*LAB #1*

## ASEPTIC TECHNIQUE AND THE TRANSFER OF MICROORGANISMS

### Introduction

In natural environments, microorganisms usually exist in mixed populations. However, if we want to study, characterize, and identify microorganisms, we must have the organisms in the form of a pure culture. A ***pure culture*** contains the organisms of interest in a sterile, nutrient-containing medium which stimulates grow. A ***sterile medium*** is free of all life forms. A sterile medium is usually prepared by heating it to a temperature at which all contaminating microorganisms are destroyed. When working with microorganisms, it is necessary to have a method to transfer the growing organisms from the pure culture (called the *inoculum*) to a sterile medium without introducing any unwanted outside contaminants. This method of preventing unwanted microorganisms from gaining access is called ***aseptic technique***, which literally means no decay or infection.

Aseptic technique was first introduced by the English surgeon Joseph Lister in the late 18th century. Lister was focused on reducing microbes in a medical setting, thereby preventing infections in wounds. His concept of asepsis was much more limited than the modern precautions that we take in the laboratory. It mainly involved disinfecting hands and the air with strong aseptic chemicals, such as phenol, prior to surgery. These techniques, and the application of heat for sterilization, form the basis for microbial control by physical and chemical methods, and are still in use today.

*Most importantly, safety is the primary concern while in the laboratory. For additional safety information, visit http://www.research.umbc.edu/~jwolf/method2.htm.*

# ASEPTIC TECHNIQUE

Aseptic techniques are precautionary measures taken to prevent the contamination of pure cultures and sterile laboratory equipment. Accordingly, the goal of such techniques is to *reduce* pathogens, and not necessarily to *eliminate* them by sterilization. Several important aseptic techniques are listed following:

- Treat all organisms as potential pathogens. Many organisms can be opportunistic in their abilities to cause infection.
- Wash hands before and after the lab. The human skin flora is diverse and omnipresent.
- Avoid placing any objects in your mouth (pen, pencils, etc.) and then laying them on the bench top.
- Microorganisms in the lab atmosphere may come to rest on the bench top between classes and overnight. Disinfect the bench top thoroughly before and after each lab period. Spray the bench top with a commercial disinfectant such as 70% ethanol or a 10% bleach solution and allow this to stand for one minute before wiping the bench clean with a paper towel.
- Petri dishes should remain covered as much as possible. If the top must be removed completely, do not lay it on the bench top. This lowers the probability of contamination and helps to prevent false positive results.
- It is important to use a sterile pipette for each different sample taken or removed because once a sterile pipette is used, it is no longer sterile. To remove a sterile pipette from its wrapping: Open the wrapping from the side at the top of the pipette. Then remove the wrapping without touching the pipette to any surfaces. Pipette out the desired volume, ensuring that you do not touch the pipette and that the pipette does not come into contact with any other object. This is important for two reasons: 1) To prevent contamination of your sample with any unwanted organisms; 2) To prevent contamination of the work space.
- Before using a wire loop, flame sterilize it, wait for it to cool for about 20 seconds, and then use it. Do not to touch the loop to anything after it has been flame sterilized.
- Pay strict attention to the disposal of used laboratory material. Anything which is used for collecting or storing a sample or bacterial culture must be autoclaved (heat sterilized) and then disposed of. Any pipettes, microtips, or filter hoses used for microbial samples must also be autoclaved, and then either disposed of or reused.

**Questions**

1. Compare aseptic techniques to sterile techniques. Provide two examples of each. 3 pts
2. Are aseptic techniques required if you are interested in conducting a chemical analysis of wastewater? Why or why not? 2 pts
3. "Petri dishes should remain covered as much as possible…to lower the probability of contamination and help prevent false positive results." Explain the meaning of "false positive results" in this case. 3 pts
4. If you already have a stock of one particular bacterial culture (a pure culture), why are aseptic techniques necessary? 2 pts

