Lab--6 -- CULTURE TRANSFER TECHNIQUES

The bacteria are (microorganisms), that are growing on or in nutrients media producing what known microbial or bacterial growth or culture. when the growth forming from **one microorganism** is called **pure culture**, if the growth is formed from **more than one microorganism** is called **mixed culture**. Indication of growth in liquid media by **turbidity** and **changing the color of indicator stain**. On plate agar by **growing colonies**.

Culture transfer

From stock culture (nutrient broth tube or slant tube). (see the figure below)

1- All instruments must be sterilized.

2- The work is always under **aseptic conditions.**

3- The stock of a liquid culture must be gently shaken in order to uniformly suspend the bacterial cells.

4- Place the stock culture tube and the tube to be inoculated in the palm of one hand (stock culture tube in distal and the inoculated tube in the proximal place). The two tube are then separated to for V-shaped in the hand.

5- By the other hand flame the loop over a Bunsen burner until the wire becomes red-hot.

6- Using the same hand that is holding the inoculating loop, remove the cap, or the cotton plugs from the two tubes and briefly flame their necks over a Bunsen burner by passing them through the flame.(do not allow the tubes to become red –hot).

7- Cool the hot loop in the broth culture. Then transfer 1 drop (loopful) from stock culture tube into the new broth tube. (By this step could be also transfer to glass slide, streak the surface of a slant agar and streak the bacterial suspension onto surface of plate agar and stab inoculation.

8- Re-flame the loop and the necks of the tubes.

9- Recap the tubes.

10-Incubate the inoculated tubes or plates at favorite temperature.

