Examination of stained microorganisms, Smear preparation, simple staining, Gram staining and Acid-fast staining

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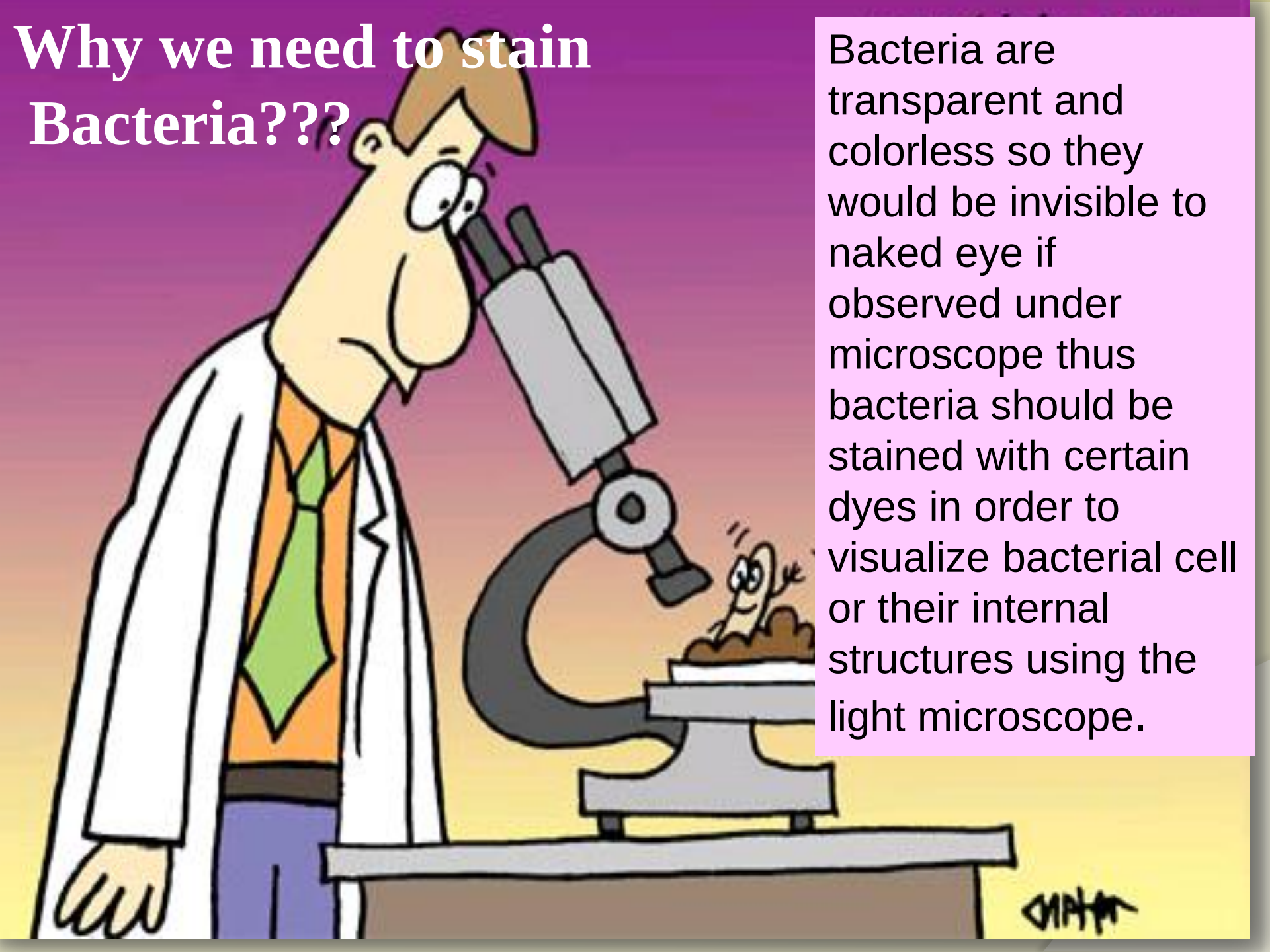
Why we need to stain Bacteria???

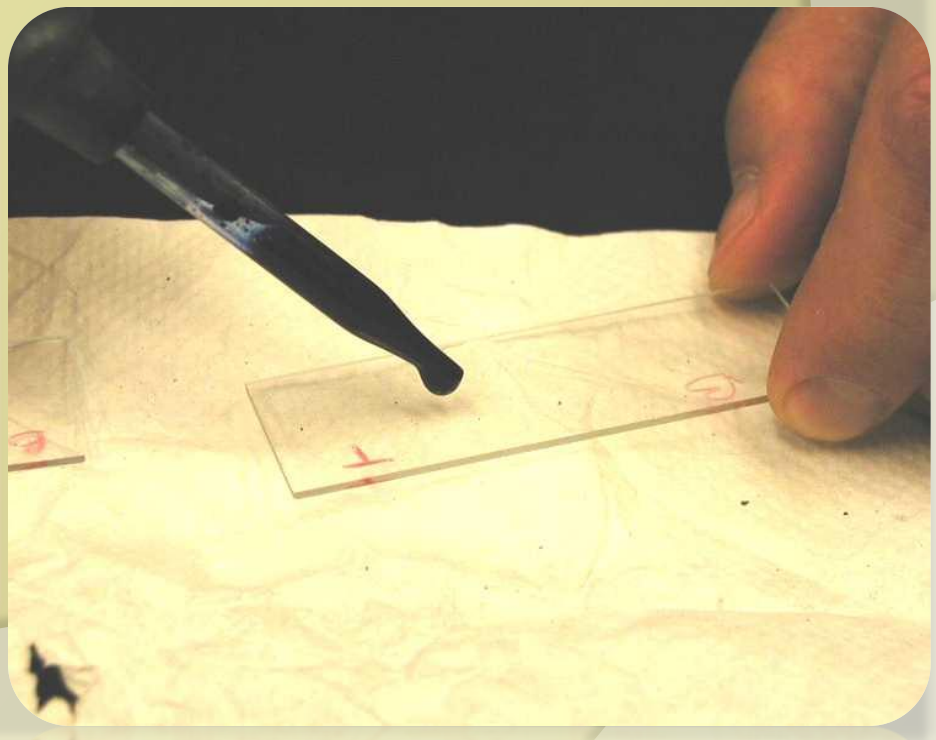


Bacteria are transparent and colorless so they would be invisible to naked eye if observed under microscope thus bacteria should be stained with certain dyes in order to visualize bacterial cell or their internal structures using the light microscope.

