Unit I: The Microbiology Laboratory & Cultivation of Bacteria

Activities:
1.1 Introduction to Culture Media (demo)  p 1
1.2 Aseptic Technique in Transferring Bacteria (Ex 1)  p 1
1.3 Hunt Microbes from the Environment (Ex 2)  p 3
2.1 Bacterial Colony Characteristics (Ex 3)  p 5
2.2 Plate Streak Method for Colony Isolation (Ex 4)  p 7
Appendix 1.1 Notebook Pre-Lab Preparation Examples  p 10

1.1 Introduction to Culture Media:

The study of microorganisms requires being able to grow them in the laboratory. Bacteria are grown in culture media, which provides the nutrients, necessary for the organisms of interest. There are different ways to classify the media:

1.- Consistency:
Liquid: is called a Broth and it is usually placed in a test tube or a flask.
Solid: Liquid media that has been solidified by the addition of agar (usually 1.5 % w/v) a complex polysaccharide. The solid media can be placed in Petri dishes (agar plates) or test tubes with a large surface area (agar slants).

2.- Type of media:
Nutrient media: is a specific chemical formulations that contain all the nutrients and minerals that a many microorganisms needs for normal growth. It is called defined when the specific nutrients and their amounts are known. An undefined media is one where the exact composition is not known.

Selective media: is a type of media that favors the growth of a specific microorganism over others. In fact others may be inhibited by media components.

Differential media: permit the recognition of specific microorganisms. Usually by taking advantage of some biochemical reaction that might produce a specific change in the media (color is often used)

1.2 Aseptic Technique in Transferring Bacteria:

One of the first requirements to study specific microorganisms is to separate them from the mixed microbial populations in which they are found in the environment. To achieve this goal microbiologists use culture media and aseptic transfer techniques.

To start, aseptic technique is used to introduce a very small sample of cells (the inoculum) into a receptacle containing nutrient or culture medium. This process is called inoculation.
The aseptic (sterile) technique is a technique designed to keep the working environment as free of contaminants as possible. This is achieved first, by sterilizing all equipment and media that will be in contact with the microorganisms. This includes minimizing the air movement on the working area. Usually the work is done within the vicinity of a flame. Aseptic technique is required for the maintenance of pure cultures and the successful isolation of specific types of microorganisms.

A pure culture is a culture that contains only one species of bacteria. A mixed culture encompasses more than one species. When isolating bacteria from the environment the microbiologist always starts with a mixed culture. A pure culture can be obtained from the mixed culture by sub-culturing and streaking for isolation.

Use of the Loop/Stab Inoculator:
Two different types of inoculators can be used depending on the purpose of the work. The loop is used to a) transfer cultures from one medium to another, b) to prepare bacterial smears, and c) to streak plates. The loop is the tool of choice for working with a liquid inoculum culture. The stab is used to prepare stab cultures and to pick single colonies from a plate.

EXERCISE 1:

Inoculating Liquid Media
For this exercise a tube containing a liquid culture will be used to provide the source of the inoculum for broth and agar slants. Work close to the flame!

1) Hold the source culture tube with your non-dominant hand and hold the inoculating loop with your dominant hand. Carefully shake the culture to make sure the cells are resuspended
2) Sterilize the inoculator by first passing the entire wire through the flame, starting at the handle end. Wait until the whole wire becomes red hot. Allow the wire to cool (without waving it on the air) for about 30 seconds.
3) Remove the cap from the source tube using the pinkie finger of the same hand that is holding the inoculator. You will hold the cap in your finger until it is time to put it back on the source tube.
4) Pass the mouth of the source tube through the flame once.
5) Without touching the walls of the tube, put the sterile inoculator into the source tube containing the culture and dip the loop into the liquid.

(NOTE: If you were using a slant or plate as a source you will simply touch the surface of the agar where the bacteria are growing with the inoculator. We will do this in the near future.)

6) Withdraw the inoculator from the source tube, and replace the cap.
7) Remove the cap of the tube to be inoculated (using the pinkie finger as before) and insert the inoculator into the tube. Don’t stir or shake the loop excessively. Flame the top of the tube and replace the cap.

(NOTE: See the specific instructions below for the different types of solid media.)

8) Flame the inoculator as before but, heat it slowly so that any material remaining on the loop does not spatter.
Inoculation of an agar slant:
Rest the inoculator gently at the lower end of the slant and withdraw it slowly upwards moving it from side to side (the surface of the agar should not be broken). This should leave a streak on the surface of the slant (In some specific experiments you may be require to stab the slant just under the agar surface, if that is the case it will be clearly specified in the instructions).

Inoculation of an agar stab:
Using aseptic technique pick a single well isolated colony with a sterile inoculating stab needle and stab the needle several times through the agar to the bottom of the vial or tube. Replace and tighten the cap. Make sure the tube and cap are well labeled. Give the stab to your instructor for storage.