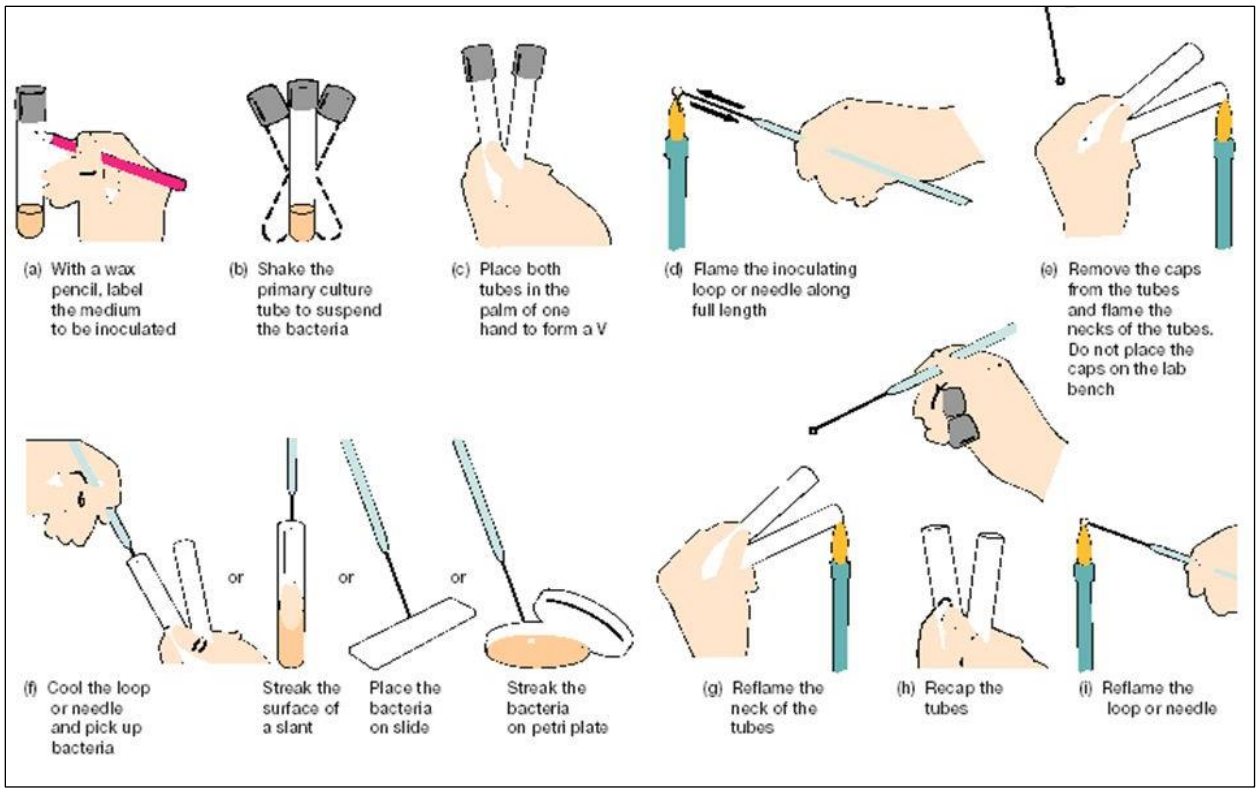

Figure (1) Aseptic technique for bacterial sub culturing

Using aseptic technique, repeat the same procedure to transfer with inoculating loop and needle to **Slant agar tube** (by undulate moving the loop or S-shaped moving from the bottom to up-side) and to agar deep tube (by stabbing) as shown in the following figure:

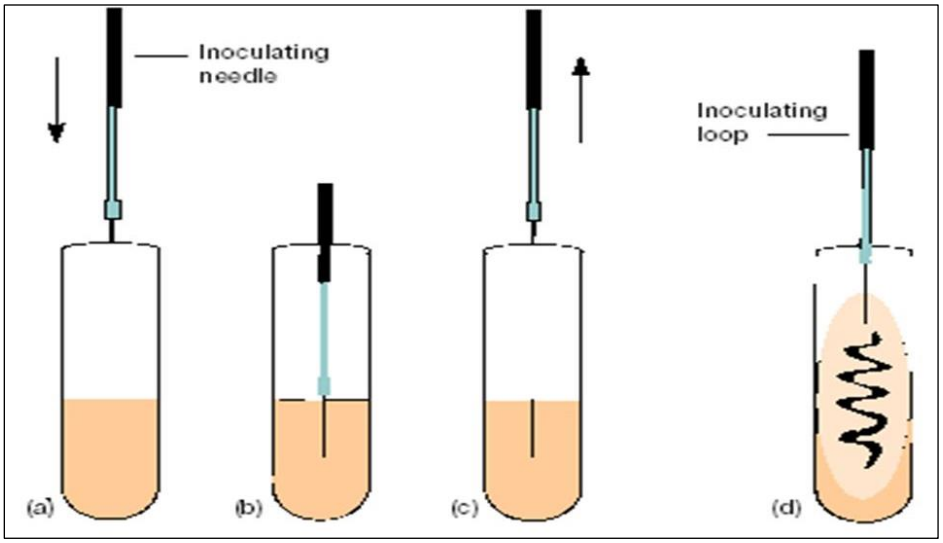

Figure (2) Transferring technique (a-inoculation, b-stabbing, c- streaking)

Pure isolated colonies

Isolation of pure colonies can be obtained by streak-plate, spread-plate, pouring-plate and dilution methods. In these techniques individual separated cells will be obtained, each cell will grow to form one colony; well isolated colonies develop in many regions of the plate. Few cells from separated colony can be picked up with an inoculating loop or needle and transferred on to any slant agar or other suitable medium for maintenance of the pure culture.

1) Streak plate technique and Streaking method:

Materials per students' group

- 24–48-hour broth culture of
- *Escherichia coli* (white colonies)
- *Bacillus subitles* (white or creamy colonies)
- *Serratia marcescens* (red colonies)
- Tryptic soy agar tubes (each 15 ml)
- Water bath (boiling)
- Water bath 48-50 °c
- Burner
- Petri-dishes 3
- Inoculating loop
- Wax pencil label strips

