Microbiology Lab Safety

# Objectives:

1. Introduce students to the lab safety procedures and rules for the semester.
2. Learn how to safely light a Bunsen Burner and correctly flame an inoculation loop
3. Complete the Glow Germ activity learning to apply why lab safety and cleanliness is important.

# Part One: Introduction to General Lab Safety

The instructions below are designed to keep you safe in the laboratory. Please read them carefully. If you have any questions about safe laboratory practices, ask your instructor.

* No eating, drinking or smoking at any time. Do not bring food or drink items into the lab. Avoid all finger/hand-to-mouth contact.
* Lab Attire- legs covered (no shorts or jeans with holes)
* Open-toed shoes (sandals, flip-flops etc.) cannot be worn in lab. NO Crocs with holes, period.
* Loose clothing and long hair must be tied back while working to avoid burning with open flames or inadvertent contamination.
* Follow all directions given by the instructor. Bring any safety concerns to the attention of the instructor.
* Come to lab on time and prepared for that day's experiments.
* Wash hands, and wipe down bench area with disinfectant prior to working. Before you leave the lab for the day wipe down your bench area with disinfectant and then wash your hands. Wash your hands at any time during the lab if you think you may have contaminated them. Wipe any surfaces or equipment with disinfectant immediately if you suspect contamination with living cultures.
* Use care with the Bunsen burners. Keep paper, alcohol and other flammable items away from the open flame
* Treat all living cultures of microorganisms (bacteria, yeast, etc.) as potential pathogens. Avoid spilling or spreading the microorganisms.
* Place all used materials in the appropriate waste containers designated for cultures (to be autoclaved).
* Use the techniques specified by the instructor for handling microorganisms. If there is a spill notify your instructor immediately.
* Know where fire extinguishers and safety equipment are located in the lab.
* To prevent contamination of these articles, books, coats, backpacks, etc. (anything you do not need for the Micro lab) must be placed in the designated area and should not be kept at the laboratory table.
* Make sure to carefully read through the entire procedure before beginning an experiment in the lab. This will help prevent you from making mistakes that could compromise your safety.

# Part Two: Instructions for Good Laboratory Practice and Care of Laboratory Equipment

Correct use and care of the laboratory equipment is considered a fundamental part of good laboratory technique. All students working in the microbiology laboratory are responsible for maintaining equipment and materials in proper working condition.

## Microscopes

The most critical (and most expensive) piece of equipment in the microbiology laboratory is the microscope. If you expect to see specimens through the microscope, it must be kept clean and in good condition. You must use the microscope assigned to your seat. Instructions for the use and care of the microscope can be found in Lab 1 of the lab manual. Report any problems with your microscope to your instructor.

## Inoculating loops and inoculating needles

Inoculating loops and needles are used to transfer bacteria into and from culture media. Inoculating loops have a loop at the end, while inoculating needles end in a point. Inoculating loops are the most common method of transferring bacteria. Inoculating needles are used when stabbing into a medium during specific inoculation procedures, or when it is necessary to pick up a small amount of bacteria from one colony on an agar plate without contacting bacteria in other colonies.

## Microscope slides

Any disposable glass slides should be discarded in the glass container. Do not discard glass slides in the waste cans.

## Petri dishes and test tubes

All materials used for handling or culturing microorganisms are to be disposed of as follows: test tubes placed in racks in a bin for autoclaving; petri dishes in the other bin for autoclaving and disposal.

## Spillage

Any living culture material that is spilled, either on tables or on the floor, is to be treated immediately with disinfectant and cleaned up with paper towels. Notify the instructor of any spills. The paper towels that you use to clean up the spill should be placed in the bin with the petri dishes for autoclaving.

## Prepared slides

Prepared slides that are used during the semester must be returned clean to the trays from which they were taken.

## Cleanliness of the room

Any papers on the floor at the end of the laboratory period are to be picked up and discarded in the wastebasket.

The same is true for your laboratory bench area.

