Staining

By
Lecturer
Anfal I. Alawadi

Types of staining methods:

Simple staining : is composed of one type of stain e.g, crystal violate, safranine. methylene blue.

Differential staining : It gives different color to different bacteria e.g.

Gram stain, and Ziehl neelsen stain.

Special staining : like Capsule stain , flagella , spores

Differential staining

A:Gram Stain

Introduction

First devised by Hans Christian Gram during the late nineteenth century, the gram stain can be used to divide most bacterial species effectively into tow large groups: those that take up the basic dye, Crystal violet (gram – positive), and those that allow the crystal violate dye to wash out easily with the decolorizer alcohol or acetone (gram – negative).

Reagents Used In Gram Stain

1. Gram Crystal Violet
2. Gram Iodine
3. Gram Decolorizer
    a. Methanol
    b. Acetone
4. Gram Safranin

Cont...

1. **CRYSTAL VIOLET**
- **Primary** stain
- **Violet** colored, stains all micro-org

2.GRAM **IODINE**
- **Mordant**
- Forms Crystal **violet iodine** complexes

3.DECOLORIZER
- Acetone + Methanol
- Removes Crystal violet iodine complex from thin peptidoglycan layers
- Dissolves outer layer of Gram negative org

4.GRAM **SAFRANINE**

- **Counter** stain
- **Red** colored
- Stains thin walled Gram negative org

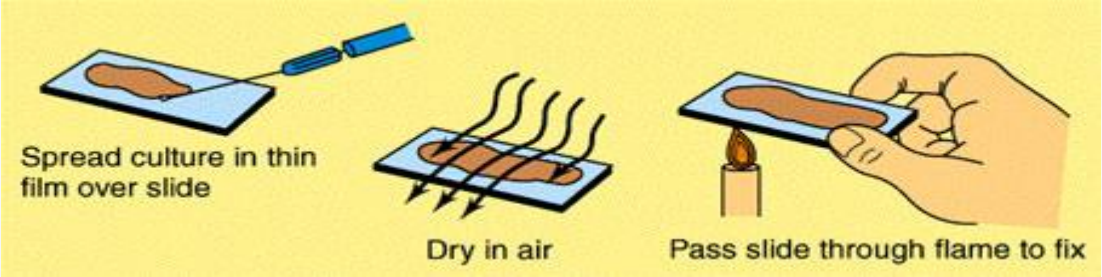
Gram Staining kit

The Gram Stain Procedure

Step 1-Prepare a Smear

“Bacteria”

Suspend some of the material to be stained in a drop of water on a microscope slide ,Then spread the drop . Fix by Heat or Allow to air dry.



Step 2-Apply the Primary Stain

Flood the Smear with **Crystal Violet**

Allow to stand for 1 min

Rinse with water to remove excess stain

Step 3-Apply the Mordant

Flood the Smear with **Iodine** solution
Allow to stand 2 min

Step 4 -Rinse

Rinse with water to remove excess Iodine

Step 5 -Decolorize

Decolorizer by alcohol
across the slide about 5 sec
The slide should appear clear

