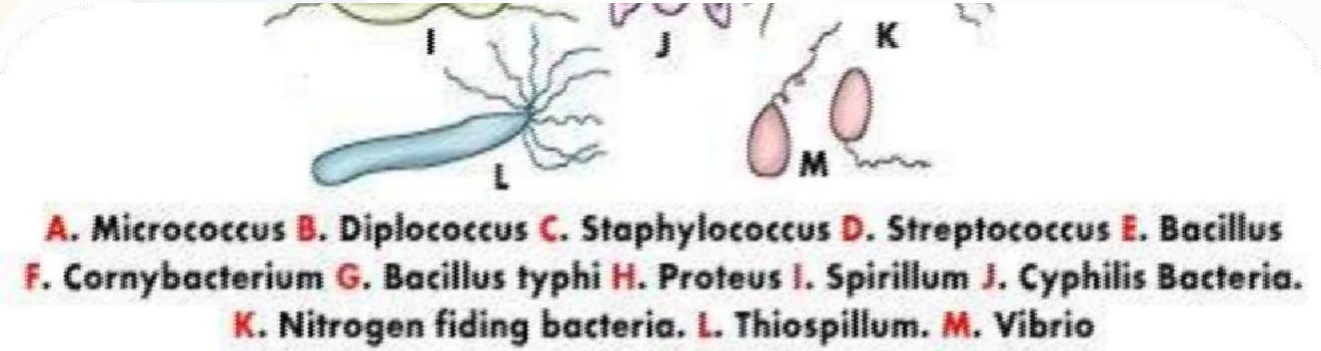


Figure 3.4

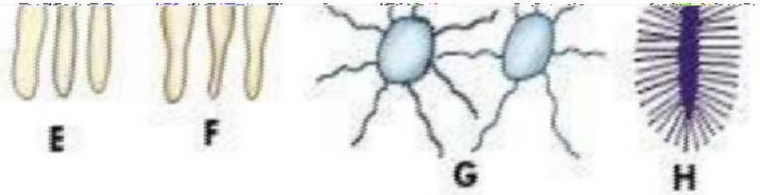
Steps in staining cells for microscopic observation.

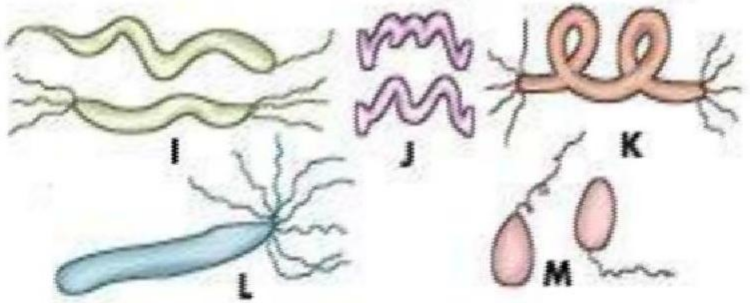
Bacterial arrangement

- **Clusters (group).**
- **Chains.**
- **Pairs (diploids).**
- **No special arrangement.**



A. Micrococcus B. Diplococcus C. Staphylococcus D. Streptococcus E. Bacillus
F. Cornybacterium G. Bacillus typhi H. Proteus I. Spirillum J. Cyphilis Bacteria.
K. Nitrogen fiding bacteria. L. Thiospillum. M. Vibrio





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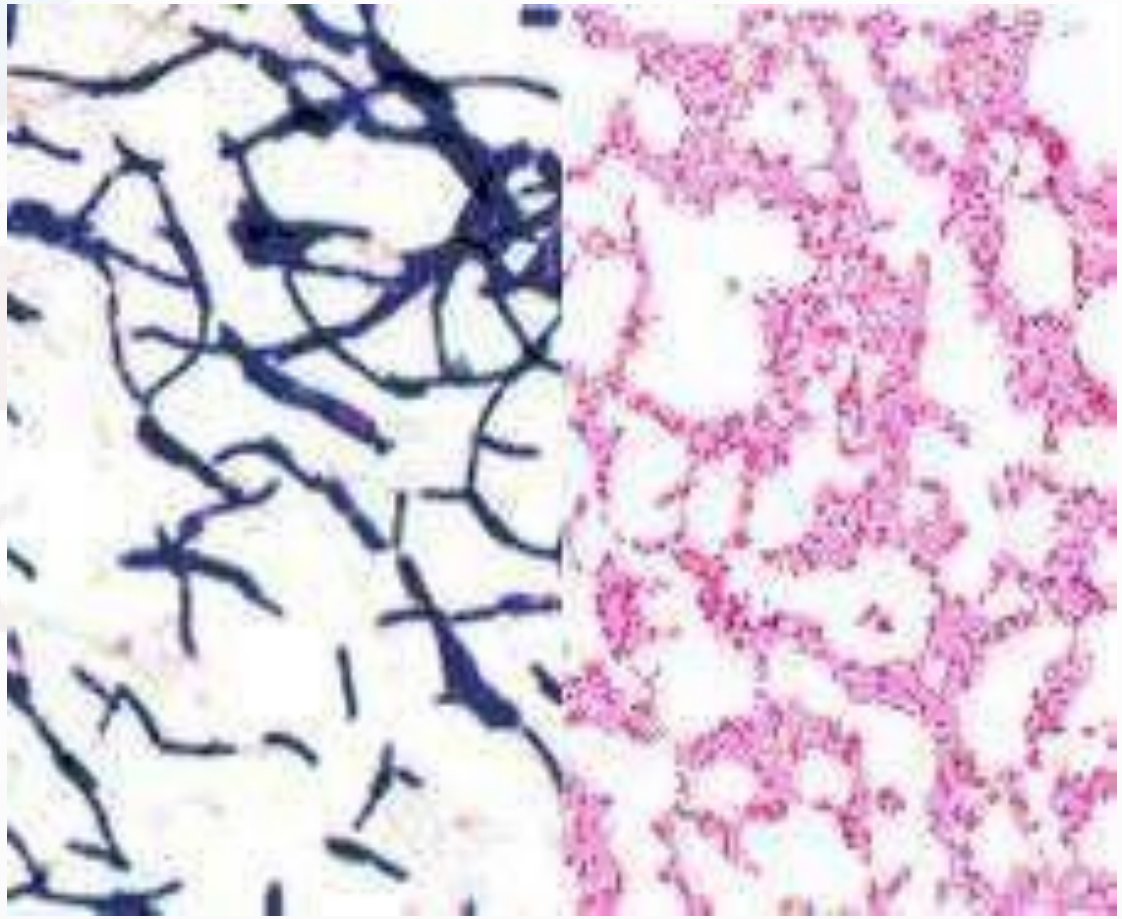
Simple Staining Easier to Perform But has Limitations

- Simple easy to use; single staining agent used; using basic and acid dyes.
- Features of dyes: give coloring of microorganisms; bind specifically to various cell structures



Differential Stains

- ☞Differential Stains use two or more stains and allow the cells to be categorized into various groups or types.
- ☞Both techniques allow the observation of cell morphology, or shape, but differential staining usually provides more information about the characteristics of the cell wall (Thickness).

GRAM STAINING

Flow Through Procedure

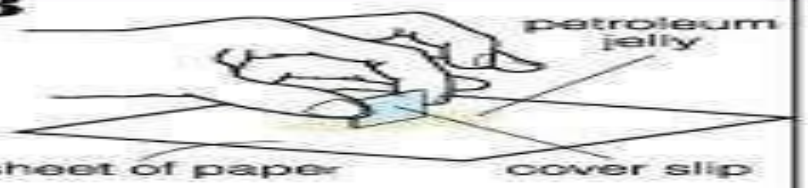
1

Wipe bottom of biofilm slide clean

2

Clean top edges of slide about 2mm

3

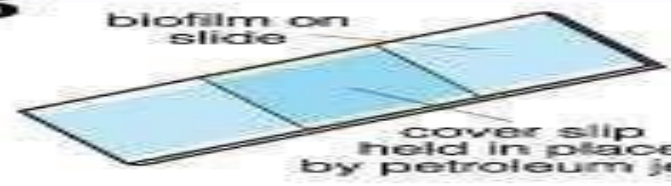


Build up a ridge of petroleum jelly on the top and bottom of a cover slip

4

Cover slip with petroleum jelly

5



Biofilm on slide with cover slip

6

Add crystal violet-wait 30 sec.

7

Wash with water

8

Add Grams Iodine -wait 1.5 min.

9

Decolorize with alcohol

10

Wash with water

11

Stain with Safranin dye-wait 30 sec.

12

Wash with water

13

Examine under oil immersion through the cover slip

Gram staining - Principles

- Gram staining is used to determine gram status to classify bacteria broadly. It is based on the composition of their cell wall. Gram staining uses crystal violet to stain cell walls, iodine as a mordant, and a fuchsin or safranin counterstain to mark all bacteria. Gram status is important in medicine; thepresence or absence of a cell wall will change the bacterium's susceptibility to some antibiotics.
- Gram-positive bacteria stain dark blue or violet. Their cell wall is typically rich with peptidoglycan and lacks the secondary membrane and lipopolysaccharide layer found in Gram-negative bacteria

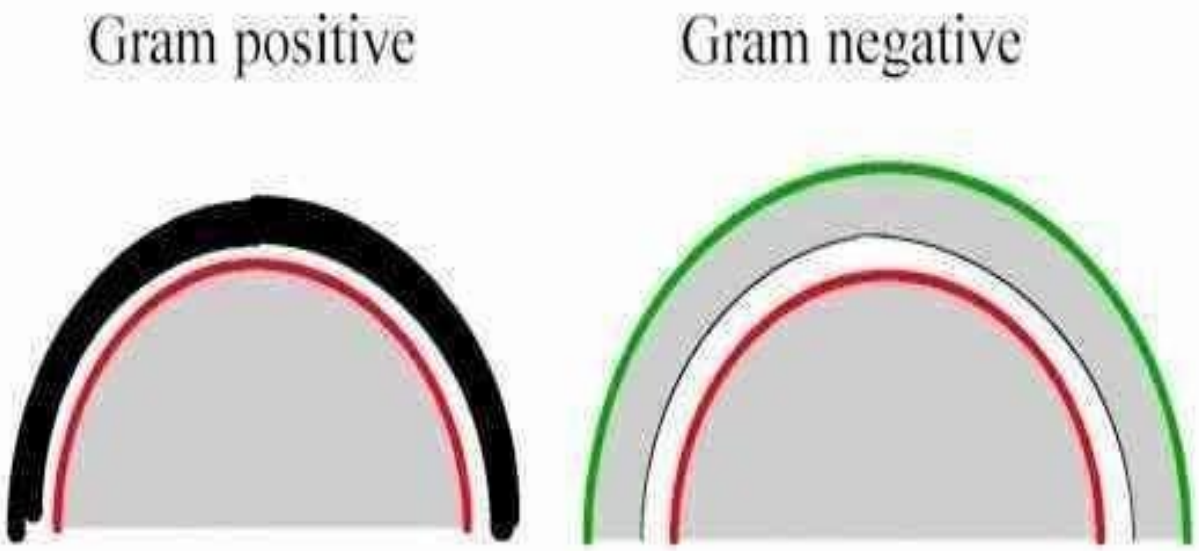
Gram Staining Steps

Gram Staining Steps

1. Crystal violet acts as the primary stain. Crystal violet may also be used as a simple stain because it dyes the cell wall of any bacteria.
2. **Gram's iodine** acts as a mordant (Helps to fix the primary dye to the cell wall).
3. Decolorizer is used next to remove the primary stain (crystal violet) from Gram Negative bacteria (those with LPS imbedded in their cell walls). Decolorizer is composed of an organic solvent, such as, acetone or ethanol or a combination of both.)
4. Finally, a counter stain (Safranin), is applied to stain those cells (Gram Negative) that have lost the primary stain as a result of decolorization