**DO NOT throw plates, tubes, swabs, slides, pipets, pipet tips, broken glass, etc. into the regular garbage. These items need to be disposed of properly. Throwing potentially contaminated items into the regular garbage is a safety issue for students, instructors, lab techs and the cleaning staff. If these items are found in the regular garbage the ENTIRE BAG OF GARBAGE must be autoclaved before disposal. If you are unsure about where an item should go, always ask your instructor.**

# Part Three: Bunsen burners

A Bunsen burner is a source of open flame that is used to sterilize loops and needles, as well as flaming the lips of test tubes during inoculations. You must always take great care when operating a Bunsen burner!

## Setup

* Check the burner and hose for any broken parts or cracked tubing
* Attach one end of the hose to the Bunsen Burner and the other end to the gas nozzle on the table. Turn the collar (air inlet) clockwise until you meet resistance and then turn slightly back to allow a small amount of air into the barrel.
* Turn the stopcock counterclockwise to close the gas intake.
* Have your striker close by. When using the striker remember to push up as you move the striker across the flint to produce sparks.

## Lighting the Bunsen Burner

* To light the Bunsen burner, turn the handle of the valve so it is in line with the tubing connecting the Bunsen burner to the gas.
* Open the stopcock at the bottom of the Bunsen burner slightly, until you can hear the sound of gas escaping (turning clockwise)
* Using a striker, light the Bunsen burner. If the Bunsen burner does not immediately light, turn off the gas and determine the cause of the problem. NEVER leave the gas on if the Bunsen burner is not lit.
* DO NOT lean over the Bunsen burner while lighting it.
* Once the Bunsen burner is lit, be careful to keep all flammable items, including lab coats, hair, shirt sleeves, scarves, tissues, alcohol, etc. away from the flame.
* To adjust the flame so that you can see the inner cone, turn the collar to allow more or less oxygen into the flame.



If you smell gas at any time, check to make sure that the Bunsen burner is still lit. If the flame goes out at any time, TURN OFF THE GAS.

## Turning off the Bunsen Burner

* Turn collar (clockwise) until orange flame appears
* Turn the stopcock (counter clockwise) until flame disappears
* Turn off gas, making sure the handle is perpendicular to the hose.
* Once Bunsen Burner is cool, remove hose and place burner, hose, and striker back into the lab bench.

## Flaming Your Loop

1. Start with a properly-lit Bunsen burner (conical blue flame):



2. Beginning where the wire and the handle meet, place the wire at the very top of the conical blue flame, as this is where the heat is greatest:



3. Hold until the wire glows orange:



4. Slowly move the wire through the flame from handle to tip, allowing each section to glow orange before moving on:



5. Once the tip glows orange, remove the loop from the flame. Letting it sit in the flame for longer will break down the metal.



After your loop has been flamed, don’t blow on it, lay it on the countertop, or touch it to anything other than sterile media or your desired culture unless you’re done using it; otherwise you have to flame it again.

# Part Four: Glow Germ

Good hygiene is very important in the lab setting as we cannot see microbes, but they are EVERYWHERE. Handwashing and keeping your lab area clean are important ways to help cut down on the possibility of spreading microbes in the lab and also once you leave the lab.

Effective handwashing is critical in the lab to prevent the transmission of potential pathogens to others, oneself, or contamination of cultures.

In this activity we will compare the degree of contamination before and after handwashing.

## Procedure

* Shake the bottle well to mix the solution
* Have your lab partner apply 2-3 drops of Glow Germ on the palms of both your hands. Be careful to not get the Glow Germ on your clothing or in your eyes or mouth.
* Rub your hand together, thoroughly covering your hand surfaces, including the back of your hands and your wrists. Lightly scratch your palms with your fingernails.
* Have your partner shine the UV light on your hands to see the extent of the coverage of the “germs”. DO NOT LOOK DIRECTLY AT THE UV LIGHT. Do not handle the light yourself to keep from contaminating it with the “germs”. Record Results (your partner)
* Wash your hands with soap and water as you would normally (recommendation is a minimum of 20 seconds). Dry your hands with a paper towel.
* Have your partner shine the UV light on your hands once again and record where any “germs” remain.
* Wash and dry your hands once more, know that you know where any germs remain.
* Repeat the experiment with your lab partner, but with the roles reversed.
* Once you have both finished the lab, shine the UV light on your lab sheets, lab bench, pencil, and sink.