EXPERIMENT 4 ASEPTIC CULTURE TECHNIQUE

Structure

4.1 INTRODUCTION

In previous experiments you learned that microorganisms thrive pretty much everywhere. It is far too easy to contaminate your lab cultures and experiments with stray microorganisms from the air, the countertop, or your tools. It is also possible to expose your surroundings or yourself to a possible pathogen. In this lab exercise, you will learn to transfer microbiological cultures from one medium to a second sterile medium without contamination of the culture, sterile medium, or the surroundings.

Objectives

After studying and performing this experiment, you should be able to:

- know how to handle microorganisms, tubed media, plated media, and inoculating tools such as loops, needles, or swabs etc.;
- learn how to transfer bacteria from test tubes or broth and agar; and
- learn how to transfer bacteria from Petri plates.

4.2 EXPERIMENT

4.2.1 Principle

Aseptic technique is a method that prevents the introduction of unwanted organisms into an environment. In order to protect sterile broth, media, plates, slants etc. from contamination we must practice aseptic i.e. sterile techniques to protect our material from contamination. By using aseptic technique only sterile surface touches other sterile surface and exposure to the non sterile environment is minimized.

Though, observing aseptic technique is the most important instruction for any microbiology experiment, some common circumstances will be discussed in this practical to make you aware of aseptic techniques.

Specific Aseptic Techniques

A) Sterilization of inoculation loop

The inoculation loop is sterilized by passing it at an angle through the flame of a gas burner until the entire length of the wire becomes orange or red hot. In this way all contaminants on the wire are incinerated. Never lay the loop down once it is sterilized or it may again become contaminated. Allow the loop to cool a few seconds to avoid killing the inoculum.

B) Transferring bacteria from broth culture to fresh broth

Requirements

- Bunsen burner.
- Inoculation needle.
- Trypticase Soy Broth cultures of *Bacillus subtilis, Escherichia coli* and *Micrococcus luteus* and *Mycobacterium phlei* – referred to as Tubes A.
- Sterile Trypticase Soy Broth tubes (4 -one for each microorganism) – referred to as Tubes B.
- Glass Marking pen.

Procedure

1. Turn on the Bunsen burner.
2. Vortex culture suspensions of Bacteria given (Tubes A).
3. Place culture suspensions tube near sterile broth tubes (tubes B). Label sterile tubes with name of microorganism and date.
4. Sterilize the inoculation loop as explained above.
5. While holding inoculation loop between thumb and first two fingers of right hand, pick up tube A with left hand and open the cap/cotton plug with last two fingers of right hand.
6. Flame the lip of test tube A.
7. Place the sterile loop into culture A and take loopful of culture.
8. While still holding the inoculum in your right hand, pick up tube B with left hand and open the cap/cotton plug with last two fingers of right hand.
9. Flame the lip of test tube B gently
10. Place the loop containing droplet of culture in tube B and gently swirl it to transfer the microbes into sterile broth..
11. Take out the loop and continue to hold it in your hand.
12. Flame the lip of test tube B gently and replace the cap/plug which should be still in your right hand. Place tube B back in the test tube rack. Like wise plug the tube A and place in a test tube rack.
13. Sterilize the inoculation loop in flame.
14. Repeat the procedure with all bacterial cultures.

Results

1. Draw and describe the growth seen in each of the four broth cultures.

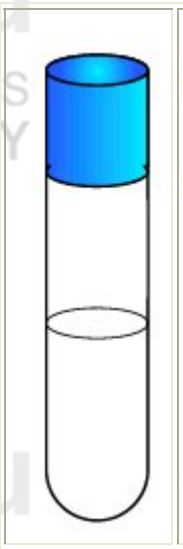	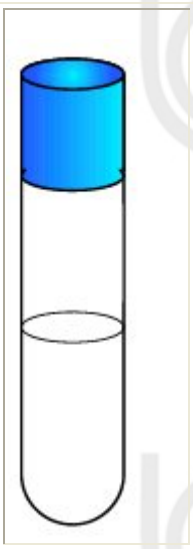	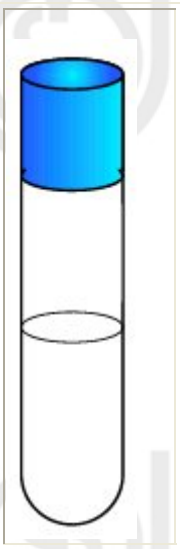	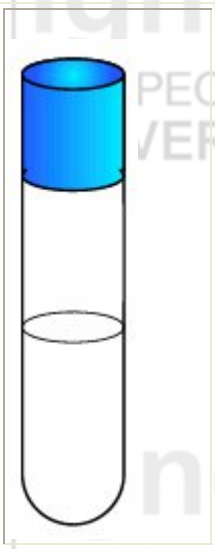
Bacillus subtilis Growth =	*Escherichia coli* Growth =	*Micrococcus luteus* Growth =	*Mycobacterium phlei* Growth =