Stains differentiates different groups of Bacteria

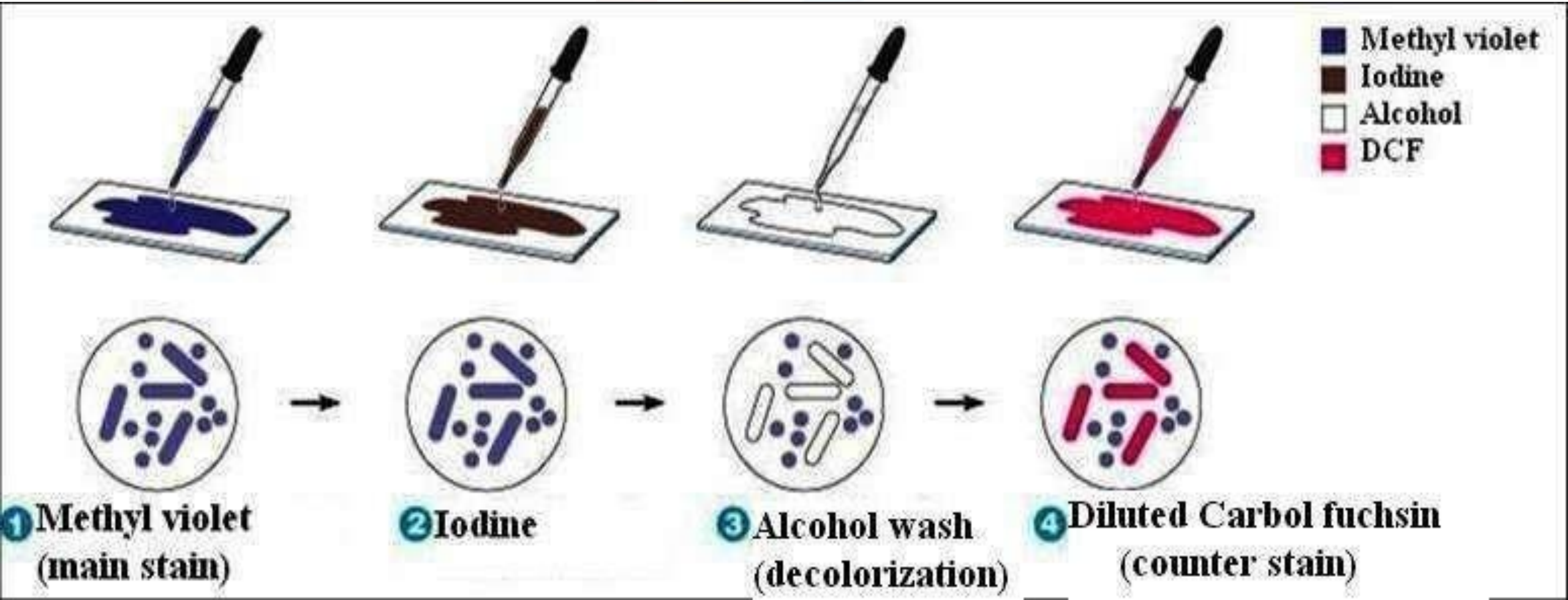
- To distinguish different kinds of bacteria into separate groups based on staining properties
- Two types: Gram stain & Acid-fast stain.



Differential Stains: Gra m Stain

	Color of Gram + cells	Color of Gram – cells
Primary stain: Crystal violet	Purple	Purple
Mordant: Iodine	Purple	Purple
Decolorizing agent: Alcohol-acetone	Purple	Colorless
Counterstain: Safranin	Purple	Red