Stains and Staining

A stain is a substance that adheres to a cell, giving the cell color. The presence of color gives the cells significant contrast so they are much more visible. A stain is a dye consisting of a colored organic compound in the form of salt composed of positive and negative ion, one of these ions is responsible for colour (a chromophore)

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains are frequently used in biology and medicine for viewing.



Based on the charges:

A stain is classified in to:-

- Basic stain/dyes – stain with +ve charge, example include crystal violate,

methylene blue and safranin

- Acidic stain/dyes – stain with –ve charge,

example nigrosin and India ink.

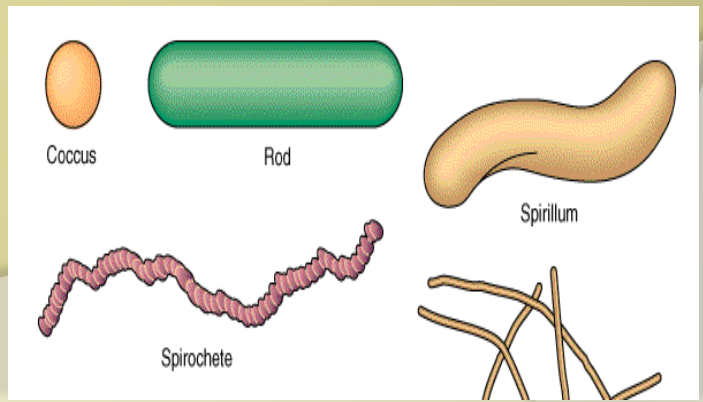
- Neutral stain/dyes – stain with both charges.

Based on function of stain:

1. Simple staining – only one dye is used- differentiation among bacteria is impossible- Eg. Simple Staining.

2. Differential staining- more than one dye is used- Differentiation among bacteria is possible- Eg. Gram's staining, Acid-fast staining.

3. Special staining – more than one dye used - Special structures are seen. Eg. Capsule staining, Spore staining



Principle of staining

- **Basic stain(+ve charge)**

To stain -ve charged molecules of bacteria

Mostly used because cell surface is –ve charge.

- **Acidic Stain(-ve charge)**

To stain +ve charged molecules of bacteria.

Used to stain the bacterial capsules.

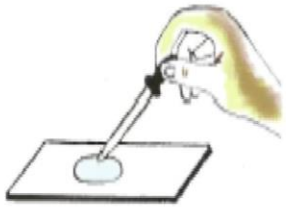
Fixation

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.

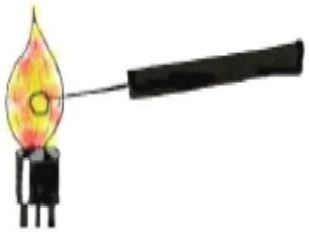
How do you prepare a smear?

- **From liquid media :**
- Sterilize the loop by Bunsen flame then let it cool.
- Shake the specimen container (broth culture) then withdraw one or more if needed loopful from the specimen and spread it on the center of a clean slide to form a thin film of 1- 2 cm in diameter, then sterilize the loop.
- Allow the smear to dry by air.
- The smear fixed by passing it (3- 4) times through the Bunsen flame then allow the slide to cool before staining.

- **From solid media (slant or plate)** :
- Sterile the loop on Bunsen flame and let it cool.
- Place a loopful of clean water on the center of a clean slide.
- Re sterilize the loop, transfer a small portion of the growth, mix it with water thoroughly and spread the mixture evenly on the slide to form a thin film of 1- 2 cm in diameter.
- Dry and fix (as mentioned above)

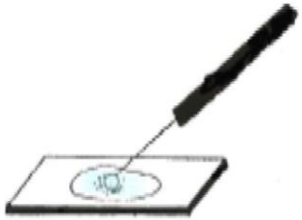


1- Place a drop of saline on the slide.



2- Sterilize loop in flame.

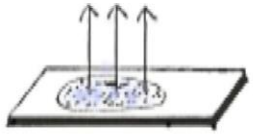
3- Select suitable colony.



4- Make suspension of colony in saline

5- Using loop, make a smear.

6- Sterilize loop in flame.



