Aseptic Technique

Aseptic - "an environment or procedure that is free of contamination by pathogens" (1)

Introduction

In the microbiology lab we use aseptic technique to:

- Prevent contamination of the specific microorganism we are working with.
- Prevent contamination of the room and personnel with the microorganism we are working with.

Many of the microorganisms we will be working with in lab are known pathogens. Proper and appropriate aseptic technique is vitally important for the safety of all lab personnel; it is also essential for the successful completion of the lab portion of this class. The skills and awareness you develop practicing aseptic technique will carry over to your career as a health professional. Many of our former students comment that this is the most important thing they learned in lab!

In this lab you will be learning standard microbiological procedures appropriate for Biosafety Level (BSL) 1 and Biosafety Level (BSL) 2 precautions. We follow the safety guidelines established by the Center for Disease Control and Prevention (CDC). Complete documentation is available at the CDC Website (2).

*BSL 1 – "suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of **minimal** potential hazard to laboratory personnel and the environment" (2)*

*BSL 2 – "suitable for work involving agents of **moderate** potential hazard to personnel and the environment" (2)*

Overview

Aseptic technique involves developing both manual dexterity in safely handling the microorganisms and mental dexterity in thinking ahead about what you are doing with the microorganism. In this lab you will learn how to:

- decontaminate your lab bench
- safely organize your workspace
- properly adjust your Bunsen burner
- properly use an incinerator
- sterilize your inoculating tools
- aseptically transfer organisms from broth/plate cultures using BSL 2 procedures
- handle biohazard spills and dispose of biohazard materials

General safety considerations

- Access to the lab is limited.
- Wear your lab coat and gloves.
- Tie back long hair.
- Leave all food and drink in your backpack. Do not chew gum in lab.
- The only thing on your lab bench should be the equipment you are working with and your lab book. Place your backpacks on the floor where you or someone else will not trip over them.
- Discard contaminated material in the appropriate container. Anything that has been in contact with microorganisms must be disinfected with a disinfectant such as Cavicide© or autoclaved.
- Clean up all spills immediately!
- Decontaminate your lab bench with disinfectant such as Cavicide© **before and after** lab.
- **Wash your hands before leaving the lab.**

Lab Bench Organization

You need to have your workspace well organized. Lab bench space is very limited. You need to have all your materials close to you. Do not stretch over the table for what you need. Each of you have your own loop and tubes, but you will be sharing a Bunsen burner and other lab equipment.

Transferring Organisms

One of your main concerns when working with microorganisms is to avoid producing aerosols that you can breathe in and droplets that can land on you, your lab partners, and your lab equipment. You will spend a lot of time in lab transferring organisms from one tube to another, or to slides or to plates. It is imperative that you do this quickly and safely. The longer your organism is exposed to the air, the more opportunities there are for it to get contaminated and/or to contaminate you, your lab partners or your equipment.

Part I - Procedures for Practice "Organisms"

After you have practiced these procedures several times your instructor or IA will assess your proficiency. It is essential that you grasp these skills **before** you proceed to working with actual microorganisms.

Materials

- Bunsen burner and striker
- Incinerator
- Inoculating loop
- 1 selective media plate
- 1 tube food dye colored water
- 1 tube sterile water
- 1 marker pen or grease pencil

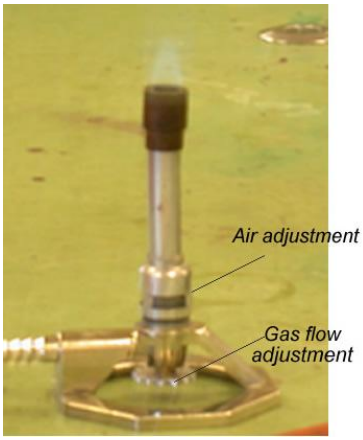

Figure 1: Bunsen burner

Using a Bunsen burner

If you can't seem to get your burner lit ask your instructor or IA if the main gas control is on.

