FE 204 Experiment 7

Preparing a Bacterial Smear and Simple Stain and The Gram Stain

Contents

- Definitions
- Smear Prepration
- Staining
- Simple Stain
- Gram Stain

Smear Prepration

- Successive microbial staining depends first on the preparation of suitable **smear** from microbial culture.
- **Smear**: It is a thin layer of bacteria placed on a slide for staining.
- Smear should be neither too thick nor too thin.

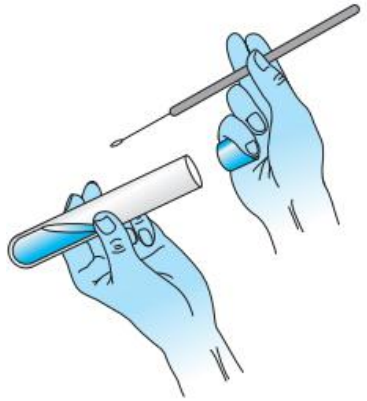
Material used in LAB

- Bacterial cultures
- Glass slides in alcohol
- Loop
- Forcep

Smear Prepration

1. Remove the slide from alcohol and pass through the flame
2. Write your sample name

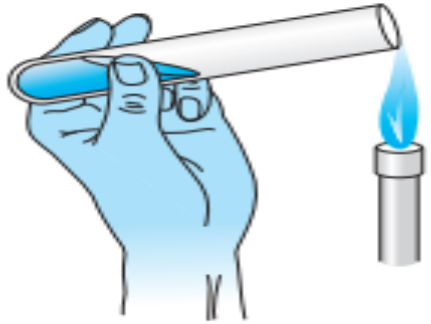