Procedures

1. Melt the **sterile**, **caped** tubes of nutrient agar by heating them in a boiling water bath.

2. Cool the tubes to 48-50°c in the water bath.

3. Pour each agar containing tube into one Petri-dish.

4. Allow the agar in the plates to cool and become solid.

5. Mark the agar plate, the name of bacterium, the name of student or group no. and date.

6. Divide the plate into 4 quadrants.

7. Sterilize the loop by flaming it and let it cool.

8. Under aseptic conditions, pick up a loopful of the bacterial mixture.

9. Streak out the loopful of bacterial suspension on the agar plate in directions as shown in figure (3) below:

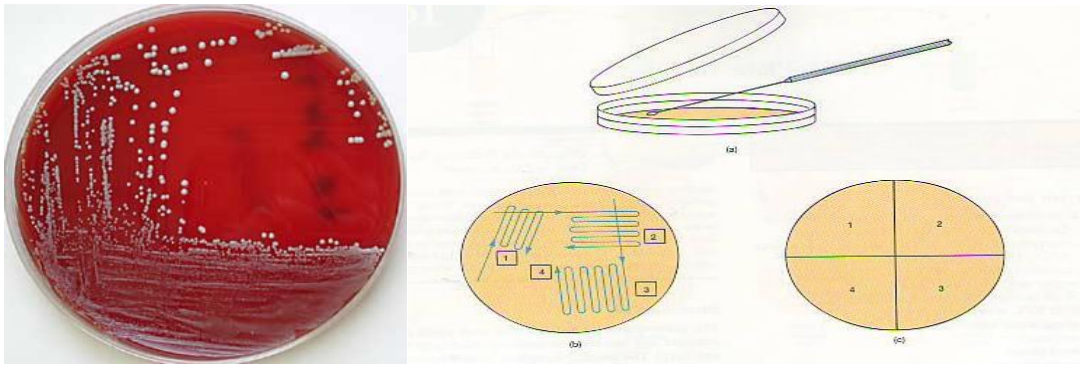

Figure (3) Streaking- plate technique

10. Start from the left by spreading the loopful suspension gently over small area (part 1).

11. Remove the inoculating loop and kill the remaining bacterial cells by flaming them, and then place the loop at the edge of the agar near area (part 1).

12. Rotate the plate anticlockwise.

13. Moving the inoculating loop from the left of part 1 and cross over the streaks in area 1 to area 2 (part 2) do the same as in area 1.

14. Remove the loop and flame it, cool in agar as previously mentioned.

15. Repeat the same above procedure to make the part 3 and 4

16. Repeat the above procedure for the other two agar plates and incubate the plates at 30 or 37°c for 24 -48 hours in inverted position. Examine each plate to determine the distribution of the colony in each part of plate.

2) Spread-plate technique

The aims of using this technique are to obtain separated colonies as pure and to study the characters of the different bacterial colonies. Which are visible to the naked eye. After incubation, the general form the shape of the edge, or margin, elevation of the colony can be determined see figure (below). In this technique a small volume of diluted bacterial mixture is transferred to the center of an agar plate and spread over the surface of agar with sterile L-shaped glass rod.

Materials:

- 24-48 hours broth culture (tryptic soy broth).
- Mixture of *Bacillus subtillus* , *Serratia marcescens* or *Micrococcus roseus* (or using soil diluted suspension).
-Bunsen burner.
-Inoculating loop.
- 95% ethyl alcohol.
- L-shaped glass rod.
- Wax pencil.
- 500 ml Beaker.
- Pipettes with pipette.
- 3 tryptic soy agar plates (or N.A).

Procedure

1. With wax pencil, label the bottom of the agar plates, the name of inoculated bacteria, the name of student or group no., and the date.

2. Pipette 0.1 ml of bacterial mixture suspension on to the center of agar plate.

3. Dip the L-shaped glass rod into ethyl alcohol and tap the rod on the side of beaker to remove the excess alcohol.

4. Briefly pass the ethanol- soaked spreader through the flame to burn off the alcohol and allow it a way of the burner, then touch it on the side surface of agar.

5. Gently spread the bacterial sample over the agar with spreader in different directions with rotating the plate to be sure that the entire surface of the plate has been covered.

6. Immerse the spreader in ethanol, tap on the side of the beaker to remove any excess alcohol and repeat flaming, then let it to cool see (fig. below).

