Lab (8)

DR.ESRA HASSAN

Bacterial Staining

Staining means coloring microorganism with a dye that emphasizes a certain structure. Staining permits the microscopic observation of the internal and external structures of microorganisms in addition to revealing their size, shape, grouping, and staining reaction .

The dyes used to stain bacteria are basic dyes. This dyes contain ionizable groups (positive charge) that are attracted to the negatively charge components of the bacterial cell.(Fig.1)

Staining bacterial cell for microscopic examination makes it possible more visible for human eye and to study unique characteristic including cell, size, shape, arrangement and other properties you can use for bacterial identification.

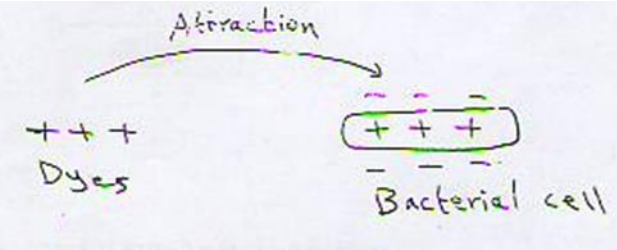


Figure (1) Attraction between the positive charge of the dyes and negative charge of bacteria .

Common Bacteria Staining Include:

- Simple Stains:
- Gram Stain.
- Acid-Fast Stain.
- Endospore Stain.
- Capsule Stain (Negative staining)
- Flagella Stain.
- Spirochete stain.

Steps of Bacteria Staining include :

A. Smear Preparation :

Smear: a thin film of material containing microorganism which is spread over the slide.

Specimens: urine , stool , blood , CSF ,Pus ,pleural fluid , broth or agar media and other.

Smear: microscopy involves collecting a biological sample (sputum or some other clinical material), fixing it thinly on a glass slide and then staining it with a dye that binds specifically to bacteria (making them easier to identify under a microscope). In a smear preparation, cells from a culture are spread in a thin film over a small area of a microscope slide, dried, and then fixed to the slide by heating or other chemical fixatives. A good smear preparation is the key to a good stain.

The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure.

A smear can be prepared from a solid or broth medium.

Below are some guidelines for preparing a smear for a Gram-stain.

Culture from solid media: Using a sterile pipette, add one drop of sterile saline or sterile water to the center of the microscope slide. Aseptically pick a small amount of an isolated colony with a loop and gently mix into the drop of sterile saline or water using circular motions. Mix evenly to make a thin smar**.**

B. Staining

Preparation of smear and bacterial staining:

To prepare smear, we need the following

Broth or solid media

Bacteriological loop

Microscopic glass slide

Bunsen burner

Procedure:

1) Sterilize the loop over Bunsen flame then let it cool.
2) A loopful of specimen and spread it over the surface of a clean slide to form a thin film of 1-2 cm in diameter (in the center of the slide)
3) Allow the film to dry by air
4) The film is fixed on the slide by passing in (3-4) time through the Bunsen flame and allow the slide to cool before staining

From solid material (colonies culture agar) (Fig.3)

1) Sterilize the loop in Bunsen flame and let it cool
2) Place a loopful of clean water on the center of a clean slide
3) Re sterilization the loop , transfer a small portion of the colony and emulsify thoroughly with water and spread the mixture on the slide to form a thin film of 1-2 cm in diameter

4) Dry and fix

Purpose of Fixation of the slide:

1) To kill the microorganism
2) To stuck the microorganism on the surface of a slide
3) To prevent the autolytic changes of M.O. (see figure)

