Lab Exercise 2: Aseptic Technique

Background

When the identification of an unknown organism is desired, whether from a patient or an environmental sample, or the characterization of that organism is necessary- having a **pure culture** of that organism is essential. The only way to maintain these **pure cultures** is by practicing **aseptic technique**: the transfer of microbial cultures from one medium to another without introducing contamination or damaging the organism of interest. Aseptic technique refers to the protocols and procedures used to ensure that no microbes contaminate an experiment and that the microbes in an experiment do not cause contamination. Because microorganisms are **ubiquitous**, by definition they are everywhere. This laboratory exercise is an introduction to the procedures required to ensure a safe and aseptic laboratory. The ability to keep cultures contamination-free is as important as keeping them from contaminating the environment.

Before beginning this laboratory exercise, become familiar with the terminology and techniques used in this discussion. **Sterilization** is typically achieved in the lab by one of two methods: **dry** or **moist heat**. Typically, dry heat sterilization is achieved by flaming the instrument in the flame of a Bunsen burner. Moist heat is most often achieved through the use of an **autoclave**. Typically, media are autoclaved to sterilize them and that sterility is maintained in the lab using dry heat sterilization of every instrument that comes into contact with the media and organism. In this way, contamination of the culture will be avoided and any growth in it will be due only to the organism which has been deliberately **inoculated (put)** into the medium.

For routine growth and testing, inoculation into a **broth culture** is usually best. For isolating specific bacteria from a mix, growth on a **Petri dish** is optimal. For long-term storage and maintenance of a culture, growth on an **agar slant** is best and for anaerobic growth, inoculation into an **agar deep** is optimal.

Work Area Disinfection

Before beginning any laboratory experiment, the lab bench must be **sanitized** (cleaned, but not sterilized) by spraying the surface down with a surface **disinfectant**. During disinfection, the lab bench is saturated with disinfectant and paper towels are used to rub it across the surface of the bench. A large portion of the disinfectant's ability to kill microbes is based on length of contact with the microbe itself, so the area can be left wet and allowed to dry evaporatively. **Remember to repeat this procedure at the end of every laboratory session**.

Media

Media comes in both liquid (**broth**) as well as solid (**agar**) form. In order for media to be a solid, a solidifying agent (most commonly a metabolically inert solidifying agent called agar) is added to the broth prior to sterilization. Solid media are prepared into **Petri plates** or in tubes as **slants** or **deeps** (Figure 1). Additionally, a **pour** can be made by pouring molten agar into a Petri dish.

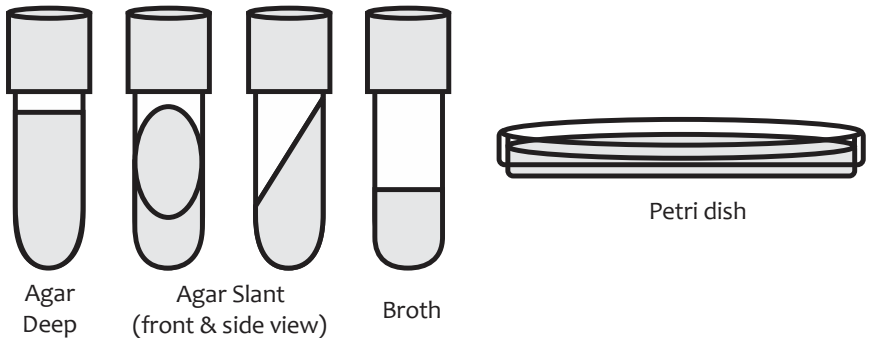

Figure 1: Types of media typically used in this microbiology lab.

Regardless of the form of the medium, the nutritional make-up of media is defined as either **complex** or **chemically defined**. Complex media have an exact chemical make-up that is unknown, while chemically defined media, as the name suggests, have a recipe that can be accounted for down to the molar amounts of each chemical added (Table 1).

Incubation

Cultivation chambers called **incubators** are used to grow microbes at specific temperatures. In this lab, incubators are set for the two temperatures most commonly used to grow typical microbes. These are **25°C** (**room temperature**) and **37°C** (**body temperature**). For culture storage, an incubator set at **4°C** (**refrigerator temperature**) is used.

Table 1: Composition of both a complex medium (left) and a chemically defined medium (right) for the growth of a chemoheterotroph. Solid media would be made by adding agar (typically 1%) to either medium prior to autoclaving.

Complex Medium (Nutrient Broth)		Chemically Defined Medium (for chemoheterotrophs)	
Peptone	5.0 g	Glucose	5.0 g
Beef extract	3.0 g	$NH_4H_2PO_4$ (monobasic)	1.0 g
NaCl	8.0 g	NaCl	5.0 g
dH_20	to 1 L	$MgSO_4 \bullet 7H_2O$	0.2 g
		K_2HPO_4 (dibasic)	1.0 g
		dH_2O	to 1 L

Transfer Instruments: Loops and Needles

Wire **inoculation loops** and **needles** are made from inert metals such as platinum. They are extremely durable and can be sterilized by **incineration**. To do this, place the loop or needle into the flame of a Bunsen burner (the hottest portion is in the blue flame). Allow the loop or needle to get red hot and then move the loop or needle slowly through the flame (ending at the loop or needle's tip), taking care to make sure that the red-hot color continues along the length of the instrument. Because this heat kills microorganisms, you must make sure that the **transfer instrument** is cool before it is inserted into a culture to inoculate media. This heat sterilization is done before and after EVERY inoculation and before an instrument is placed on the countertop or other surface.

