Lab–3

Handling of Bacterial Cultures

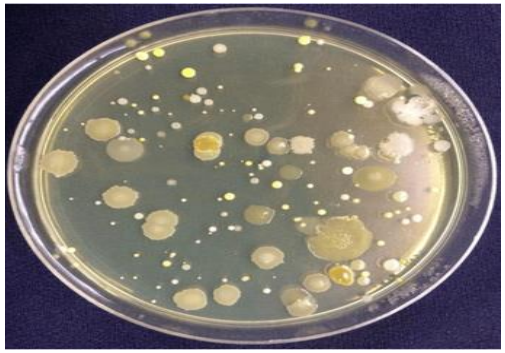
Cultures of MO are important to:

- **Study the biological characteristics of MO**
- **Provide pure colonies of single MO**
- **Permit the subsequent steps of diagnosis that include staining and biochemical reactions**

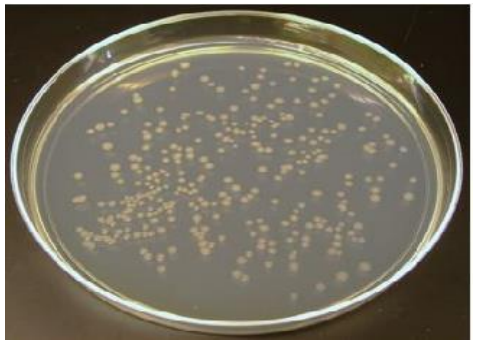
In Solid medias (Petridish agar plate or slant tube)

- Individual organisms will multiply at individual sites until a visible aggregate called a **colony** is formed.
- Coloneal morphology can be easily studied
- Isolation of more than one type of bacteria growing together in a specimen (called mixed culture).
- Pure culture obtained by using loop & transferring single species to another medium, where it will grow as a pure culture, and can be studied as such.

A **colony** is a large number of bacterial cells on solid medium, which is visible to the naked eye.

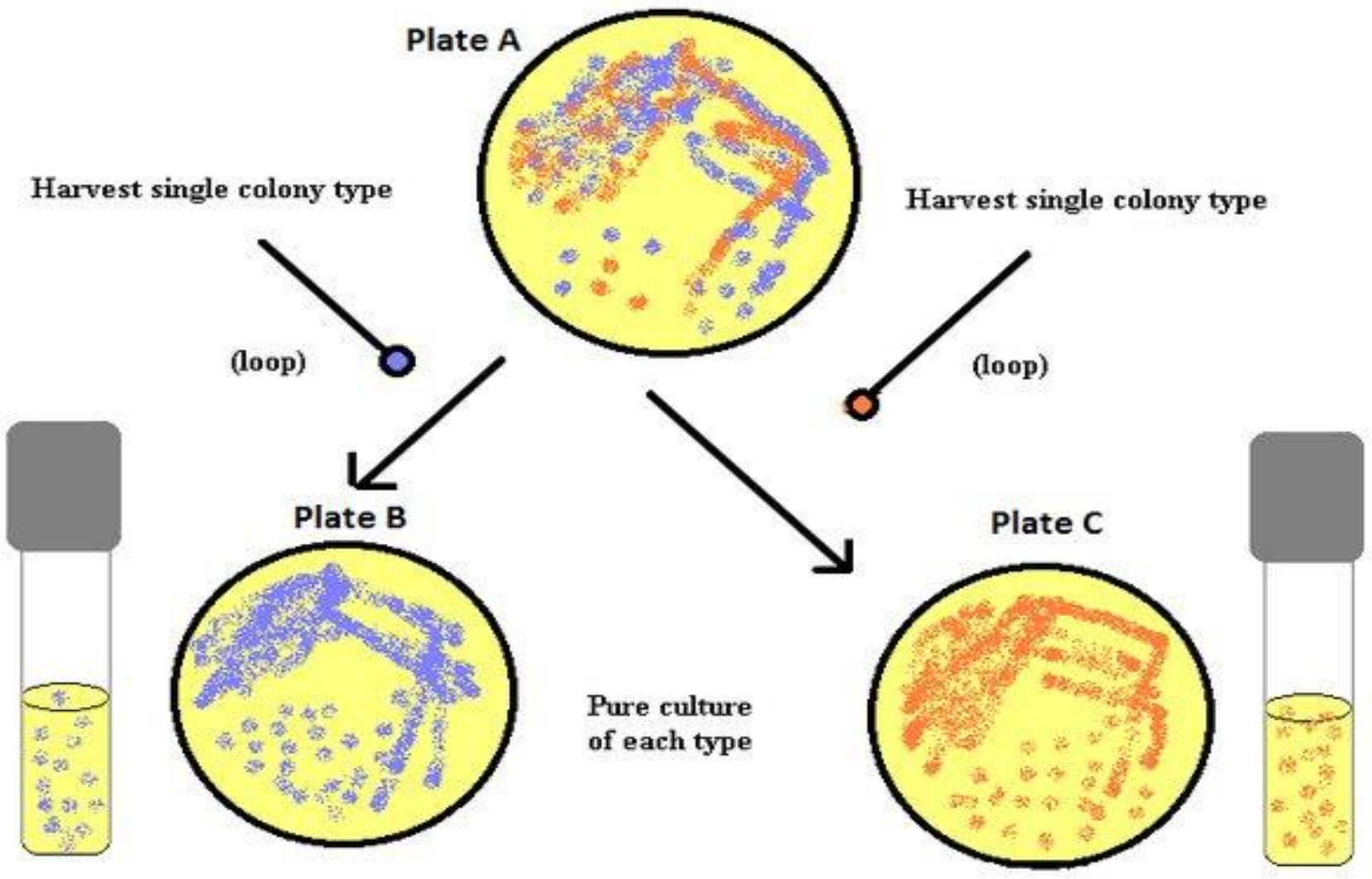


Mixed culture



Pure culture

Mixed and pure cultures



In liquid media (broth)

-Bacteria may grow easily in a diffuse manner, producing uniform clouding, or may looks very granular in broth.

-Layering of growth at the top, center, or bottom of a broth tube reveals something of the organism's oxygen requirements.

-Coloneal morphology cannot be studied

Culture Techniques

Culturing of MO involve the following steps

First step: Transfer MO by using loop

Materials;

- MO that want to study it which may be taken from: petridish, slant or broth
- Wire inoculating loop
- Bunsen burner

Method:

-Flaming the loop by Bunsen burner then lets it cool.

-With a sterile cool loop, pick one isolated bacterial colony from solid media (dish or slant) or from broth by introducing the loop inside the broth (technique will be illustrated by the following slides)

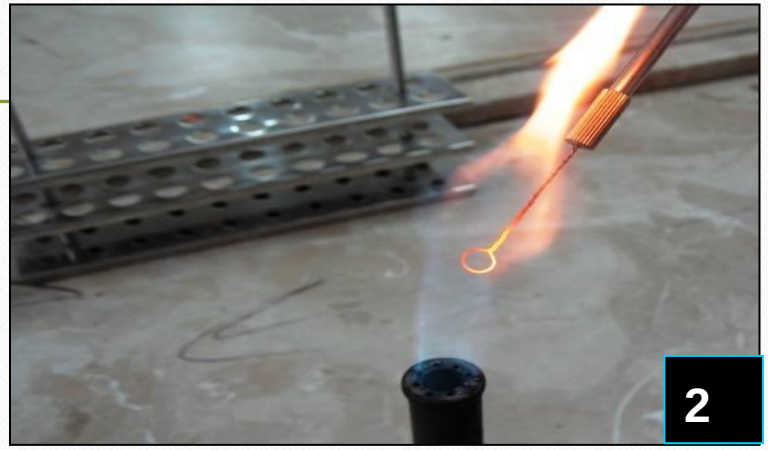
Second step:

Loop with MO then can be inoculated to already prepared culture medias

A. Solid media (petridish or slant)
B. Semi solid media
C. Broth tube

First step: Transfer MO by using loop

Disinfect the area with 70% alcohol.



Flame the loop.

In case of tube (slant, broth)

Hold the culture tube cap with the right hand.

Open the culture tube.

Flame the mouth of the culture tube.

Transfer a loopful of organisms.

Flame the mouth of the culture tube again.

Return the cap.

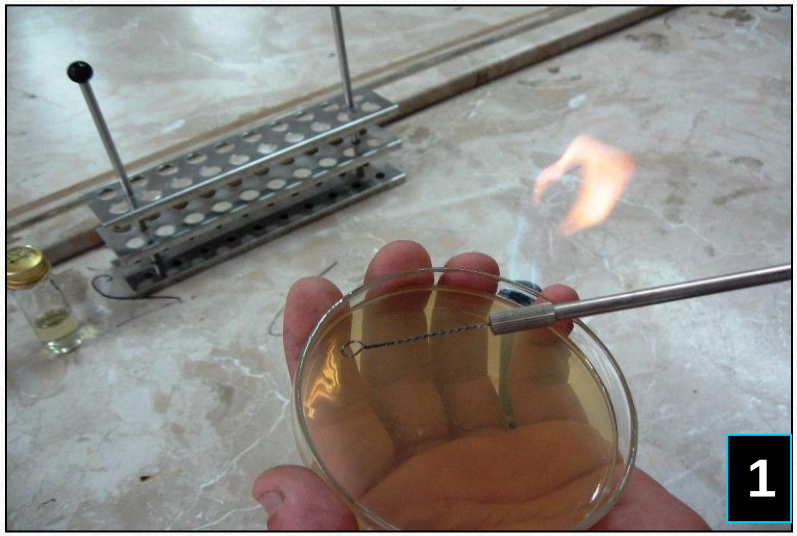
In case of Petridish (agar solid media)

Place the dish in an inverted position (base spueriorly) Hold the base by left hand then with the sterile cool loop, pick one isolated bacterial colony then replace the base.

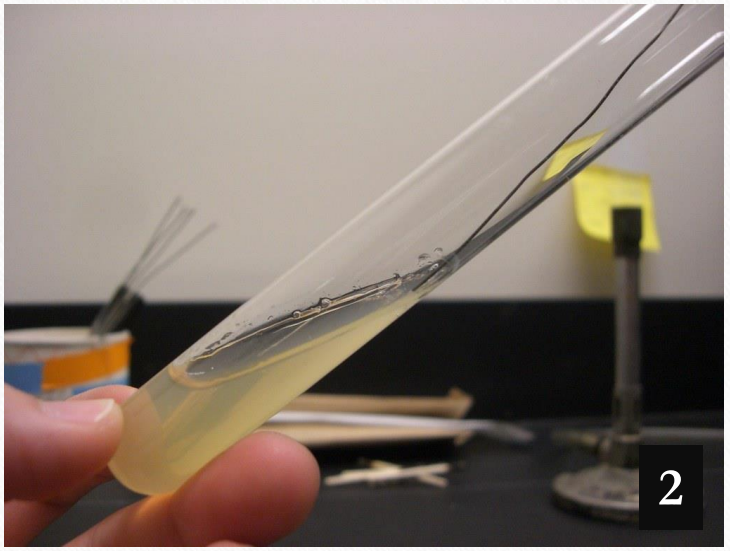
Second Step

Inoculation on agar medium

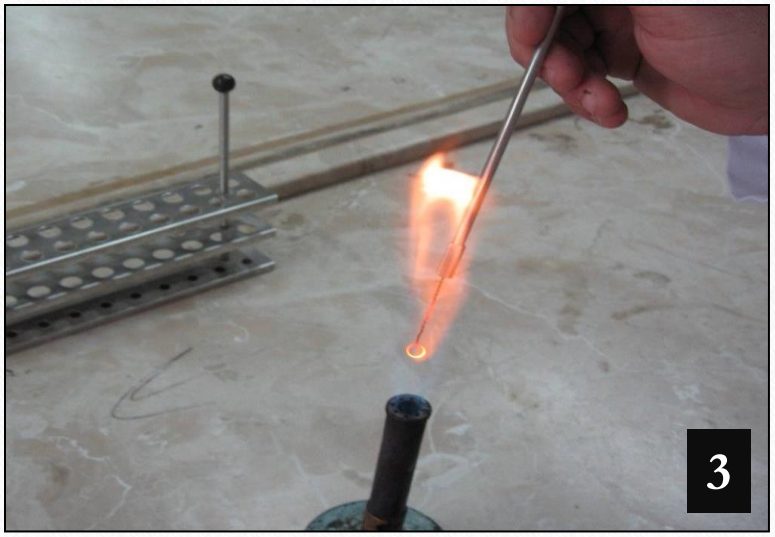
A. Inoculation of the infected loop in solid media (petridish and slant)



Streak the plate with one of streaking patterns.

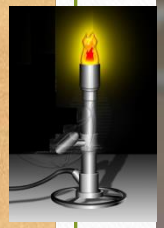


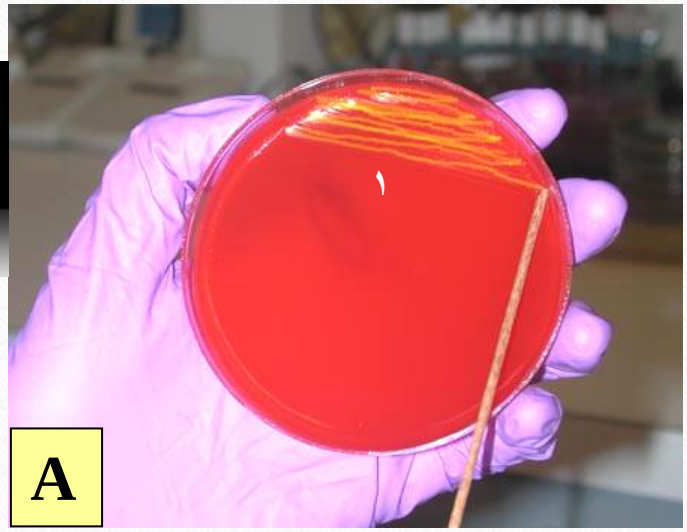
Streak the slant by single streak line . Flame te mouth before and after streaking



Flame the loop before placing it down.

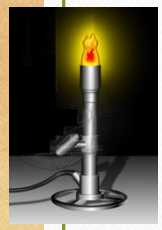
Streaking Method

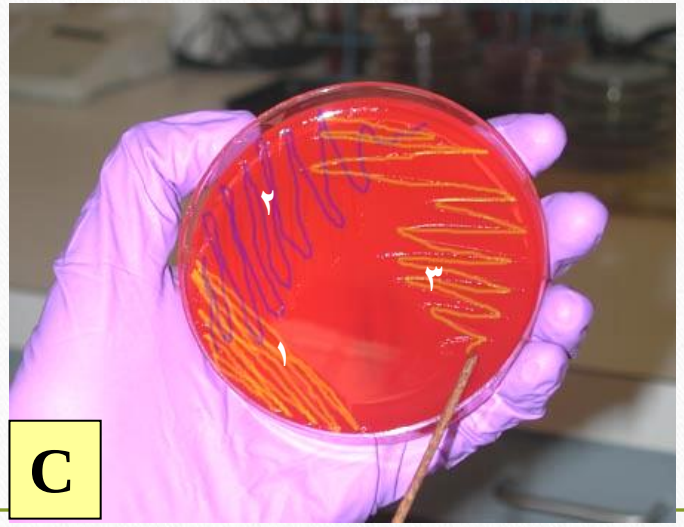




A

B

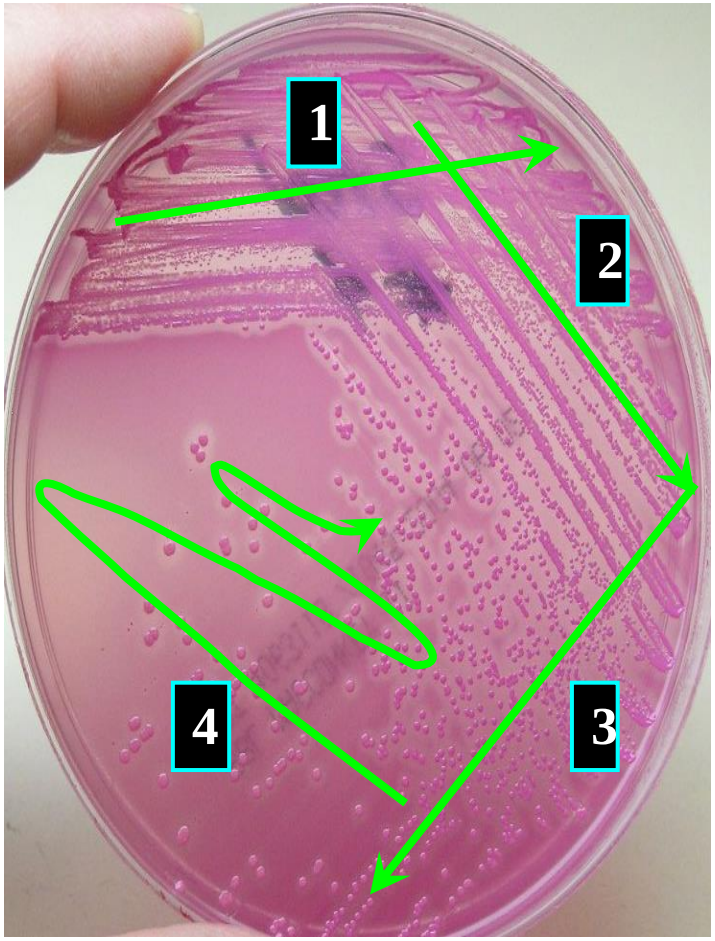




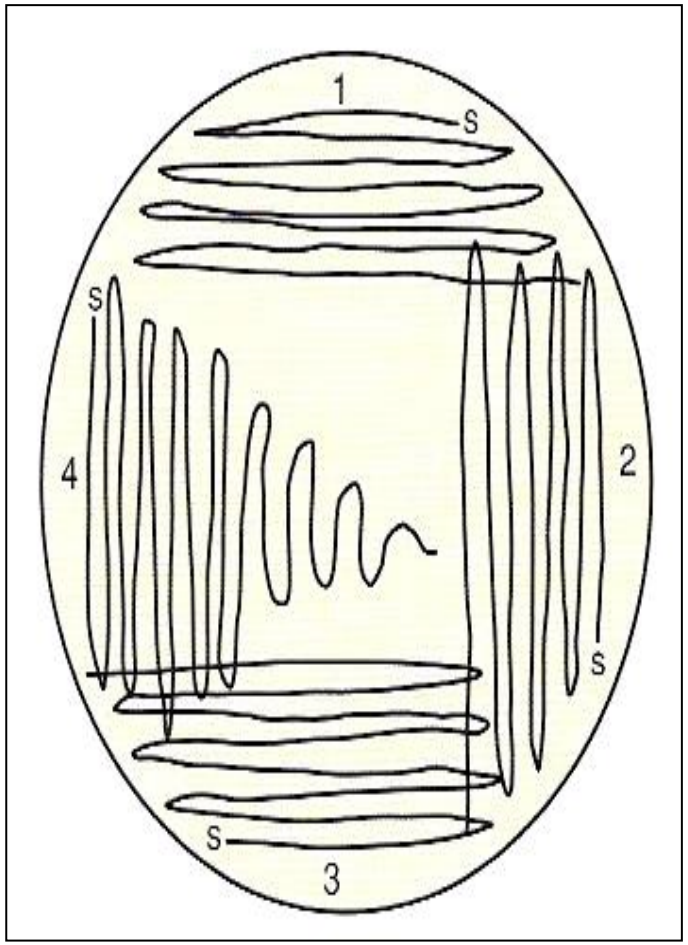
C

D

Streaking on an agar plate for obtaining isolated colonies



MacConkey medium

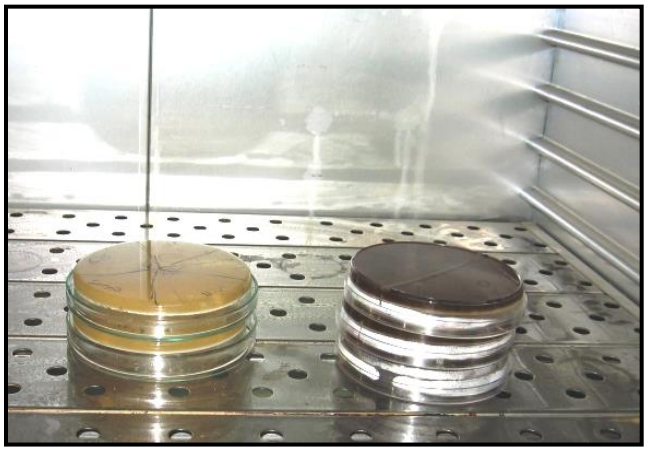


Streaking