Step 6 -Rinse

Rinse with water to remove excess alcohol

Step 7-Counter stain

Flood the slide with **Safranin** solution

Let stand for 2 minutes

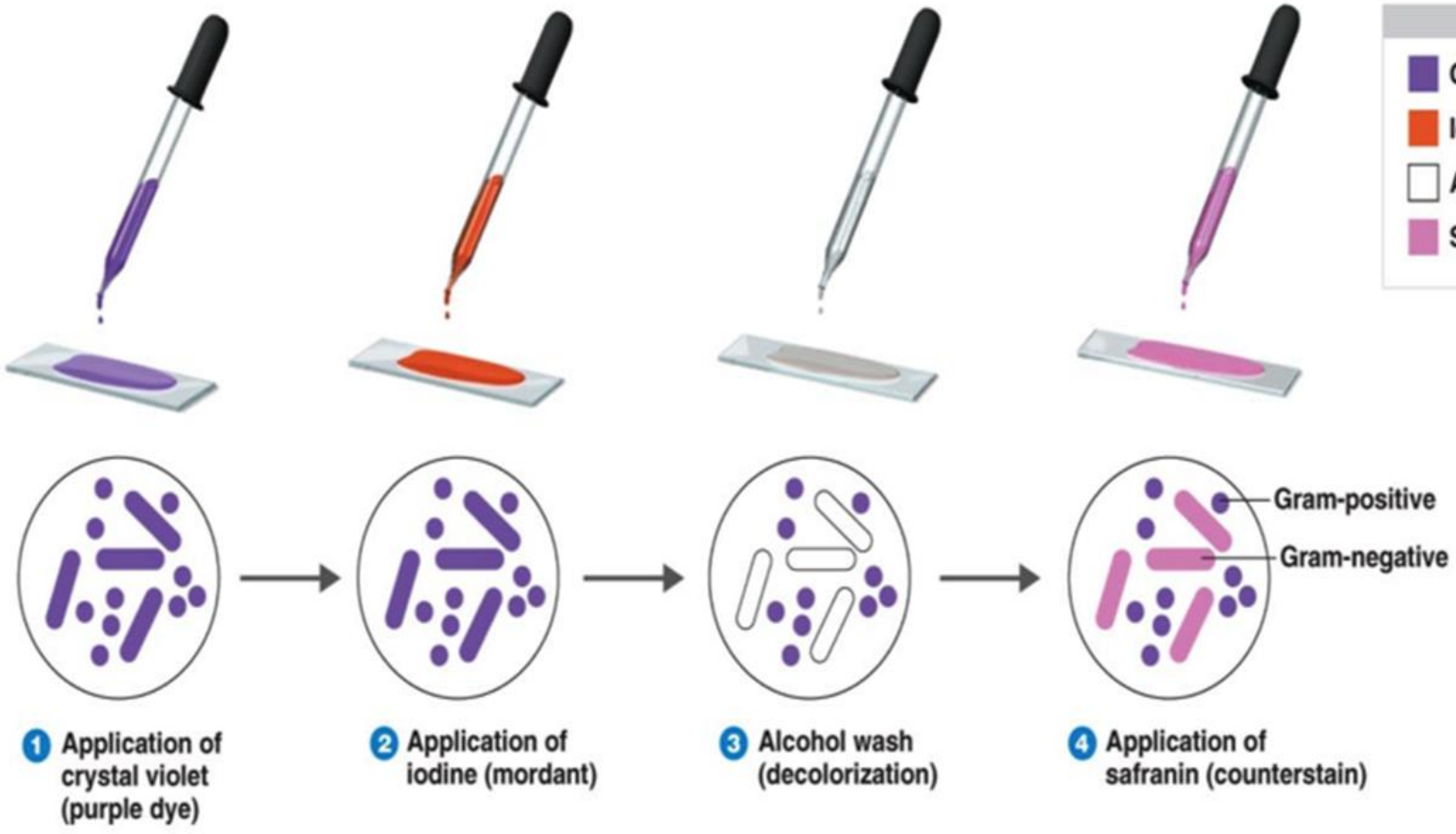
Step 8 –Rinse , Dry and Observe

Gram-Positive Gram-Negative

