

Practical No.3

Handling of Bacterial Cultures

Microscopic examination of microorganisms provides information about their morphology but does not tell us much about their biological characteristics. To obtain information about the latter it is necessary to observe microorganisms in culture.

If we are to cultivate them successfully in the laboratory, we must provide them with suitable nutrients, such as protein components, carbohydrates, minerals, vitamins, and moisture in the right composition. This mixture is called a culture medium (plural, media). It may be prepared in liquid form, as a broth, or solidified with agar (a nonnutritive solidifying agent extracted from seaweed). Agar media may be used in tubes as a solid column or as slants. They are also commonly used in Petri dishes (named for the German bacteriologist who designed them) or plates, as they are often called.

Solid media are essential for the isolation and separation of more than one type of bacteria growing together in a specimen(called mixed culture). If a mixture of bacteria is spread across the surface of a plated agar medium, individual organisms will multiply at individual sites until a visible aggregate called a colony is formed. One colony of a single species can then be separated from the rest and transferred to another medium, where it will grow as a pure culture, and can be studied as such.

In liquid media, some bacteria may grow diffusely, producing uniform clouding. Others may look 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.. Observation of such features, and others, can also be helpful in recognizing types of organisms.

Student must learn how to handle cultures aseptically. The organisms must not be permitted to contaminate the worker or the environment and the cultures must not be contaminated with extraneous organisms.

It would be advisable, before you begin, to reread the opening paragraphs of part one dealing with safety procedures and general laboratory directions.

To make aseptic transfers of pure cultures and to examine them for important gross features.

- Materials;
- tubes with nutrient broth
 - slants of nutrient agar
 - different cultures of organisms
 - Wire inoculating loop
 - Bunsen burner (and matches)
 - China-marking pencil (or labels)

Procedures:

A. Transfer of a slant culture to a Nutrient Broth.

- 1- The procedure will be demonstrated. Watch carefully and then do it yourself, following direction below.
- 2- 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.

B- Transfer of a slant culture to a Nutrient Agar Slant

- 1- Start again with flaming the loop.
- 2- Pick up the slant culture and take a scraping by the loop following the same procedure as above.

- 3- Pick up a sterile nutrient agar slant (keep the charged loop steady meantime). Open and flame it as before.
- 1- Introduce the charged loop into the fresh tube of agar, and without touching any surface, pass it down the tube to the deep end of the slant. Lightly touch the loop to the surface of the agar and draw a zigzag from bottom to the top of the slant. Lift the loop from the agar surface and withdraw it from the tube without touching tube surfaces.
- 2- Flame, close, and replace the inoculated tube in the rack; then sterilize the loop as before.
- 6- Label the freshly inoculated tube with your name, the name of the organism, and the date.

C- Transfer of a single Bacterial Colony on a Plate Culture to a Nutrient Broth or/ to a Nutrient Agar Slant

- 1- Start as before with 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, as in procedure A, steps 7 through 10.
- 5- Flame the loop again and pick one colony as in steps 1-3 above.
- 6- Inoculate the slant as in procedure B steps 4-6.
 - After you have done the procedure A,B & C, put the inoculated tubes in a test tube rack, and the plates in a tray and put them in the incubator at 35 – 37⁰C.
 - Read the results after 24 hours.

D- Streaking on an agar plate for obtaining isolated colonies;

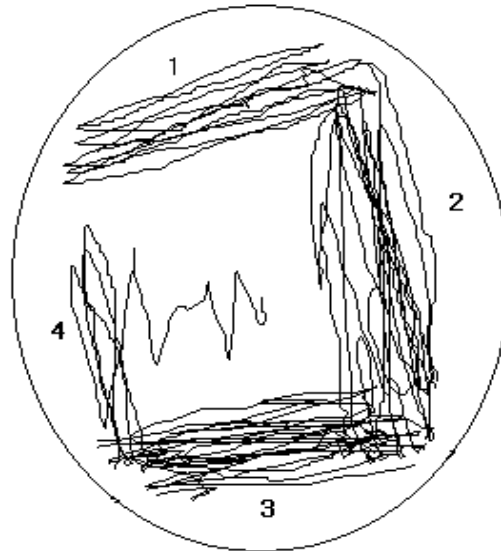
Materials: broth culture of mixed bacteria, a sterile agar plate.

Procedure;

- 1- Take a loopful of broth culture following the same procedure as shown above
- 2- From the inverted agar plate, pick the part containing the agar by the left hand, and bring it close to the flame in an angle position, while the other hand standstill holding the charged wire loop.
- 3- While still close to the flame, make several zigzags of about 2 cm. wide and about 1/4 the length of the plate, without touching the edges of the plate.
- 4- Flame the loop to kill the excess of the organisms, cool it on the agar plate away from the streaked area, now using the loop, take from tips of the first streaked area and make another several zigzags which will become in a right angle to the first one.

- 5- Repeat step 4 where this zigzag will become in a right angle to the second one.
- 6- Repeat step 5 by making another zigzag with a right angle to the third one, and finally draw a simpler zigzag from the fourth one to the center of the plate without touching any previous zigzags.
- 7- Put the streaked plate in the incubator, and read the results after 24 hours.
- 8- It depends on the density of the culture and the accuracy of the procedure, isolated colonies may appear in the 3rd, or the 4th zigzag, or may be in the final line in the center of the plate.

Flame the loop after each step



*1st streaking
heavy growth*

*4th streaking
isolated colonies*



*2nd streaking
heavy growth*

*3rd streaking
lighter growth*