1. Firmly attach the hose to the tapered gas line. The burner has two adjustments. The knob underneath adjusts the amount of gas going into the burner tube. The barrel of the burner turns to adjust the amount of air going into the burner. Before lighting a Bunsen burner, set the air and gas adjustments to a minimal open position. Turn the air adjustment clockwise to decrease the air (a more purple flame) and counterclockwise to increase the air (a more yellow flame). ***Refer to Figure 1.***
2. Look at the underneath of the striker. There is a roughened rod against which a small flint rubs when you push the handle. If you are pushing firmly and do not get a spark, you probably need a new flint. Turn the gas on partway when initially lighting your burner.
3. Hold the cup part of the striker at an angle slightly above the opening of the burner and push the handle to generate a spark. You may need to repeat this several times before your burner lights. Alternatively, there may be butane lighters available in lab to light your Bunsen burner.

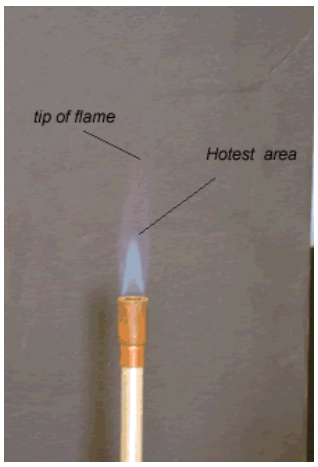

Figure 2: Good Flame


Figure 3: Bad Flame

4. Modify the air and gas adjustments until you get a hissing, small, blue flame within a taller lighter blue/violet flame. ***Refer to Figure 2.*** If your flame is just one violet flame you either have too much air or gas. If your flame is yellow and blows in the air currents in the room, there is too little air in your flame. ***Refer to Figure 3.*** Fiddle around with both of these adjustments until you get the proper flame. There are many other classes using the same equipment. You will most likely never have your burner the way you left it!
5. The tip of the little inside flame is the hottest part of the flame (1560°C). This is the area where you will want to run the top of your tubes through to maintain sterility.
6. **NEVER leave a lit burner unattended!**

Using an incinerator

Special precautions must be taken to prevent the formation of aerosols when working with BSL 2 organisms. Instead of using a Bunsen burner to "flame" loops and other inoculating utensils, BSL 2 procedures require the use of an incinerator. An incinerator sterilizes inoculating utensils much the same way as a Bunsen burner does except the risk of aerosol production is reduced.

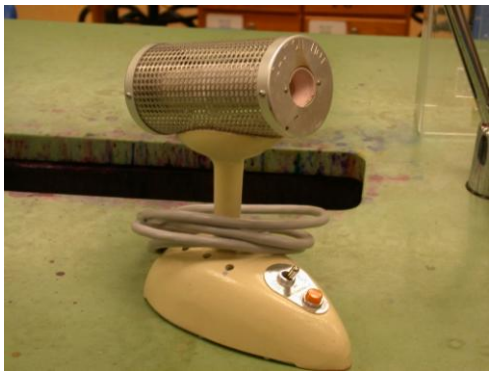

Figure 4: Incinerator

Broth to Broth Transfers Using BSL 2 Procedures

If you are using screw cap tubes be sure to loosen them **before** you start this procedure.