Rinse with water to remove excess stain

Blot dry

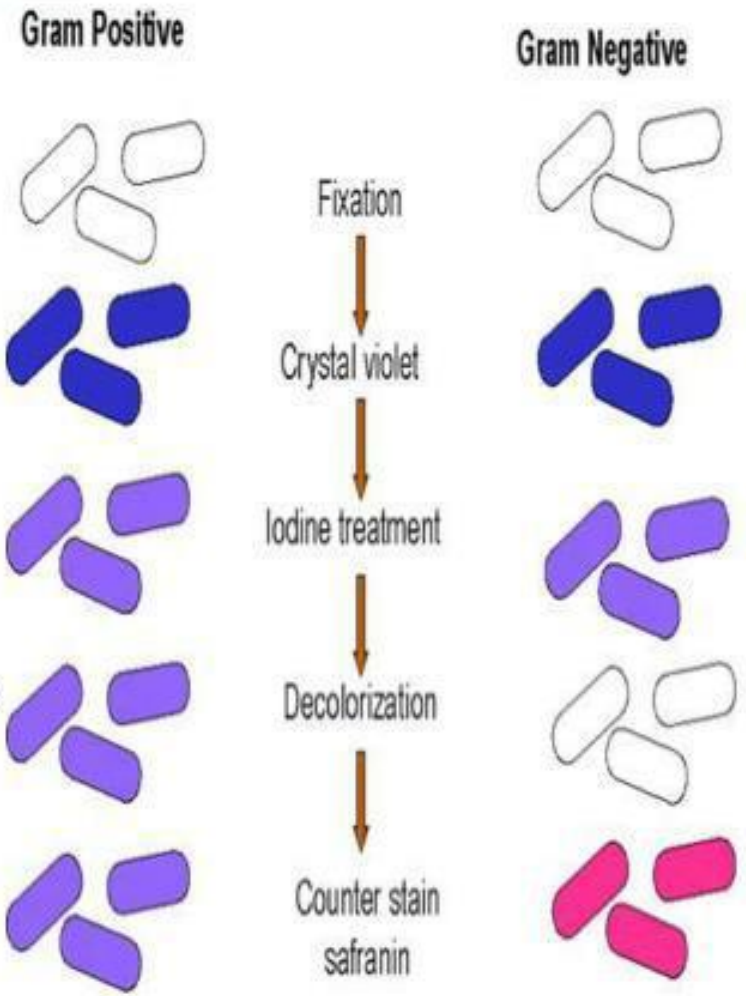
Observe under Oil Immersion

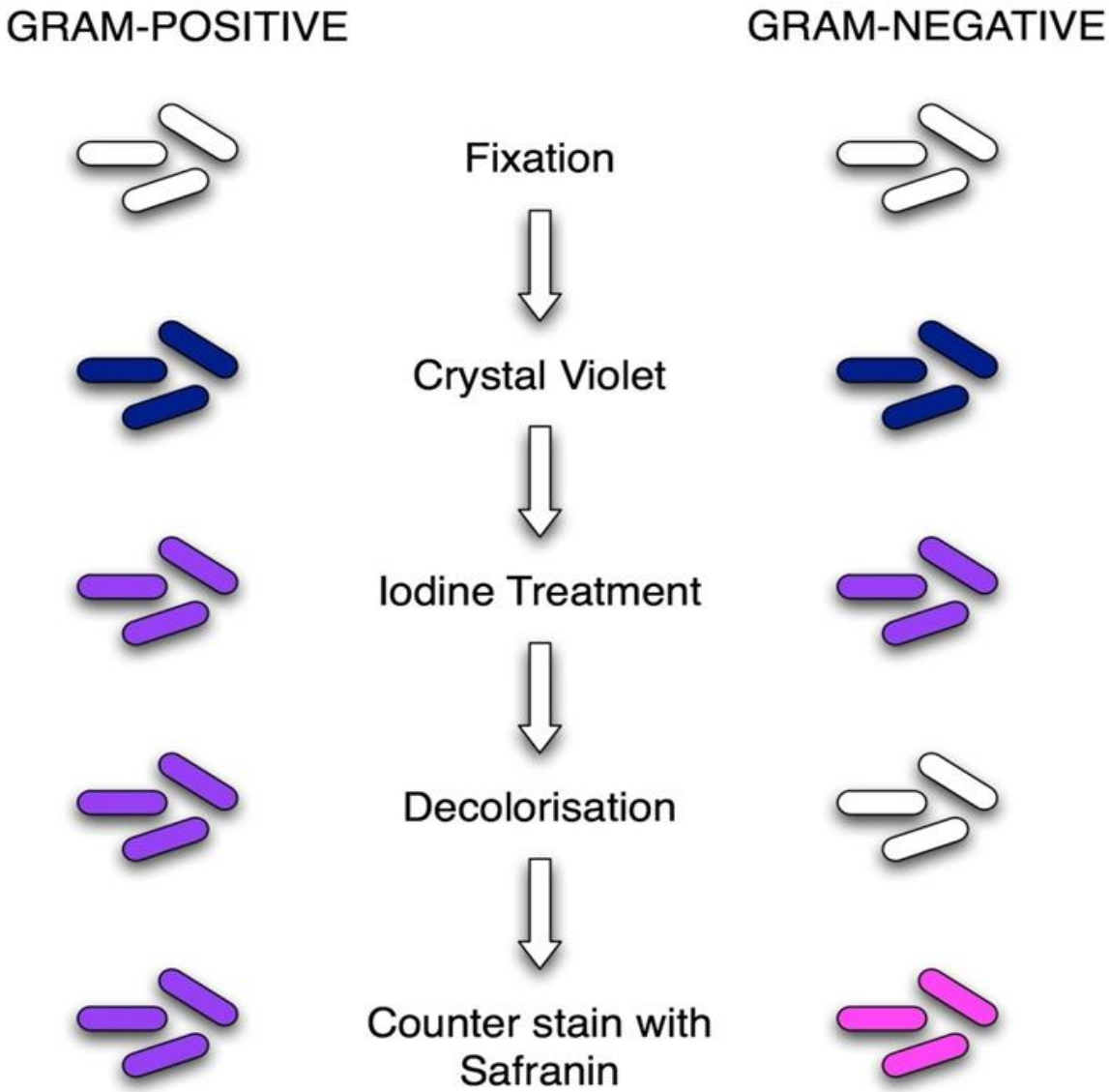

KEY
Crystal violet
Iodine
Alcohol
Safranin
Gram-positive
Gram-negative
1 Application of crystal violet (purple dye)