Gram Staining technique



Gram Staining Procedure

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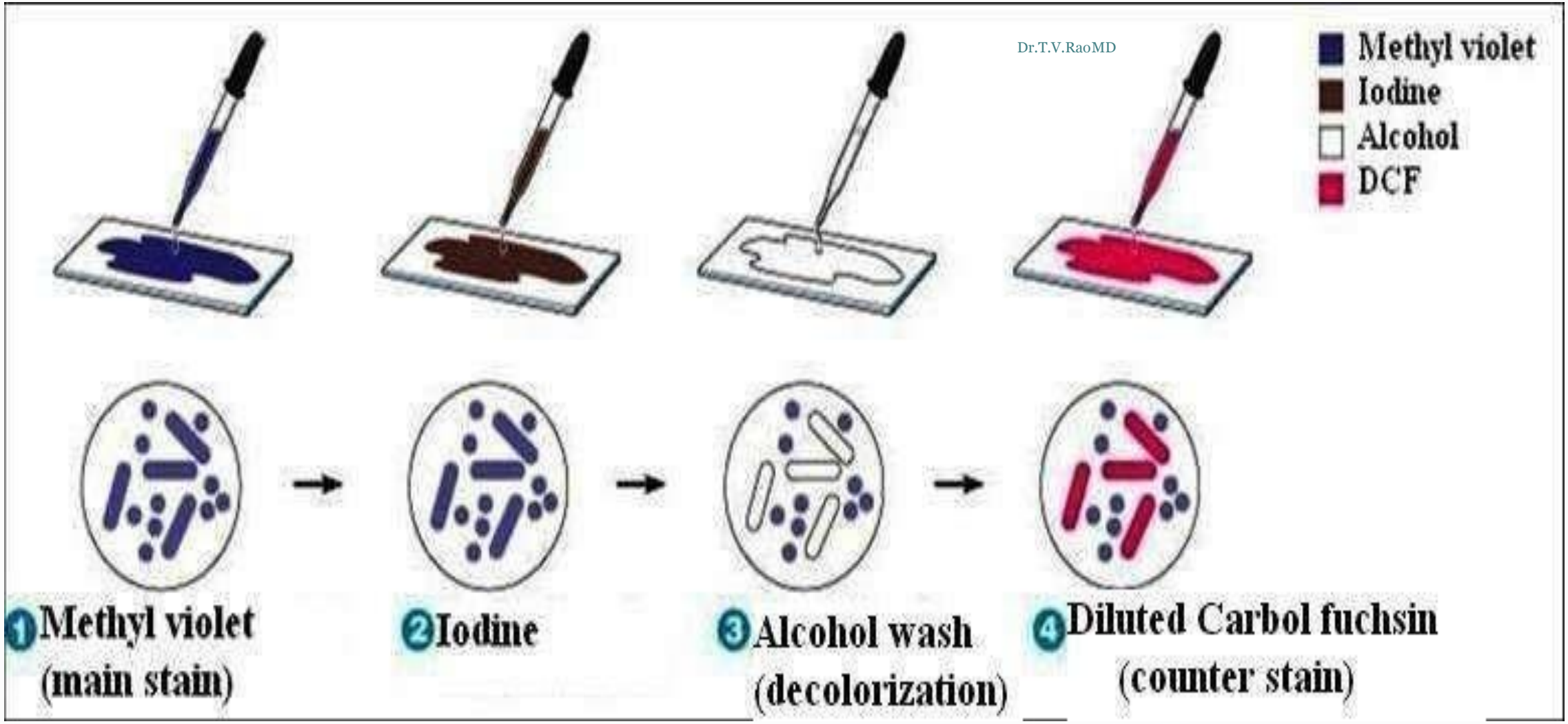