Culture Tube Flaming

Prior to inserting a cooled transfer instrument into a culture tube, the cap is removed and the lip of the tube is flamed. The warm air from the flame will create airflow out of the tube and will help prevent contamination with transient and airborne microbes. This will be done before and after any contact with a transfer instrument. It is only necessary to pass the tube through the flame, thorough heating may cause the tube to crack or to melt its cap.

Culture Tube Inoculation

Broth: using aseptic technique, deliver microbes using a **loop**, make sure there is a film of the liquid culture in the loop. Twist the loop several times in the sterile medium to ensure microbes are inoculated.

Agar Slant: using aseptic technique, deliver microbes using a **loop** by swiping across surface of slant in an overlapping zigzag pattern from the bottom to the top.

Agar Deep: using aseptic technique, deliver microbes by using a **needle** and stabbing the deep down the center of the agar.

Agar Plate Inoculation

Solid agar media can also be placed in Petri dishes. These are never flamed, so extra care must be taken to avoid contamination with airborne microbes. Always work near an open flame and keep the lid on the Petri dish as much as possible. When taking an inoculum from an agar plate, place the dish upside down and grab the bottom (media-containing portion) and touch the surface of the colony/ sample with your loop.

Introduction

In this lab activity, the aseptic transfer of *Escherichia coli* from various media types will be practiced. Pay particular attention to creating and maintaining an aseptic environment as well as aseptically transferring cultures. Good habits formed now will be essential to success as the semester progresses.

Objectives

1. Compare the different types and forms of media. Understand the uses of each media type.
2. Explain how to sterilize media and inoculate various media types.
2. Carry out aseptic technique for the removal and transfer of microorganisms for culturing.
3. Correctly sterilize and flame transfer instruments and tubes.

Protocol

Team Supplies	Individual Supplies
Escherichia coli slant	Inoculating Loop
E. coli Petri plate	Bunsen burner & striker
E. coli broth	2 general nutrient agar slants
	2 general nutrient agar broths

Aseptic Transfer Technique Protocol

Keep the following in mind as transfers are performed. If a solid culture is used, touch the loop to the surface of the agar. It is not necessary to take a large chunk of the culture. If a liquid culture is used, make sure that the loop is full of liquid. **Make sure to read through all of the steps of this exercise before beginning any of the aseptic transfers.**

Inoculations
Escherichia coli slant → Agar slant
E. coli plate → Broth tube
E. coli broth → Agar slant
E. coli broth →Broth tube

REMEMBER NOT TO TILT THE TUBES OR TO GRAB THEM BY THEIR LID

1. Prepare the lab bench by removing extraneous items and cleaning the surface with table disinfectant.
2. Collect the appropriate sterile media and cultures from the Back Bench, using a test tube rack.
3. Label all sterile tubes with paper *Inoculation Labels* (found at the beginning of this manual).
4. Hold the inoculating loop in the dominant hand. Sterilize the loop by heating it until it turns bright red. Make sure to allow it to cool before proceeding.
5. With the free hand, obtain the proper *Escherichia coli* culture. Use the little finger of the dominant hand to uncap the tube and hold the lid. Do not put the lid down.
6. Quickly pass the lip of the tube through the flame.
7. Insert the cooled loop into the tube and collect an inoculum of the culture. If a solid medium is being used, only touch the surface, do not break through the agar, in order to pick up sufficient inoculum. If the culture is liquid, make sure the loop has a film of broth in it.
8. Flame the mouth of the tube and replace the cap.
9. Place the culture tube into the test tube rack.
10. Inoculate the tubes according to the table above, using the sterile technique outlined in the steps above, with the following steps specific to the media types.
    Broth Tube: using aseptic technique as outlined above, deliver the microbes using a loop. Twist the loop in the sterile broth to ensure microbes are deposited into liquid medium.
    Agar Slant: using aseptic technique as outlined above, deliver the microbes using a loop by swiping the loop across the surface of the slant in a zigzag pattern from the bottom to the top as illustrated below.

11. Place all tubes into the assigned test tube rack in the 37°C incubator.

Data Collection & Analysis (To be completed after the cultures have incubated and examined microscopically.)

1. Were all the media successfully transferred without contamination? What signs would indicate contamination?

Discussion

1. Give an example when the Petri dish, broth and slants would be used in routine microbiological inoculations.
2. Why is aseptic technique essential when handling microbial cultures in the lab?
3. How is air contamination prevented when an inoculating loop is used to introduce or take a bacterial sample to/from an agar plate?
4. Where and how should a label be written on an agar plate? What about on a test tube?
5. Should plates be incubated agar side up or down? Why?