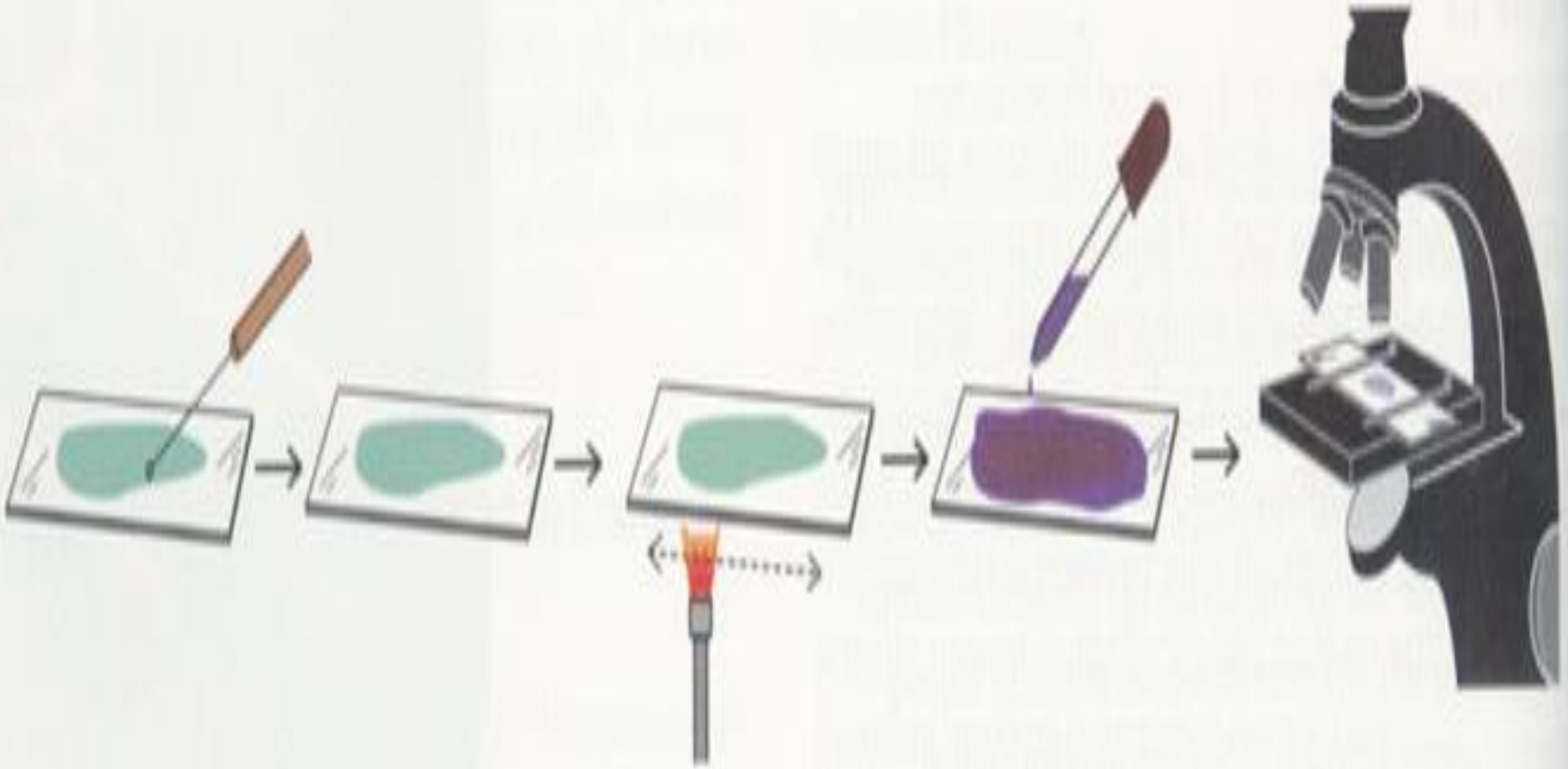
7- Dry at room temperature.

8- Fix slide by passing through flam 3x .

Type of staining

a. Simple Staining:

This procedure uses only one basic dye e.g. **Crystal violet** or **Methylene blue** or **Safranin** to stain bacteria. The bacteria will simply take the color of the dye.



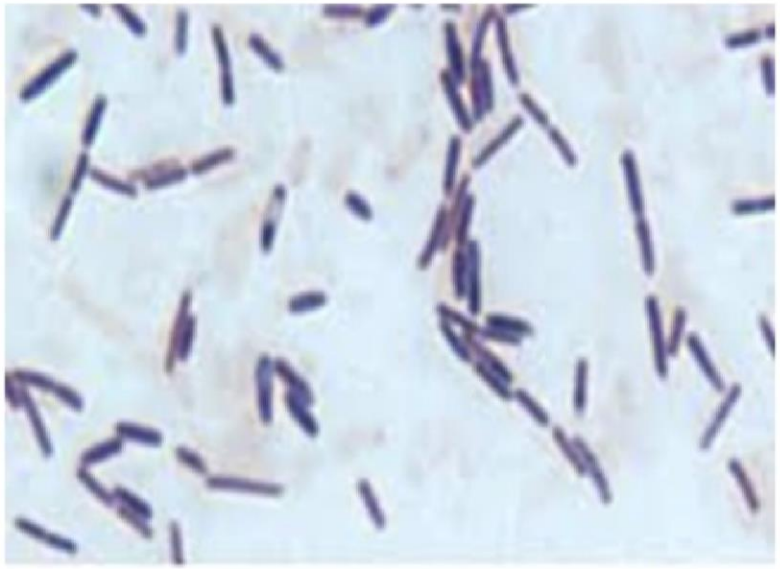
Spread thin film of culture over slide

Allow to air dry

Pass slide through flame to glue the bacteria to slide

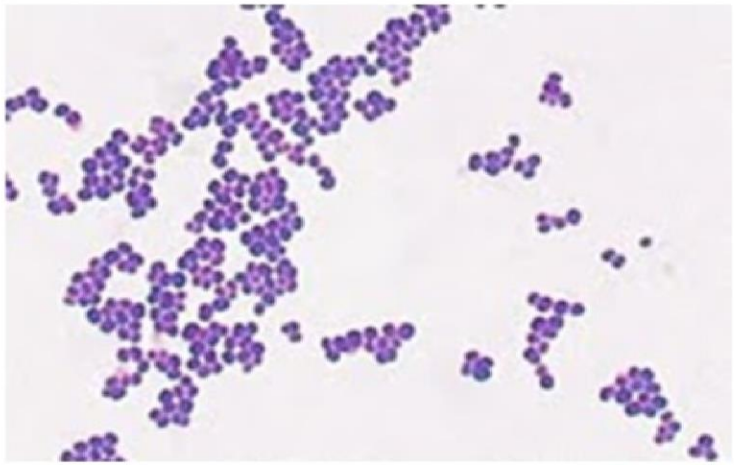
Flood with stain, rinse and dry

Place drop of oil on slide and examine with microscope



Bacillus subtilis stained with crystal violet

Saccharomyces stained with crystal violet



Staphylococci stained with crystal violet

b. Differential Staining:

These involve more than one dye solution. The dyes may be added in several steps according to the procedure.

