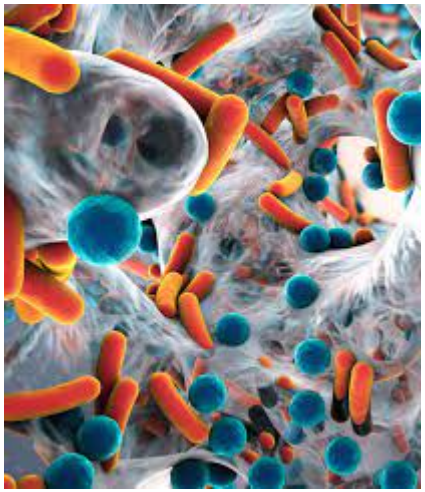
STAINING METHODS



Dr. Orass Madhi

STAINING METHODS:

A - Simple staining technique:

- Simple stains are used to demonstrate the presence of organisms and the nature of any cell present in the smear by applying only one dye.

-Stains in general can be divided into three groups: Basic , Acidic and Neutral .

Acidic Stains	Basic Stains	Neutral Stains
Nigrosin	Crystal violet	Giemsa
Malachite green	Methylene blue	Leishman
Acid fuchsin	Safranin	Wright
	Basic fuchsin	

-As bacterial cells are rich in nucleic acid (which has a negative charge) it will follow that “basic stain ,bearing its coloring matter in the positive charge , will be attracted to the organism and stain it” . Acid stain ,however, will not stain the bacteria ; they are used mainly for staining the background material a counterstaining color.

Procedure of simple staining:

1. Flood the slide with Loffler's methylene blue for 5-10min.

2. Wash off the stain with slowly running tap water.

3. Allow the to slide to dry in air or placed it between two sheets of filter paper.

4. Examine under oil immersion.

B.Differentional (compound) staining technique:

- It consists of more than one dye used successively to identify organisms according to their type of reaction.

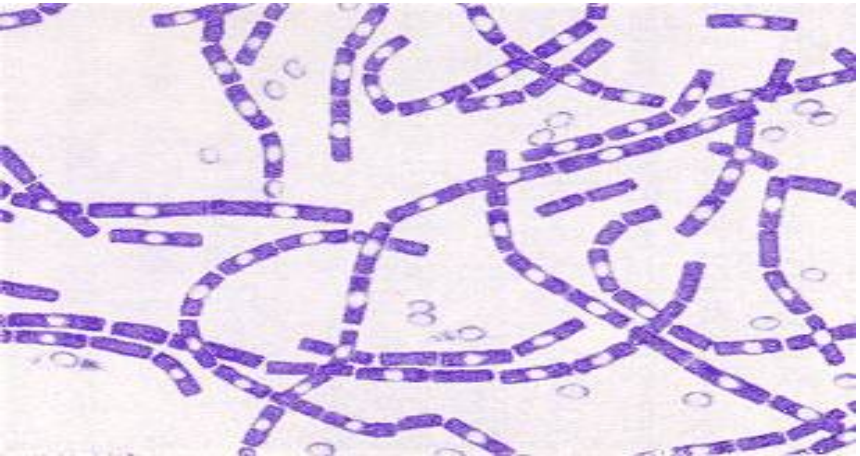
- The most important examples for this group of stains are:

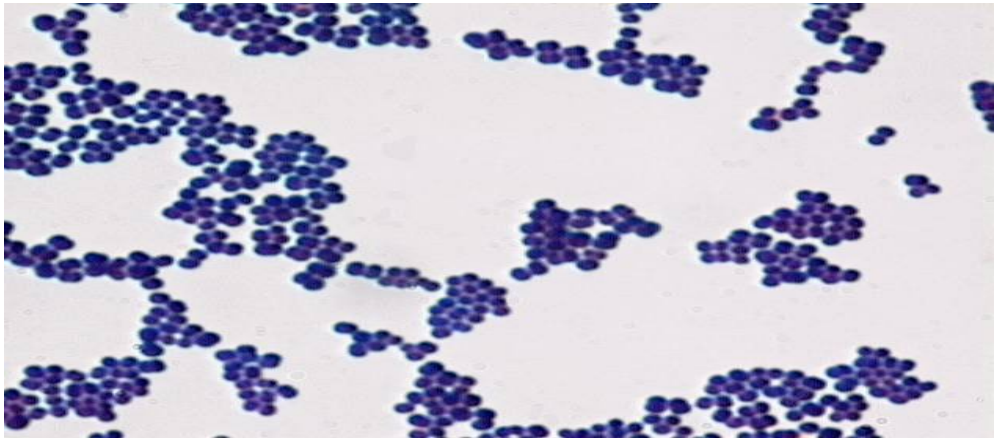
1. Gram's staining method
2. Acid fast stain (zeihl neelsen methods)
3. Spore stain