Inoculation of an agar plate:
Working with agar plates is bit different than working with media in tubes in that you have a wide lid instead of narrow cap. This means there is a greater surface area of sterile media that can be exposed to contaminations in the atmosphere. The key is to keep as much of the lid over (covering) the open agar plate as possible. Never set the lid down on the lab bench when in an open contaminating environment. Who the agar in a plate is inoculated depends on the goal. In the next exercise we will swap the plate to get as many possible microbes across the surface as possible. Next lab period we will try to isolate a single colony of a bacterium, in which case we use the “streaking for isolation” technique.

1.3 Hunt Microbes from the Environment:
Fomites are inanimate objects, which carry viable pathogenic organisms. Equipment and objects used by many individuals such as restaurant’s eating utensils, public phones, ATM machines, and money are some of the common objects that may act as vehicles of dissemination for some bacteria.

Public health microbiologists routinely examine utensils used in public eating establishments and are able to assess the sanitary conditions prevailing based on the results obtained from such examination. There are various procedures employed in the examination of utensils. One of these employs a cotton swab soaked in a phosphate buffer. The swab is rubbed over the surface of the object(s) under study, then returned to the test tube of buffer in which it was originally soaked. The top of the swap is broken off into the buffer while the portion contaminated by handling is discarded. Portions of the liquid from the tube containing the swap tip are plated on solid nutrient medium and colony counts obtained indicate the degree of contamination present.

In this exercise we will use a slightly different method. You will use a sterile swab, which you will rub over the surface to be tested, and then is streak over the surface of a plate containing nutrient agar and a selective medium.
EXERCISE 2:

**Examination of fomites for bacterial contamination:**

**MATERIALS:**
1. Sterile cotton swabs.
2. Tube of sterile distilled water.
3. Petri dishes with nutrient agar
4. Object to be tested: Your group will be assigned one of the following
   a) ATM machine versus Public Phone
   b) Money (coins versus paper)
   c) Bathrooms (male versus female)
   d) Tables on cafeteria versus benches on the microbiology lab

Your instructor will give you specific instructions for each location.

**PROCEDURE:**
1. Carefully withdraw a sterile cotton swab from its container, making sure that the cotton is moisten (but not too wet) with the sterile water.

2. Rotate the side of the swap, rather than the tip, over the portion of the object to be tested.

3. Smear and rotate the swap thus contaminated over the surface of the agar on the Petri dish.

4. Label the Petri dish to indicate the object tested.

5. Incubate the inoculate dish upside down at room temperature for three or more days.

6. In the next lab period you will be asked to exam the dish, count the colonies, and grade the level of contamination as follows:
   0 to 10 colonies/dish - Good (low contamination)
   11 to 50 colonies/dish - Fair (some contamination)
   Over 50 colonies/dish - Poor (heavy contamination)

7. Next lab period, also describe colony morphology for your two media types as indicated in Unit 1, Exercise 2 (above).

8. Discuss which fomites were most contaminated and offer an explanation.

9. Discuss differences between media types.
2.1 **Bacterial Colony Characteristics:**

A bacterial colony is a very homogeneous population formed by the progeny of a single bacterium. The colony becomes visible with the naked eye after several millions of individual are produced. Since all the members of the colony are derived from a single cell, all the characteristics of the bacteria within that population are essentially the same. Different bacteria (i.e. different species, and sometimes strains) have specific colony characteristics on specific media. Thus, evaluation of colony characteristics is one of the first steps in the process of identifying bacteria. Always select a typical well isolated colony for your observations. Crowded colonies are going to be small due to competition for nutrients between adjacent colonies.

There are **seven macroscopic characteristics of bacterial colonies** that must be observed and recorded. (Figure 1.3) These are:

1- **SIZE**: whenever possible measure colony diameter and express it in millimeters mm. If the bacteria colony is too small to be measured you may describe it as punctiform.
2- **SHAPE**: look at the whole colony. Describe the shape using the terms: circular, irregular, filamentous, or spindle as indicated in figure 2.1.
3- **MARGIN**: Describe the outer edge of the colony as entire/smooth, undulate/wavy, filamentous as indicated in figure 2.1
4- **SURFACE**: select one of (a) and one from (b)
   a) smooth, wrinkled, rough, concentric rings
   b) dull (matte), or shiny (glistening)
5- **PIGMENTATION**: Describe the color as precisely as possible
6- **TRANSMITED LIGHT**: hold the plate up to the light. Call it translucent if the light passes through the colony or opaque if the light does not pass through the colony
7- **ELEVATION**: hold plate to the side and note if the colonies are flat, raised, convex, umbonated or craterform.