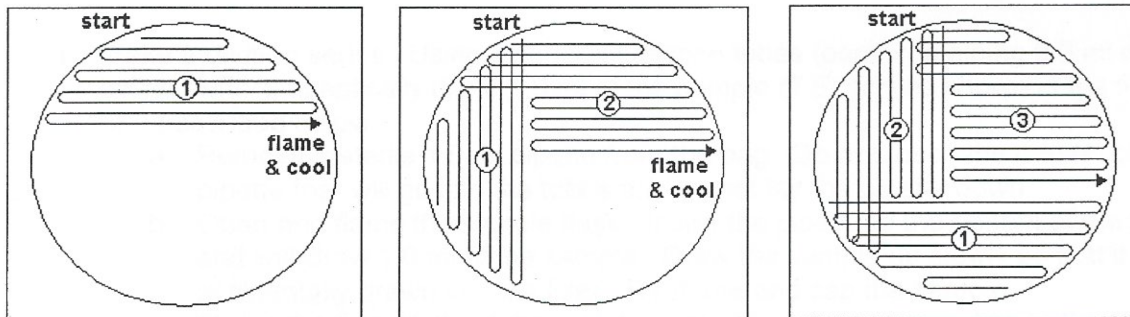
# MICROORGANISM ISOLATION USING THE STREAK PLATE METHOD

## Introduction

The streak plate method is used to systematically decrease the number of microorganisms such that you can obtain a single isolated colony. Thus, the purpose of the method is *isolation* rather than *identification*.

## Procedure

1. Sterilize the inoculating loop.
   a. The inoculating 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 from heat. This incinerates all of the contaminants on the wire.
   b. Allow the loop to cool for about 20 seconds to avoid killing the inoculum. **Never lay the loop down once it is sterilized or it may become contaminated**.
2. Lift the edge of the lid just enough to insert the loop.
3. Streak the loop lightly across the surface of the agar medium. Always start streaking at the "12:00 position" of the plate and streak side-to-side as you pull the loop toward you. Each time you flame and cool the loop between sectors, rotate the plate counterclockwise so that you are working in the 12:00 position of the plate.
4. Remove the loop and close the lid.
5. Re-sterilize the inoculating loop.




## Questions

5. What is the main goal of the streaking plate technique? 1 pts
6. Describe two aseptic techniques that should be used when preparing a streak plate. 2 pts

# MICROORGANISM ENUMERATION USING THE SPREAD PLATE METHOD

## Introduction

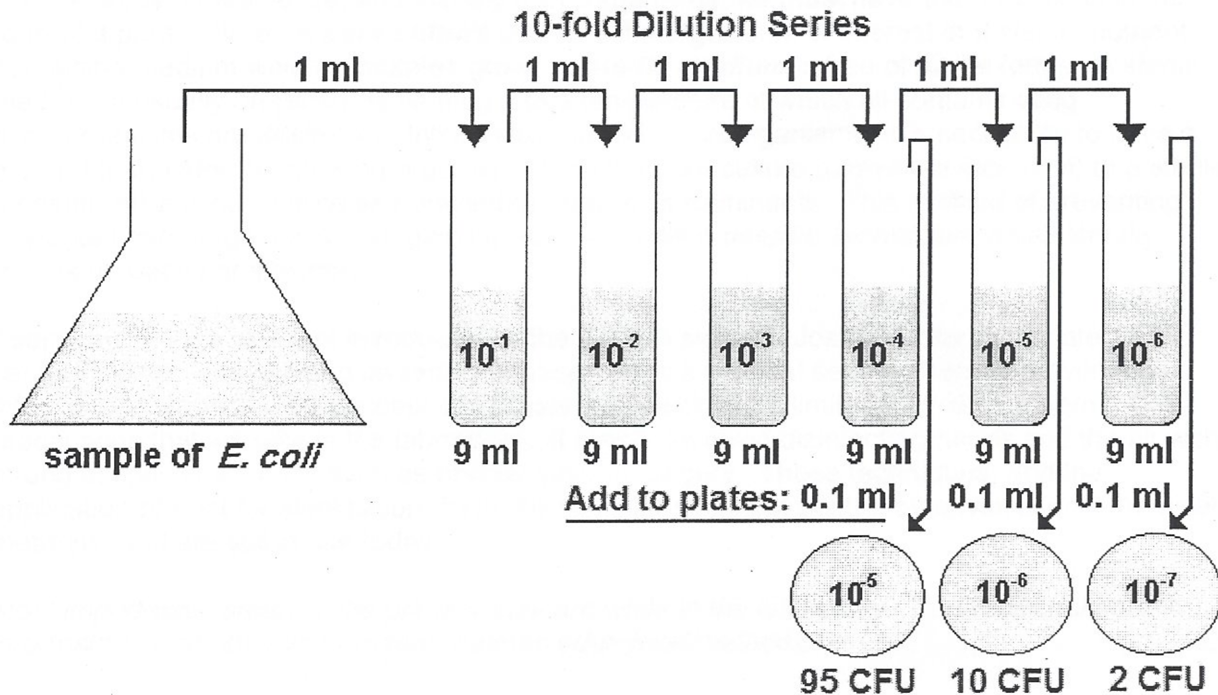
The number of bacteria in a given sample is usually too many to be counted directly. However, if the sample is serially diluted and then plated on an agar plate, the number of colonies can generally by counted in order to determine the number of viable (living) cells. Normally, the bacterial sample is diluted by factors of 10 and is plated on a nutrient agar plate. After incubation, the number of colonies on a dilution plate can be determined. Plates with between 30 and 300 colonies are considered to be statistically significant. If there are less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants may have a drastic effect on the final count. Likewise, if there are more than 300 colonies on the plate, there will be poor isolation and colonies may have grown together.