GRAM STAINING METHOD:

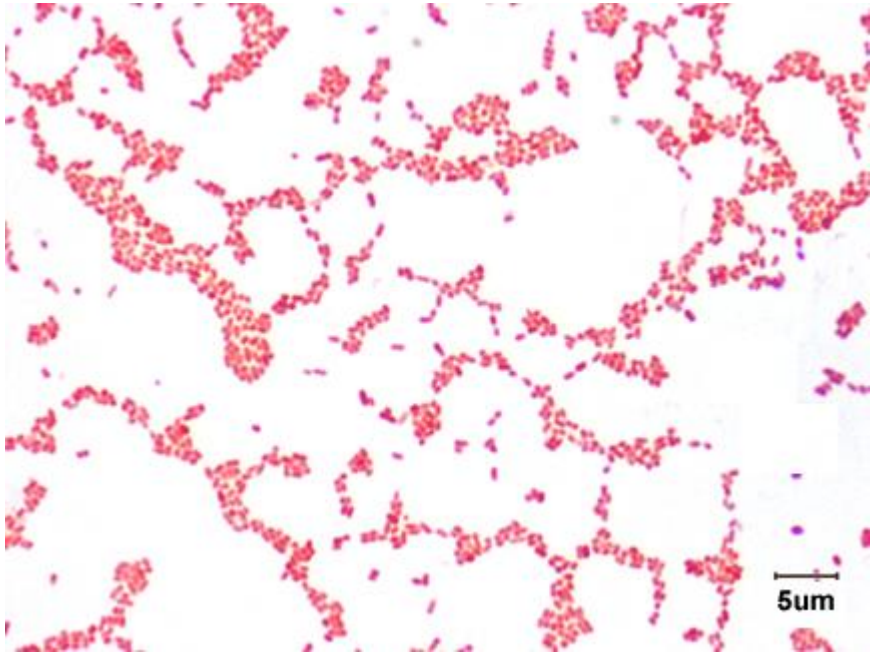
- It is one of the most important methods widely used in bacteriology dicovered in 1884 by gram (a Danish physician), using two dyes in sequence each of different color. He found that bacteria fall into two different categories:

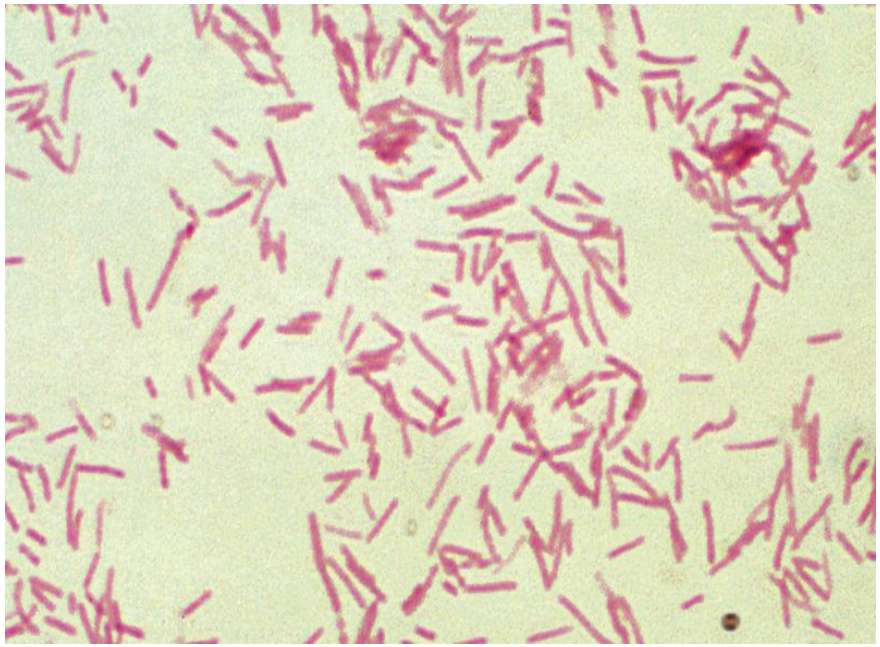
A) Those that retained the first dye **(crystal violet)** throughout the staining procedure are known as **"GRAM POSITIVE"**



