Culture Media

A- Natural Media

The media about which one cannot be certain of the exact components or their quantity are called Natural Meida for Bacteria.

(a) Beef Broth:

Since the time of Pasteur and Koch, microbiologists have used media such as beef broth for the laboratory cultivation of bacteria.

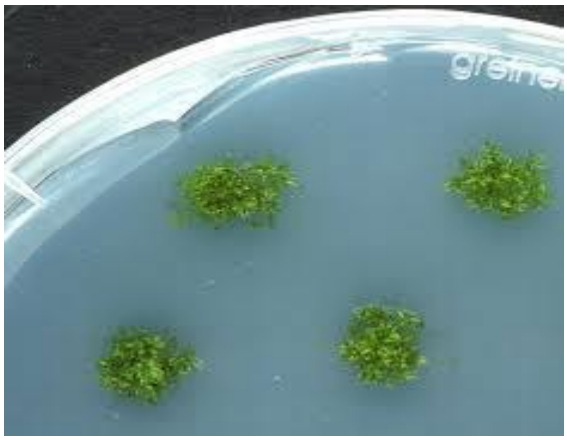
(b) Nutrient Agar

The modern form of this liquid medium, called nutrient broth, consists of:

1-Water, beef extract, and peptone. Peptone is a protein supplement from plant or animal sources.

2 -Agar. **Agar** is a polysaccharide derived from marine algae. It adds no nutrients to the medium but only serves to make it solid so that bacteria can be cultivated on the surface.

(c) <u>Enriched medium:</u>



Most common bacteria grow well in nutrient broth and nutrient agar, but certain fastidious bacteria may require specially enriched media.

Enriched media are:

(i) Blood Agar:

Some the streptococci that cause strep throat and scarlet fever grow well when whole blood is added to the nutrient medium. In this instance, the medium is called blood agar.

(ii) Chocolate Agar:

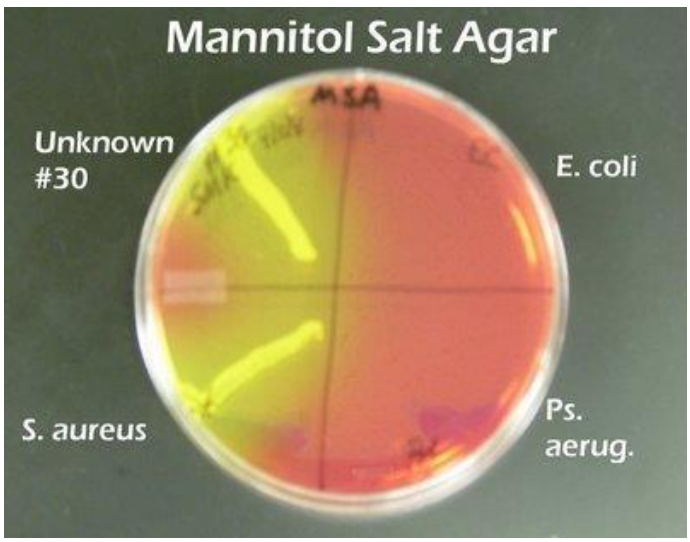
To encourage the growth of Neisseria species, blood agar is heated before solidification. Heating disrupts the red blood cells and releases the hemoglobin. The medium is now termed chocolate agar because of its charred brown appearance.

(e) <u>Slective Media</u>:

Selective media contain ingredients to inhibit the growth of certain bacteria in a mixture while permitting the growth of others. For example,

(i) <u>Mannitol Salt Agar:</u>

Staphylococci are cultivated-on **<u>mannitol salt agar.</u>** This medium contains mannitol and a high salt concentration that inhibits most other bacteria. Mannitol is an alcoholic carbohydrate fermented by staphylococci.