Sterilize the loop



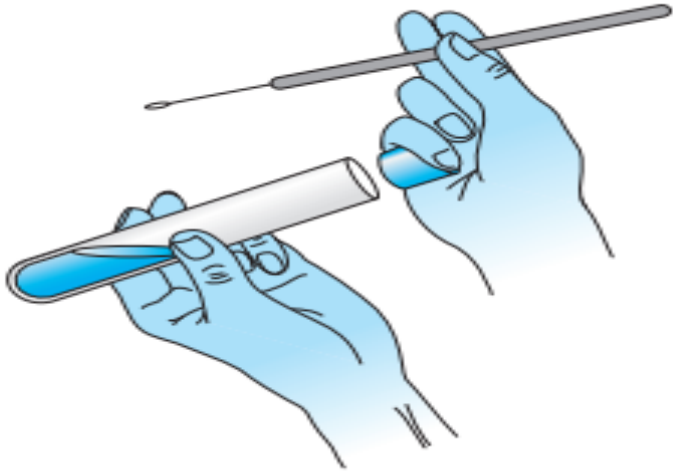
Remove the cap of tube

Flame the neck of the tube

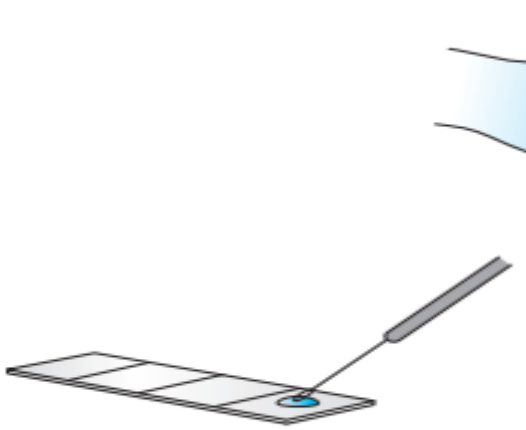
Remove one loopful culture



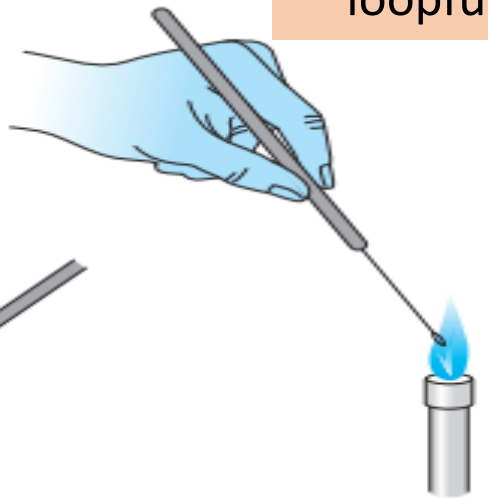
Flame the neck of the tube



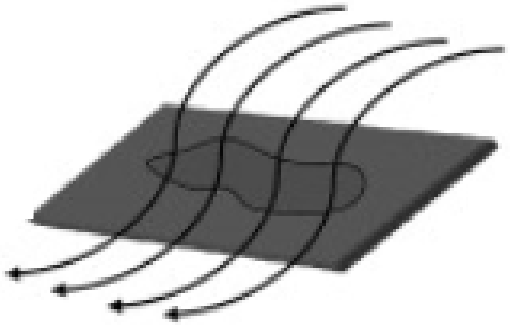
Replace the cap of tube



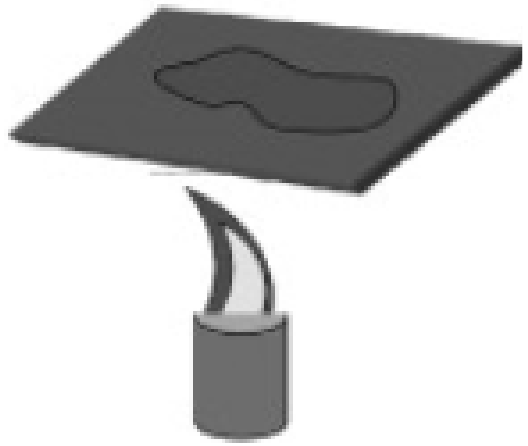
Place culture and spread with loop



Sterilize the loop



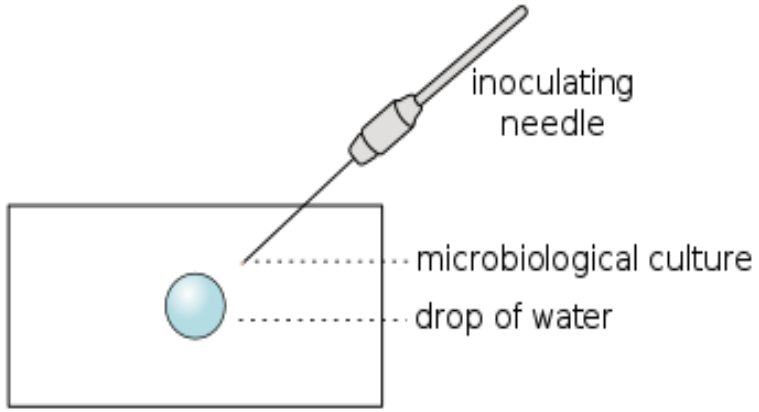
Waiting for completely dry



Rapidly pass the smear through the flame three times

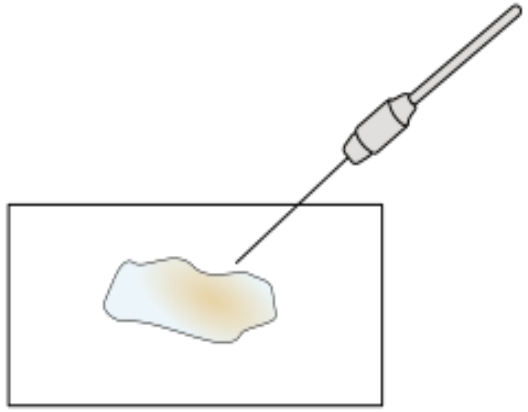
Smear
from solid medium

1



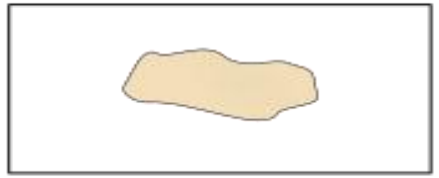
Place a drop of water in the center of the slide