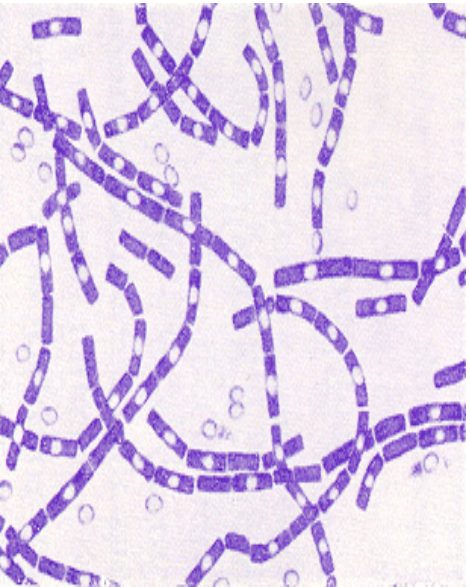


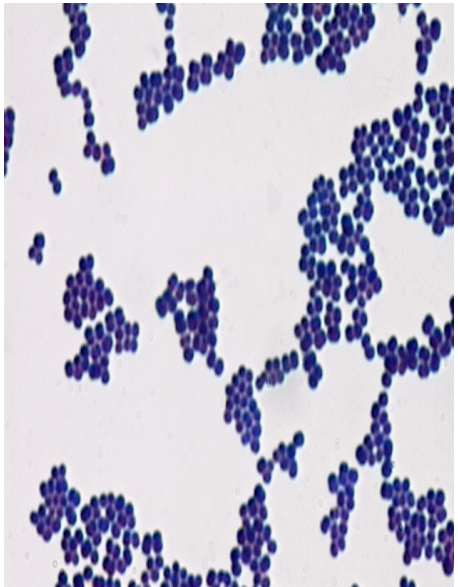
B) Those that lost the first dye **(crystal violet)**after washing with adecolorizing solution and stained with the second dye (safranine) are known as **“GRAM NEGATIVE”**

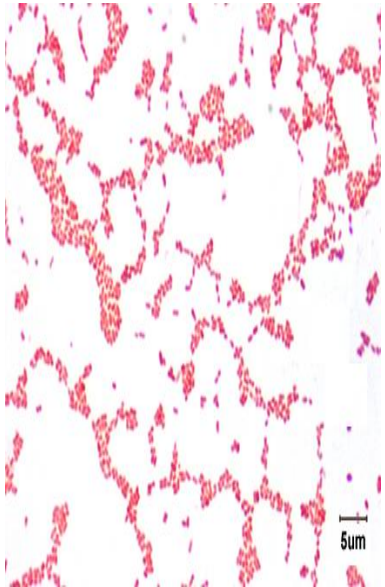


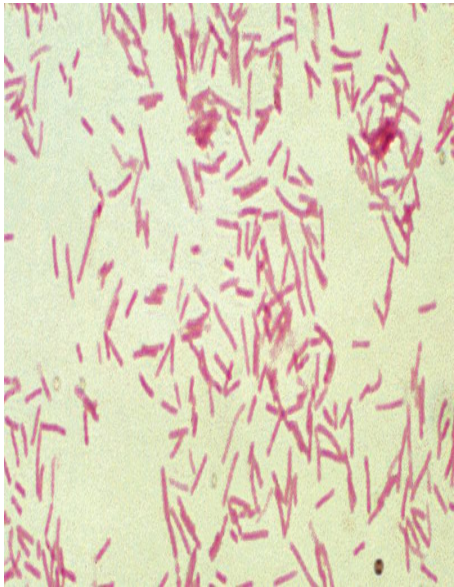


- IN CONCLUSION, the gram positive bacteria appear **violet** ,while gram negative bacteria are **red** in color .therefore ,it is possible to differentiate between bacteria of the same morphology. furthermore, it can be used to determine the relative number and morphology of bacteria in a smear taken directly from a patient









PROCEDURE OF GRAM STAINING:

1 - Flood the slide with **crystal or gention violet**,leave to act for 1-2min. ,wash with tap water.

2 - Apply gram's iodine (a mordant),leave to act for one minute ,wash with tap water.

3 - Apply 95%ethyl alcohol (a decolorizer).leave to act for 20-30 seconds ,wash with tap water.

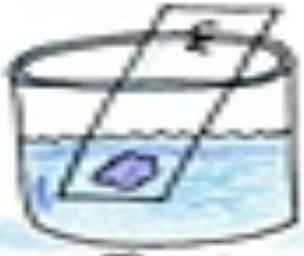
4 - Apply saffranin (the counter stain), leave to act for 1-1.5min. , wash with tap water, blot, dry in air and examine with oil immersion lens.

① Prepare Slide: bacteria dried onto slide

② Stain with crystal violet (1-2 minutes)

③ Pour off stain and rinse with water

④ Stain with Gram's iodine (1-2 minutes)



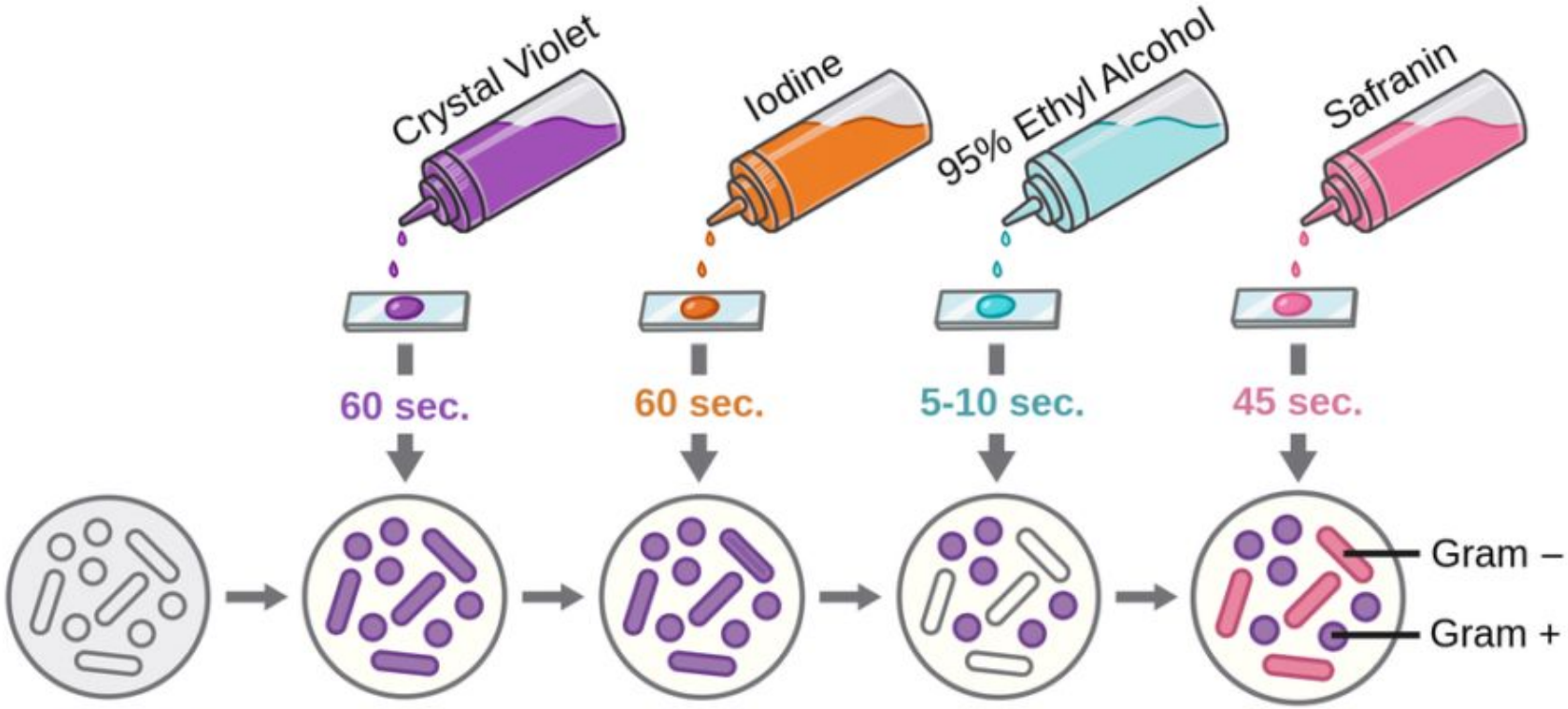
⑤ Pour off iodine and rinse with water

⑥ Rinse with 95% alcohol (50 seconds)