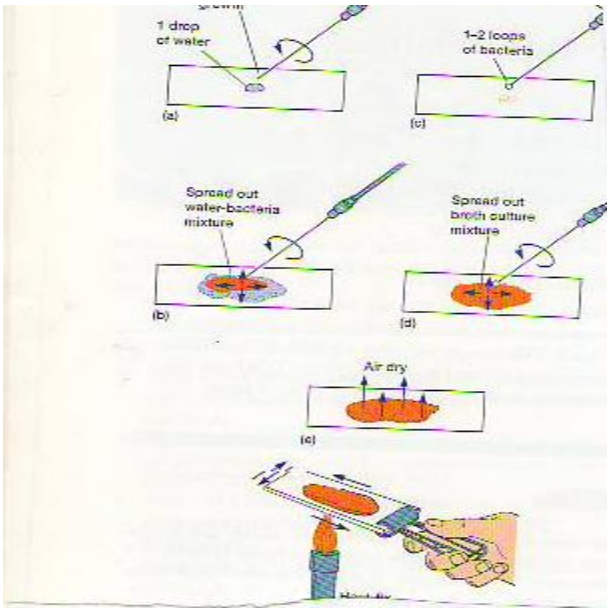


Figure (2) Bacterial smear preparation

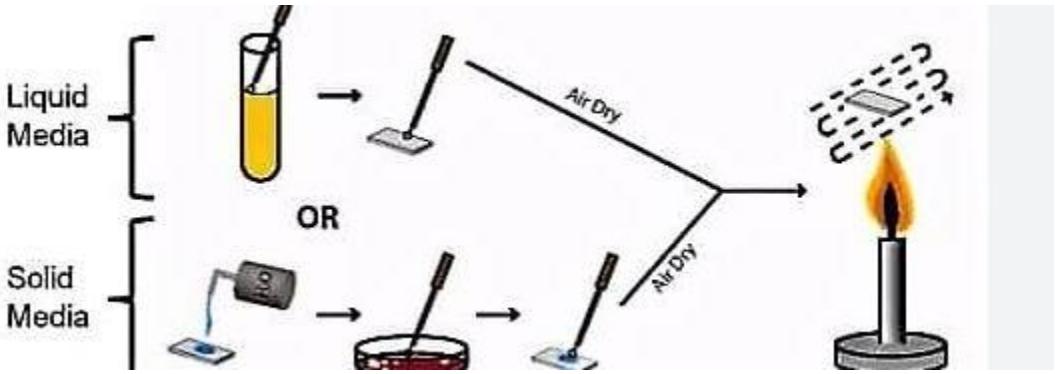


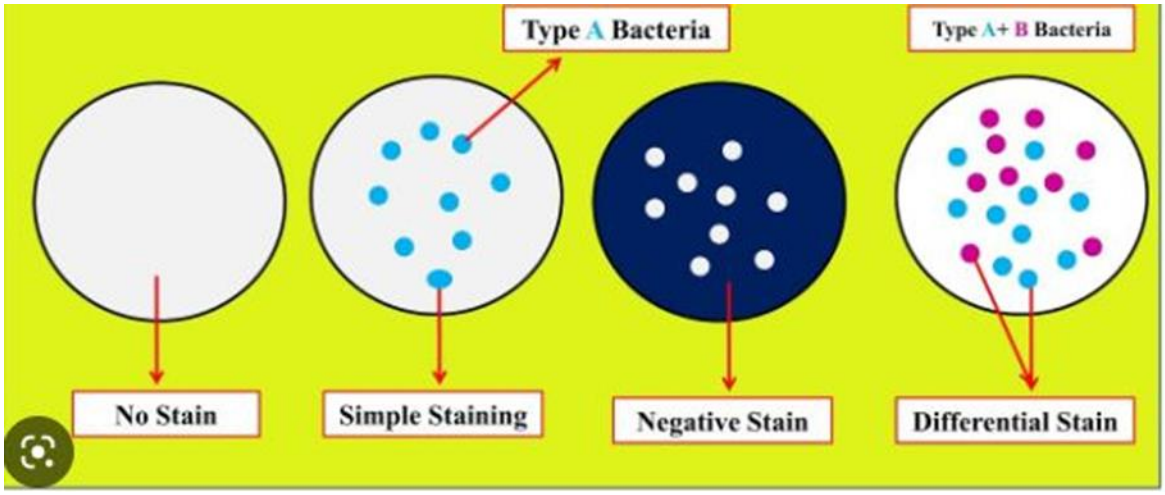
Figure (3) Bacterial smear preparation from liquid or solid media

Types of stain:

I. Simple stain

II. Differential stain

III. Structural stain



Also, can classify Based on the nature of chromogen, there are three types of stain.

- Acidic stain (Anionic stain)
- Basic stain (Cationic stain)
- neutral stain.

Acidic (-ve) stains:

- Nigrosin
- Malchite green

Basic(+ve) stains:

- Crystal violet
- Methylene blue
- Safrinin
- Basic fuchsin

Simple Stain:

.**Simple stain**: using one stain only such as safranin , crystal violete or methylene blue and appears in one color.

Generally ,Simple staining include directly staining the bacterial cell with a positively charged dye in order to see bacterial detail such as size morphology of cells , in contrast to negative staining where the bacteria remain unstained against a dark background.

It is used to get the following:

- Size of microorganism
- Shape: spherical (cocci), rod (bacilli), pleomorphic (coccobacilli) and spiral(spirillum)
- Grouping or arrangement : single, pairs, chain and cluster (see figure).