2



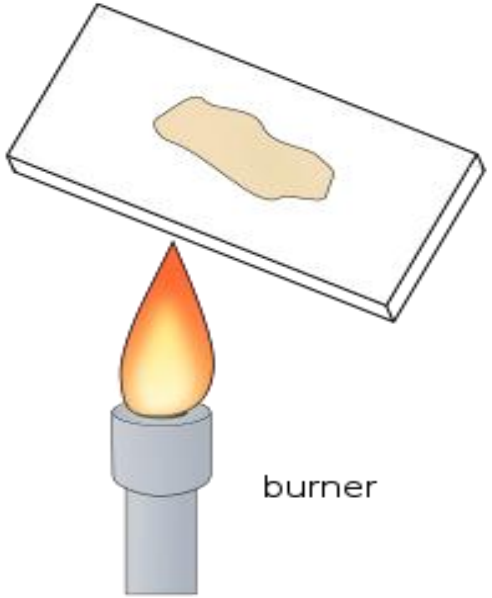
Mix a very small amount of culture
into the drop of water and spread it out thinly

3



Allow the smear to air dry

4



Pass through flame several times
to kill and fix microorganisms to the slide

Simple Stain and Gram Stain

Simple Stain and Gram Stain

- The chemical compunds used to stain bacteria are called **dyes**.
- With staining, bacterias are made more visiable.
- In microscoping observation, stained bacteria are most often used.

Simple Stain and Gram Stain

Most often dyes are;