⑦ Rinse with water

⑧ Stain with Safranin

⑨ Pour off stain and rinse with water



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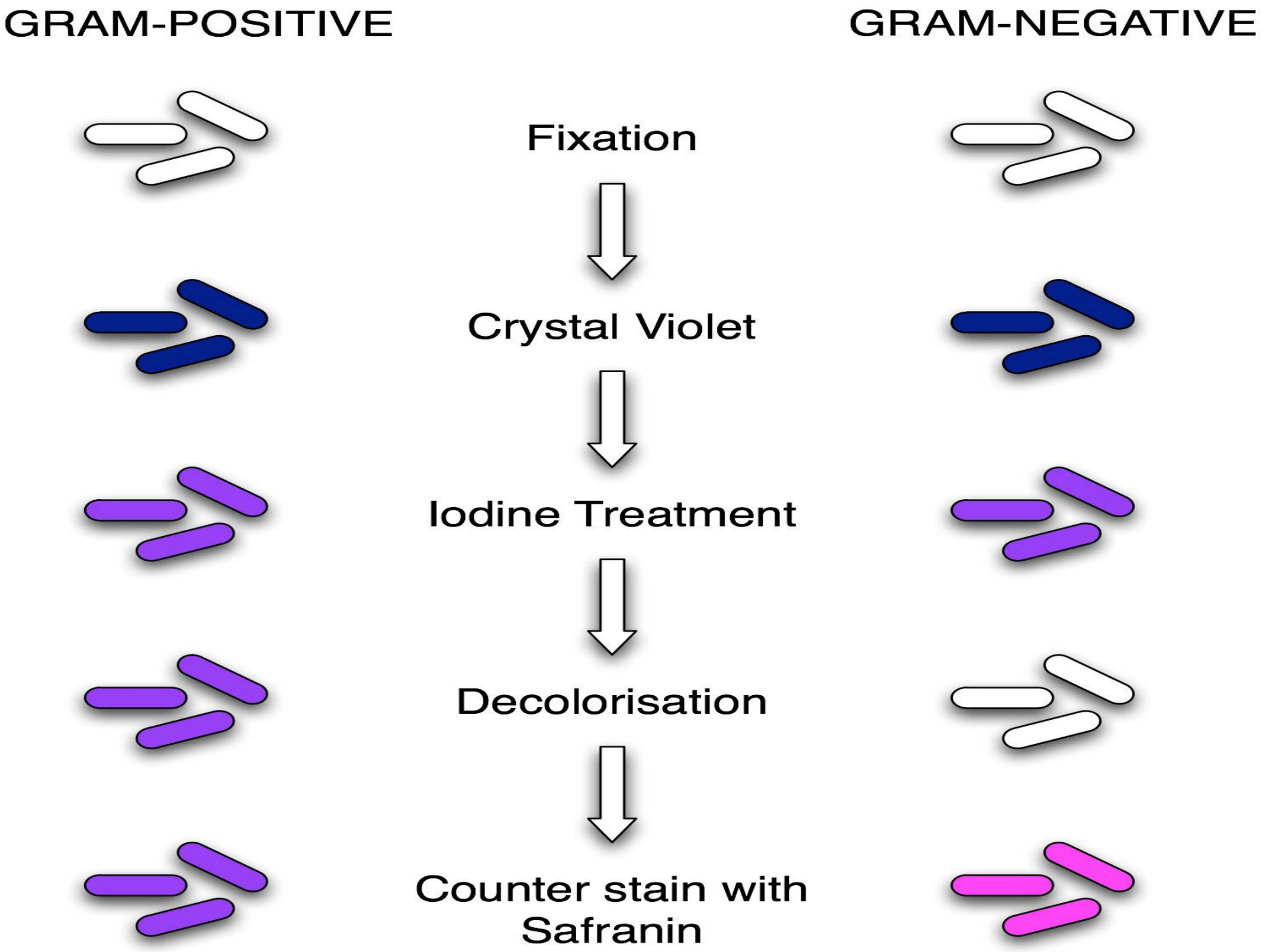