7. Incubate the plates at 30 °C or at room temperature in inverted position.

8. In the next session examine the colonies and characterize their morphology (shapes, edges, elevations, colors, transparencies): choose different colonies

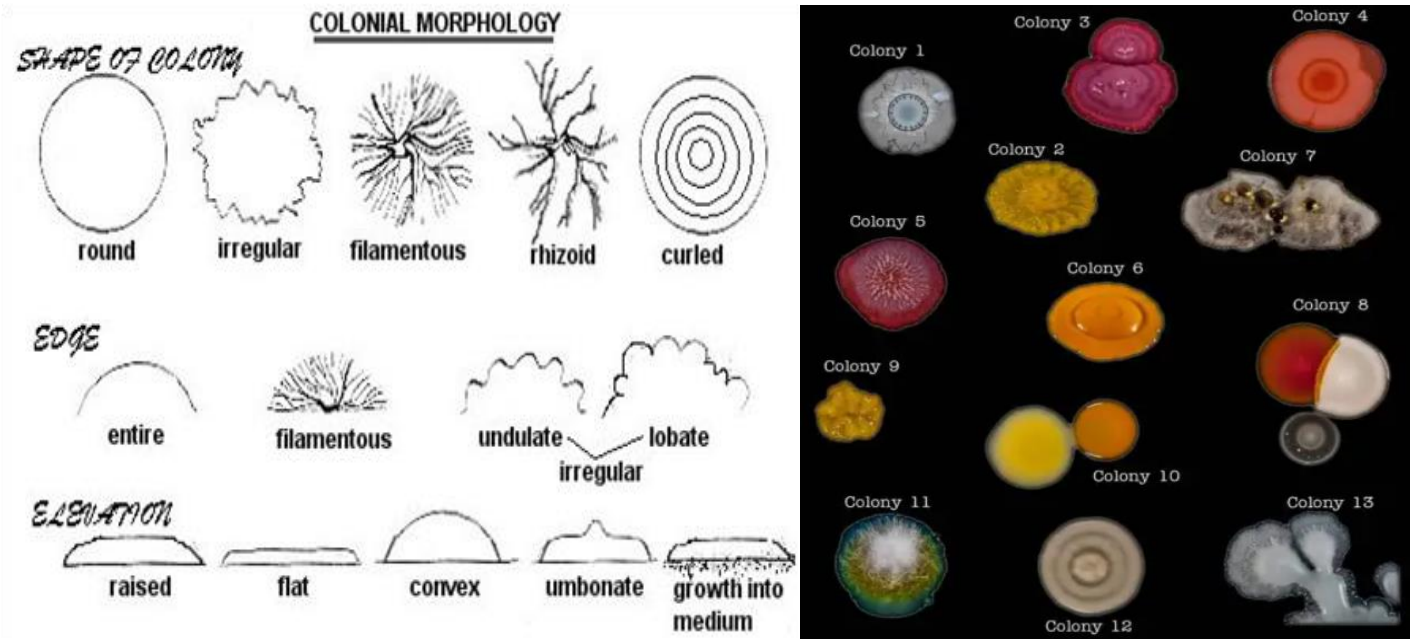

Figure (4) colony morphology

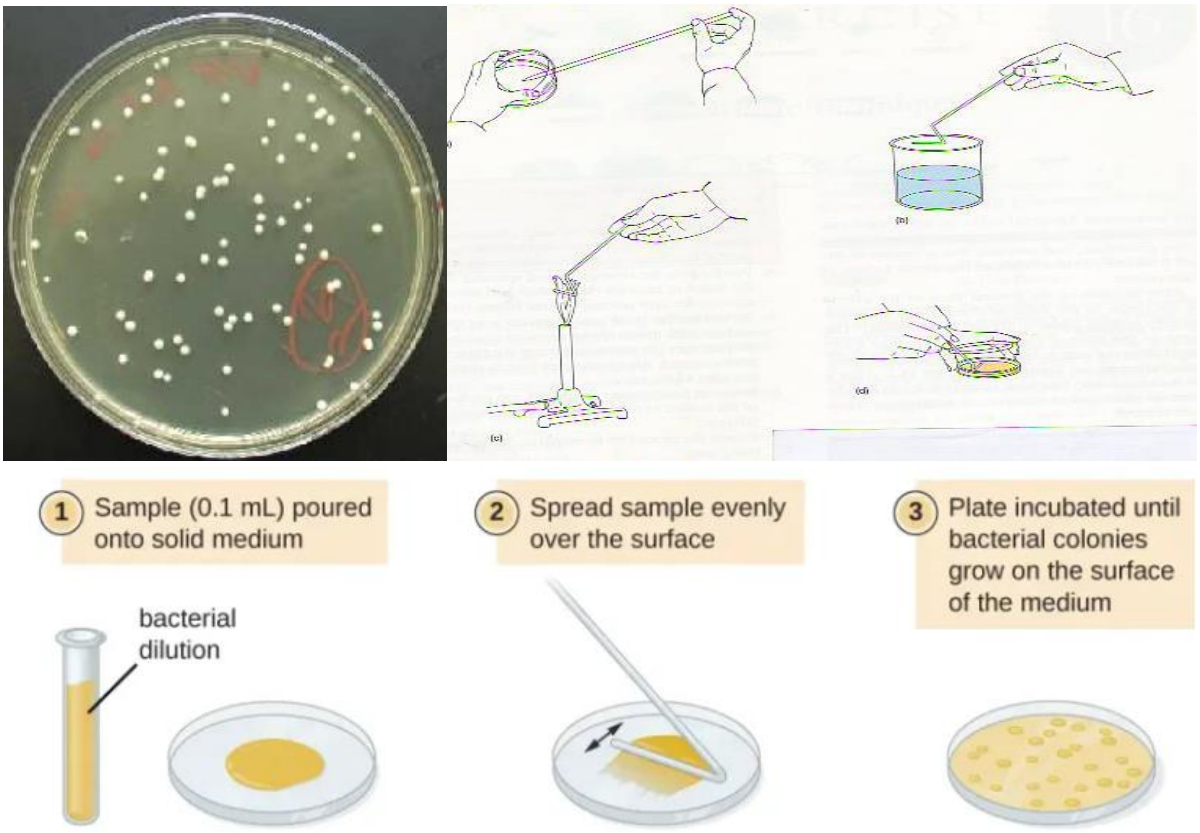

Figure (5) Spreading-plate technique

3) Dilution and pour plate technique

This is another method that is used to obtain well isolated pure colonies. pour-plate technique will yield also isolated colonies and has been extensively used to obtain pure culture of bacteria and fungi. The original mixed culture is diluted several times to reduce the microbial cells density. Small volumes of serially diluted samples are added to sterile Petri plates, and then mixed with melted agar that has been cooled to about 48-50°c. The mixing is done by rotating the poured agar immediately on the bench (clock and anti-clockwise). Then let the agar plate to become solid.

Materials

- 24-48 hour mixed broth culture
- N.A tubes
- Sterile 0.9% NaCl (saline) 9 ml in each tube (3 or 4 tubes).
- Water bath (boiling).
- Water bath 45-50°c.
- Inoculating loop.
- Sterile pipettes (3-4).
- Wax pencil (or label papers).
- Bunsen burner.
- Sterile petri-plates (3-4).

Procedure

1. Label three or four saline tubes (9 ml in each one) serial dilution 10^{-1},10^{-2}, 10^{-3}, 10^{-4}.

2. Label three-four Petri-plates with dilutions (10^{-1},10^{-2}, 10^{-3}, 10^{-4}).

3. The original sample is diluted several times, with pipette take 1 ml from the mixed culture broth after shaking, added to tube no.1 (10^{-1}) mix gently and take 1 ml from this tube (no.1, 10^{-1}), add it to the next tube (no.2, 10^{-2}) mix well and take

1 ml from it to another tube gain (10^{-3}) and so on up to desired dilution, as shown in (figure 6).

4. Take 1 ml from the first diluted tube (10^{-1}) and add that amount to the sterile plate marked (10^{-1}), take the same quantity from the second tube (no.2 , 10^{-2}) and add it to the next plate (10^{-2}). Complete the same process up to the last tube 10^{-4}. Follow the steps as demonstrated in the (figure 6).

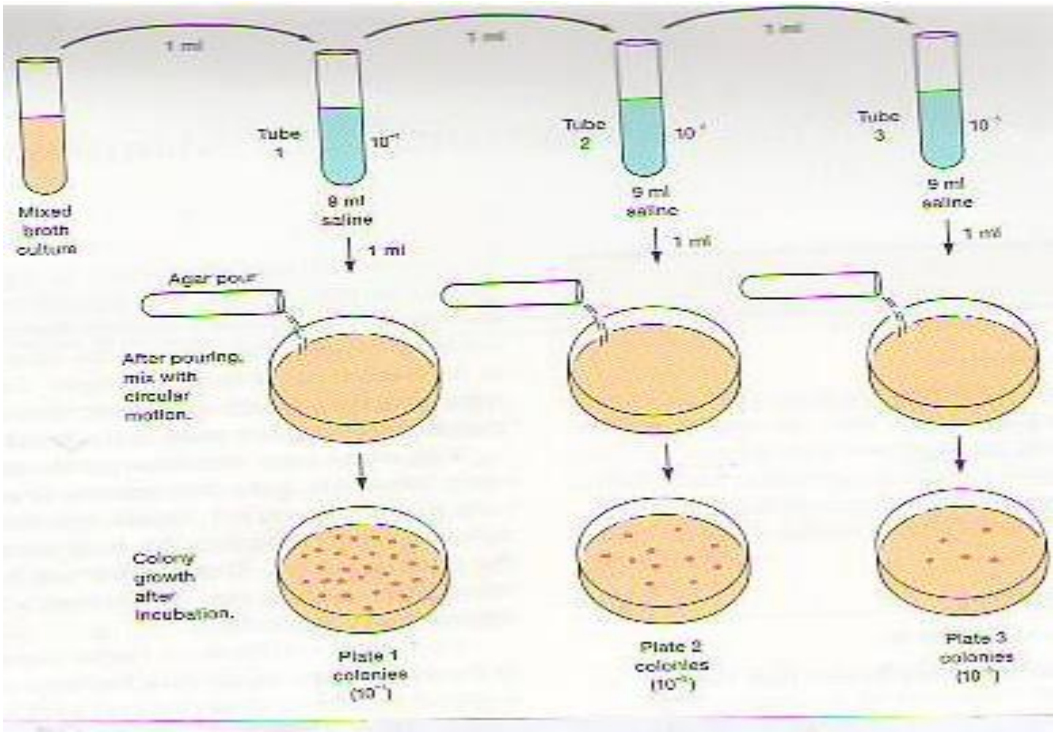

Figure (6) Dilution and pouring plate technique.

5. Melt the agar in boiling water bath, then cool it in water bath ≈ 50°c.

6. Add 20 ml of the melted agar to each plate and mix each agar plate with rotating it gently clock and anticlockwise on the bench (as shown in figure 7), do not allow the agar to splash over the side of the plate. Set the plate aside to cool and to be solidified. Then incubate the plates at 30°c for 24-48 hours.