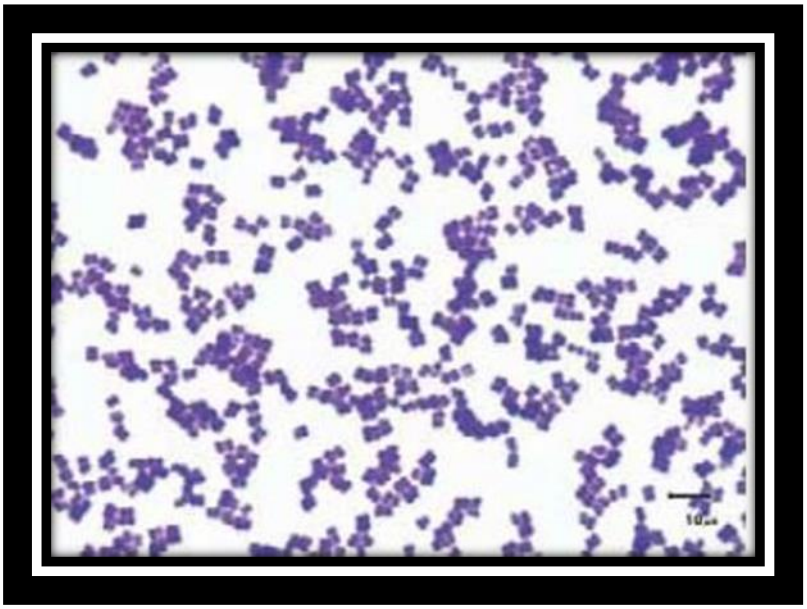


Fig.4 : Simple stain for micrococcus

Procedure: (Fig.5 and 6)

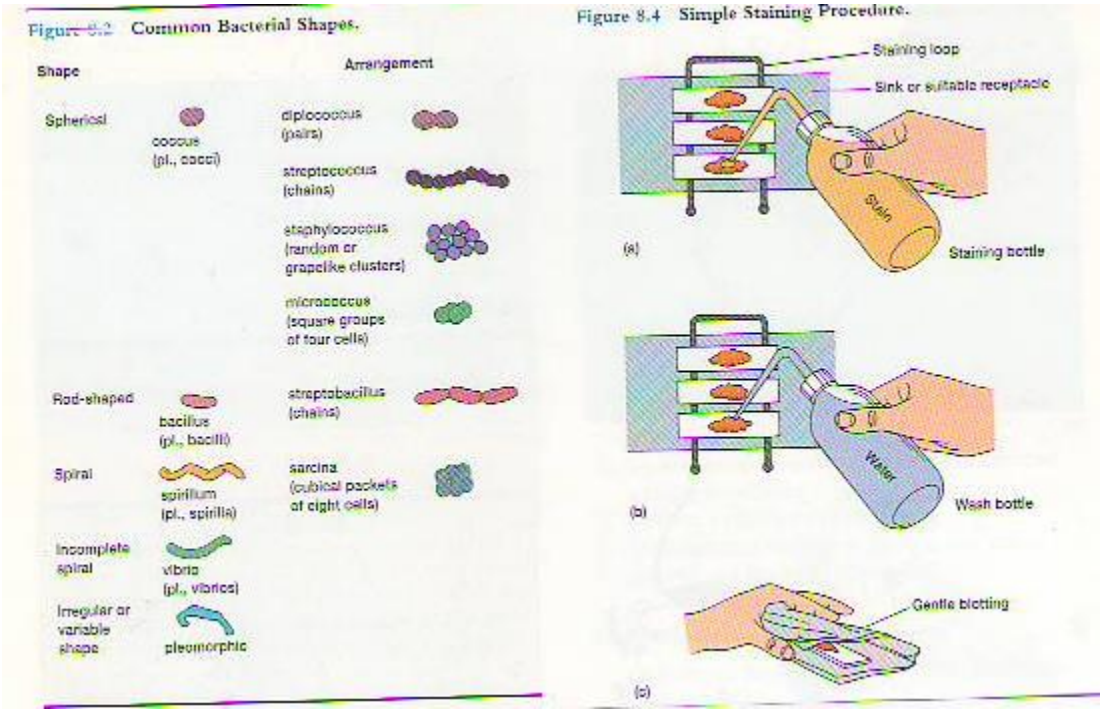
1- Prepare a fixed smear of bacteria.

2- Flood the slide with simple stain for 1minute.

3- Wash off the stain with tap water.

4- Dry the slide (place the slide between the layers of filter papers).