GRAM-POSITIVE
GRAM-NEGATIVE
Fixation
Crystal Violet
Iodine Treatment
Decolorisation
Counter stain with Safranin

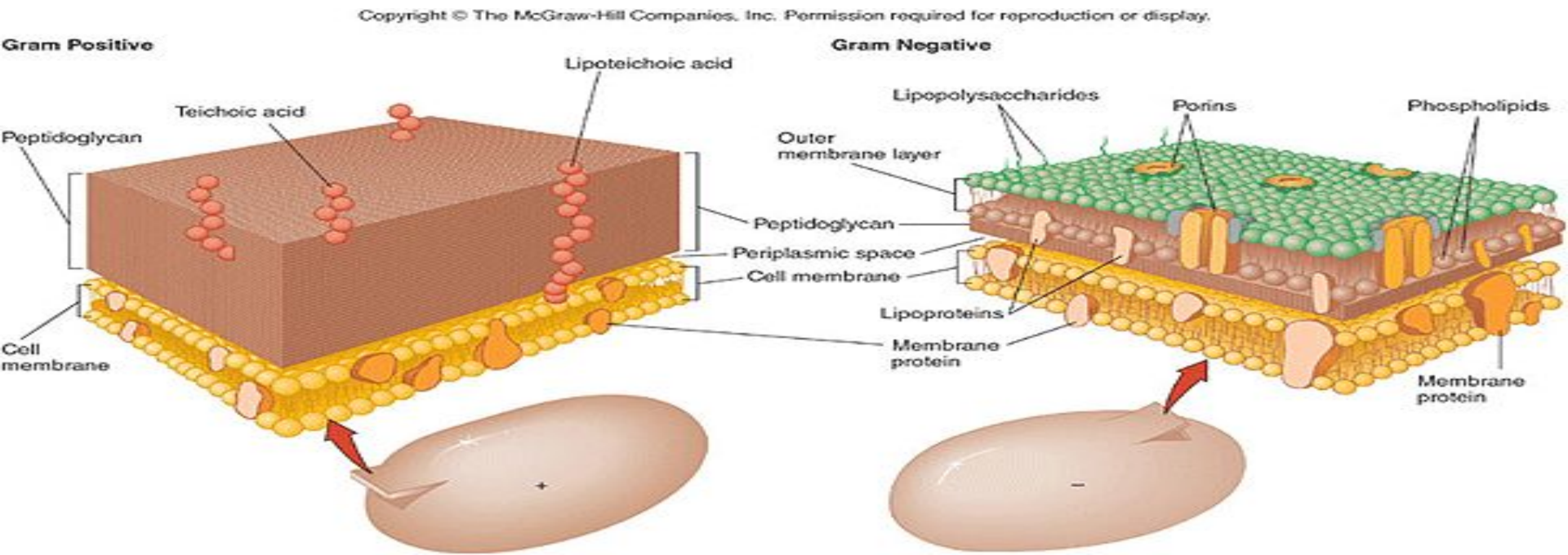
MECHANISM OF STAINING:

The division of bacteria into two categories, indicates a basic chemical differences between gram positive and gram negative bacteria. The most important differences are:

1 - The cell wall of Gram-negative organisms have relatively little peptidoglycan and mainly consist of lipoproteins and polysaccharides. While in Gram positive organisms the peptidoglycan comprises the major part of the cell wall rendering them more rigid than Gram negative cells and less permeable for the dye iodine complex to diffuse freely out of the cell during the process of decolourization.

2 - The more acid charater of the protoplasm of Gram positive bacteria which is enhanced by treatment with iodine may partly explain their stronger retention of the basic dye.

3 - Integrity of the cell wall.



Acid-Fast Stain

The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups.
This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid-fast staining.

Summary of Acid-Fast Stain

Application of	Reagent	Cell colour	
		Acid fast	Non-acid fast
Primary dye	Carbol fuchsin	Red	Red
Decolorizer	Acid alcohol	Red	Colorless
Counter stain	Methylene blue	Red	Blue

Procedure of Acid-Fast Stain