(ii) <u>Eosin Methylene Blue Agar :(EMB).</u>

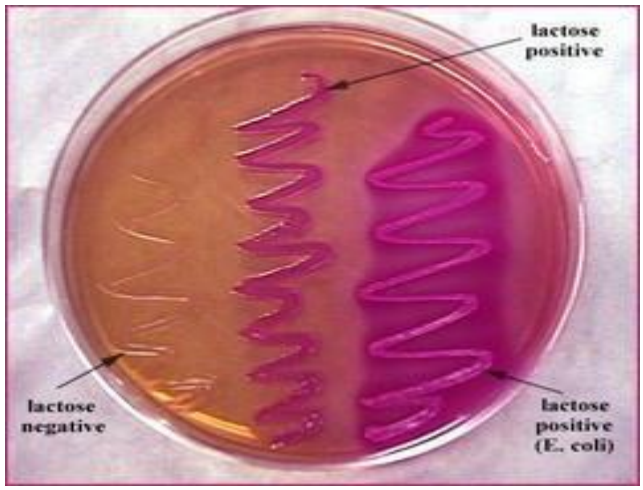
This selective medium has carbohydrates fermented by E. coli and other Gram-negative bacteria. It also contains eosin and methylene blue, two dyes that inhibit Gram-positive bacteria.

The _____ dyes select for the growth of Gram-negative organisms. Organisms capable of fermenting _____ form dark purple colonies that sometimes have a metallic sheen.

(f) <u>Differential Medium:</u>

Another type of medium is the **differential medium.** This medium makes it easy to distinguish colonies of one organism from colonies of other organisms on the same plate.

(i)Mac Conkey agar is typical. It contains



1-The dyes neutral red and crystal violet

2- Carbohydrate lactose. Those bacteria that ferment the lactose take up the dyes and form red colonies; other bacteria show up as colorless colonies

3-MacConkey_agar contains bile salts that inhibit the growth of Gram-positive bacteria.

This medium is thus selective as well as differential.

B-Synthetic Media

Synthetic media are chemically defined. Here the nature and amount of each component are known. Such a medium might contain glucose, ammonium phosphate, potassium phosphate, magnesium sulfate, and sodium chloride.

1) The glucose supplies energy to the cell;
2) The ammonium ions are a source of nitrogen for amino acid arid nucleic acid formation;
3) Phosphate is used in DNA and RNA synthesis;
4) Sulfur from magnesium sulfate is valuable for enzyme formation;
5) Sodium chloride maintains a stable internal environment in the cytoplasm.