- Incubate the plate **inverted,** at 37° C for 24 hours.

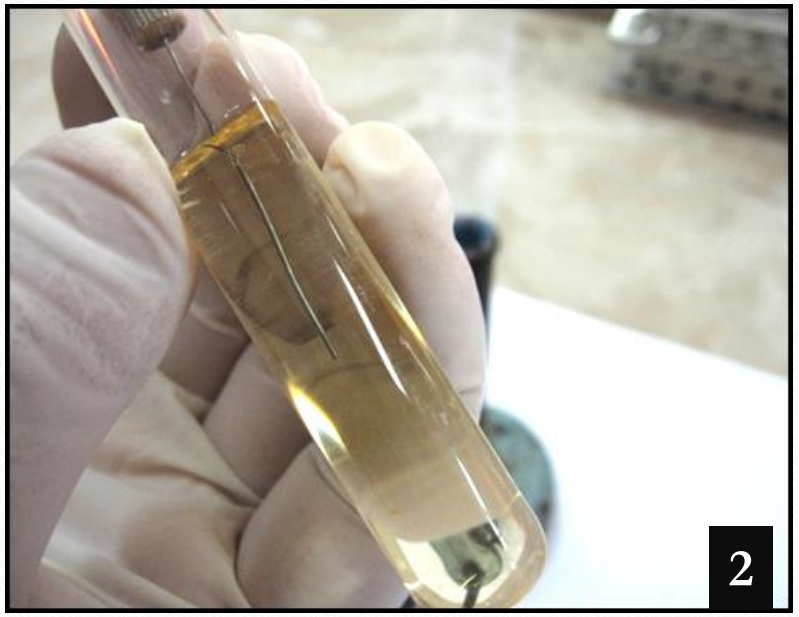
- By incubating plates upside down will **minimize condensation of moisture on the cover.**

- **Any moisture on the cover is undesirable** because if it drops down on the colonies, the organisms of one colony can spread to other colonies, defeating the entire isolation technique.