2 Application of iodine (mordant)
3 Alcohol wash (decolorization)
4 Application of safranin (counterstain)

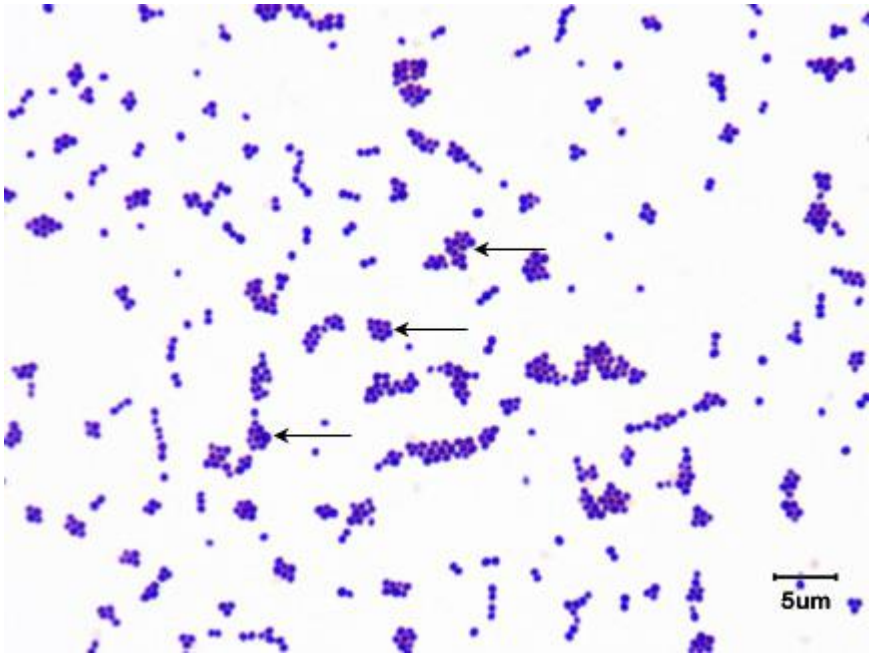
Gram Stain

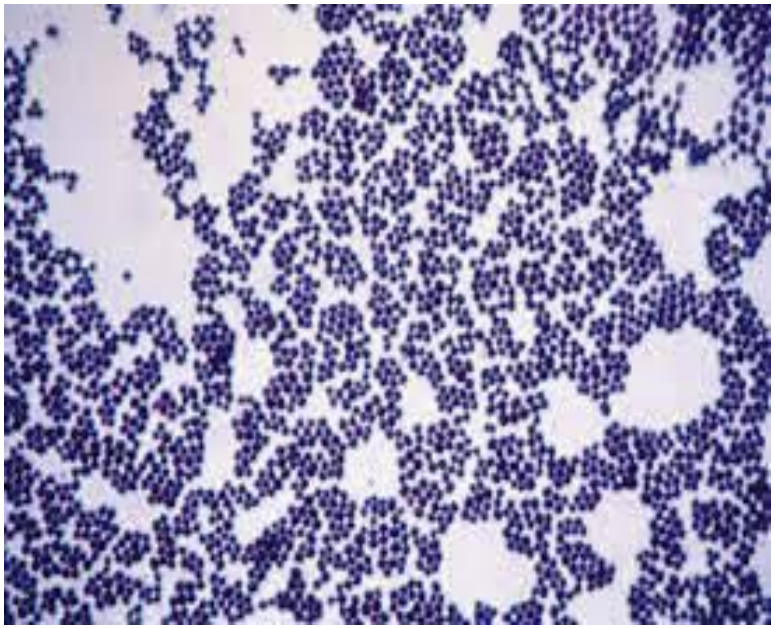
Principle of staining technique:

