**Aseptic Technique**

# Background:

In the early days of surgery the most important consideration when choosing a surgeon was how fast could he perform the surgery. There was no anesthesia or sterile operating rooms. Surgery was messy and often patients died of shock or post-operative infections. Where did the infections come from you might ask? Our current understanding of the ubiquitous nature of microorganisms and the role that some play in disease was unknown at the time, so surgeons had no concept that by not properly sterilizing their instruments or preparing the patient they were contributing to the spread of disease and death. It would not be until the mid to late 1800’s before our modern germ theory came to be widely accepted and the consequences of not using aseptic technique were fully understood.

The word aseptic literally means without sepsis. Sepsis is a word that means infection. Therefore if a surgeon was practicing aseptic technique they would be operating in such a manner as not to transmit an infectious agent or cause an infection to occur. In today’s operating rooms, aseptic techniques are strictly adhered so as to prevent infection. The techniques include the use of antiseptics, which are substances when applied to the tissues of the body prevent infections, proper sterilization of surgical instruments, proper hand washing, and the use of barriers such as gloves. These measures have no doubt reduced suffering and saved many lives.

In the laboratory the aseptic technique is less concerned with the transmission of

infectious agents as it is with the contamination of laboratory cultures and personnel working in the lab.

Contamination is the introduction of an unwanted organism or organisms into a culture, environmental space, other organism (such as your lab partner) or yourself. You will be working with a variety of bacterial cultures during the course of the semester. It is important that you work with the organisms safely and not contaminate them or yourself.

To accomplish this you will need to keep a few things in mind.

You must be aware of the sources of contamination. The first is the environment. We are literally living in a sea of microorganisms. There are billions of bacteria on most environmental surfaces as well as fungal spores, viruses and perhaps a few protozoan parasites. If these organisms were to enter your culture they may begin to grow and your pure culture will no longer be pure. In the laboratory exercises we will be conducting it is important that we have pure cultures to achieve proper results.

A second source of contamination is what we refer to as cross contamination. This occurs when organisms from one culture are introduced into another. This can be deliberate or unintended, but the result is the same, a mixed culture. You will need to see that this does not happen.

The last thing to remember is that if you spill any of your organisms onto your lab bench or other surface you must

properly clean up the spill immediately. While the bacteria we will be working with are not likely to cause problems if properly handled, they have the potential to cause infections if you contaminate yourself with them. **Remember safety first.**

# Basic Principles of Aseptic Technique:

There are a few basic considerations you need to keep in mind when working with microorganisms. If you learn these principles and apply them, you should have no problem keeping your cultures pure and achieving good results in the lab. It should be noted that these principles can be extended to the medical and health care arenas, as well as the kitchen, bathroom, and to some extent the bedroom.

The first principle is that you should consider **EVERYTHING** to be contaminated with microorganisms. This is not meant to frighten you, as most bacteria and fungi you will encounter in your day to day life are harmless to you, but if these critters are not accounted for, they could contaminate a laboratory culture. In the medical world, some of these organisms if given the opportunity could cause an infection.

The second principle is to never work with microorganism unless you know that the tools you are working with and the medium you are using are sterile. In this lab you can assume that the media I will supply is sterile, but there are times it may become contaminated, so look it over before you use it. Culture medium should not be fuzzy or come with bacterial colonies on the surface.

The third principle is that cultures should be axenic, which means they contain only one species of bacteria. On occasion mixed cultures may be used for a very specific reason. Since most of the cultures are axenic when given to you, they should remain that way.

# Aseptic Technique

Now that you have a background concerning the basic principles of aseptic technique, it is time to outline how you are to apply these principles in the laboratory.

1. the first thing you do upon entering the laboratory is to wipe down your work area with disinfectant. This will not eliminate all the microorganisms on the lab bench, but remove most, particularly any potential infectious agents.
2. the second thing you do is to pay careful attention to all instructions given concerning the laboratory procedures you will be asked to perform.
3. when you actually begin to work with the microorganisms, you need to following the following steps and protocols:
4. light your Bunsen burner. Be sure it is in a safe place and will not cause a fire. You will be instructed on the proper use of the Bunsen burner.
5. obtain your inoculating loops and flame them. The entire wire portion of the loop must be allowed to remain in the flame until it glows red. The loop should be held on a slight angle. see Figure 1



Figure 1 Flaming the loop

1. once the loop is flamed, it is sterile. **DO NOT** touch the loop, set it down on the lab bench, or allow it to come into contact with anything. It is now ready to be placed into the culture to remove a small amount of the organisms.
2. carefully remove the cap on the culture. **DO NOT** set the cap onto the lab bench; hold onto it in such a manner as it is held right side up.
3. pass the mouth of the culture tube through the flame several times. The idea here is to remove any microorganisms on the lip of the tube and to warm the tube. **DO NOT** leave the tube in the flame until it glows red. If you do, you will not like the results. Also, **DO NOT** hold the tube vertical. Always keep the tube on a slight slant.
4. once the tube has been passed through the flame, insert the sterile loop into the culture tube and down into the culture. Gently remove a

loopful of the culture. The loop is now referred to as being charged and contains thousands of microorganisms**. DO NOT** touch anything with the loop or shake it around.

1. before putting the cap back onto the culture tube, pass the mouth of the tube through the flame several times. Replace the cap.
2. at this point, you are ready to transfer the bacteria on the loop to a microscope slide or to culture medium – broth or solid medium.
3. after the transfer, you must flame the loop again to remove any remaining microorganisms. Once flamed the loop can be set aside. Remember it is hot. **DO NOT** touch the loop or touch anything with it that could be burned, i.e. your lab partner or lab manual, etc…
4. each time you are going into or out of a culture to either remove organisms or to inoculate the medium, you must flame the loop and the mouth of the tube.
5. after you have finished all the transfers and are ready to leave the laboratory, wipe down your work area with disinfectant and wash your hands. It is good practice to wash your hands every time you leave the laboratory. You really do not want to take any of the microorganisms with you. They do not make good pets.

# NOTES:

**Exercise in Aseptic Technique**

This exercise is designed to help you practice your aseptic technique.

* 1. obtain two tubes of medium from the instructor
	2. label the tubes #1 and #2 with your wax pencil
	3. open tube #1 and insert your loop into the tube without flaming it or the tube mouth. There is no need to be careful here. You may even allow the tube to remain uncapped for several minutes if you like
	4. using the aseptic technique described above repeat the

process in step 3. This time be careful to flame the loop, and tube. Do not allow the tube to remain uncapped for any longer than necessary to place the loop into the medium and remove it.

* 1. place both tubes into a rack as instructed. The tubes will be incubated overnight and examined for growth.

# Day Two

1. examine the tubes for growth. Growth will be evidenced by cloudiness in the medium.
2. record your results in Table 1.

# Table 1 - Results

|  |  |
| --- | --- |
| **Tube #1** | **Tube #2** |
|  |  |

Was there a difference between the two tubes?

If there was a difference, how can this be explained?