B. Inoculation of the infected loop in semisolid media Stabbing method

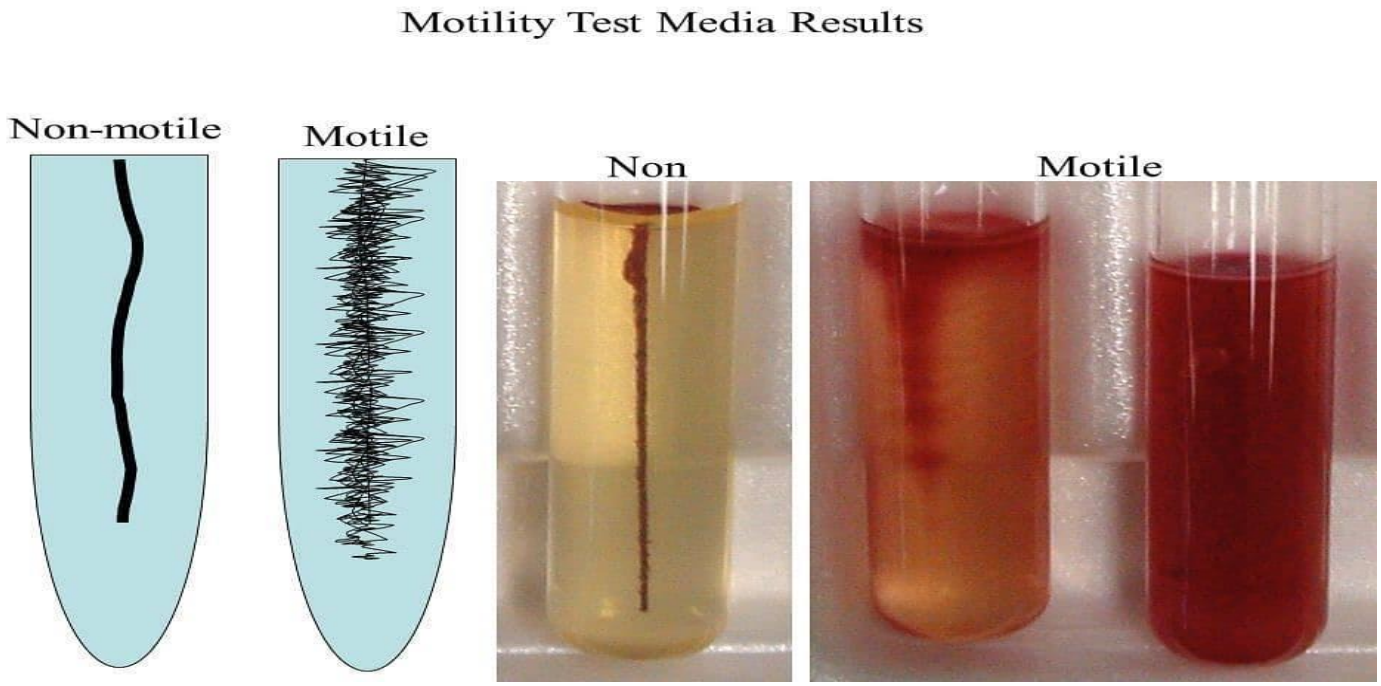
Open the tube and flame its mouth



Make a deep stab inoculation with tested bacteria into the gelatin.

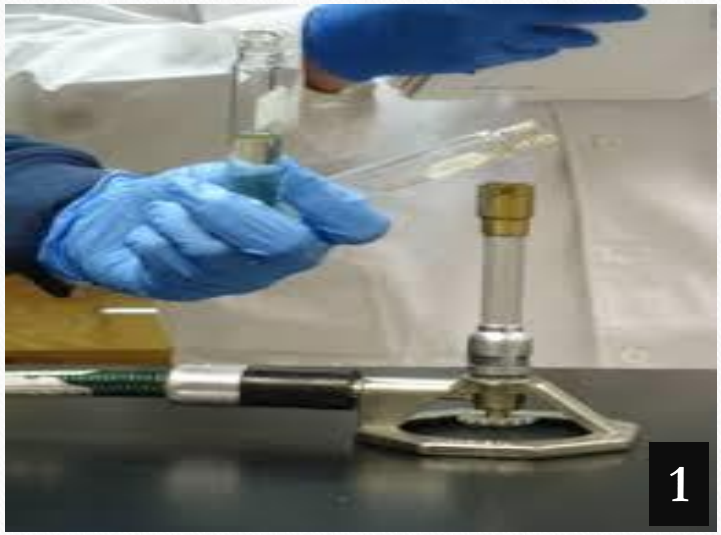
3. Re-flame the tube moth
4. Recap the tube
5. Burn the loop before lets it down

Motility test, to determine whether certain bacteria are motile



Inoculation on agar medium

C. Inoculation of the infected loop in a liquid broth media



Open the media tube and flame its mouth

Insert the loop inside the media

3. Re-flame the tube moth
4. Recap the tube
5. Burn the loop before lets it down

Colonial Morphology

1. Color : some bacteria may produce pigments that colors the colony only without coloring the surroundings, and it is called **non diffusible pigment**. The other type of pigment produced by bacteria is that which is produced extracellularly and diffused in the surrounding medium leading to its coloration, and this type is called **diffusible pigment.**

2. Consistency: this character depends on the ability of the bacteria to produce large capsule, small capsule, slime or do not produce any.

Therefore it makes the colony either; viscous, slightly viscous, or even watery.

3. Surface texture the colonial surface may be either; smooth, rough, granular, radiated….etc.

4. Shape could b

e regular, irregular, lobulated, radiated…etc.

5. Elevation could be flat, slightly elevated, dome-shape.

6. Margin Either smooth,, undulated or irregular.

7. Size This is by measure incarnated the diameter of the colony using a small ruler.

8. Odor Some organisms produce colonies with a very distinctive odor that could be very helpful in identification, such as that produced by Proteus, Pseudomonas, and yeasts….etc.

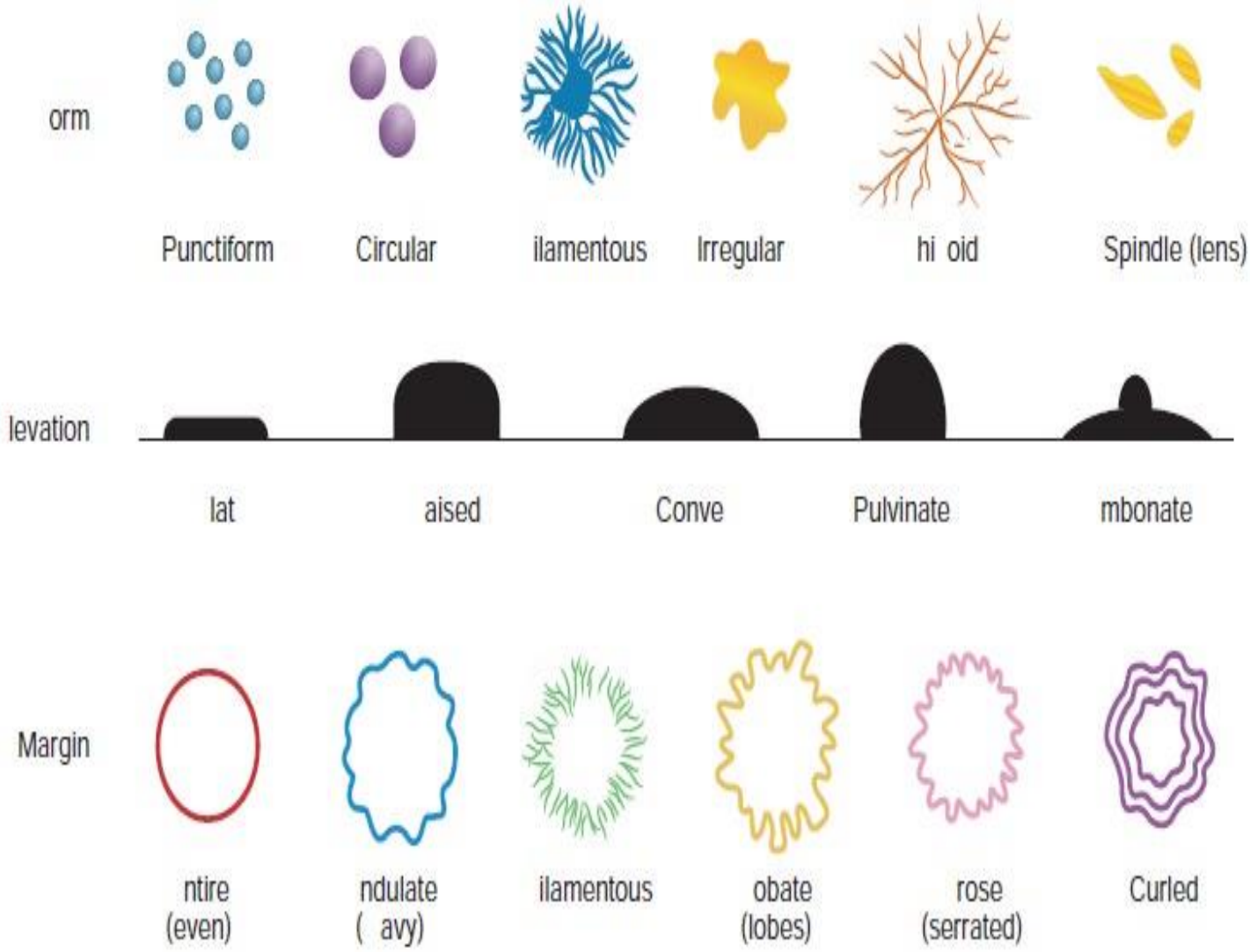


Figure 5.13: Colony morphology of bacteria

Manual of some culturing techniques

A. Transfer of a single Bacterial Colony on a Plate Culture to a Broth

1. flaming the loop.

2. Hold the sterile, cooling loop in one hand and with the other hand turn the assigned plate culture so that it is positioned with the smaller part of the dish (containing the culture) up. Lift this part of the dish with your free hand and turn it so that you can clearly see the isolated colonies of the desired bacteria growing on the surface of the plated agar.

3. With the sterile cool loop, pick one isolated bacterial colony. Withdraw the loop and replace the bottom part of the dish in the inverted top lying open on the table.

4. Now inoculate a sterile nutrient broth with the charged loop.

5. Flame the loop again.

6. put the inoculated tubes in a test tube rack, and plates put them in the incubator at 35 – 37^0C.

7. Read the results after 24 hours.

B. Transfer of a single bacterial Colony on a Plate Culture to an Agar Slant

1. flaming the loop.

2. Hold the sterile, cooling loop in one hand and with the other hand turn the assigned plate culture so that it is positioned with the smaller part of the dish (containing the culture) up. Lift this part of the dish with your free hand and turn it so that you can clearly see the isolated colonies of the desired bacteria growing on the surface of the plated agar.