**EXERCISE 3:**

**Macroscopic Examination of Bacterial Cultures:**
You will be given several plates, including those from the microbe hunt, to observe and describe the cultures that you see. Include the name of the media (e. g. Nutrient agar, etc.) since the colony morphology is affected by the media used. In your lab notebook take note of the following for each plate: Is this a pure or mixed culture? Select a typical colony and describe its seven macroscopic characteristics. If there is a mixed culture with more than one type of colony, select one of each type and proceed to describe each one individually.

(Please refer to the appendix at the end of this lab for samples of flow charts relating to the different experiments that should be used in your lab notebook)
Figure 1.3. Bacterial colony morphology characters and descriptions.
2.2 **Plate Streak Method for Colony Isolation:**

The purpose of this procedure is to separate the cells so that some of them will produce well isolated colonies. One common technique is to achieve this is shown below (Figure 1.4):

1. Place the plate to be inoculated upside down labeled it carefully and draw a line through 1/3 of the plate.
2. Using aseptic technique remove a sample from the source culture.
3. Pick up the bottom ½ of the plate to be inoculated in your hand and hold it at about 45 ° degree angle
4. Place a loopful of the organism in the 1/3 area and streak the culture back and forth across the plate until you have covered the entire 1/3 of the plate.
5. Re-sterilize the loop, cool it (both by waiting the customary time and by dipping it on the agar as shown by the instructor).
6. Turn the plate (about ¼ of a turn) and draw two separate parallel lines at about 45° angle through the first 1/3 into the remaining 2/3 area.
7. Continue streaking separate parallel lines without entering the first 1/3 section. Cover approximately a ¼ of the plate.
8. Repeat steps 5 to 7 until the plate is completely streaked, replace the lid and sterilize the loop.
9. Incubate the streaked plate at the indicated temperature in an inverted position to prevent drops of condensed water falling onto the surface and smearing the colonies.
10. Next day examine the plates for isolated colonies. Score them according with the scale shown on Figure 1.4 (below)

**EXERCISE 4:**

**Streaking Plates for Isolation of Bacterial Colonies:**

**Materials:**
1. Overnight mixed broth culture containing *Proteus vulgaris*, *Bacillus sp.*, and *Staphylococcus aureus*; approximately 2 ml per student.
2. Nutrient agar plate with a mixed population of *Proteus vulgaris*, *Bacillus sp.*, and *Staphylococcus aureus*.
3. Nutrient agar plates per student (about 4 plates per student)

**THIS EXERCISE IS TO BE PERFORMED BY EACH STUDENT INDIVIDUALY**

Always start by labeling the bottom of your plates to be used for all exercises (your name, date, and name of the bacterial culture)

5A) Use the mixed broth culture to streak for isolation on a nutrient agar (NA) plate as described earlier.
5B) From the mixed plate culture:
Use the streak technique you learned to produce three plates each having a pure culture of one of the three organisms found in the mixed plate. To do this pick a well isolate colony of *Staphylococcus aureus* (the yellow colonies are *S. aureus*) and streak a new plate of nutrient agar. Repeat the procedure for *Proteus vulgaris* (the smooth white colonies) and for *Bacillus sp.* (the rough white colonies) using a separate plate for each.
Now, invert all your plates and incubate them in the 37 °C incubator. MAKE SURE THEY ARE PROPERLY LABELLED!
Within the next couple of days examine your plates and record your results on your lab notebook. Score the plates according with the scale shown in Figure 1.4 (below).
Figure 1.4. Streaking for isolation protocol and performance scoring guide.
Appendix 1.1:
DEMO FLOW CHARTS RECOMMENDED FOR PRE-LAB PREPARATION TO BE PLACED IN YOUR LAB NOTEBOOK.

Unit 1: Exercise 3  Macrophscopic Observations Of Culture Plates

Culture plates given by instructor
↓
Fill the following information on lab notebook
↓
Media type and name
↓
Determine if culture is
Mixed or pure
↓
Select an isolate colonies and describe the following
Size
Shape
Margins
Surface
Pigmentation
Transmitted light
Elevation
Unit 1: Exercise 4: Streaking for Isolation from a Mixed Broth Culture to a Nutrient Agar Plate:

Obtain a NA plate, on outside of the bottom half (the part that contains the media) label it with Name, Date, Culture source, draw a line through 1/3 of the plate ↓

Take the source broth culture tube and mix gently to resuspend the cells ↓

Sterilize inoculating loop and let it cool ↓

Open source culture and flame the mouth of the tube ↓

Dip the sterile loop into liquid culture ↓

Remove loop from source culture, flame mouth of tube and replace cap ↓

Place the loopful of the organism in the market 1/3 area of the nutrient agar plate and streak the culture back and forth ↓

Flame loop and cool it ↓

Turn plate ¼ of a turn, pass the loop once over the previously streaked area and proceed to streak a new area of the plate without entering the first 1\3 section ↓

Repeat the last two steps until the plate is completely streaked ↓

Replace lid, sterilize the loop, incubate plate upside down (agar side up)