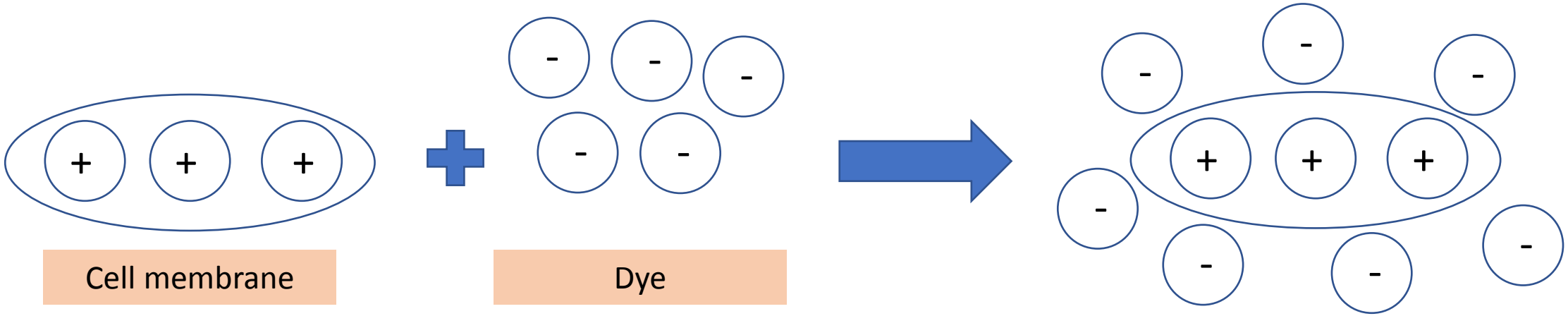
Methylene blue

Safranine

Crystal violet

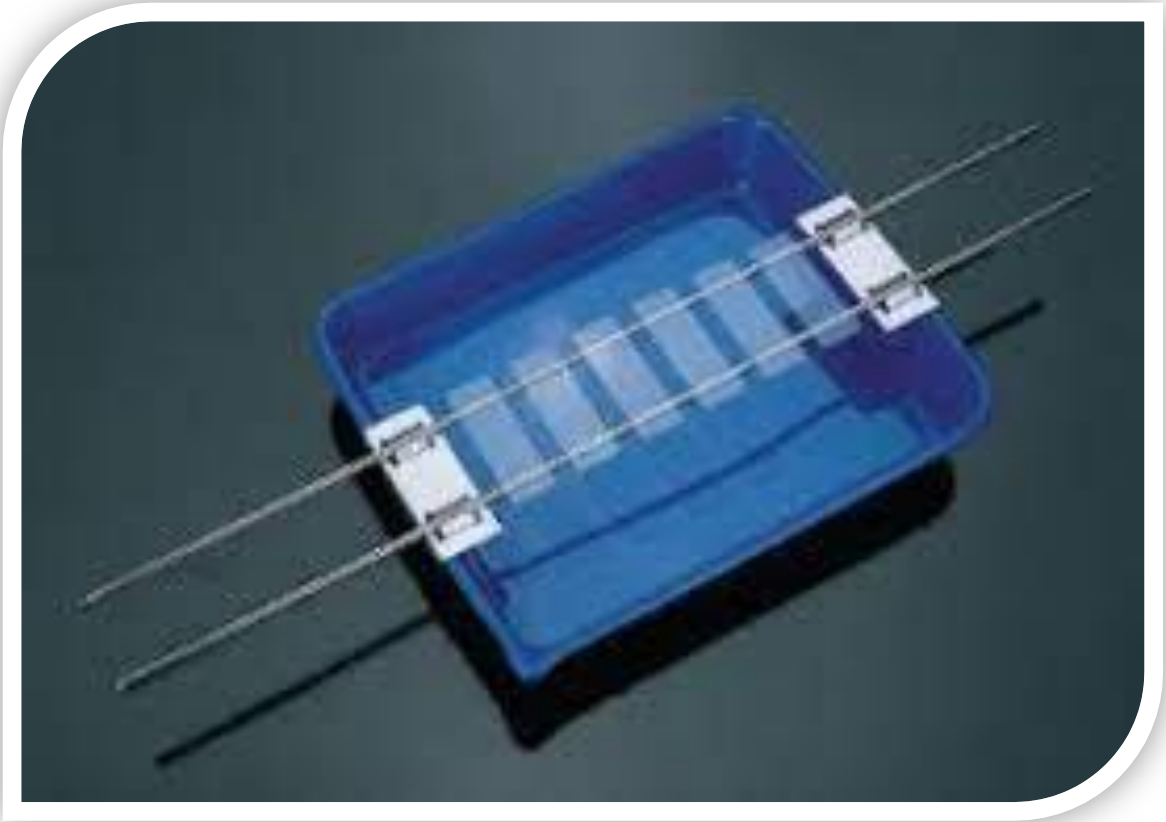
Simple Stain and Gram Stain

- Dyes can be acidic or basic.
- Acidic dyes have a strong affinity for basic portions of the cell.
- The staining of bacteria is an ion exchange reaction.
- Positive and negative charges exchanges between molecules from dyes and cell structure to form an ionic bond.



Material used in LAB

- Bacterial cultures
- Glass slides in alcohol
- Simple stains
- Dyes
- Loop
- Forcep
- Staining rack
- Microscope

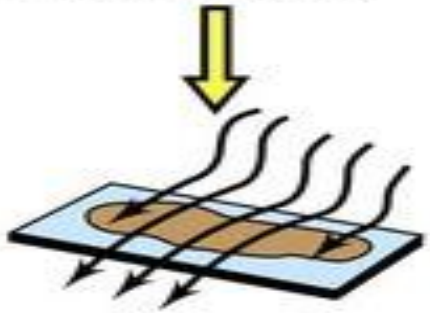


Simple Stain

1

Spread culture over slide

2



Dry in air

3

Pass slide three times through flame

4

Flood the smear with dye

Dye on the slide is washed off with water.

