Microbiology Laboratory 1

Laboratory Safety

Safety is a priority in the microbiology lab. We will work with living organisms, we will generate chemical solutions, and we work with glassware and open flames. It is essential to consider the best practices to avoid injury. Although we do not use known pathogenic cultures or biohazardous materials, we still are careful to handle and dispose of living bacterial cultures. You may recognize the international symbol for biohazard material, developed in 1966 by a Dow Chemical worker to serve as a warning for materials that carry significant public health risk, such as human viral samples or hypodermic needles.

Since we will be working with living organisms in the laboratory the primary goal is "containment". We want to keep the microorganisms where they are and where we want them. It is necessary to transfer microorganisms to determine their metabolic capabilities and to view them under the microscope. The principle laboratory procedure we will use is **aseptic technique**. The goal of aseptic technique is to transfer microorganisms without contaminating two things:

- yourself
- cultures

We don't want the microorganisms to escape and infect the environment, the student, or others. In addition it is important that the organism you are working with does not become contaminated with another organism. Aseptic technique involves many different aspects of laboratory work. You will learn and develop these practices throughout the semester. Most involve common sense but it is challenging to be utilizing these techniques while concentrating on what must be accomplished during that day's laboratory assignment.

Biosafety Levels (BSL)

The Centers for Disease Control has categorized microorganisms into four biosafety levels. These levels take into account factors such as virulence, pathogenicity, antibiotic resistance, vaccine and treatment availabilities, plus other factors. The divisions are as follows:

BSL 1 - This includes organisms not known to consistently cause disease in immunocompetent (healthy) adults. No safety equipment is required. Work may be conducted on open bench tops following standard microbiological practices (SMP).

BSL 2 - Organisms in this category are associated with human disease. For the laboratory SMP practices apply plus limited access, biohazard signs, sharps precautions, and contaminated waste is autoclaved.

BSL 3 - This level includes indigenous/exotic organisms that may have serious or lethal consequences. They also have the potential for aerosol transmission. Laboratory precautions include all in BSL 2 plus decontamination of all waste and lab clothing before laundering. All personnel must have baseline antibody titers determined. The facilities must also have negative airflow, separation from access corridors, and double door access.

BSL 4 - This category includes dangerous and exotic agents with a life threatening nature or have an unknown risk of transmission. Of course all BSL 3 precautions are required plus clothing change before entering the laboratory and showering before leaving the lab. A positive pressure personnel suit is required for entry. The facility must be a separate building with dedicated air supply and the exhaust air must be decontaminated.

In our laboratory we will be using only BSL 1 and BSL 2 organisms. Although some of the organisms we will work with are classified BSL 2, these organisms are laboratory strains which do not have the same threat of infection as primary isolates. Therefore we can handle these organisms with normal SMP procedures. But please note that many bacteria are opportunistic pathogens and therefore should be handled with the proper techniques and precautions.

Good Microbiological Practice (GMP) = Standard Microbiological Practice (SMP)

- When you enter the laboratory
  - Always download and read the laboratory manual before the start of the laboratory period.
  - Drop off extra books, coats, and other materials at the front of the lab or in the lower drawers at your work station.
  - Wipe down your workstation with disinfectant and paper towels.
  - At the start and end of each laboratory session, students should clean their assigned bench-top area with a disinfectant solution provided. Your work area should then be kept neat, clean, and uncluttered throughout each laboratory period.
- During the laboratory exercise
  - Good personal habits: Tie back long hair neatly, away from the shoulders. Keep fingers, pencils, and such objects out of your mouth. Do not smoke, eat, or drink in the laboratory. Do not wander about the laboratory. Unnecessary activity can cause accidents, distract others, and promote contamination.
  - Discard all cultures and used glassware into the designated container. (This container will later be sterilized.) Never discard contaminated liquids or liquid cultures in the sink.
  - Never place contaminated pipettes on the bench top.
  - Broken glassware should be placed in the glass discard box. Glassware should never be placed in the regular trash can.
  - If you should spill or drop a culture or if any type of accident occurs, call the instructor immediately. Place a paper towel over any spill and pour disinfectant over the towel. Let the disinfectant stand for 15 minutes, then clean the spill with fresh paper towels. Finally, wash your hands thoroughly.
  - Report any injury to the instructor either before the laboratory session begins or during the session.
  - Never remove specimens, cultures, or equipment from the laboratory under any circumstances.
- At the end of the laboratory
  - Return all materials and equipment to their proper location.
  - Place labeled cultures at the appropriate location (usually in the incubator).
  - Place your workstation stool under the bench.
  - Wipe down your workstation with disinfectant and paper towels.
  - Wash you hands with soap and water.

Workstation drawer contents

Familiarize yourself with the items in the top drawer of your workstation.

Inoculating loop	Bibulous paper
Inoculating needle/wire	Bunsen burner
Tweezers / forceps	Striker
Dropper bottle of water	Foam test tube rack
Oil (for oil immersion microscopy)	Clothespin (for holding hot slides)
Sharpie or wax pencil	Slide box with slides and cover slips
Lens cleaning solution	Eye protection
Lens paper	

In the second drawer you will find a staining tray and rack.

On the bench tops you will find, distilled water, sand for extinguishing small fires, alcohol, and disinfectant.

<u>Lab Assignment</u> - Light a Bunsen Burner

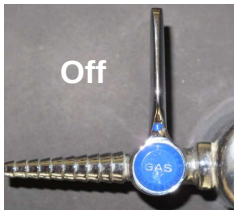
Set up:

- Remove the Bunsen burner from your drawer and place it on the lab bench near the gas jet.
- Remove the striker from your drawer as well.
- Inspect the tubing to make sure there are no holes or cracks in the tube.
- Connect the tube to the gas jet, a pointed nozzle with a handle labeled GAS.

Before turning on the gas - Safety first:

- Make sure the area around the Bunsen burner is cleaned up. Be especially aware of flammable items such as paper and alcohol.
- Make sure long hair is pulled back and long sleeves are not hanging down.
- You must have safety glasses on when ever working with open flames.
- Once lit never leave the burner unattended. If you need to leave the burner, for any reason, extinguish the flame by turning the gals valve handle to the OFF position.
- Always shut the gas when the Bunsen burner is not in use.

Lighting the Bunsen burner:



- With your dominant hand hold the striker over the top of the Bunsen burner. Please note the striker is made of metal. It will not catch on fire. The striker can be in the flame.
- With your other hand turn on the gas valve to the correct position.
  - The off position has the valve handle turned 90° form the outlet.
  - The on position has the valve in line with the gas outlet.
  - You do not want the handle to be completely in line with the gas outlet. That position would allow far too much gas to the burner for you to light it.
  - You want to turn on the gas valve about 4/5th of the way on.

On

- With the gas on hold the striker just above the barrel top and squeeze the striker handle to create a spark that will light the gas.
- Once lit remove the striker and place it back in your drawer so it isn't cluttering your work space.

Adjusting the Bunsen burner:

- You can adjust the flame height by adjusting the amount of gas coming from the jet by turning the valve at the gas jet.
  - You want a flame that is not too high and not too small. Aim for a flame that is between one and two inches high from the tip of the Bunsen burner.
- Adjust the air vents at the bottom of the Bunsen burner so that they are fully open.
  - You want to see a clear blue flame surrounding an inner blue cone.
  - The tip of the inner blue flame is the hottest part of the flame. That is where you will want to flame your loop and needle to glowing red to incinerate (sterilize) them.

Lab Assignment - Skin Microbes

- Obtain one Nutrient Agar petri dish for every two students.
- Draw a line on the bottom of the plate dividing the plate into two halves.
- Label the bottom of the plate so you know which plate is yours next week.
- Flip the plate over.
- Tilt the lid to partially expose the sterile nutrient agar.
- Gently place three of your fingers on the agar.
- Close the lid.
- Have your lab partner press their finger tips to the other half of the plate.
- Invert the plate and place in the incubator.

Lab Assignment - Ubiquity of Microorganisms

Items needed for every two students:

- Two sterile Nutrient Agar (NA) petri dishes
- Two Potato Dextrose Agar (PDA) petri dishes
- Two unopened sterile cotton swabs
- One tube of sterile water

Procedure:

- Wet a swab by dipping it in the water. Then swab a surface in the building. Rotate the swab to pick up as many microorganisms as you can. Next swab the NA plate. Be careful the agar is only semisolid. Try to swab across as much as the surface of the agar as possible while rotating the swab.
- Using the same swab, immediately swab the PDA plate in the same way.
- Now repeat the same procedure with a new swab (but with the same sterile water tube) on a different part of the building.
- Label your plates on the bottom and incubate them inverted in the incubator.

There are many different types of media used to grow microorganisms. Certain media are favored by certain microbes. In this lab, NA supports the growth of bacteria best. PDA supports the growth of fungal organisms best. But this is not in total. Some bacteria will grow on PDA and some fungi will grow on NA.

After you are finished:

- Pick up your work space by returning any equipment to the appropriate storage space.
- Disinfect your lab bench.
- Wash your hands.
- Finally take all your belongings with you.