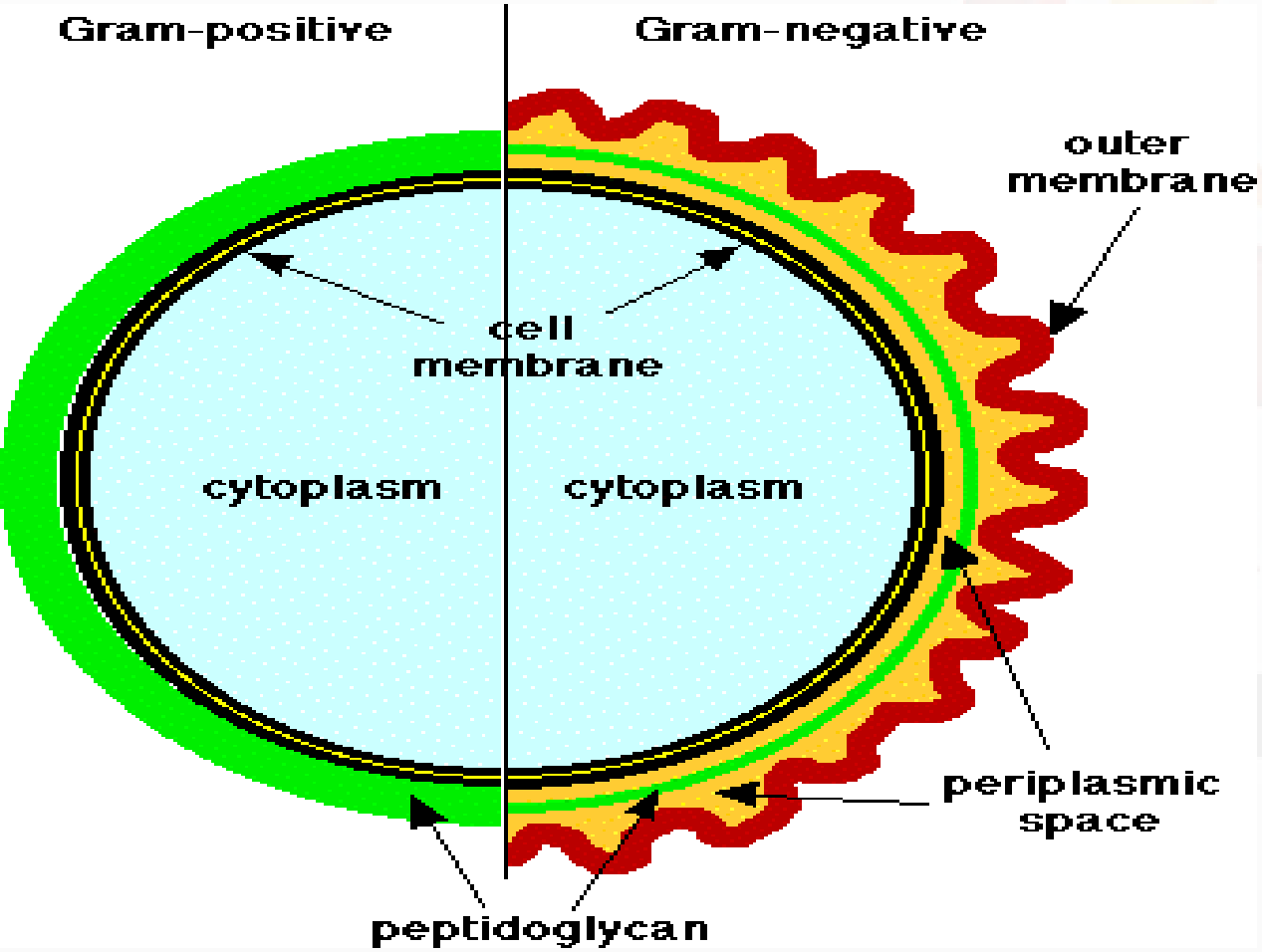
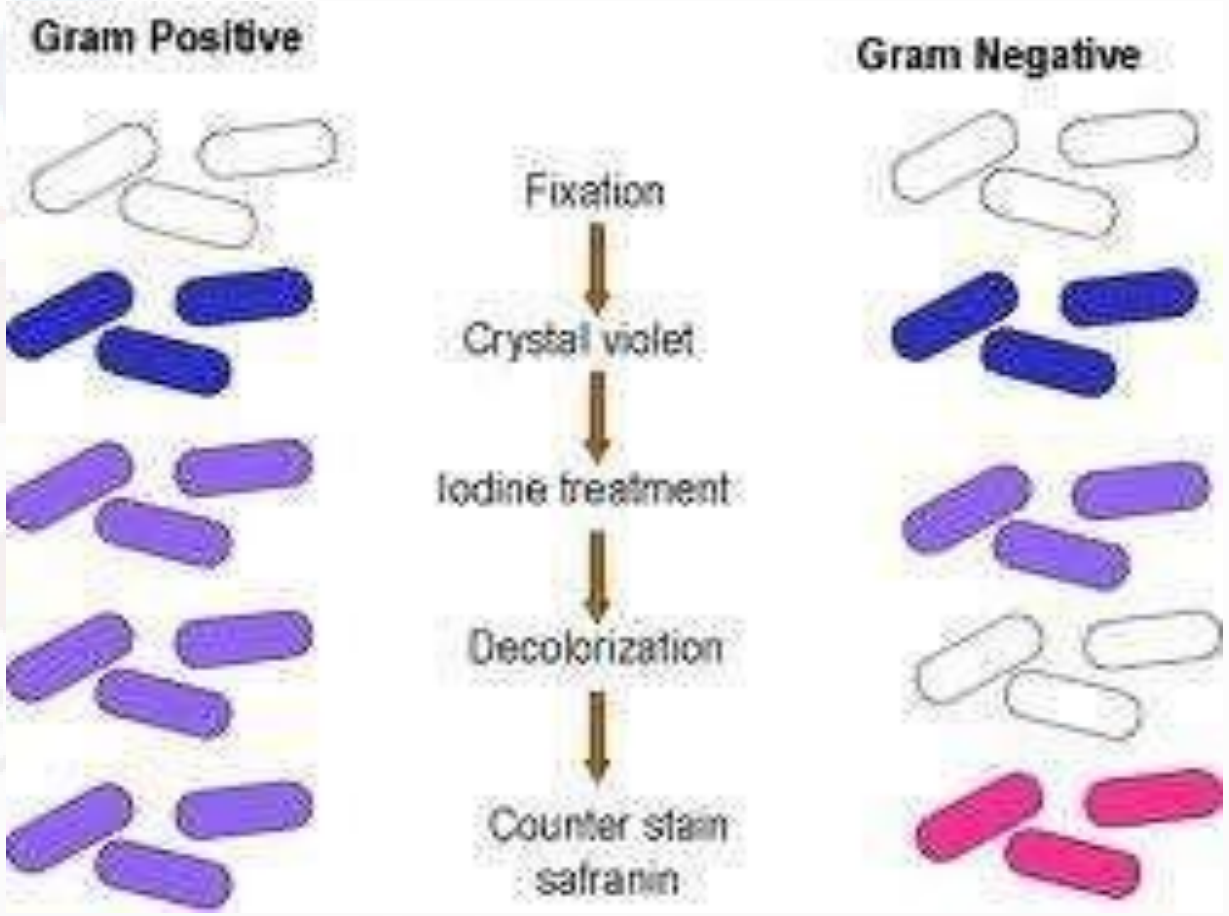
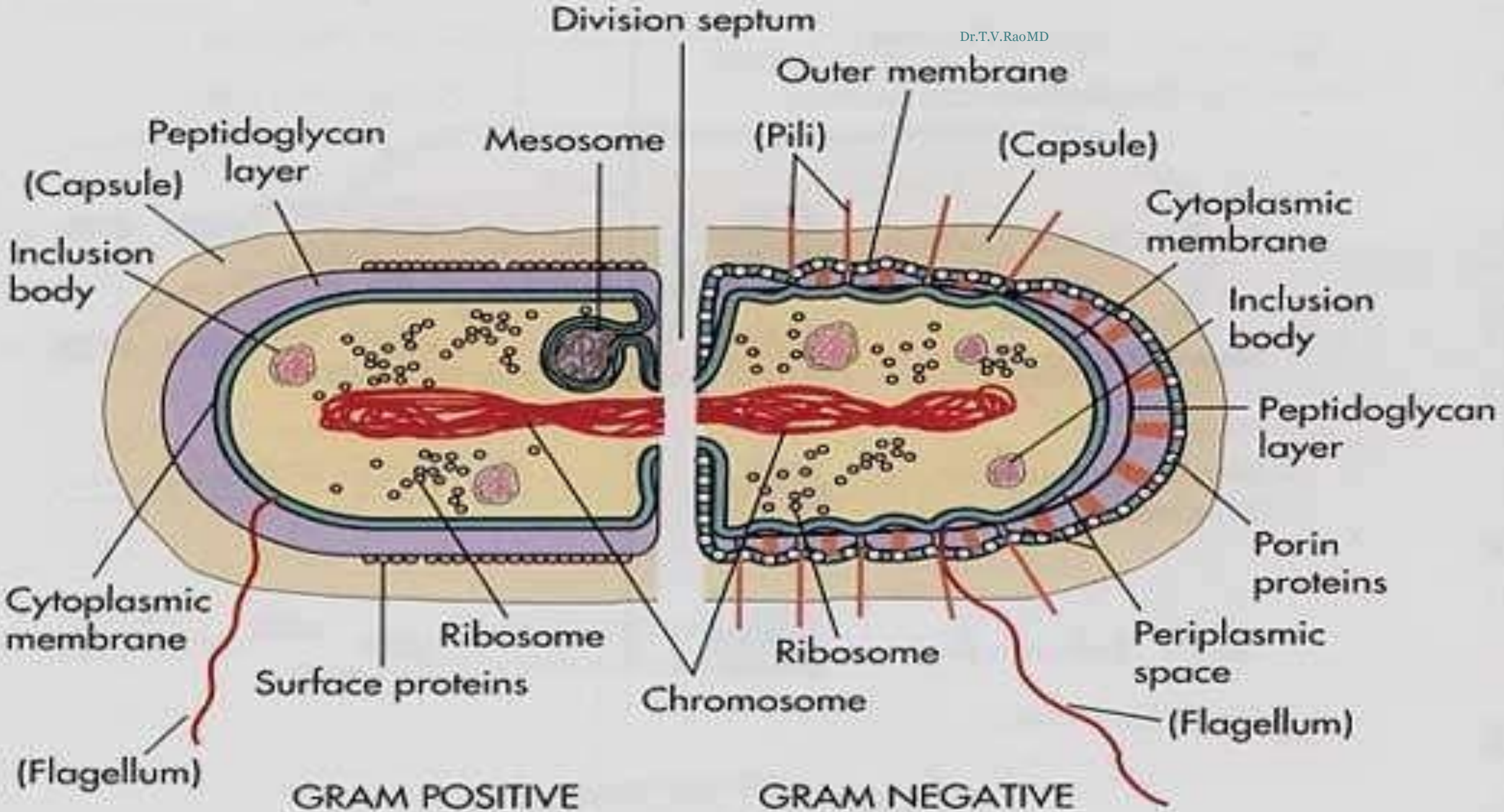