5

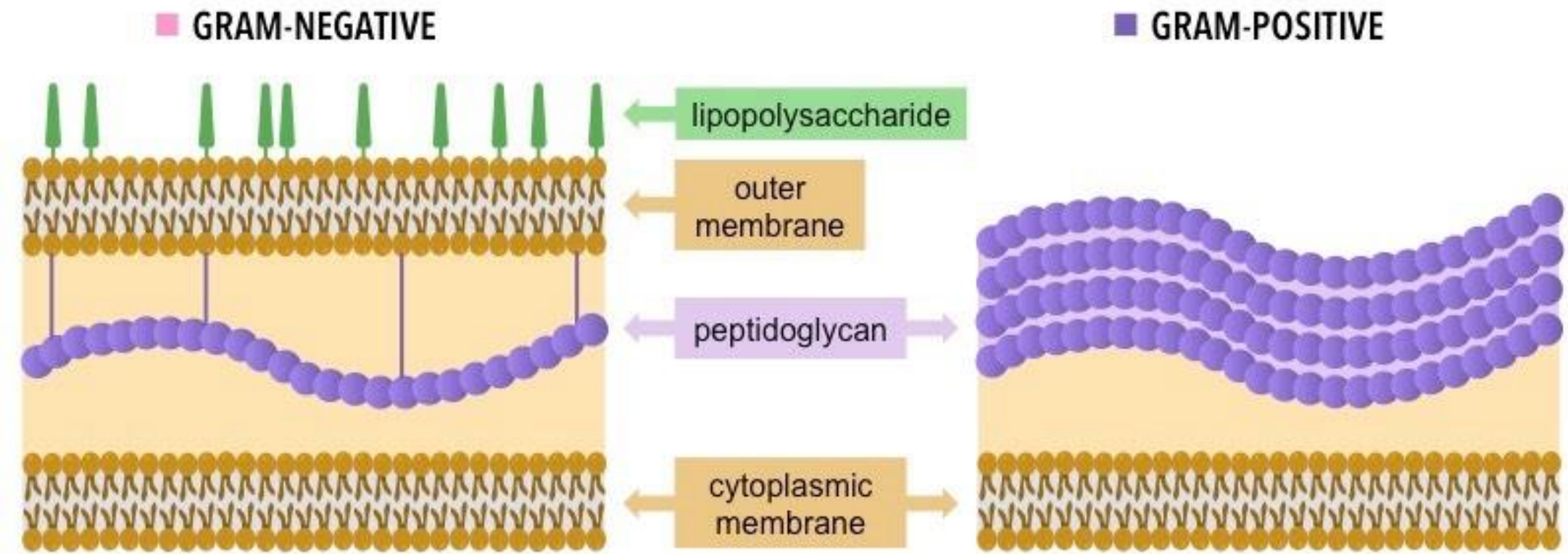
Exemine with microscope

Gram Stain

- The Gram stain allows one to distinguish between Gram-positive and Gram-negative bacteria on the basis of differential staining with a crystal violet-iodine complex and a safranin counterstain.

- The cell walls of Gram-positive organisms retain this complex after treatment with alcohol and appear purple, whereas gram-negative organisms decolorize following such treatment and appear pink

- The peptidoglycan structure in the cell wall is stained in Gram stain.

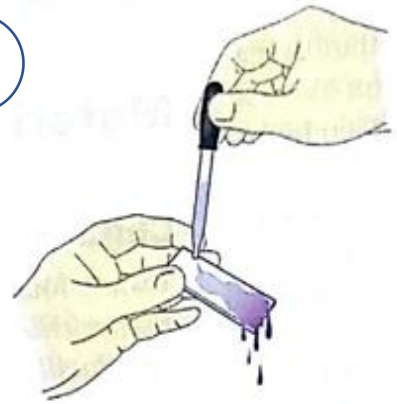

GRAM-NEGATIVE
GRAM-POSITIVE
lipopolysaccharide
outer membrane
peptidoglycan
cytoplasmic membrane

Gram Stain

1

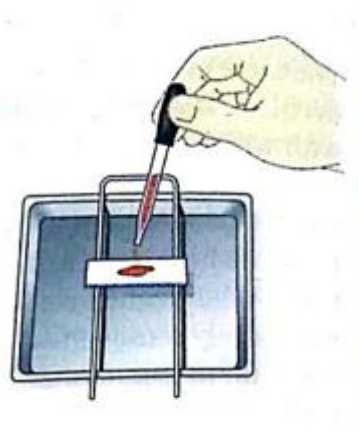
Cover the smear with crystal violet stain and leave 1 minute

