

## Lab Exercise 2: Aseptic Technique

### Background

**Aseptic technique** is one of the most fundamental techniques in all of microbiology. The ability to keep your cultures contamination-free is as important as keeping you from being contaminated- for different reasons, of course. When you are attempting to identify an unknown organism, whether from a patient or an environmental sample, or you're trying to characterize an organism that you've already identified- having a pure culture of that organism is paramount. 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.

Sterilization is typically achieved by one of two methods: **dry or moist heat**. Typically in a microbiology lab, dry heat sterilization is achieved by flaming your instrument in the flame of a Bunsen burner. Moist heat is most often achieved through the use of an **autoclave**. Typically you will use media that have been autoclaved to sterilization and you will maintain this sterility by dry heat sterilizing every instrument that you use which comes into contact with the media. In this way, you will avoid any contamination and any growth in your culture will be only the organism which you have deliberately **inoculated** into the medium.

Depending on your needs, bacteria can be cultured onto different types of media. For routine growth, oftentimes inoculation into a **broth culture** is 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.

### Aseptic Technique

**Aseptic technique** refers to the protocols and procedures you use to ensure that no microbes contaminate your experiment and that the microbes you are working with do not cause contamination. Because microorganisms are **ubiquitous**, you need to assume that every environment is replete with them. This laboratory exercise will introduce you to the procedures required to ensure a safe and aseptic laboratory. Before beginning today's laboratory exercise, you should become familiar with the terminology and techniques discussed below.

### Work Area Disinfection

Before beginning any laboratory experiment, you must spray down the lab bench with a surface disinfectant, such as **Cavicide**. Saturate the bench with the solution and rub it in to cover the area. Do not completely dry the surface with paper towels, instead let the disinfectant evaporate. A large portion of the disinfectant's ability to kill microbes is based on contact with the microbe itself. You must 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 agar) is added to the broth prior to sterilization. Solid media is 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.

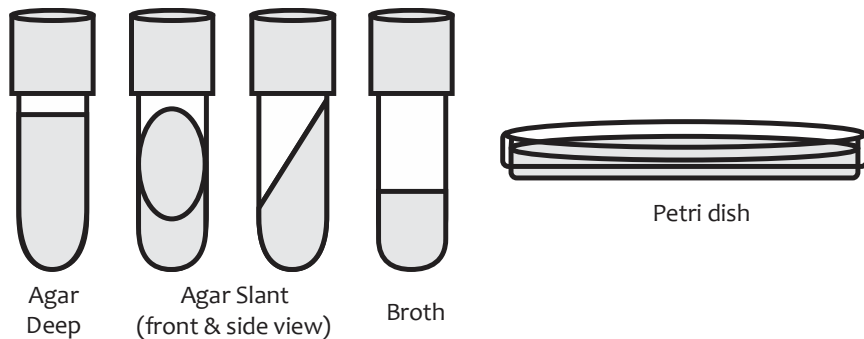


Figure 1: Types of media typically used in this microbiology lab.

Regardless of the state of the medium, media are 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).

Table 1: Composition of both a complex medium (left) and a chemically defined medium (right) for the growth of a chemoheterotroph.

Complex Medium (Nutrient Broth)		Chemically Defined Medium (for chemoheterotrophs)	
Peptone	5.0 g	Glucose	5.0 g
Beef extract	3.0 g	NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> (monobasic)	1.0 g
NaCl	8.0 g	NaCl	5.0 g
dH <sub>2</sub> O	to 1 L	MgSO <sub>4</sub> •7H <sub>2</sub> O	0.2 g
		K <sub>2</sub> HPO <sub>4</sub> (dibasic)	1.0 g
		dH <sub>2</sub> O	to 1 L

## Incubation

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

## 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 is continuous along the length of the instrument. Because this heat kills microorganisms, you must make sure that your **transfer instrument** is cool before you insert it into a culture to inoculate media. This heat sterilization is done before and after EVERY inoculation.

## Culture Tube Flaming

Prior to inserting a cooled transfer instrument into a culture tube, you need to remove the cap and flame the lip of the tube. This warm air will create airflow out of the tube and will help prevent contamination with transient and airborne microbes. You will do this before and after any contact with a transfer instrument. It is only necessary to pass the tube through the flame (you're not sterilizing it). Thorough heating may cause the tube to crack or to melt its cap.

## Culture Tube Inoculation

To inoculate a culture tube, you must determine the type of culture tube being used and follow the appropriate protocol:

**Broth:** using aseptic technique, deliver microbes using a loop, twist the loop several times to ensure microbes are deposited into the liquid medium

**Agar Slant:** using aseptic technique, deliver microbes using a loop by swiping across surface of slant in a 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, lift the lid slightly and touch the surface of the colony/ sample with your loop. Remember, microbes are microscopic! You do not need to scoop up a large chunk of bacteria to have enough to transfer. Too much can be problematic.

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## Introduction

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

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## 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.
3. Carry out aseptic technique for the removal and transfer of microorganisms for culturing.
4. Correctly sterilize and flame transfer instruments and tubes.

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**Prior to beginning this experiment, make sure that you have downloaded a copy of the *Laboratory Organism List* and the *Inoculation Labels* from the Course Website.**

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

## Aseptic Transfer Technique Protocol

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

Inoculations
<i>Escherichia coli</i> slant → Agar slant
<i>E. coli</i> plate → Broth tube
<i>E. coli</i> broth → Agar slant
<i>E. coli</i> broth → Broth tube

1. Prepare your lab bench by removing extraneous items and cleaning the surface with table disinfectant.
2. Label all sterile tubes using proper technique and paper labels.
3. Hold your inoculating loop in your dominant hand. Sterilize your loop by heating it until it turns bright red. Make sure to allow it to cool completely before proceeding.
4. With your free hand, obtain the proper *Escherichia coli* culture. Use the little finger of your dominant hand to uncap the tube and hold the lid. Do not put the lid down. Quickly pass the lip of the tube through the flame.
5. Insert the cooled loop into the tube and pick up some of the culture.
6. Flame the mouth of the tube as before and replace the cap. Place the culture tube into your test tube rack.
7. Inoculate your tubes according to the table above, using the sterile technique outlined in steps 1–6 above.
  - i. **Broth Tube:** using aseptic technique as outlined above, deliver microbes using a loop. Shake the loop in the broth to ensure microbes are deposited into liquid medium.
  - ii. **Agar Slant:** using aseptic technique as outline above, deliver 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.



8. Place all tubes into the 37°C incubator.

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### Data Collection & Analysis

1. Did you successfully inoculate your media without contamination? What signs would indicate contamination?
2. Were all the transfers successful?

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### 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. How should agar plates be incubated? Why?