3. With the sterile cool loop, pick one isolated bacterial colony. Withdraw the loop and replace the bottom part of the dish in the inverted top lying open on the table.

4. Inoculate the slant Lightly touch the loop to the surface of the agar and draw a zigzag from bottom to the top of the slant.

5. put the inoculated tubes in a test tube rack, and plates put them in the incubator at 35 – 37^0C.

6. Read the results after 24 hours.

C. Transfer a colony on solid culture to a broth.

1. Take up the inoculating loop by the handle and hold it as you would a pencil, with the loop down. Hold the wire on the flame of a Bunsen burner until it glows red. Remove loop from flame and hold it for few moments until cool. Avoid touching it to anything.

3- Pick up the slant culture with your left hand. Still holding the loop like a pencil, but more horizontally, in your right hand, use the little finger of the loop hand to remove the closure (cotton plug; slip-on or screw cap) of the culture tube. Keep your little finger curled around this closure when it is free, do not place it on the table.

4- Pass the neck of the open tube rapidly through the Bunsen flame two or three time (do not over-heat; if it is glass, it could crack or burn you later; if it is plastic, it could melt). This flaming sterilizes the air in and around the mouth of the tube.

5- Insert the loop into the open tube (holding both horizontally). Touch the loop (not the handle:) to culture. Don't dig the loop into the agar; merely scrape a small surface area, gently.

6- Withdraw the loop slowly and steadily, being careful not to touch it to the sides or the mouth of the tube. Keep it steady, and do not touch it to anything (it's loaded) while you replace the tube closure and put the tube back in the rack.

7- Still holding the loop quietly in one hand, use the other hand to pick up a tube of sterile nutrient broth from the rack. Now remove the tube closure, as you did before, with the little finger of the loop hand (don't wave or shake the loop). Flame the neck of the tube; insert the loop into the tube and down into the broth. Gently rub the loop against the wall of the tube (don't agitate or splash the broth), making sure the liquid does not touch the loop handle.

8-As you withdraw the loop, touch it to the inside wall of the tube (not the tube's mouth) to remove excess fluid from it. Pull it out without touching it, again flame the tube neck, replace the closure, and put the tube back in the rack.

9- Now carefully flame the loop, holding it first in the coolest part of the flame (yellow), then lower it to the blue cone until it glows. Be sure all of the wire is sterilized, but do not burn the handle. When the wire has cooled, the loop can be placed on the bench top.

10- Label the tube you have just inoculated with your name, the name of organism, and the date.

Thank You