1. Primary stain:- Crystal Violet
2. Mordant(fixes the dye):- Iodine
3. Decolorizing agent:-Alcohol/Acetone
4. Counter stain;- Safranin

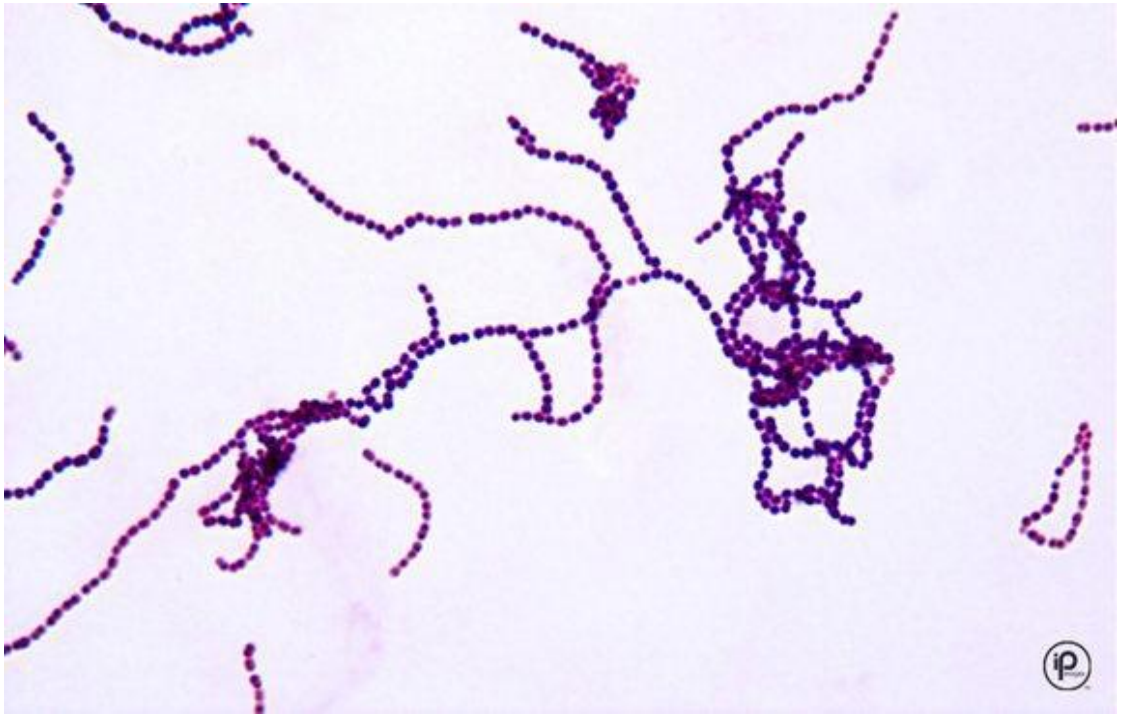


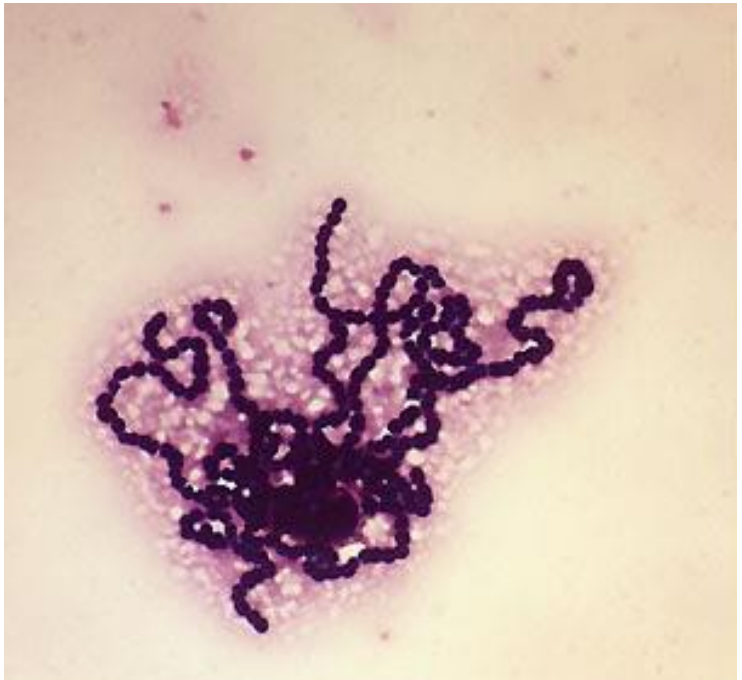

GRAM-POSITIVE
GRAM-NEGATIVE
Fixation
Crystal Violet
Iodine Treatment
Decolorisation
Counter stain with Safranin