2



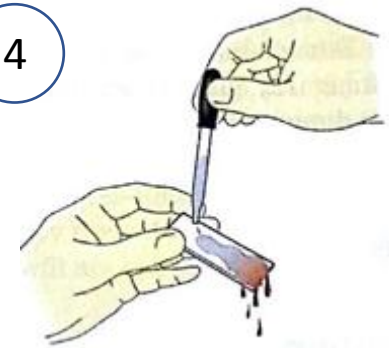
Wash off the stain with tap water drain off excess water

3



Cover the smear with iodine solution and leave 1 minutes

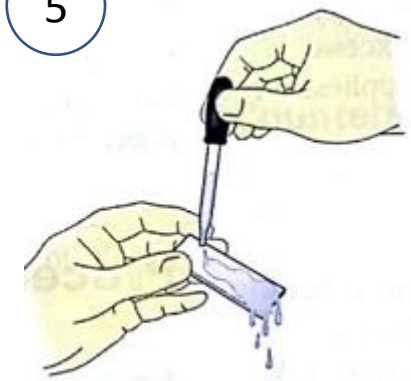
4



Wash off the stain with tap water drain off excees water

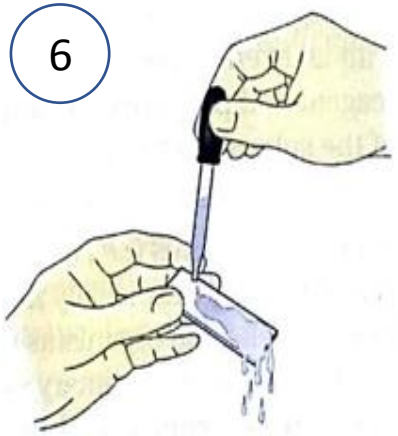
Gram Stain

5



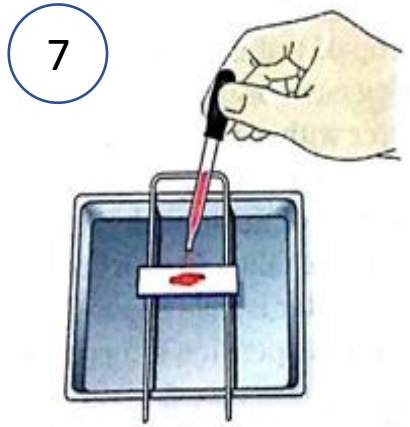
Add %95 alcohol drop by drop until the runs almost clear

6



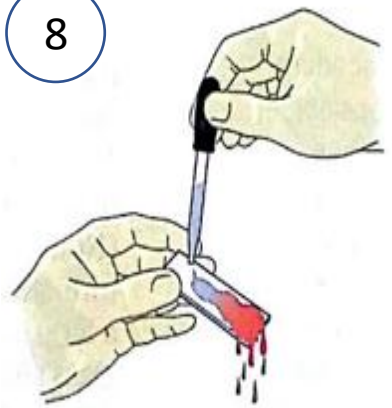
Wash off the stain with tap water drain off excess water

7



Cover the smear with safranine stain and leave 1 minute

8



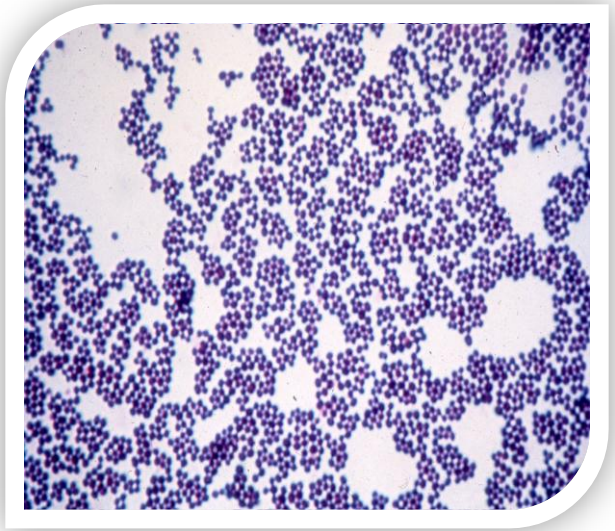
Wash off the stain with tap water drain off excees water

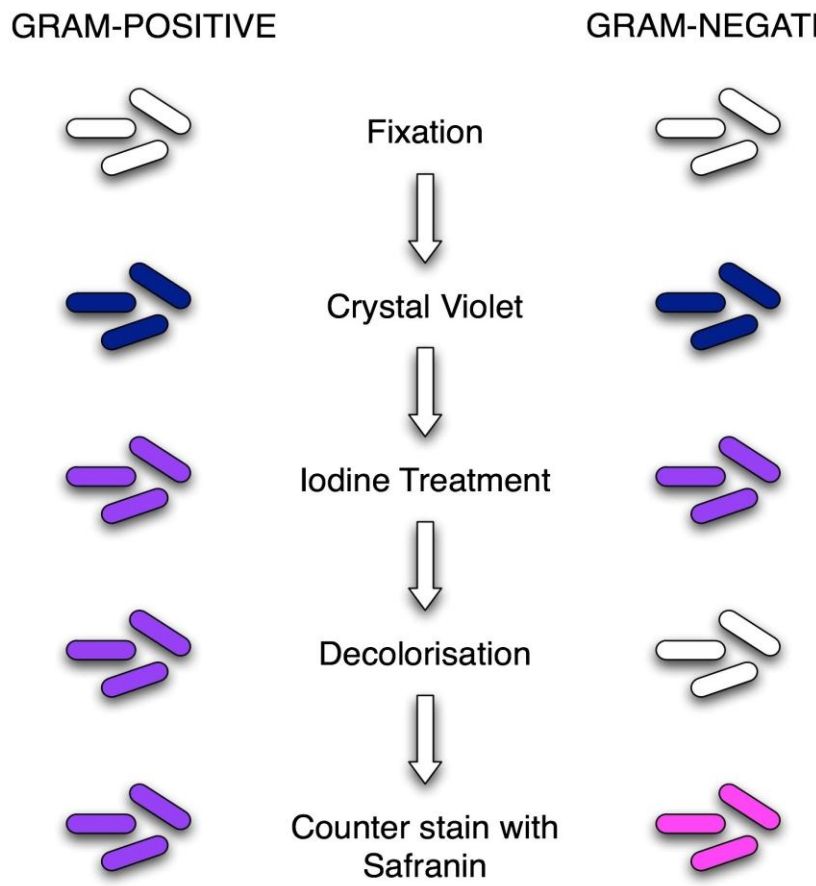
Gram Stain

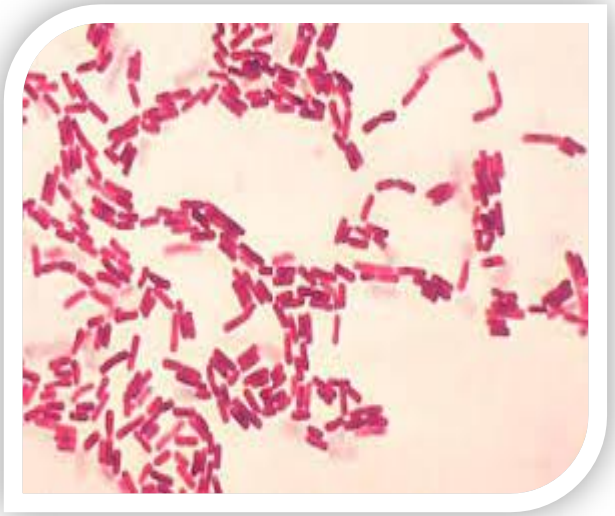
Some factors can results in the variable gram reactions:

- Improper heat fixing of the smear
- An extremely thick smear
- The amount of water remaining on the smear
- Time of applied
- Age of culture

Results




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



Results