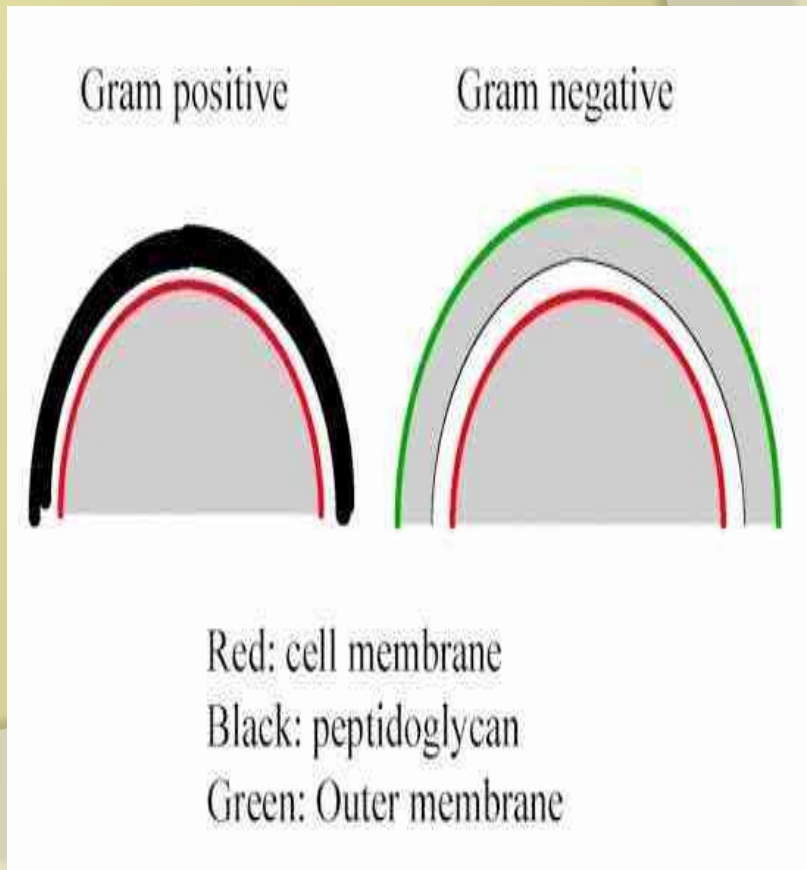
Gram Stain

Gram stain is used to stain bacteria. Bacteria stain either Gram-positive or Gram negative on the basis of the differences in their cell wall composition

What is the different between Gram positive and Gram negative bacteria ?????

Gram positive species have a thick peptidoglycan layer and large amount of teichoic acid and are therefore unaffected by alcohol decolourization and retain the initial stain (crystal volet) giving the organism violet apperance .

Gram negative cell wall have a single peptidoglycan layer. The outer membrane is damaged by alcohol decolorizer allowing crystal violet- iodine complex to take out and be replaced by the counter stain (safranine) giving the organism a pink/ red appearance.



Major Steps of Gram Stain

Gram-Stain step 1

Here crystal-violet dye is applied to the slide specimen beginning the Gram staining procedure.

Gram-Stain step 2

The slide is washed with water for 10 sec. after having stained it with crystal-violet.

Gram-Stain step 3

Gram's iodine is applied, and after one minute it is then washed off with tap water.

Gram-Stain step 4

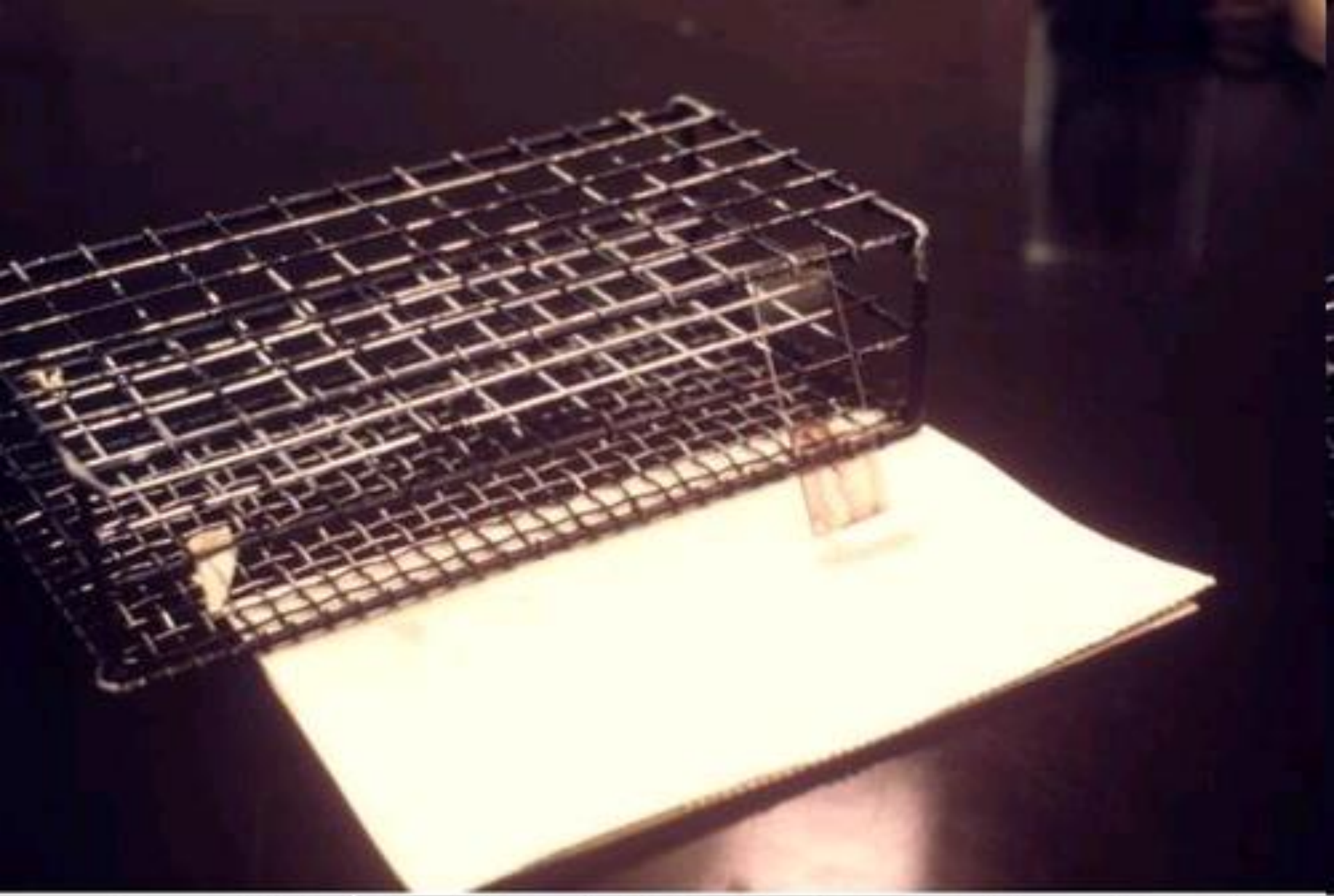
After the slide has been treated with Gram's iodine, it is washed off under tap water.

Gram-Stain step 5

Here the slide is being "decolorized" using a mixture of ethanol and acetone.

Gram-Stain step 6

The counterstain Safranin is applied to the slide, imparting a pink color to Gram-negative bacteria.



Gram-Stain step 7

The slides are finally dried after being stained, but a plastic rack is now used instead of metal.

The reports

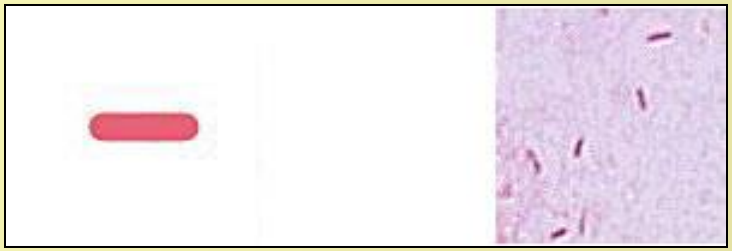
1. If no microorganisms are seen in a smear of a clinical specimen, report “No microorganisms seen.”

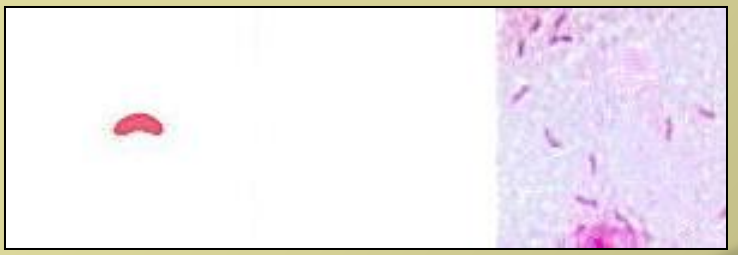
2. If microorganisms are seen describe morphology.

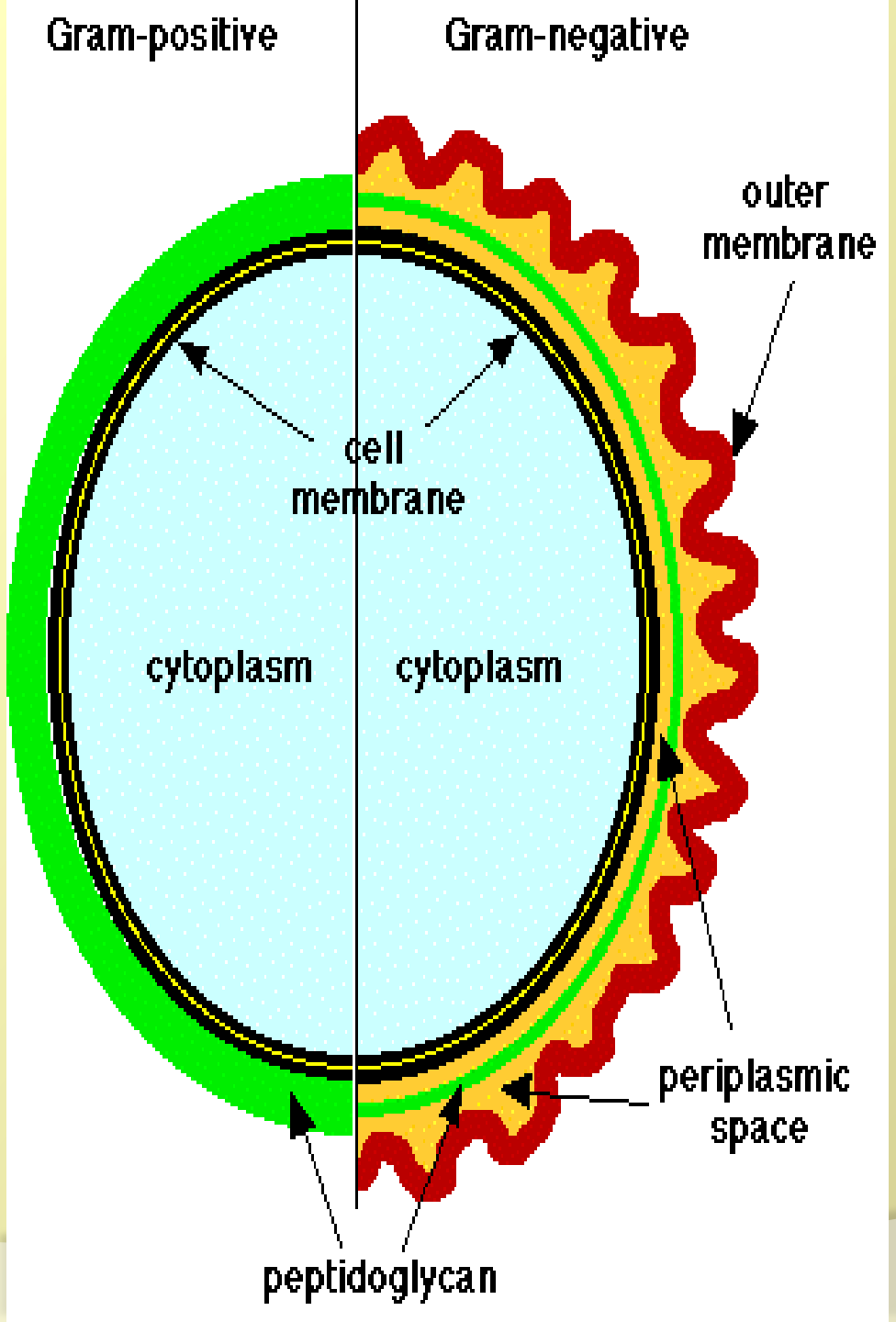
	Color of Gram + cells	Color of Gram – cells
Primary stain: Crystal violet	**Purple**	**Purple**
Mordant: Iodine	**Purple**	**Purple**
Decolorizing agent: Alcohol-acetone	**Purple**	**Colorless**
Counterstain: Safranin	**Purple**	**Red**

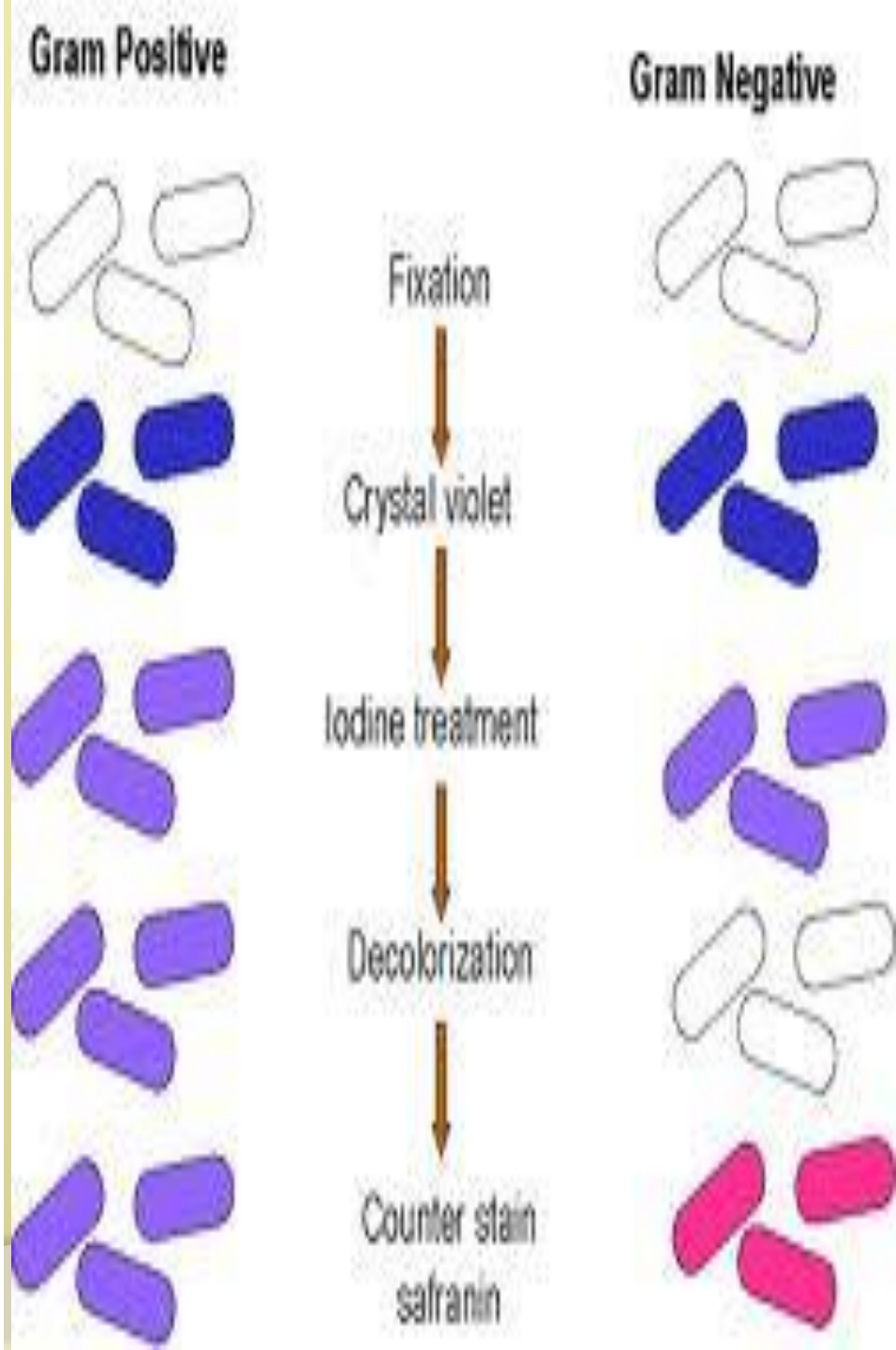
Gram Staining – Gram +’ve

Gram Staining – Gram -’ve

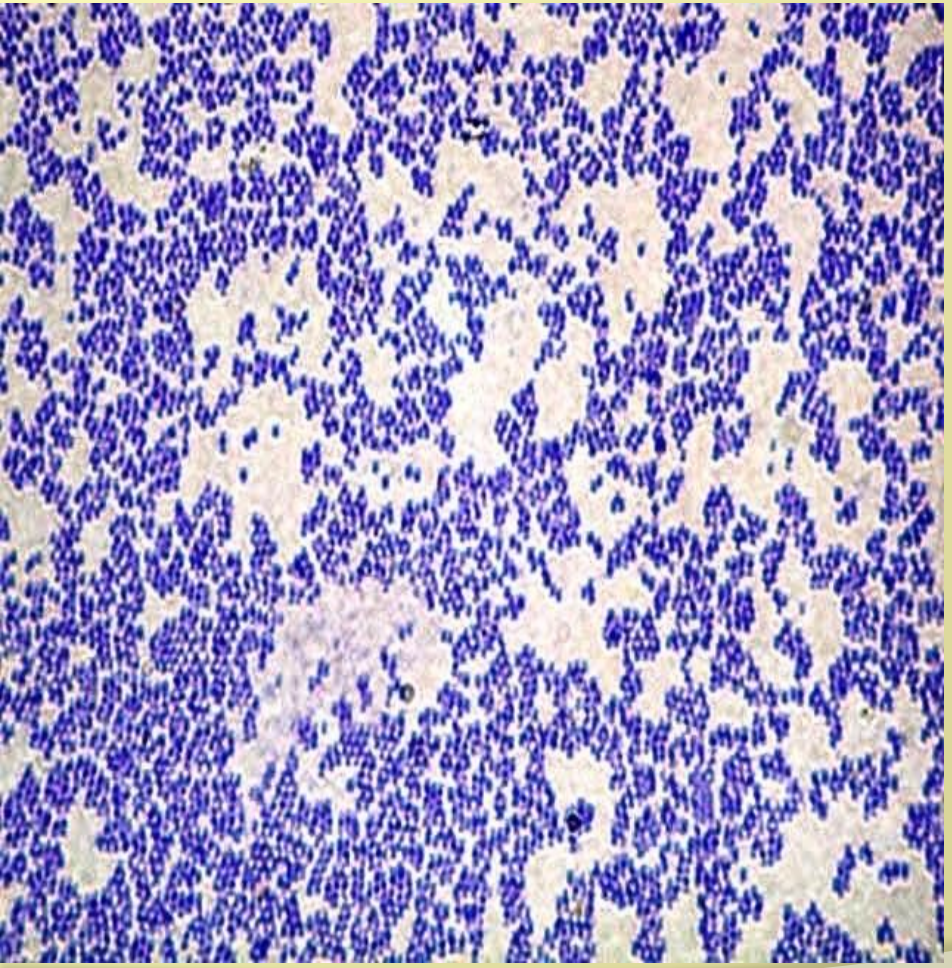


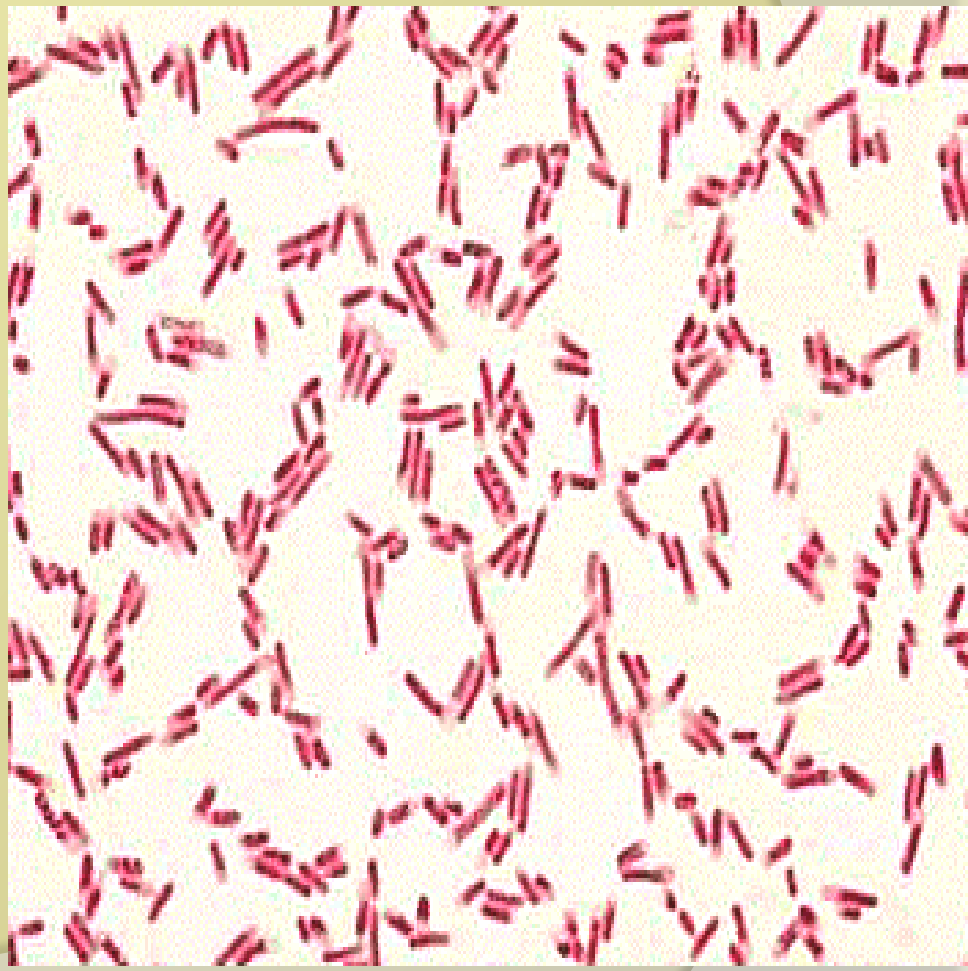


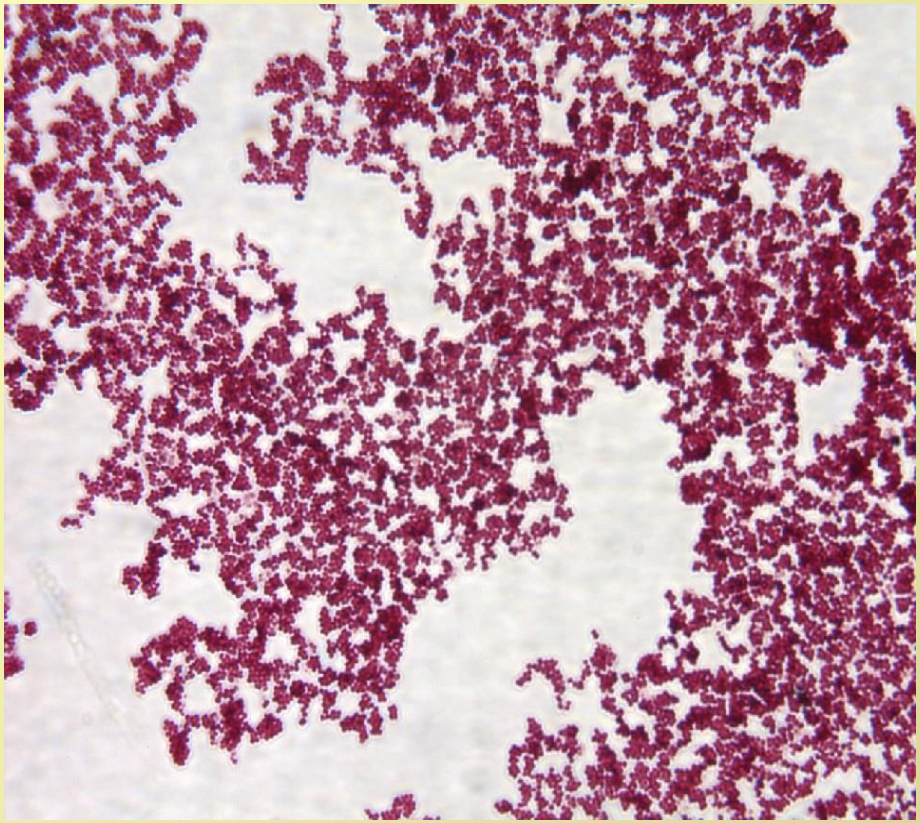

Gram-positive
Gram-negative
outer membrane
cell membrane
cytoplasm
cytoplasm
periplasmic space
peptidoglycan


Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin

Gm+ve cocci & Gm-ve bacilli







Gram-positive bacteria

Streptococcus
Staphylococcus
Lactobacillus
Bacillus
Clostridium

Gram-negative bacteria

- *Escherichia*
- *Salmonella*
- *Vibrio*
- *Treponema*

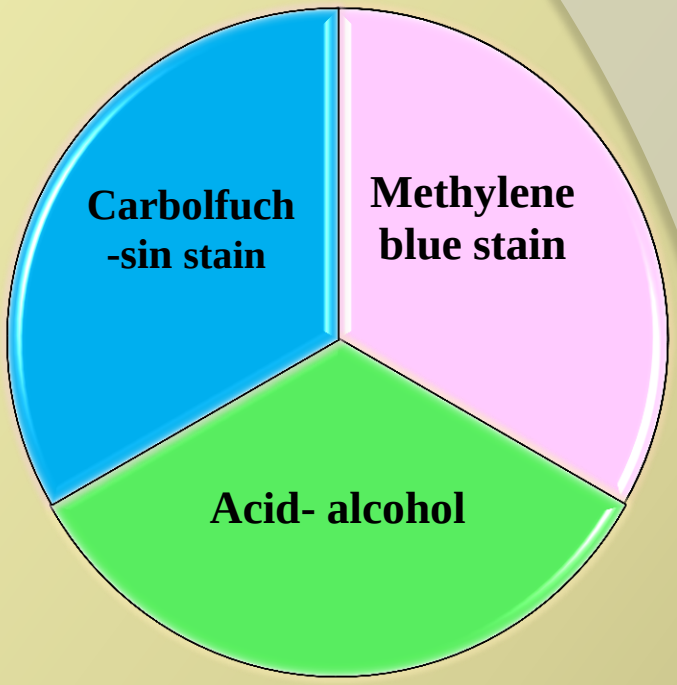
ACID FAST STAIN (ZIEHL- NEELSEN STAIN)

The acid fast stain is one of the most medically important stains.

Purpose:
Used in the demonstration of acid-fast bacteria belonging to the genus 'mycobacterium', which contain fatty acids (mycolic acid) in their cell wall.

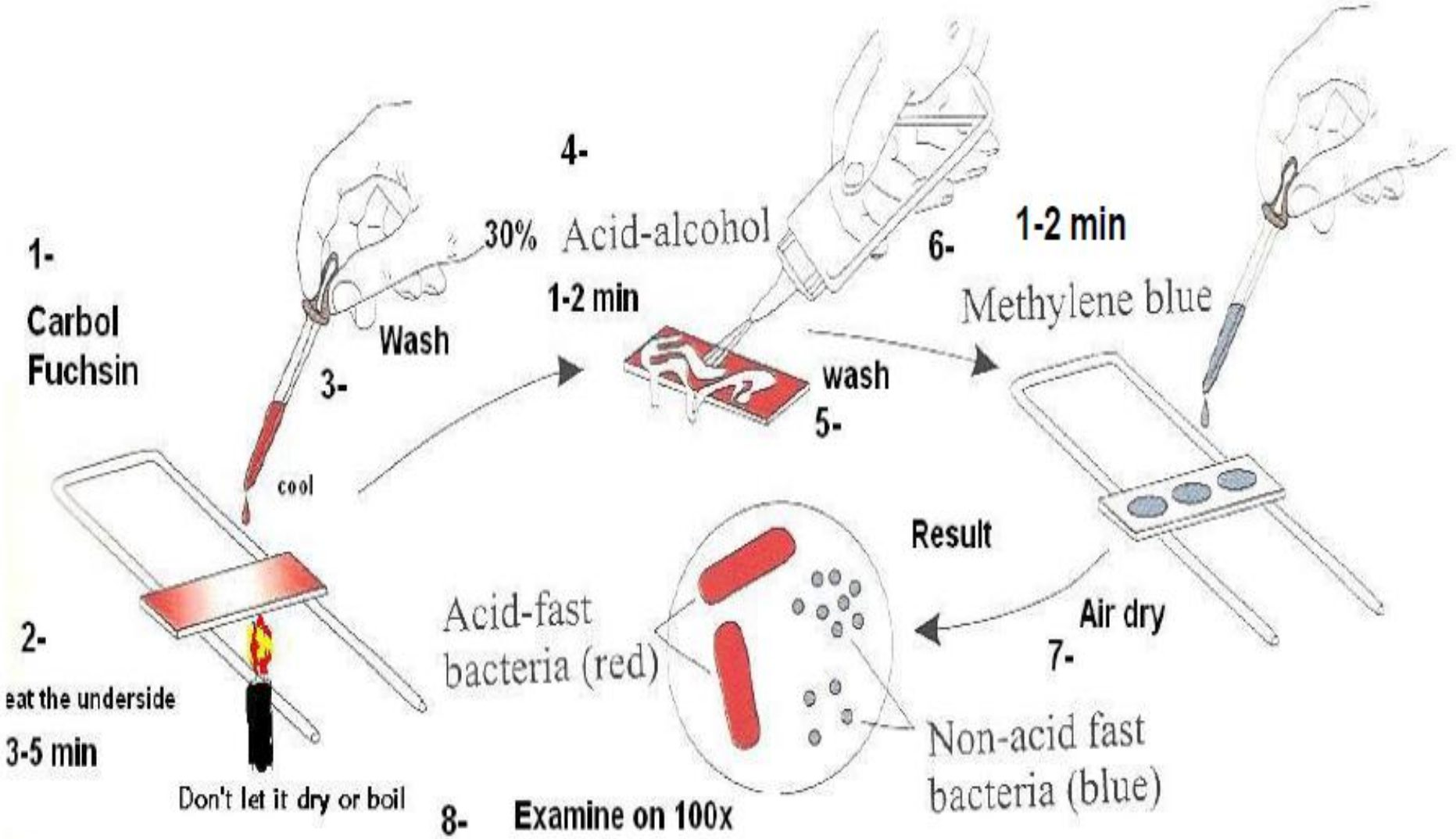
materials

the principle



The carbolfuchsin dye penetrates the cell wall and stains the bacteria. The slide must be heated to melt the mycolic acids. The mycolic acid does not allow the acid alcohol to penetrate, so the cell resists de-colorization and remains a bright pink or red.

Zeihl-Neelsen Staining Procedure



Named this staining Technique