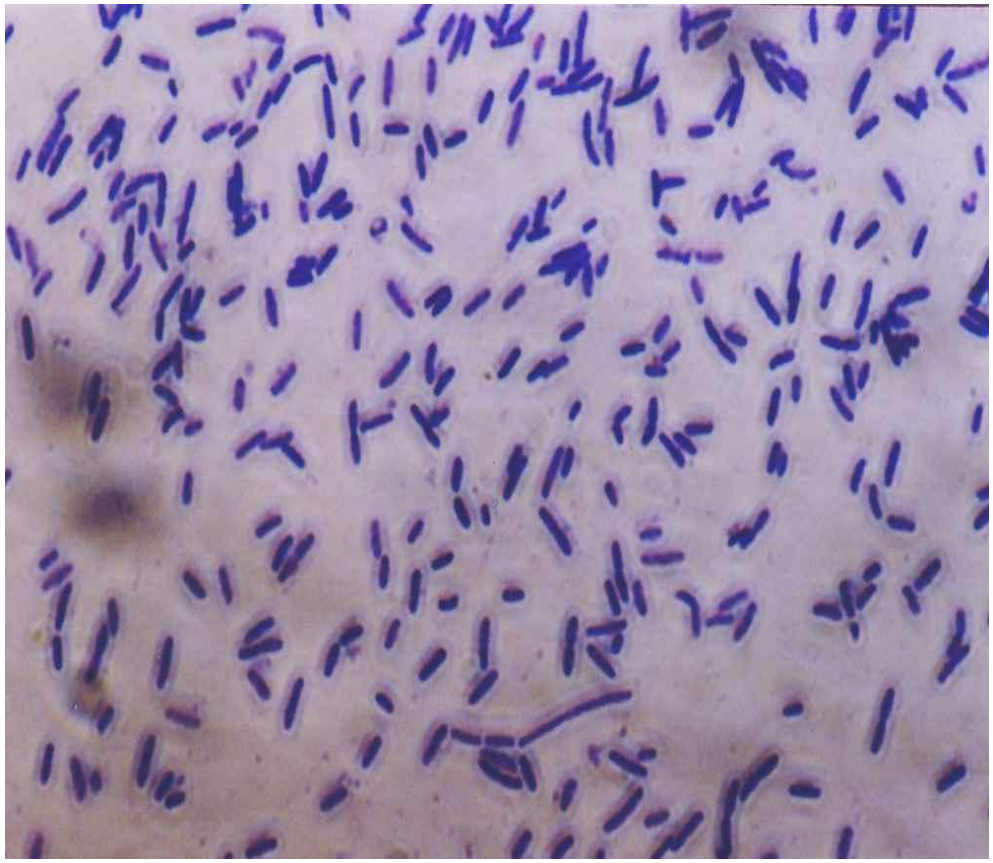


Gram Positive
Staphylococci

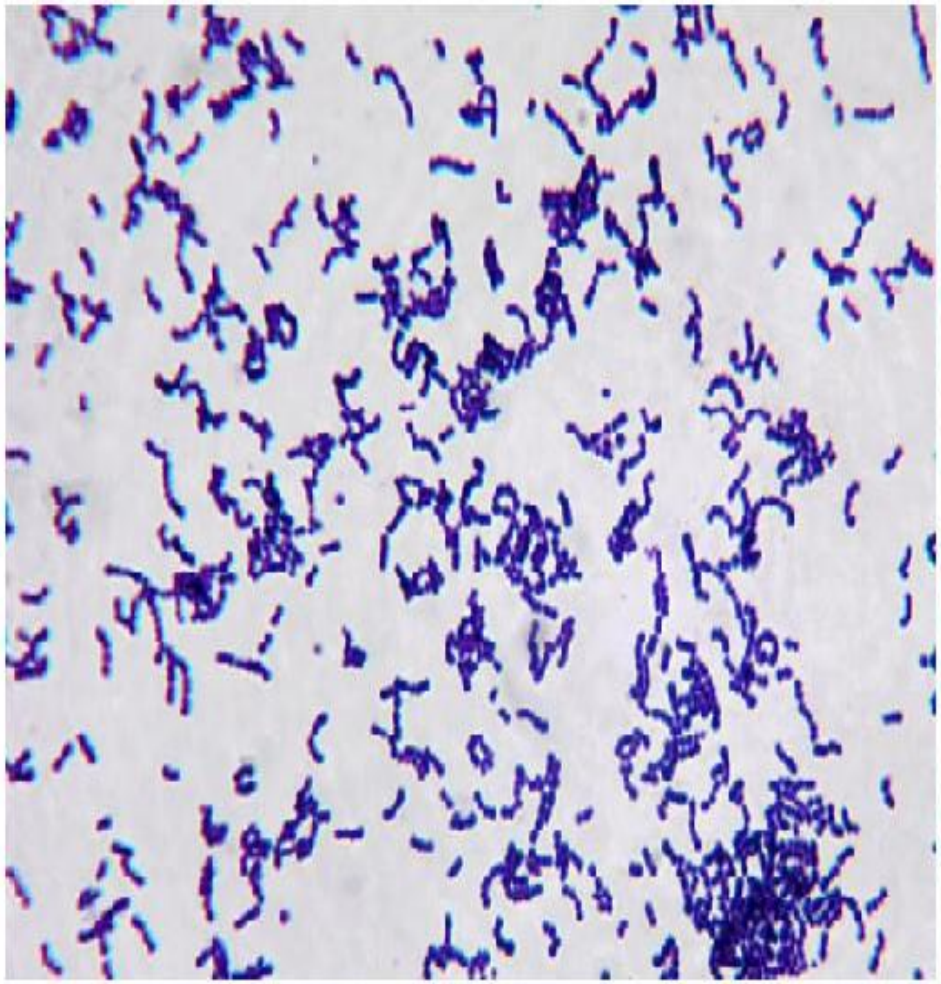




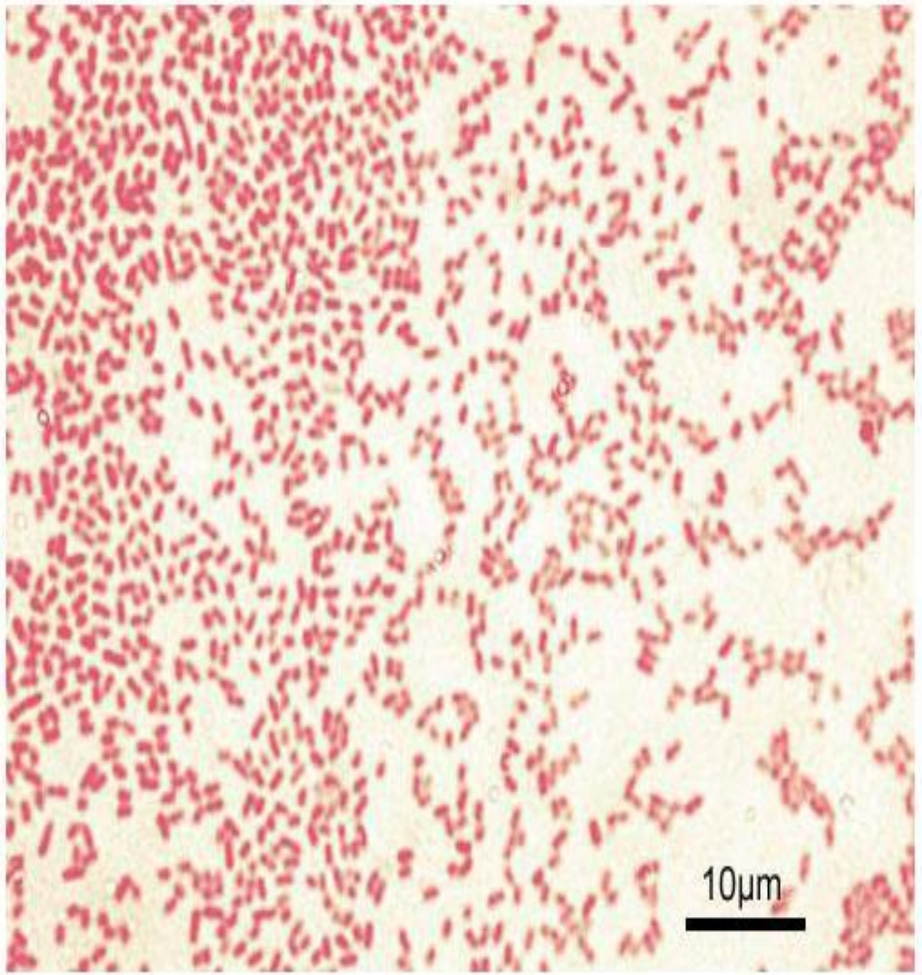
Gram Positive
(*Streptococci*)



Gram Positive
Bacilli

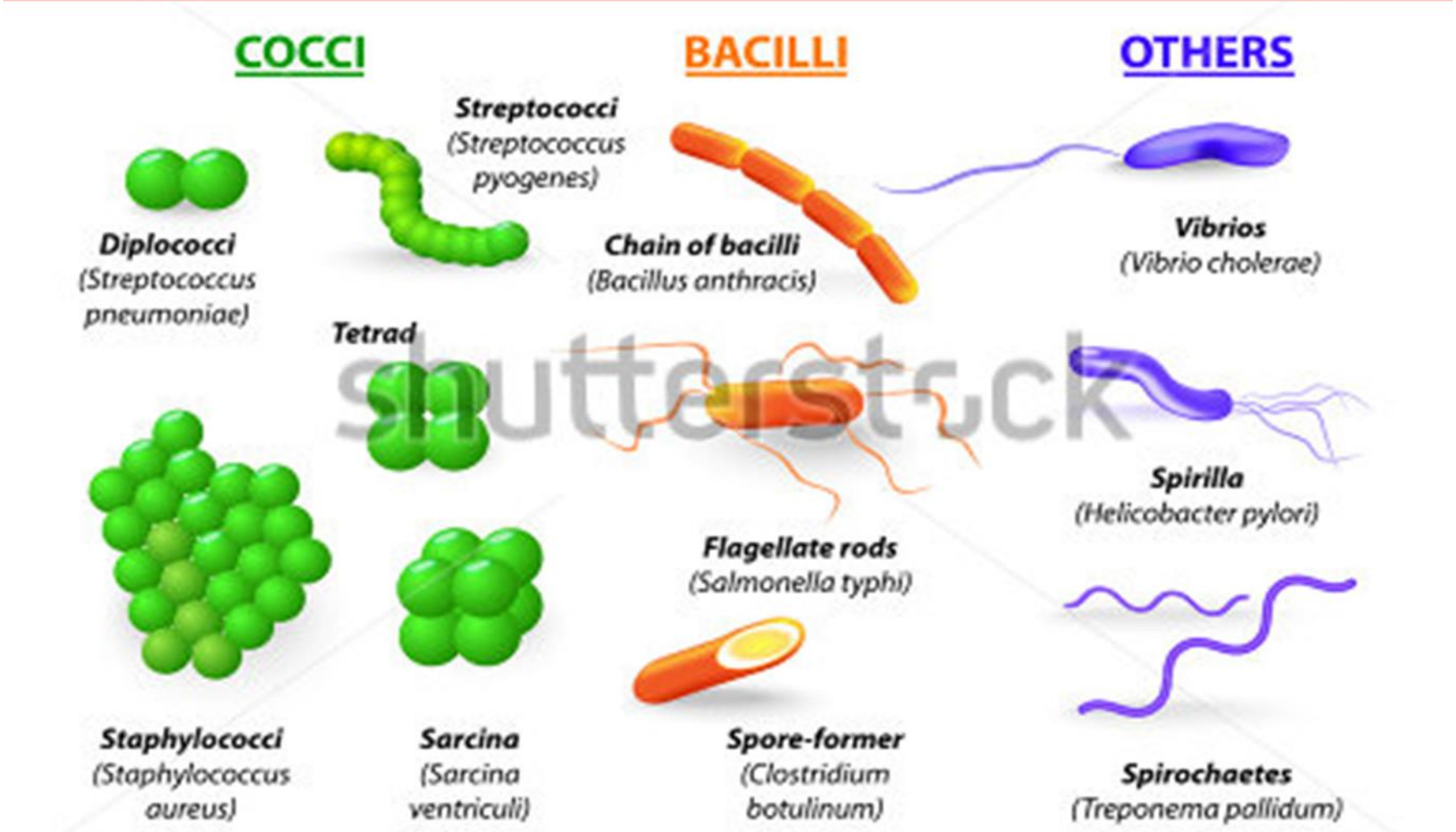


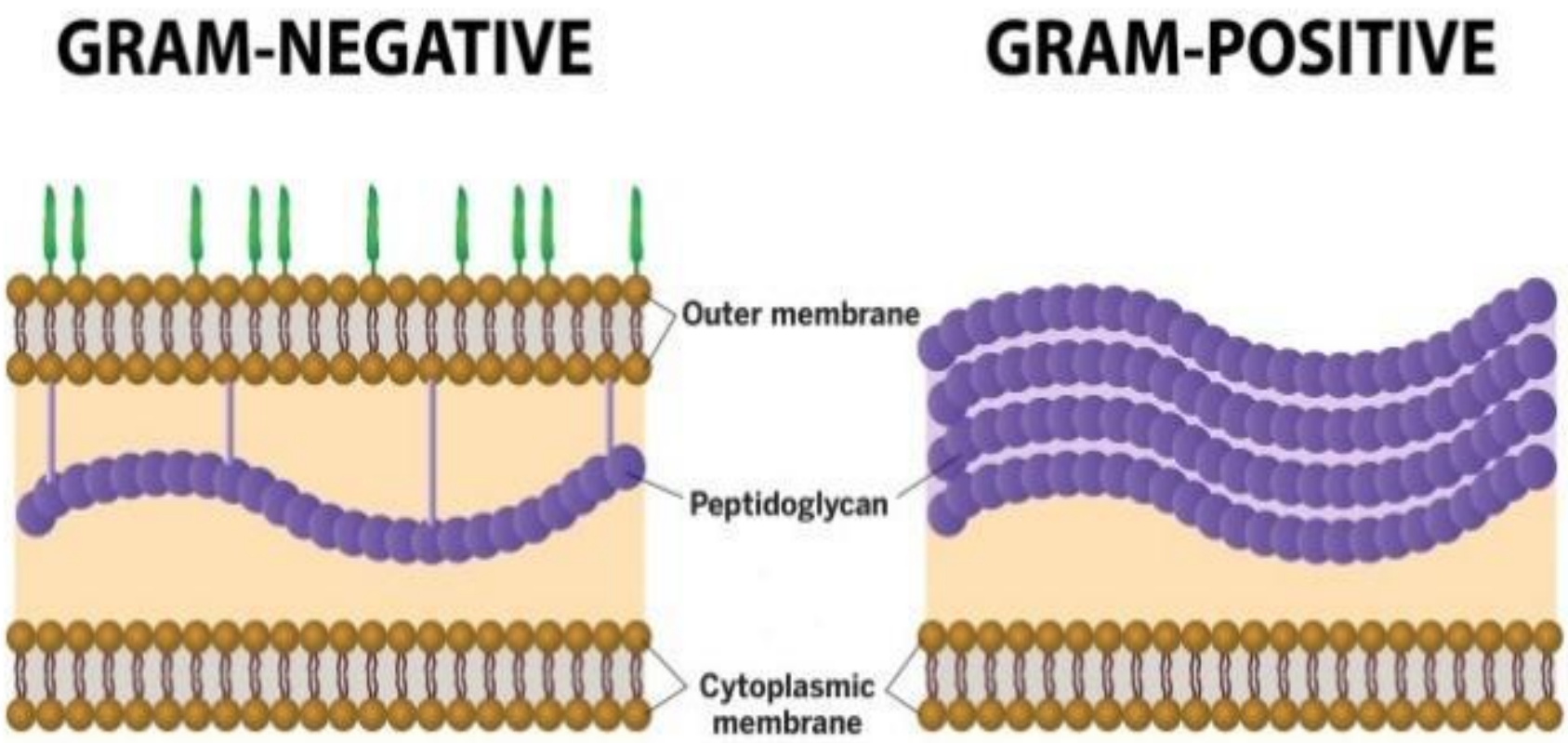
Gram Positive Bacteria



Gram Negative Bacteria

SHAPES OF BACTERIA




GRAM-NEGATIVE
GRAM-POSITIVE
Outer membrane
Peptidoglycan
Cytoplasmic membrane

CELL WALL IN GRAM +VE AND GRAM –VE BACTERIA

Cell Wall Structures	Gram Positive organisms	Gram Negative organisms
Inner cytoplasmic membrane	Present	Present
Peptidoglycan layer	Thick	Thin
Teichoic Acid	Present	Absent
Outer membrane layer	Absent	Present
Lipid A, LPS , Lipo-protien components	Absent	Present
Peri-plasmic space	Absent	Present

Errors in Gram stain technique:-

1. Over decolorization and conc. of alcohol

2. Old culture

3. Thick smear

4. More exposure to heat (during the fixation of smear)

B- Acid fast stain(Ziehl- Neelsen stain) :-

Most bacteria in the genus Mycobacterium contain very high percentage lipid in their cell wall.