Generally, one wants to determine the number of colony-forming units (CFUs) per milliliter of sample. To find this, the number of colonies (on a plate having 30 – 300 colonies) is multiplied by the number of times the original milliliter of bacteria was diluted (the dilution factor of the plate counted). For example, if a plate contains 150 colonies and dilution factor is $10^{-6}$, then the number of CFUs per milliliter of the original sample is determined by multiplying the count by the dilution factor, or $150\times10^{6}$ CFU/ml (reported as $1.50\times10^{8}$ CFU/ml). For a more accurate count, it is advisable to plate each dilution in duplicate or triplicate and then to report the average number of CFU/ml.

## Procedure

1. Make a dilution series. Using a series of dilution tubes (each containing 9.0 ml of sterile saline buffer), aseptically dilute 1.0 ml of the sample of *E. coli*, as shown in the figure, and described below.
    a. Remove a sterile 1.0 ml pipette from the bag. Do not touch the portion of the pipette that will go into the tubes and do not lay the pipette down.
    b. Open and flame the sample flask. Insert the pipette to the bottom of the flask, and withdraw 1.0 ml of the sample. Draw the sample up slowly so that it isn't accidentally drawn into the filter. Re-flame and cap the sample.
    c. Flame the first dilution tube and dispense the 1.0 ml of sample into the tube. Draw the liquid up and down in the pipette several times to rinse the pipette and mix the sample. Re-flame and cap the tube.
    d. Mix the tube thoroughly by holding the tube in one hand and vigorously tapping the bottom with the other hand or by using a vortex mixer. This is to ensure an even distribution of the bacteria through the solution.
    e. Using the same procedure, aseptically withdraw 1.0 ml from the first dilution tube and transfer into the second dilution tube. Continue transferring from tube to tube as shown in the figure until the dilution is completed. Discard pipettes in the biowaste disposal containers.
2. Prepare the spread plate.
    a. Using a new 1.0 ml pipette, aseptically transfer 0.1 ml from the last dilution tube onto the surface of the plate. Note that since only 0.1 ml of the bacterial dilution (rather than 1.0 ml) is placed on the plate, the bacterial dilution on the plate is an additional 1:10 dilution.
    b. Sterilize the glass rod by dipping the bent portion in alcohol and igniting the alcohol with the flame. Let the flame burn out.

c. Immediately spread the solution over the surface of the plates.
d. Replace the lid and re-sterilize the glass rod.
e. Discard the pipette in the biowaste disposal containers.
f. Incubate the agar plates upside down at 37°C for 24 hours. Place the used dilution tubes in the disposal baskets.




7. Why is it necessary to perform a 10-fold dilution series for the spread plate technique? 2 pts
8. A sample of *E. coli* is diluted as shown in the above diagram. The number of colonies counted is shown on the petri plates. How many CFUs are there per milliliter of the original sample? Explain how you arrived at your answer. 3 pts
9. Why would one perform the streak plate method as opposed to the spread plate method and vice versa? 2 pts