BACTERIAL CULTURES

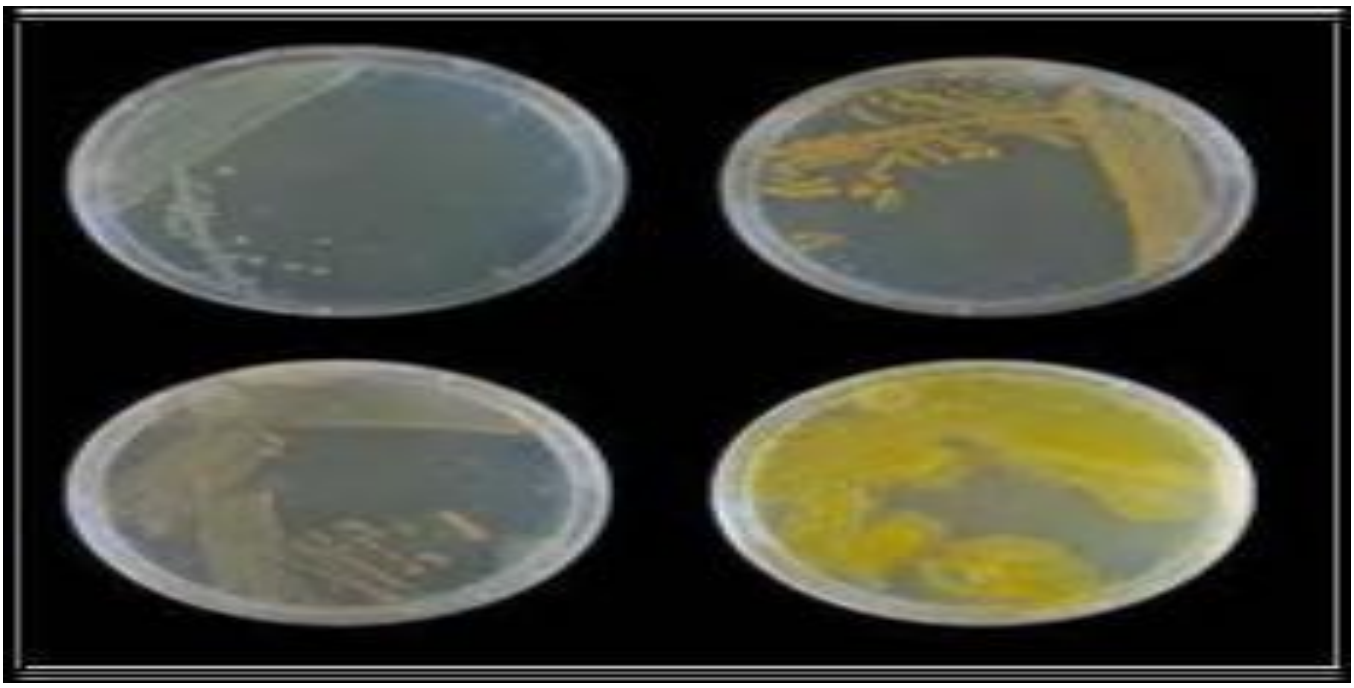
Bacteria or other microorganism grow on a laboratory they are referred to as a culture.

PURE CULTURE

A pure culture consists of a population of cells which are derived from a single cell.

METHODS OF ISOLATION OF PURE CULTURE

I. THE STREAK PLATE TECHNIQUE



The procedure of streaking a plate with an inoculating loop is used to spread millions of cells over the surface of a solid medium so that some individual cells are deposited at a distance from all others. These cells grow and reproduce, forming an isolated colony.

One or more colonies will be well Separated from all others and "represent a source of a pure culture.

The procedure is similar to the one used for streaking from the collection swab.

MATERIALS:

- Streak plates
- Nutrient agar plates (NA)
- Bunsen burner
- Bacteriological loop

STUDENT DIRECTIONS

- Examine the streak plates prepared and locate a number of well-isolated colonies.
- Now you must transfer a portion of each colony to a separate agar slant.
- To "pick" a colony you will be using an inoculating loop.
- Sterilize the loop in the burner flame, let cool 3-5 seconds then touch the end of the loop to the isolated colony, picking up the microorganisms from the colony.
- Now recover the streak plate and pick up one NA plate.
- You will now be holding the inoculating loop in your right hand and the fresh NA plate in your left hand (lefties reversed).
- Remove the lid from the plate, place the inoculating loop at one edge of the plate and with a sweeping stroke, and inoculate the agar using the

same tri-streak method as used for the initial isolation.

- Replace the lid.
- Flame the loop and proceed to inoculate another plate from different colonies.
- Try to use colonies that are visibly different in morphology.
- Incubate the plates in the 37°C incubator.

2) POUR PLATE TECHNIQUE

PURPOSE

- The pour plate technique can be used to determine the number of microbes/ml or microbes/gram in a specimen.
- It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs.

PROCEDURE

The principle steps are to

1) Prepare/dilute the sample.