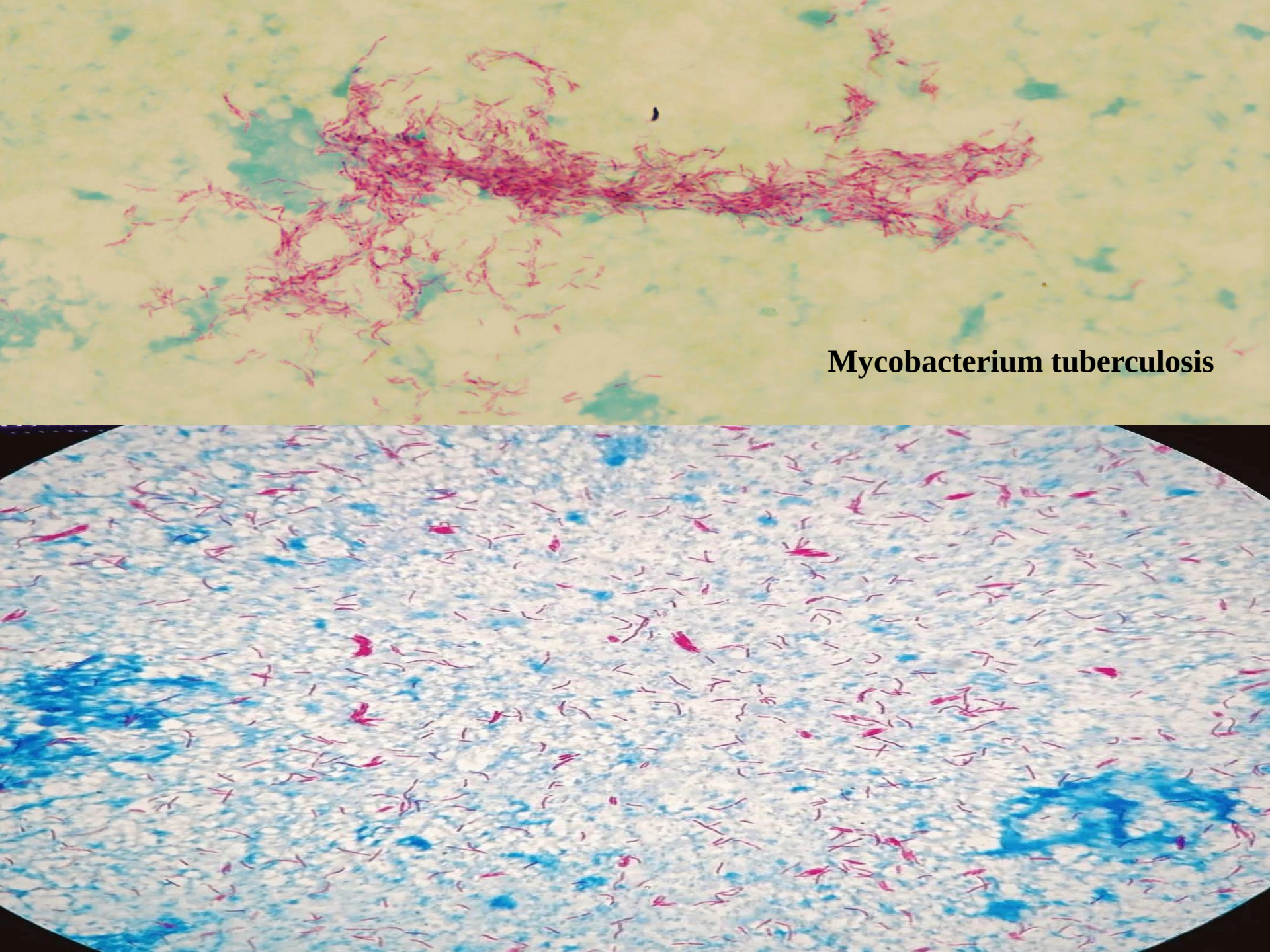
This stain is used in the identification of the tuberculosis bacillus , **Mycobacterium tuberculosis** , and the leprosy organism , **Mycobacterium leprae** .

The Ziehl-Neelsen stain composed of

Carbol fuchsin solution (red color).
Ethanol 95% acidified with 20% HCL or H2SO4.
Methylene blue solustion

Procedure:-

1-Prepare microbial **smear (the sputum) specimen** & fixed it.
2-Flood the slide with **carbol fuchsin** stain then heat it on the Benson burner for 5minutes.
3-Wash the slide with water.
4-Decolorize with the **acid- alcohol for l0-20seconds**.
5-Wash the slide with water.
6-Add **methylene blue** for 30 seconds .
7-Wash the slide with water & dry it with bibulous paper.
8-Examine the prepared slide under the microscope.


Mycobacterium tuberculosis


Thank
you!