Gram Staining technique

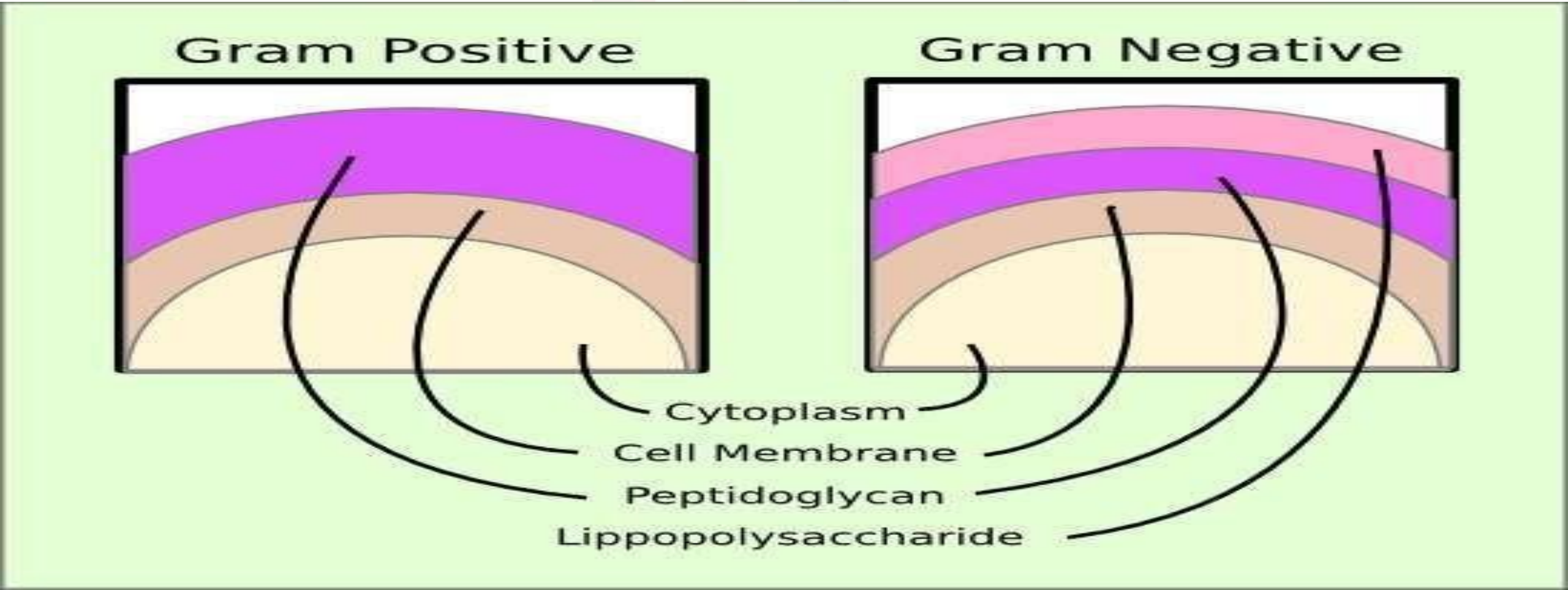
Structure and Reactivity to *Gram Staining*.