7. Examine the growth and counting the no. of the colonies in each plate, record your results in report.

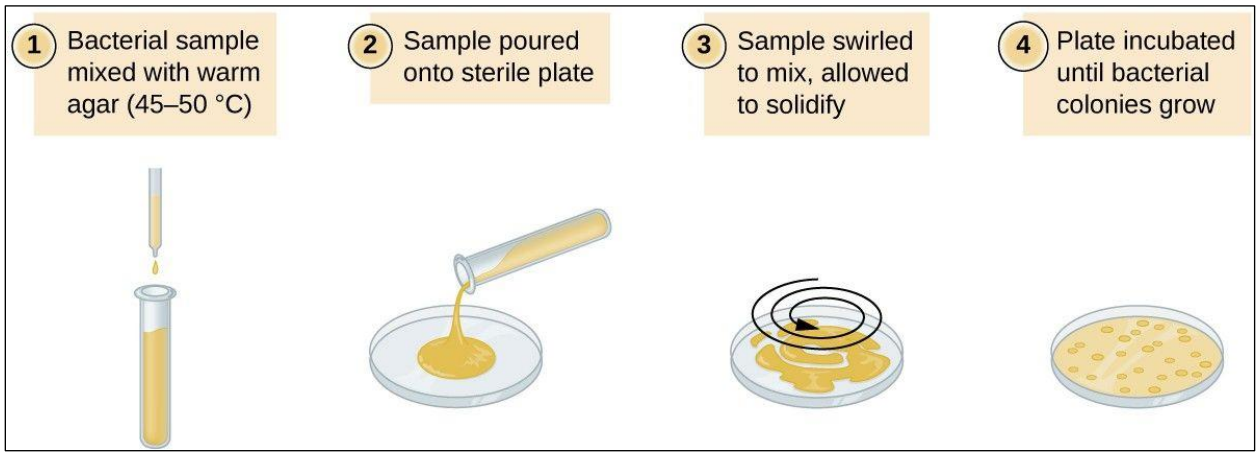

Figure (7) Pour plate Method.

Maintenance of pure culture

By the previously described methods , it is possible to gain isolated colonies of microorganisms, an isolated colony represents the growth of one cell.

Maintenance should be performed For further more purposes such as characterization (cell morphology, staining, Biochemical tests, immunological tests for the identification of the pure isolation of known and unknown microbes. The pure culture from well-separated colony are transferred into agar slant ,that represents the growth of single genus of microorganisms and is called pure or stock culture.

The storing of stock culture for several weeks or months needs periodically sub culturing the growth of microorganisms and storing in refrigerator or by desiccation For short-term maintenance (3-6 weeks) or more. Aerobic bacteria can often be achieved by storing slant culture in the refrigerator at 4-10°c.

Procedure

1. With a wax pencil, label the agar slants agar and broth tubes.

2. Under aseptic condition, select a well-isolated colony and pick off from the center of the colony into agar slants, then from that colony into broth medium. Repeat this step from other different colonies.

3. Incubate at 25-30°c 24-48 hours.

4. Observe the broth culture of each organism (take care not to agitate the tubes) see (figure 8).

5. Place the pure cultures in the refrigerator for later use.

6. Draw and write a report on your results.

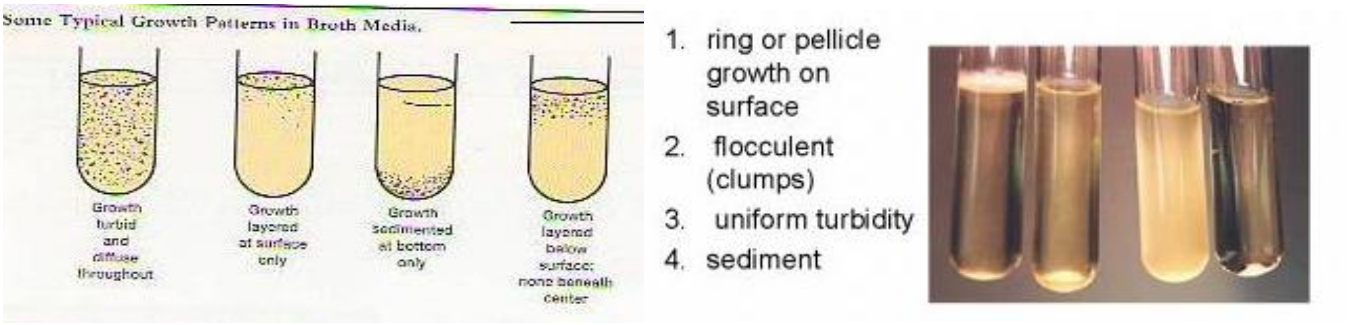

Figure (8) -Some typical growth patterns in broth media.