Figure 4.1: Growth of bacterial culture

C) Streaking plating bacteria

a) From broth culture to sterile medium plate
b) From one petridish to fresh sterile medium plate

Requirements

- Bunsen burner.
- Inoculation needle.
- Trypticase Soy Broth cultures of bacteria (*Bacillus subtilis and Escherichia coli*) to be transferred (Tubes A).
- Trypticase Soy Agar plate cultures of bacteria (*Bacillus subtilis and Escherichia coli*) to be transferred.
- Sterile petridish having Trypticase Soy Agar medium (4 no. Two for each bacterium).
- Glass Marking pen.

Procedure

Removing inoculum from a broth culture

1. Label the plates and tubes.
2. Turn on the Bunsen burner.
3. Loosen the top of the bottle/ Tube containing the inoculum.
4. Hold the loop in the right hand.
5. Flame the loop and allow to cool.
6. Lift the bottle/test tube containing the inoculum with the left hand.
7. Remove the lid/cotton wool plug of the bottle/test tube with the little finger of the left hand.

8. Flame the neck of the bottle/test tube.
9. Insert the loop into the culture broth and withdraw. At all times, hold the loop as still as possible.
10. Flame neck of the bottle/test tube.
11. Replace the lid/cotton wool plug on the bottle/test tube using the little finger. Place bottle/test tube on bench.

Removing inoculum from a plate culture

1. Sterilize the inoculating loop in the flame of a gas burner.
2. Lift the lid of the culture plate slightly and stab the loop into the agar away from any growth to cool the loop.
3. Scrape off a small amount of the organisms and close the lid

Transferring the inoculum into a petri plate

1. Partially lift the lid of the Petri dish containing the solid medium.
2. Hold the charged loop parallel with the surface of the agar; smear the inoculum backwards and forwards across a small area of the medium
3. Remove the loop and close the Petri dish.
4. Flame the loop and allow it to cool. Turn the dish through 90º anticlockwise.
5. With the cooled loop streak the plate from area A across the surface of the agar in three parallel lines. Make sure that a small amount of culture is carried over.
6. Remove the loop and close the Petri dish.
7. Flame the loop and allow to cool. Turn the dish through 90º anticlockwise again and streak from B across the surface of the agar in three parallel lines.
8. Remove the loop and close the Petri dish.
9. Flame the loop and allow to cool. Turn the dish through 90º anticlockwise and streak loop across the surface of the agar from C into the centre of the plate
10. Remove the loop and close the Petri dish. Flame the loop.
11. Seal and incubate the plates inoculated with *Bacillus subtilis and* *Escherichia coli* **at 37°C upside down** (lid on the bottom) to prevent condensing water from falling down on the growing colonies and causing them to run together in inverted position.

Results

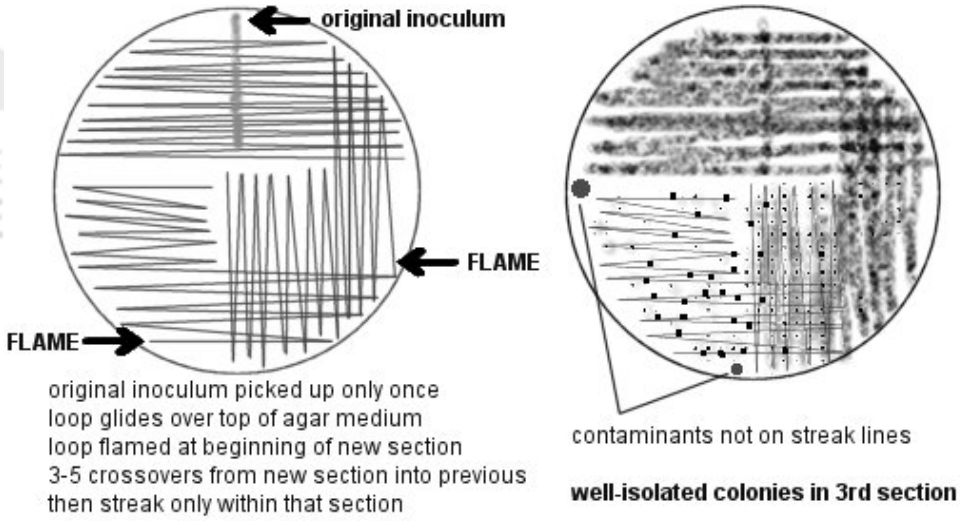

Figure 4.2: Streak plate technique

Expressing results

Bacterial colonies contain millions of cells and exhibit diverse morphologies; however, all isolated colonies produced on streak plates arise from a single bacterial cell. When evaluating colony morphology, use specific terms to describe the shape, elevation, colony margin shape, and surface texture (Figure 4.3). Colony size and colour are also useful features that are noted. All of these characteristics may be useful in the initial identification of unknown bacteria. Colonies that have different morphologies may be considered to contain different bacterial species. However, colonies that appear to be similar in morphology are not always the same bacterial species.

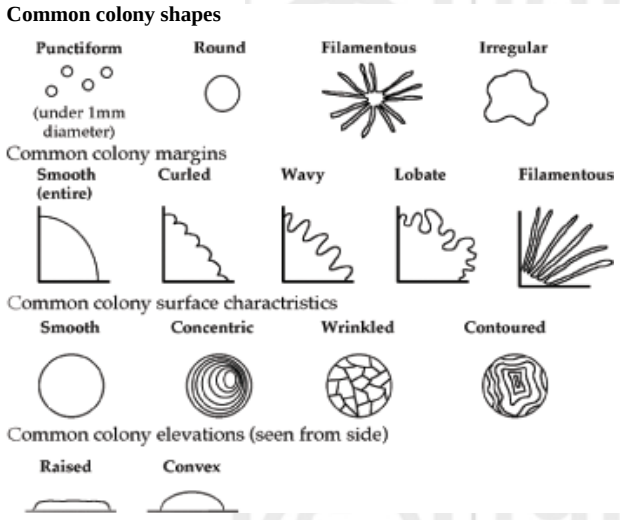

Figure 4.3: Terminology used to describe colony morphology

4.2.2 Observations

Obtain your streak plate from the incubator and visually examine the different regions:

1. Notice a dilution effect as you move from region to region.
2. Look for isolated individual colonies present.
3. Note different types of colony morphologies present.
4. Measure the size (diameter or length) and record the colony colour in Table 1.

Table 1: Colony characteristics of two bacterial colonies isolated using streak plating

Colony Size (mm)	Colour	Shape	Margin	Surface	Elevation
1.					
2.					

4.3 PRECAUTIONS

- Operations must not be started until all requirements are within immediate reach and must be completed as quickly as possible.
- Carry out all microbiological operations in a laminar flow hood.
- Wear gloves and lab coat to protect yourself but also to prevent dry skin and microorganisms from contaminating your samples.
- Use plugs made of non-absorbent cotton wool in test tubes and pipettes to prevent microorganisms from passing in or out and contaminating either the culture or the environment. The cotton wool must remain dry because this filtration property is lost if the cotton wool becomes moist – hence the use of non-absorbent cotton wool.
- For use in test tubes a plug should be properly made to ensure that it can be held comfortably without being dropped and its shape and form are retained while being removed from and returned to a test tube several times.
- Disinfect all surfaces prior to use with a disinfectant solution.
- Swab down the working surface liberally with 70% ethanol.
- Periodically spread a solution of 70% ethanol over the exterior of gloves to minimize contamination. Replace them if torn.
- In case of any spill, spread a solution of 70% alcohol and swab immediately with non-linting wipes.
- Discard gloves after use and do not wear them when entering any other lab area.
- Bring into the work area only those items needed for a particular procedure.
- Leave a wide clear space in the centre of the hood (not just the front edge) to work on. Do not clutter the area to prevent blockage of proper air flow and to minimize turbulence.
- Swab with 70% alcohol all glassware (medium bottles, beakers, etc.) before placing them inside the hood.
- Arrange the work area to have easy access to all of it without having to reach over one item to get at another (especially over an open bottle or flask).
- Use sterile wrapped pipettes and discard them after use into a biohazard waste container.

- Check that the wrapping of the sterile pipette is not broken or damaged.
- Vessels must be open for the minimum amount of time possible and while they are open all work must be done close to the Bunsen burner flame where air currents are drawn upwards.
- Discard any contaminated material immediately.
- Never perform mouth pipetting. Pipetting aids must be used.
- When handling sterile containers with caps or lids, place the cap on its side if it must be laid on the work surface.
- Make sure not to touch the tip of the pipette to the rim of any flask or sterile bottle.
- Clean the work area when finished by wiping with 70% alcohol.