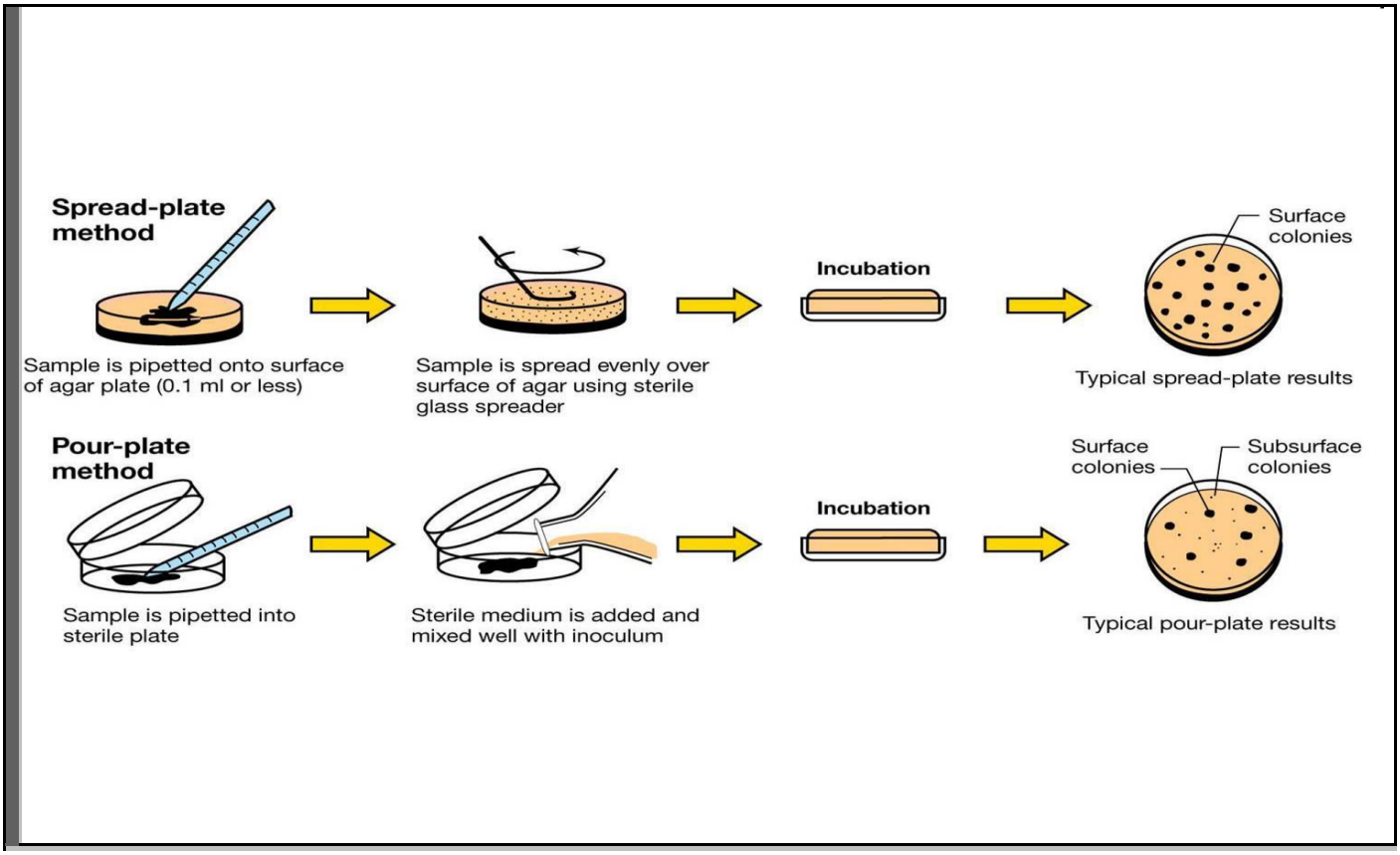
2} Place an aliquot of the diluted sample in an empty sterile plate.

3) Pour in 15 ml of melted agar which has been cooled to 45° C, swirl to mix well

4) Let cool undisturbed to solidify on a flat table top

5) Invert and incubate to develop colonies.

Each colony represents a "colony forming unit" (CFU). For optimum accuracy of a count, the preferred range for total CFU/plate is between 30 to 300 colonies/plate



DISADVANTAGE

- One disadvantage of pour plates is that embedded colonies will be much smaller than those which happen to be on the surface, and must be carefully scored so that none are overlooked.
- Also, obligate aerobes may grow poorly if deeply imbedded in the agar.

3) SPREAD PLATE METHOD

- By means of a transfer loop, a portion of bacterial specimens is spread over the surface of a solid medium.
- After appropriate incubation, growth from each culture should be checked microscopically and culturally to verify that it a pure culture.

LIMITATION:

Very small amount of specimen can be spread over the agar.

ADVANTAGE:

Minimal amount equipment s required,

4) ENRICHMENT CULTURE TECHNIQUE

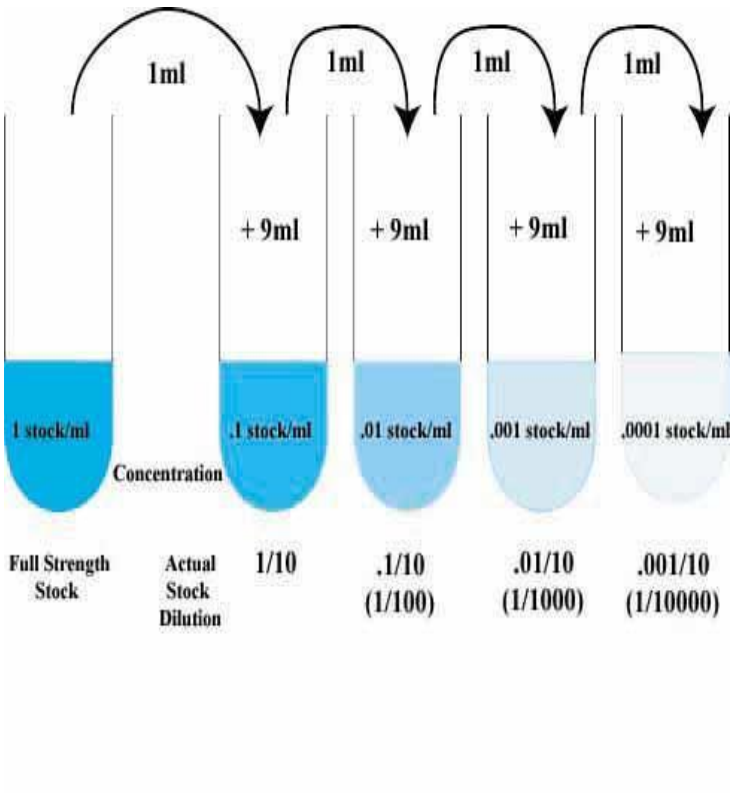
Enrichment cultures are generally used when the type of bacterium to be isolated is present in relatively small number and grows more slowly than many other species in the inoculum.

5) SERIAL DILUTION TECHNIQUE

1) If the organism in a mixed culture in present in greater number than any other organism, it may be possible to obtain it in pure culture by a series of dilution in tubes of appropriate medium,

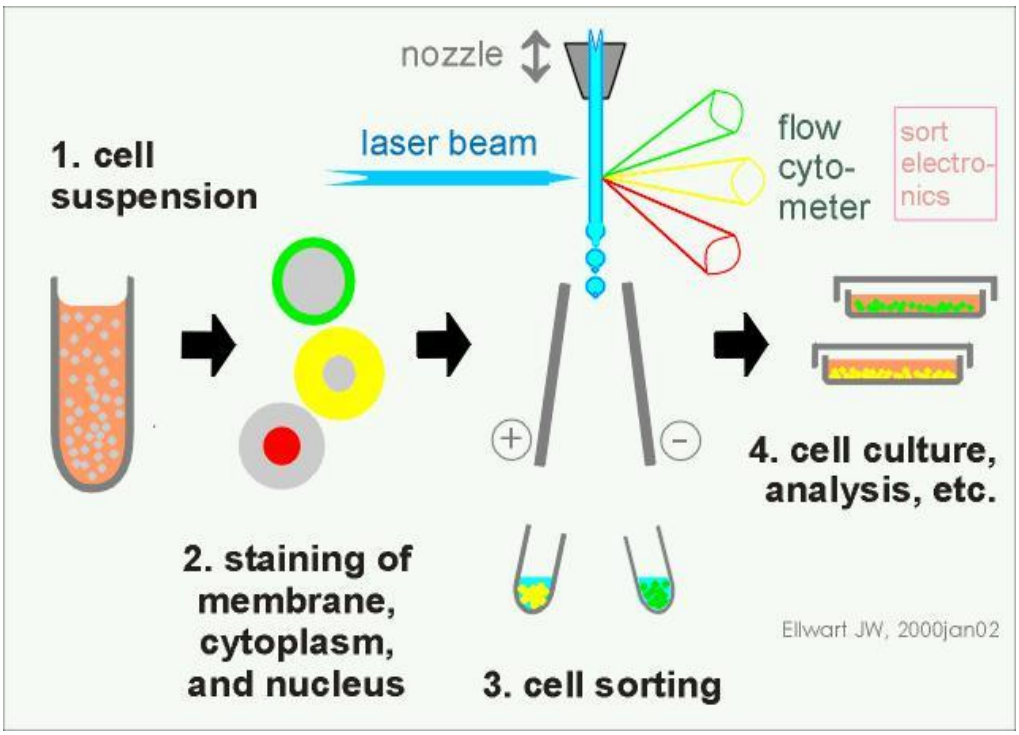
2) When greatly diluted, the specimen contains only the one specie

3) It is advisable to confirm the purity of a culture isolated in this fashion, by a plating procedure,



6) SINGLE CELL ISOLATION TECHNIQUE

- Special equipment, the micromanipulator, can be used in conjunction with a microscope to pick a single organism from a hanging drop preparation.
- The micromanipulator permits the operator to control the movement of a micropipette in the hanging drop so that a singe-cell can be taken into the tube and transferred to a suitable medium for growth.
- The technique is reserved for use in highly specialized studies as it requires skilled operator.

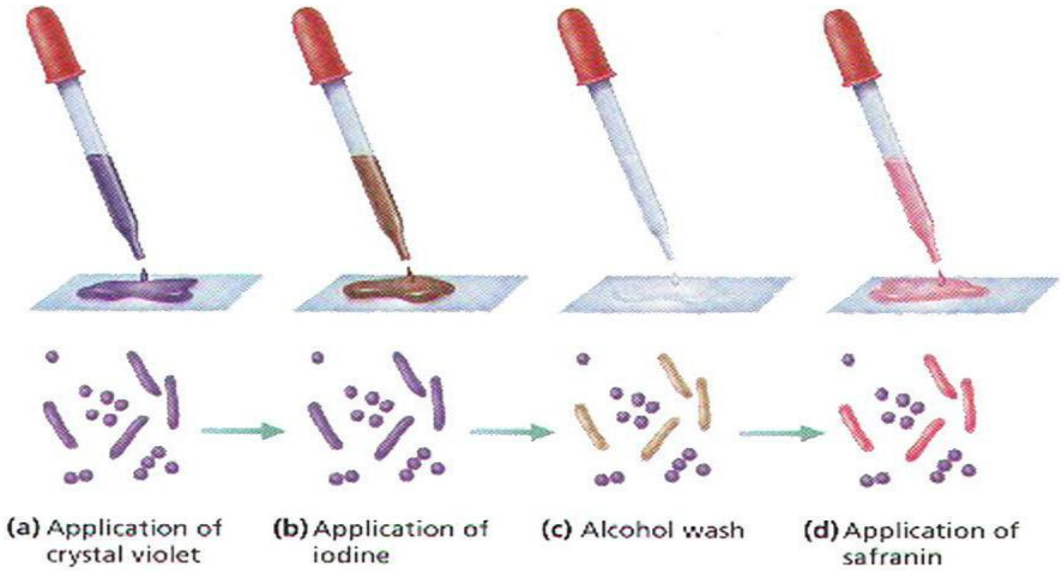


Staining Techniques

1) Gram's Stain

This is the most widely used but not a fully understood technique various theories put forward are.

- It is has been shown that gram-positive organisms contain a substance known as magnesium ribonucleate which gram positive bacteria they will react as gram negative organisms.
- When iodine is applied for staining with crystal violet or another stain of that group a compound is formed which is insoluble in water but soluble in alcohol or acetone .It is said that the more permeable the organism the more likely It is to be gram-negative since the acetone or alcohol has easier access to the compound which it will dissolve.
- It is also through that the pH of the organism has at least some influence on the reaction .Gram –positive bacteria have a more acid cytoplasm and this is increased by the addition of iodine. According to this school of through it is the acidity of the cytoplasm which helps the organism to retain the stain.



http://www.sirinet.net/~jgjohnso/monerans.html

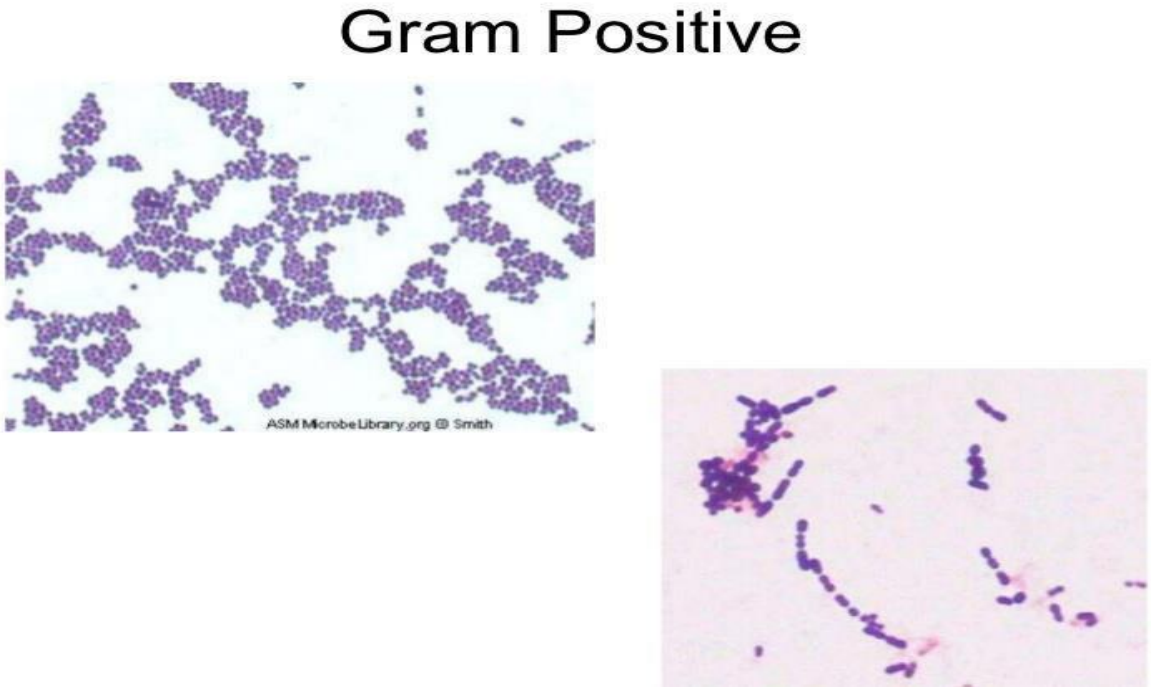
Method

- Make a thin smear of the material or culture let it dry at room temperature .Heating should be avoided as this interferes with the staining reaction

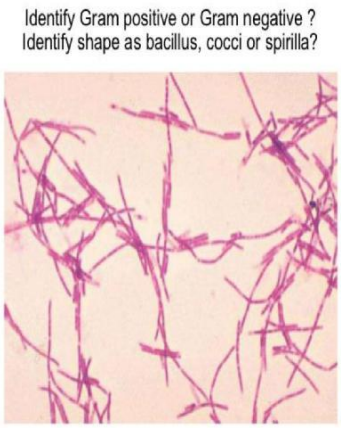
- Pass the slide through a flame once or twice or until it feels comfortable warm on the back of the hand
- Place the slide on the rack and flood with the crystal violet or gentian violet stain-stain for one minute.
- Was off the stain with gram's or Lougol's iodine and leave the slide covered with iodine for one minute.
- Rinse in water.
- Pour on acetone or alcohol till no more violet color comes from the slide
- Rinse in water again
- Stain with one of the following counter-stains; Safrainin ,Neutral red or 1:10 Carbolfuchsin
- Rinse in water and allow it to dry by standing it vertically or by blotting it with filter paper.

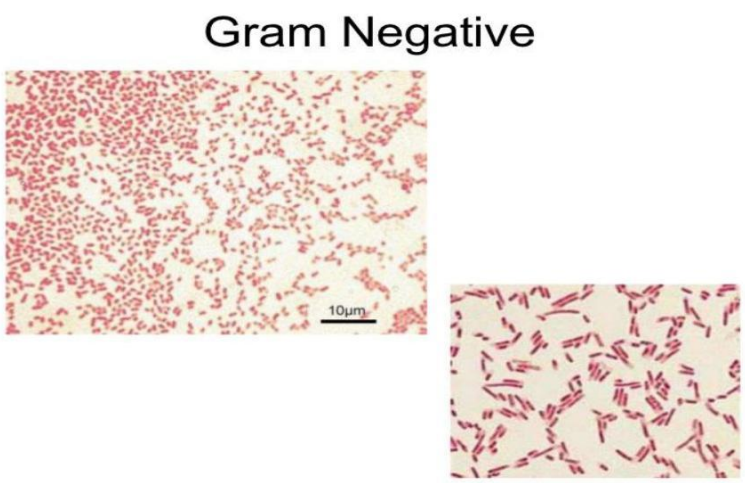
Results

Because the gram-positive organisms retain the crystal violet after decolorized they appear violet in color



.The gram negative organism are decolorized and take up the counter-stain and therefore appear pink in color .





- Crystal violet -0.5 % solution in distilled water.
- Iodine –(Lugol's)-10 gm iodine 20gm potassium iodine in 1000 ml of distilled water .Dissolve the potassium iodide in 250 ml water and then add 10 gm of iodine when dissolved make up to 1000 ml with distilled water
- Mixture of Acetone and Alcohol
- Counter stain
    - 1gm neutral red
    - 2 ml 1%Acetic acid
    - Distilled water to make 1000 ml
- Safranin
    - 1.7 gm safranin
    - 50 ml alcohol
    - Distilled water to make 500 ml
- Dilute carbolfuchsin
    - 1:10 dilution of strong carbolfunchsin

ZIEHL-NIELSEN STAIN /ACID-FAST STAINING

This stain is another method of categorizing certain bacteria dependind on their ability to resist decolourization by acid and alcohol a very strong stain is used basic fuchsin in a phenol solution and heat is applied in order that the stain can penetrate the waxy covering of certain bacteria.

Method

- Make a smear of the material and allow drying at room temperature.
- Flood the whole slide with strong carbolfuchsin and heat gently underneath the slide until steam is seen rising from the slide
- Rinse in water and flood the slide with 25% sulfuric acid .Leave the until the smear is pale pink in color.
- Rinse in water and pour on alcohol for a few minutes.
- Counter stain with malachite green ethylene blue or picric acid
- Dry by staining the slide vertically do not blot dry as the tubercle organism.may get attached of the paper a later may get transferred to another slide.

Result

The tubercle bacillus resists decolorizing by acid and alcohol it will remain bright red while all other organisms and material will take on the colour of the counterstain.