1. Usually when you are working with a broth culture, the organisms will have settled to the bottom.
   - You need to first re-suspend them in the broth.
   - Do **NOT** invert the tube! Most of the tubes we use have slip caps with a space at the top to allow air into the tubes. If you invert the tube you will pour bacteria all over. If you invert a screw cap tube, the liquid now in the inside of the lid will drip all over when you remove the lid.
   - Hold the tube near the top and flick the bottom of the tube with your other hand. Continue flicking until the organisms are re-suspended.
2. Place both your colored practice tube and your sterile water tube in your left hand with the lids pointing up.
   - Hold the tubes closer to the bottom so that your hand will not be close to the flame when you sterilize the mouth of the tubes.
3. Grab the inoculating loop far back on the handle as if you were going to write with it.
4. Place your loop in the mouth of the incinerator briefly for 2-4 seconds to sterilize it.
   - Do NOT leave your loop in the incinerator for more than 10 seconds, you will destroy the loop!
5. Wait about 10 seconds for your loop to cool.
6. While still holding the inoculating loop, use the lower part of your hand to grab both of the slip caps and pull them off. Keep them tucked in your hand.
   - Yes, this is as tricky as it sounds.
7. Sterilize the mouth of the tubes by passing them through the flame 2-3 times.
   - This kills organisms on the opening of the tubes.
8. Hold the tubes slightly tipped to minimize microorganisms in the air from falling into the open tubes.
   - Insert the loop into the colored tube without touching the sides of the tube.
   - As you pull it back out you will notice a film across the loop, just like when you blow soap bubbles.

9. Carefully withdraw the inoculating loop without touching the sides of the tube and insert it into the sterile water tube without touching the sides of the tube.
    - Watch your droplet while you are transferring to see if it is still in the loop. If you are successful, you should see some color in your water tube. You have now transferred your organism to a fresh tube.
    - Shaky hands or air currents can make the drop fall off onto the bench top, you, or your neighbor. Be careful!
10. When you withdraw your inoculating loop from the second, freshly inoculated tube, be sure to touch it against the inside of the tube to knock off excess fluid.
11. Flame the mouth of your tubes and replace the caps.
    - Do **NOT** miss your caps and push the mouths of the hot tubes into the palm of your hand.
12. Place your loop in the mouth of the incinerator briefly for 2-4 seconds to sterilize it.
    - Do NOT leave your loop in the incinerator for more than 10 seconds, you will destroy the loop!
13. **NEVER** lay your loop or other inoculating equipment on the counter without first sterilizing it.
14. Have your instructor observe and record your technique.

Broth to Plate Transfers Using BSL 2 Procedures

This is a little easier because you only have to hold one tube in your hand.

1. Be sure you have your practice plate on the lab bench ready to use.
2. Label the bottom with the date, the organism, your initials and lab section number.
    - **Write small and along the edges. Why?**
3. Follow the same procedure as above (steps 2-7) to get your sample from the colored “broth” tube.
4. While holding the loop with the practice "organism" (colored broth), flame the mouth of the tube and replace the lid. Return the tube to the rack.
5. Hopefully your loop still has its contents, if not where did it go?
6. Open the lid of your dish with your left hand and hold it ajar. Touch the surface of the loop to the agar surface. Refer to Figure 5 on the next page.
    - Lightly drag your loop back and forth across the surface of the agar being careful not to gouge the surface. Think of agar as really firm Jell-O.
    - The more your drag the more bacteria you deposit. The general idea is to decrease the bacterial concentration with each swipe. Four to five zigzags seems to work well. Experiment with your different plates. Be sure to keep track of what you did on each plate.
7. Sterilize your loop. Do **NOT** go back into the original broth tube.
    - Wait about 10 seconds for your loop to cool.
    - Touch the agar surface against the far end of your first streak.
    - Repeat by dragging back and forth. Do not drag into the center of your plate. You should be able to see the faint indentations of your streaking line on the agar surface.
8. Sterilize your loop and cool. Repeat the procedure on your second streak.
9. Sterilize your loop and cool. Repeat the procedure on your third streak. Zigzag the last part into the center of the plate. You should end up with isolated colonies somewhere in your last streak.
    - This procedure is called “streaking for isolation”
10. Replace the lid on your plate.
    - The longer the plate is open to the room air, the greater your chance of contamination.
11. Sterilize your loop in the incinerator.
12. **NEVER** lay your loop or other inoculating equipment on the counter without first sterilizing it.
13. Have your instructor observe and record your technique.