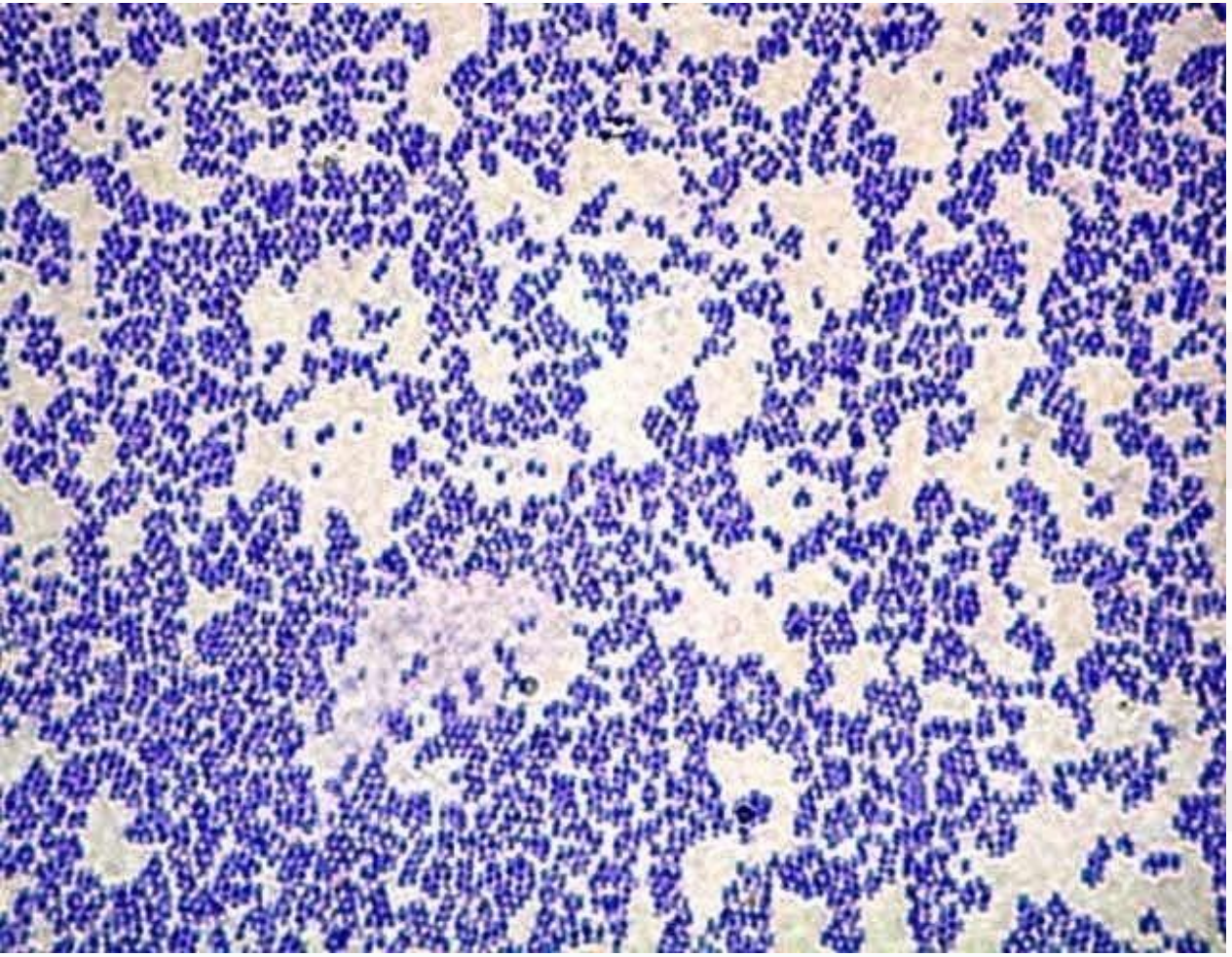


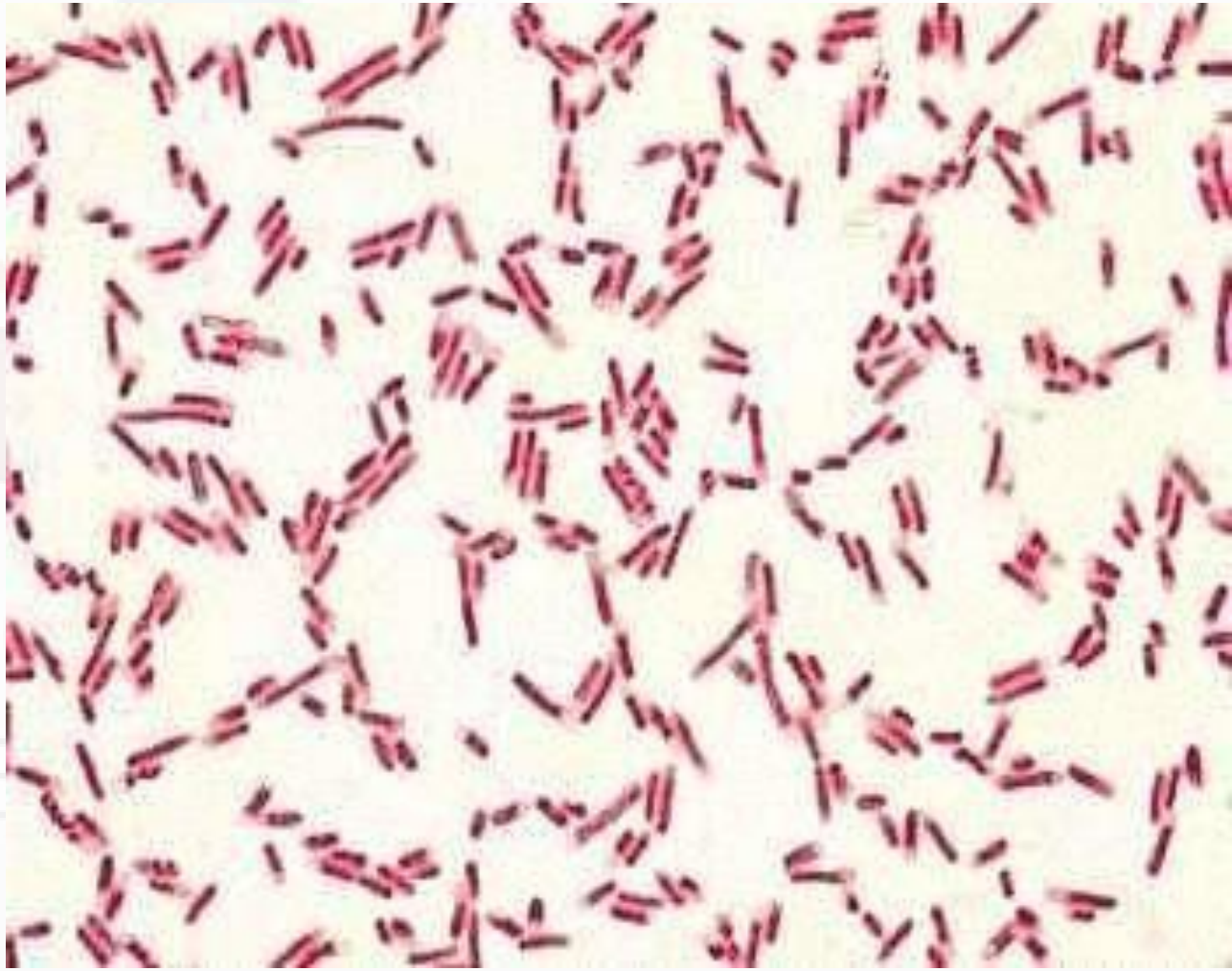

Division septum
Outer membrane
Peptidoglycan layer
Mesosome
(Pili)
(Capsule)
(Capsule)
Cytoplasmic membrane
Inclusion body
Inclusion body
Peptidoglycan layer
Porin proteins
Cytoplasmic membrane
Ribosome
Surface proteins
Chromosome
Ribosome
Periplasmic space
(Flagellum)
(Flagellum)
GRAM POSITIVE
GRAM NEGATIVE

Cell structure differentiates Gram positive from Gram Negative



Gm+ve cocci & Gm-ve bacilli



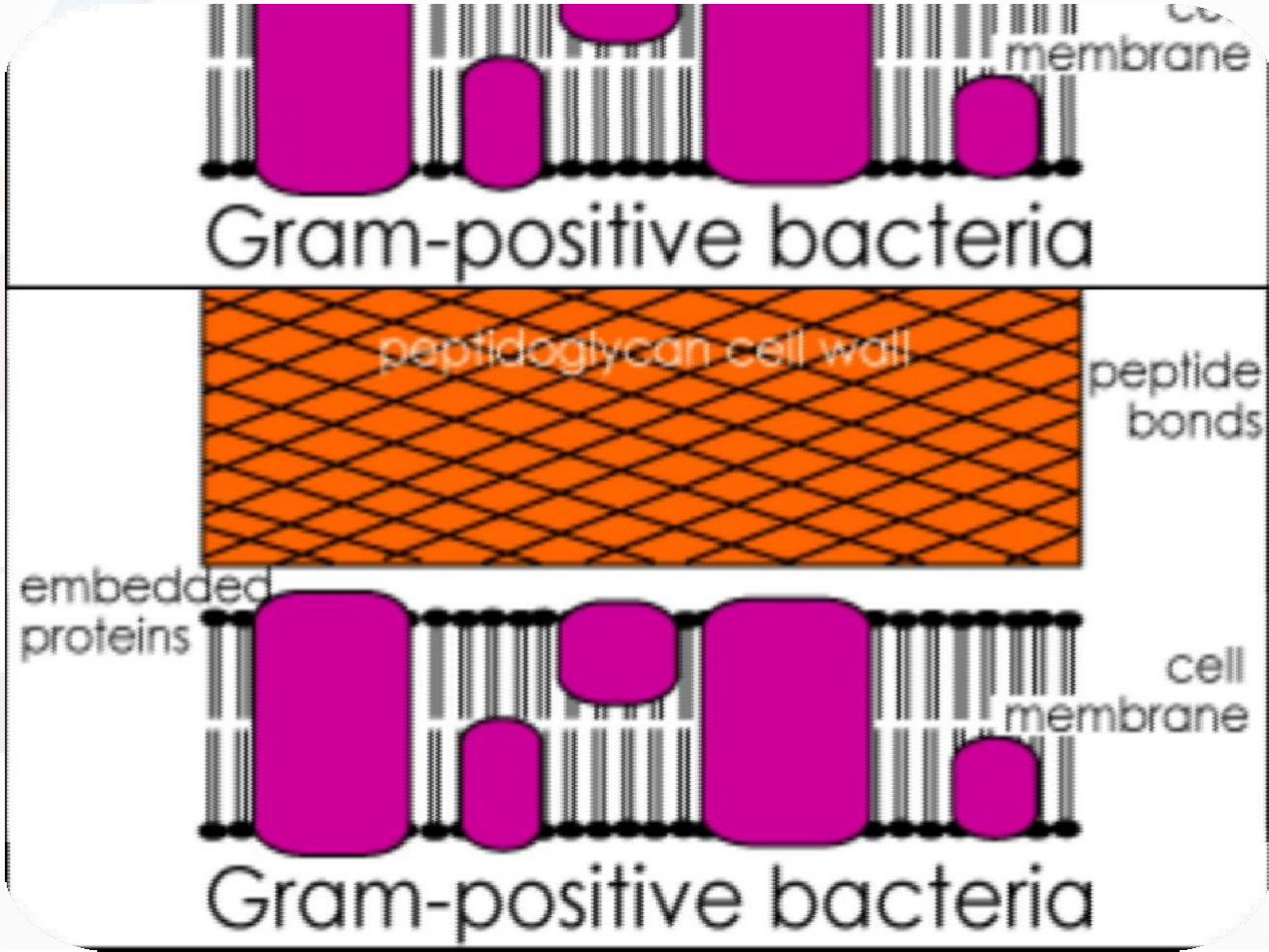


Gram-positive

- **Gram-positive** bacteria are those that are stained dark blue or violet by Gram staining. This is in contrast to Gram-negative bacteria, which cannot retain the crystal violet stain, instead taking up the counter stain (safranin or fuchsine) and appearing red or pink. Gram- positive organisms are able to retain the crystal violet stain because of the high amount of peptidoglycan in the cell wall. Gram-positive cell walls typically lack the outer membrane found inGram-negative bacteria.

GRAM-POSITIVE BACTERIA

- GRAM-POSITIVE BACTERIA are characterized by having as part of their cell wall structure peptidoglycan as well as polysaccharides and/or teichoic acids. The peptidoglycans which are sometimes also called murein are heteropolymers of glycan strands, which are cross-linked through short peptides.



What are Gram Negative Bacteria

- **Gram-negative bacteria** are those bacteria that do not retain crystal violet dye in the Gram staining protocol. In a Gram stain test, a counter
stain (commonly safranin) is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color. The test itself is useful in classifying two distinct types of bacteria based on the structural differences of their cell walls. On the other
hand, Gram-positive bacteria will retain the crystal violet dye when washed in a decolorizing solution.

Gram negative bacteria

- On most Gram-stained preparations, Gram-negative organisms will appear red or pink because they are counterstained. Due to presence of higher lipid content, after alcohol-treatment, the porosity of the cell wall increases, hence the CVI complex (Crystal violet - Iodine) can pass through. Thus, the primary stain is not retained.