5- Examine under oil immersion lens.



- **Figure (5) simple stain and bacterial shape**

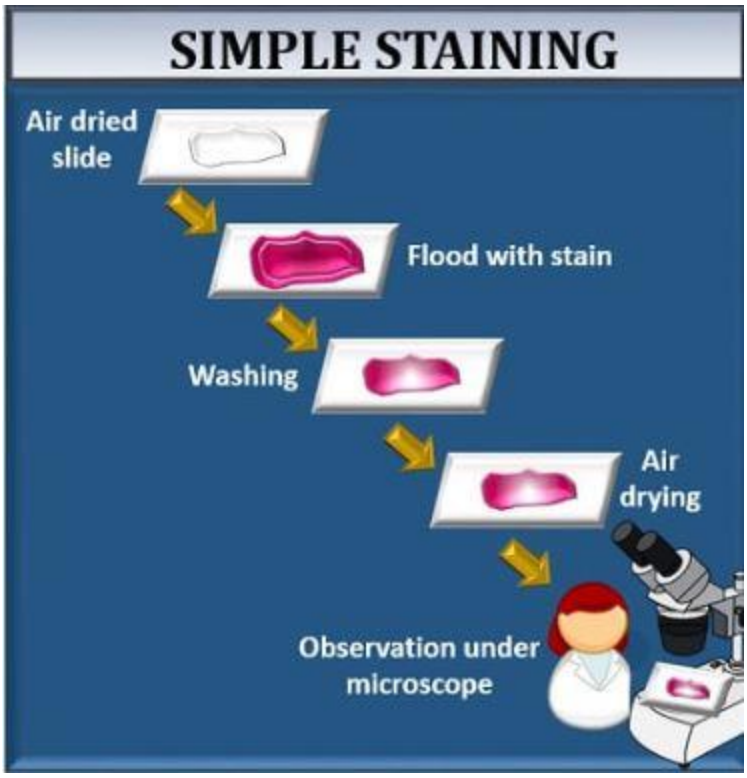


Fig.(6) :Simple stain procedure

2. Differential stain

Using two or more dyes successively to differentiate between organisms according to their response to these dyes include :

1. Gram stain
2. Acid fast stain

Gram Stain:

The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884.

The Gram stain is the most common differential stain used in microbiology. The unique cellular components of the bacteria will determine how they will react to the different dyes

Differential stains use more than one dye.

Also this method of staining used to classify bacterial species into two large important groups, Gram-positive bacteria such as *Saphylococcus* aureus and Gram-negative bacteria Such as E.coli and Klebsiell . in which Gram positive appear (dark) purple and Gram negative have a (pink).

Bacteria that retain the primary dye (crystal violet) appear dark purple and are called gram positive .The bacteria that lose the primary dye after the application of the decolorizer agent , take up the counter stain (safranin) and appear red are termed gram negative .

The ability to resist decolorization is related to the chemical composition and structure of cell wall (fig.7).

Grams iodine is a mordant that increase the affinity of the primary stain to the bacterial cells. Counter stain the safranin will stain the colorless , gram negative bacteria pink but does not alter the dark purple color of the gram positive bacteria . The decolorization step is the critical step that differentiate the bacteria

So, final step in Gram staining is to use **basic fuchsin stain** to give decolorized Gram-negative bacteria pink color for easier identification. It is also known as counterstain. Some laboratories use safranin as a counterstain; however, basic fuchsin stains gram-negative organisms more intensely than safranin.(Fig.8)

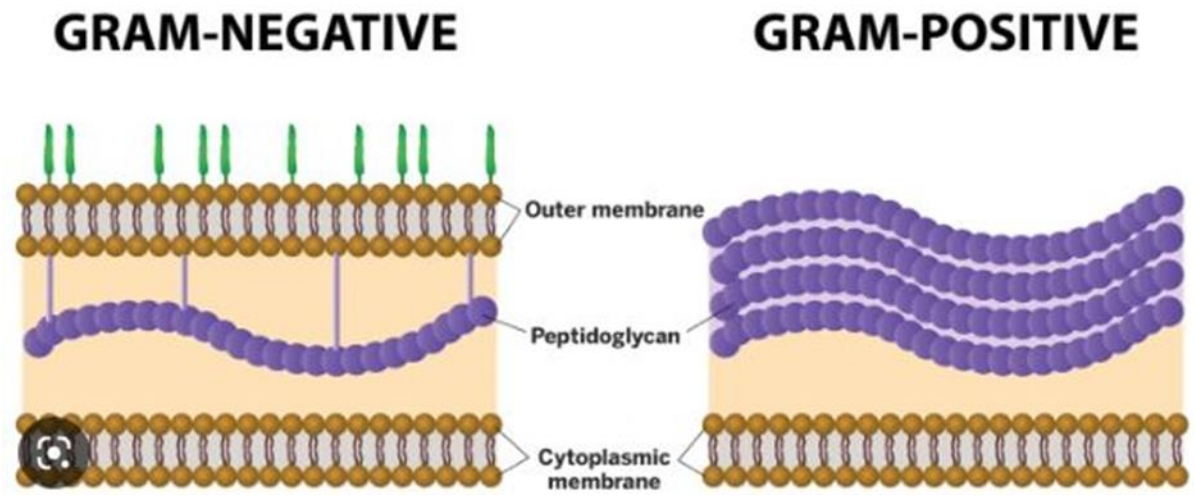


FIG.(7) : Cell wall structure of Gram positive and negative bacteria

. so in general, Gram staining process includes four basic steps, including:

1. Applying a primary stain (crystal violet).
2. Adding a mordant (Gram's iodine).
3. Rapid decolorization with ethanol, acetone or a mixture of both.
4. Counterstaining with safranin.

Procedure: (fig.8 and9)

1- Prepare a fixed smear.

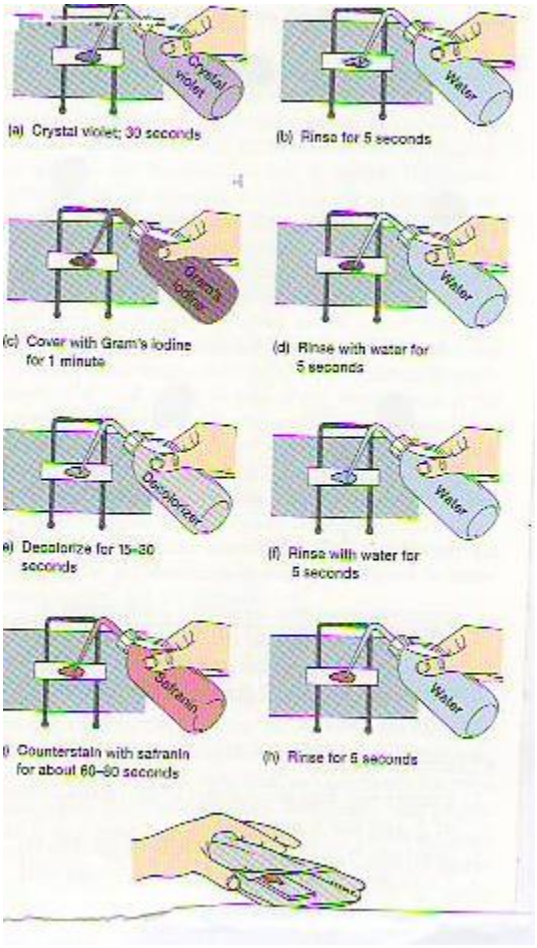
2- Flood the smear with crystal violet for 1 minute , wash with water.

3- Flood the smear with grams iodine for 1 minute ,wash with water.

4- Decolorize with ethyl alcohol by adding drops of solution until no more color appear (30 seconds). The time may by vary depending on the thickness of the smear .Wash with water.

5- Counterstain with dilute carbol fuchsin (1%) or safranin for 1 minute ,wash with water.

6- Observe under oil immersion ,the gram positive bacteria are dark purple and the gram negative are red or pink in colour.



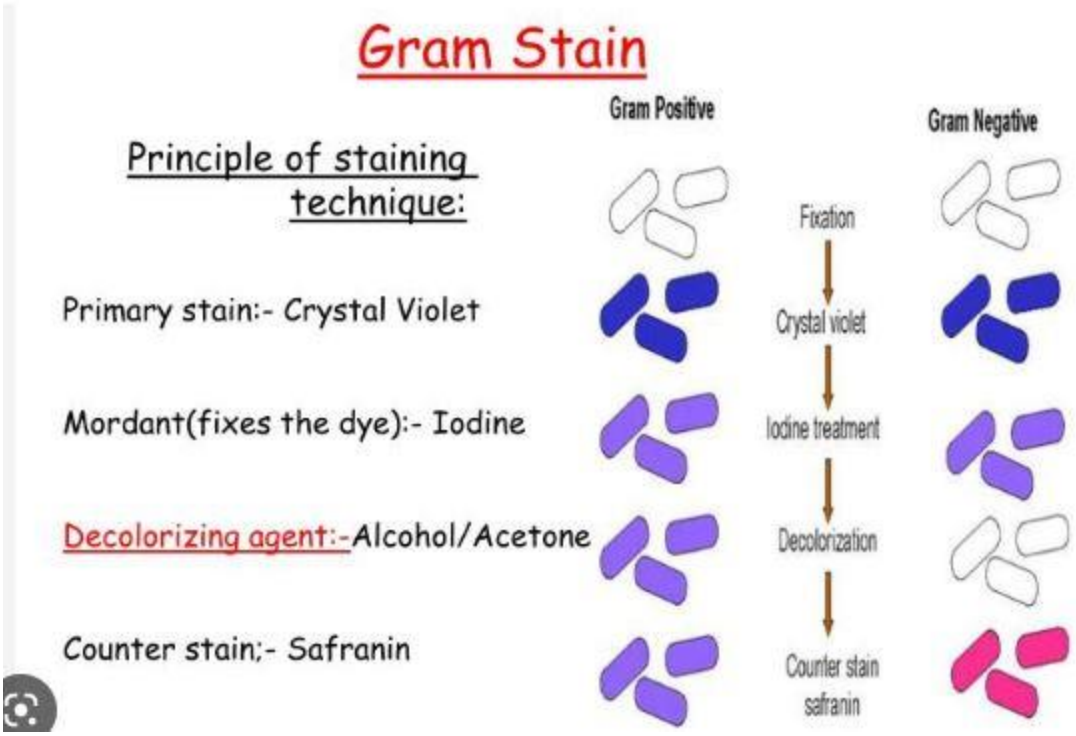
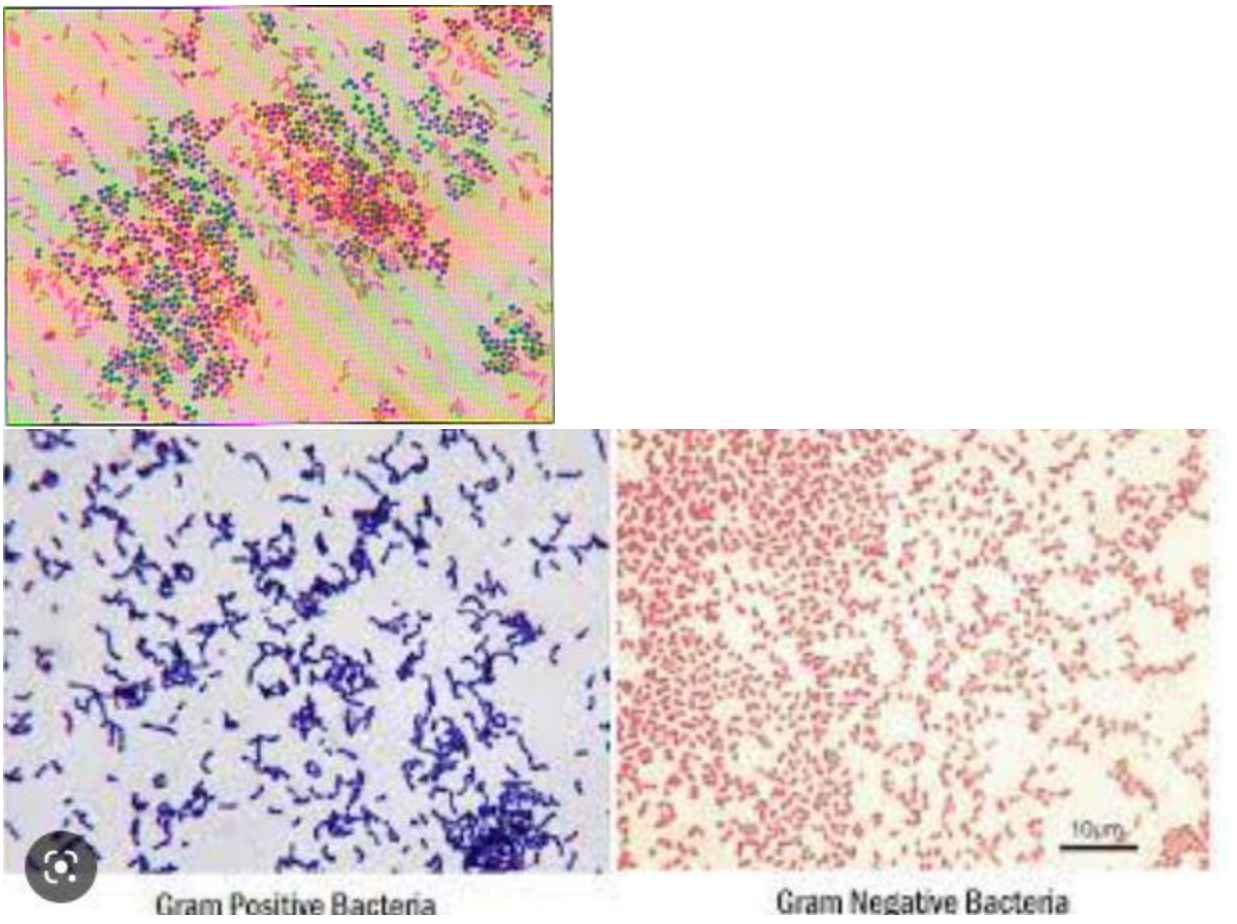


Fig.(8 and 9): Procedure Gram staining



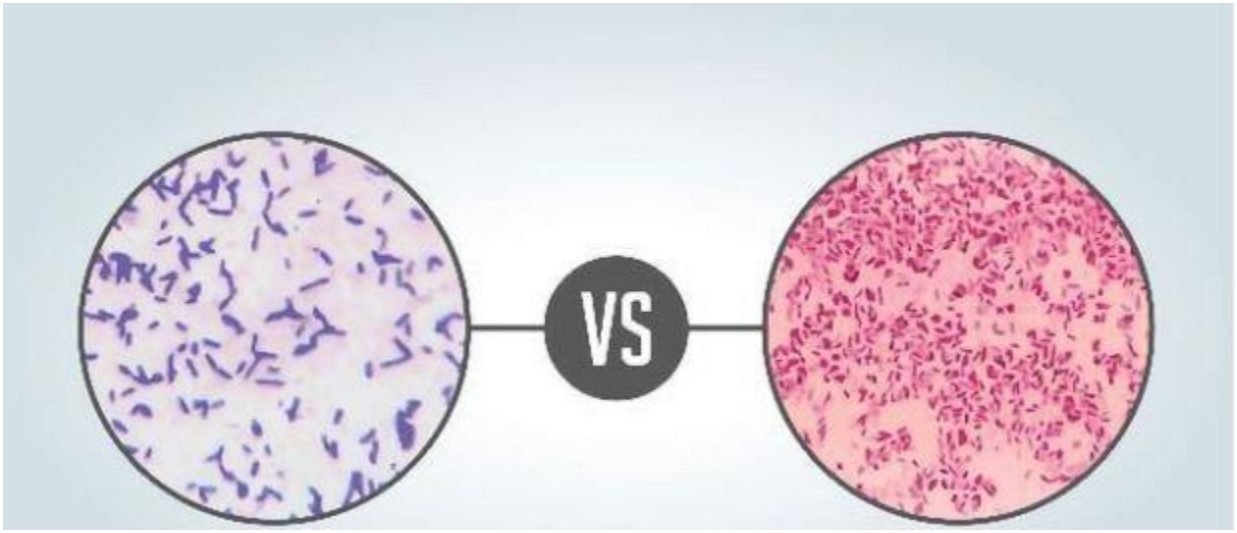


Fig.(10,11,12):Gram stain results (positive and negative)

2. Acid fast stain

The acid-fast stain is a laboratory test that determines if a sample of tissue, blood, or other body substance is infected with the bacteria that causes tuberculosis (TB) and other illnesses stain also called,

Acid-Fast Bacteria—Ziehl– Neelsen Stain

Is a differential stain that is used in the identification of Mycobacteria and other acid fast bacteria .Acid – fast means the ability of a microorganism to resist depolarization by acid alcohol after primary staining .Acid alcohol resistant is due to the presence of a high lipid content (40-60%) in the cell envelope (gram negative bacteria have no more than 20% while gram positive bacteria have 1-4% lipid in their cell envelope).The acid – fast staining procedure also called the ziehl-neelson technique

The lipoid capsule of an acid-fast organism stains with carbol-fuchsin and resists decolorization with dilute acid rinse. The acid-fast bacilli will stain bright red, and the background will stain blue.

Purpose of using : Used in the demonstration of acid-fast bacteria belonging to the genus 'mycobacterium', which include the causative agent for tuberculosis.

Principle: The lipoid capsule of the acid-fast organism takes up carbol-fuchsin and resists decolorization with a dilute acid rinse (Fig.13 and 14)

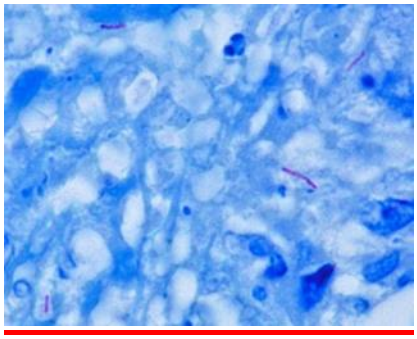


Figure (13 and 14) : Acid Fast Bacteria (AFB)

Technique(fig,15,16)

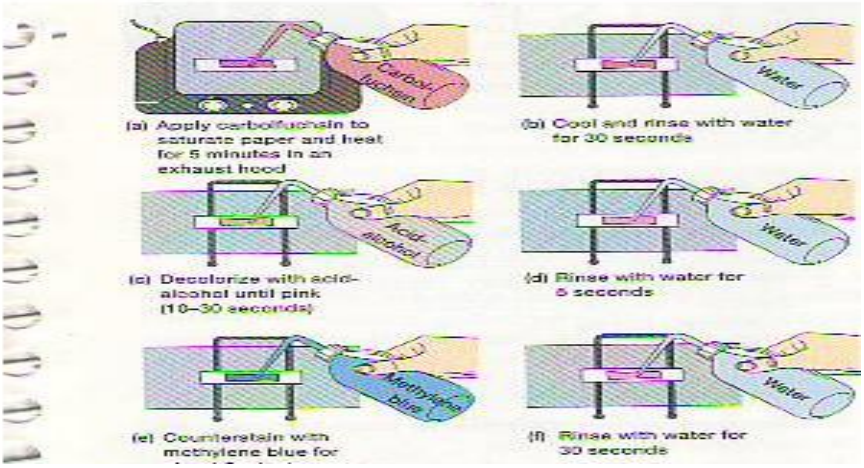
1- Prepare fixed smear of a mixture of two bacteria for example E.coli and mycobacterium on the slide.

2- Flood the smear with concentrated carbol fuchsin with flaming until steaming for 5 minutes wash with water.

3- Add the decolorizer acid alcohol (20% H_2SO_4 in ethanol or 3% HCL in ethanol), until no more color appears. Wash with water.

4- Flood with methylene blue for 1 minute.

5- Wash with water, dry the slide and observe under oil immersion. Acid fast bacteria appear red and non-acid fast bacteria appear blue. Notify fig. (15 and 16).



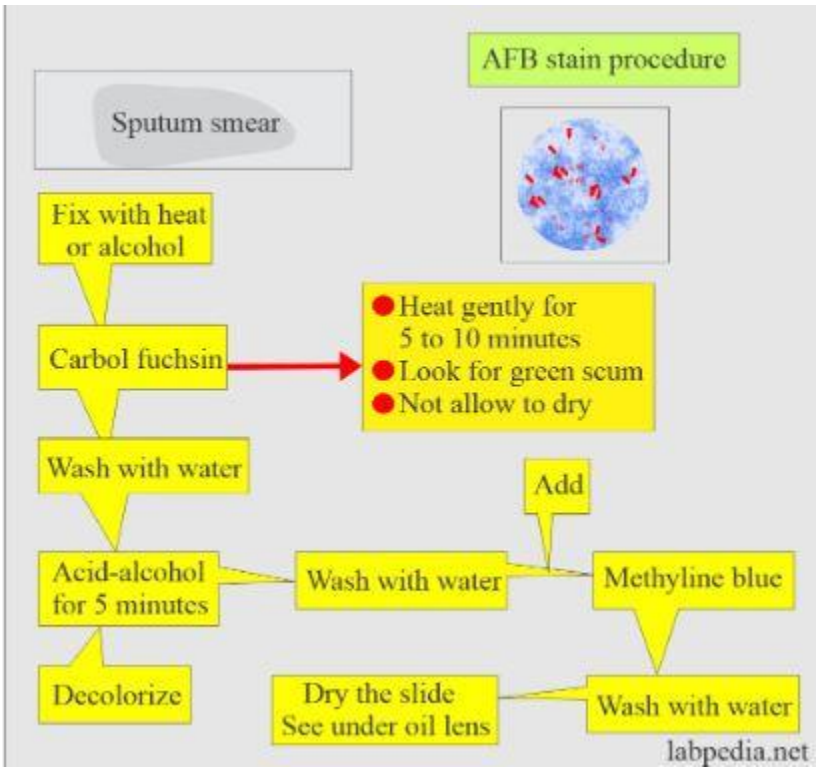


Figure (15 and 16): Technique OF AFB Stain