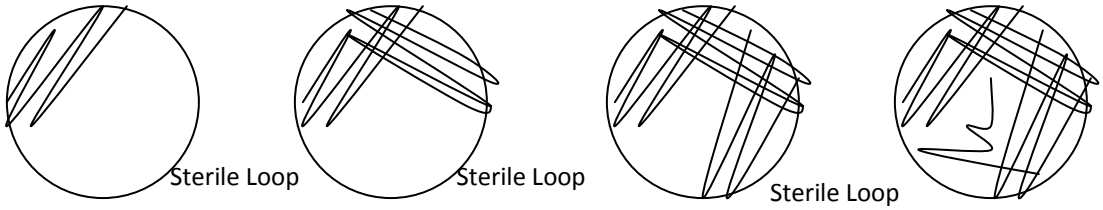

Figure 5: Streaking for isolation using the quadrant streak method.

Part II - Procedures for BSL 2 Organisms

Once your instructor or IA has observed your aseptic technique using the practice materials, you can begin to work with real organisms. The next step is learning proper aseptic technique for handling BSL 2 organisms.

Materials:

- Bunsen burner
- Striker
- Incinerator
- Inoculating loop
- 1 tube tryptic soy broth (TSB)
- 1 TSA plate
- 1 plate of *E.coli* K-12

Plate to Plate Transfers Using BSL 2 Procedures

Have your two plates on your lab bench. You will be given a plate with streaked organisms on it. These are the real thing. Look closely at it and select an area that has individual colonies. They will look like small dots on your plate. Each dot represents one or a few cells that multiplied to form a colony – also called a colony forming unit (CFU).

1. Place your loop in the mouth of the incinerator briefly for 2-4 seconds.
    - Do NOT leave your loop in the incinerator for more than 10 seconds, you will destroy the loop!
2. Wait about 10 seconds for your loop to cool.
3. Open the lid of the plate with the bacteria.
4. Gently scoop up a single well-isolated colony. Close the lid of the plate.
5. Open the lid of the labeled new plate and streak for isolation.
6. Close the lid and sterilize your loop in the incinerator.
7. Place your labeled inoculated plate upside down in the rack for incubation. Incubate at 37°C for 24 hours.
8. You will check them next lab period for growth.

Plate to Broth Transfers Using BSL 2 Procedures

Use the same plate of bacteria you did for your plate-to-plate transfer. Find another well-isolated colony. You want to know where you're working from in your plate so you aren't standing there with a cooling loop wondering what colony would work the best.

1. Place your loop in the mouth of the incinerator briefly for 2-4 seconds.
    - Do NOT leave your loop in the incinerator for more than 10 seconds, you will destroy the loop!
2. Wait about 10 seconds for your loop to cool.
3. Open the lid of the plate with the bacteria.
4. Gently scoop up a single well-isolated colony. Close the lid of the plate.

5. Grasp the lid off the tube and flame the mouth using the Bunsen burner.
6. Insert the loop into the broth without touching the sides of the tube. Twirl the loop like a swizzle stick to dislodge the bacteria.
7. Touch the loop to the side of the tube by the mouth to remove excess fluid.
8. Use the incinerator to sterilize the loop.
9. Flame the mouth of the tube with the Bunsen burner and replace the cap.
10. Place your labeled broth tube in the rack for incubation. Incubate at 37°C for 24 hours.
11. You will check them next lab period for growth.

Disposal Instructions

- Put the colored practice tubes in the appropriate rack on the "Save" tray on the front bench.
- Place the plates of *E. coli* on the "Save" tray
- Place the practice water tubes in the rack on the "Kill" tray.
- Place the practice plates on the "Kill" tray
- Place your inoculated plates and tubes in the appropriate racks on the "Incubate" tray.

1. Bauman, Robert W. *Microbiology* San Francisco: Pearson Education Inc., 2004.
2. http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm