Lab Basics

Performing scientific research in a laboratory can be an exciting and rewarding experience. As a research scientist in this program, you will learn scientific techniques and protocols that are often used in microbiology and molecular biology research. Before you begin, however, you will need to understand the fundamentals of working in a lab that are critical to your safety and success.

Lab Safety

While every laboratory has its own specific rules and regulations, there are some universal guidelines that you should always follow. They are designed to keep you and your lab mates safe and to comply with state and federal regulations. Please read the list below carefully and talk to your instructor about any specific rules that must be adhered to in your workspace.

General Rules

• No eating or drinking.
• No open-toe shoes.
• Never work alone in a lab.
• Tie back long hair.
• Avoid wearing baggy clothing.
• Never leave a lit Bunsen burner unattended.
• Keep workspace clear of clutter.
• Minimize loud talking and distractions.
• Know the location of fire extinguishers, emergency eye wash stations, and emergency showers.
• Properly dispose of waste generated from an experiment. Not everything can go down the drain or in a garbage can.
• No mouth pipetting!
• Wear safety glasses at all times.
• Wear gloves when working with bacteria.
• Wash your hands before leaving lab.
• If you are not sure, ask first!

Guidelines for working with bacteria

The primary hazard of working with the pathogenic bacterial species is from exposure via mucosal membrane routes (eyes, mouth and nose), broken skin, or ingestion. Therefore, you should wear safety glasses at all times, wear gloves when handling bacteria, avoid generating aerosols of bacterial cultures, and avoid touching your face, applying cosmetics,

adjusting contact lenses, biting your nails etc., while in lab. Consult your instructor if you have health concerns about working with bacteria.

Record Keeping

Laboratory Notebooks

When performing scientific research it is important to keep a record of your work that follows the principle of autonomous replication. Basically, anyone should be able to repeat your experiments by using your notes and obtain the same results. Therefore, maintaining a detailed and descriptive laboratory notebook is a key part of laboratory research. To fulfill the requirement for the principle of autonomous replication, your notebook should follow these basic guidelines:

A) The level of detail should be high enough that you can go back at a later time and troubleshoot your procedures if, for some reason, a procedure does not work or yields questionable results.

B) Jargon, personal shorthand, and personal abbreviations should be kept to a minimum.

C) Drawings, figures, and tables are encouraged. They must contain enough information so that another scientist can interpret them without extensive reference to the text.

Results

1) Primary data (e.g., numerical values, photographs, printouts, observations, drawings) are *results* of an experiment.

2) Negative results are results too! Be sure to include them.

3) All results must be described comprehensively and accurately.

4) Any loose data (e.g., photographs, printouts) must be securely taped into the notebook, dated, and properly labeled.

5) Anyone should be able to read the results of an experiment and know exactly what happened.

6) Data tables are labeled with a descriptive legend *above* the table.

7) Figures are accompanied by a descriptive legend *below* the figure.

G) **Analysis and interpretation**.

1) The results of an experiment must be analyzed and an objective interpretation documented in the notebook. Even if the results indicate a simple “yes” or “no” answer, it is important to state that explicitly in this section.

2) Report any unexpected findings or problems during the course of the experiments. This can act as a rationale for additional experiments, for changing the protocol or materials, or for altering the path your research has been following.

3) Interpretation of data can sometimes be subjective, but your reasons for a particular interpretation *must* be stated here.

Labeling in the Laboratory

Maintaining a good laboratory notebook isn’t the only important record keeping that happens in a lab. You also need to be able to identify your experimental samples when they are placed in an incubator or freezer with hundreds of other identical-looking samples. Just as with keeping a notebook, every scientist has his or her preferred way of labeling things

like Petri plates, microcentrifuge tubes, and reagents—but here are some general strategies to follow.

A) Each set of tubes, plates, and bottles should be labeled with
• Your name or initials
• The date
• The contents (i.e., DNA)

B) Any media or other solutions, tubes, cultures, or stocks that are *exclusively* yours should be labeled with
• Your name or initials
• The contents
• The date(s) received, prepared, and opened

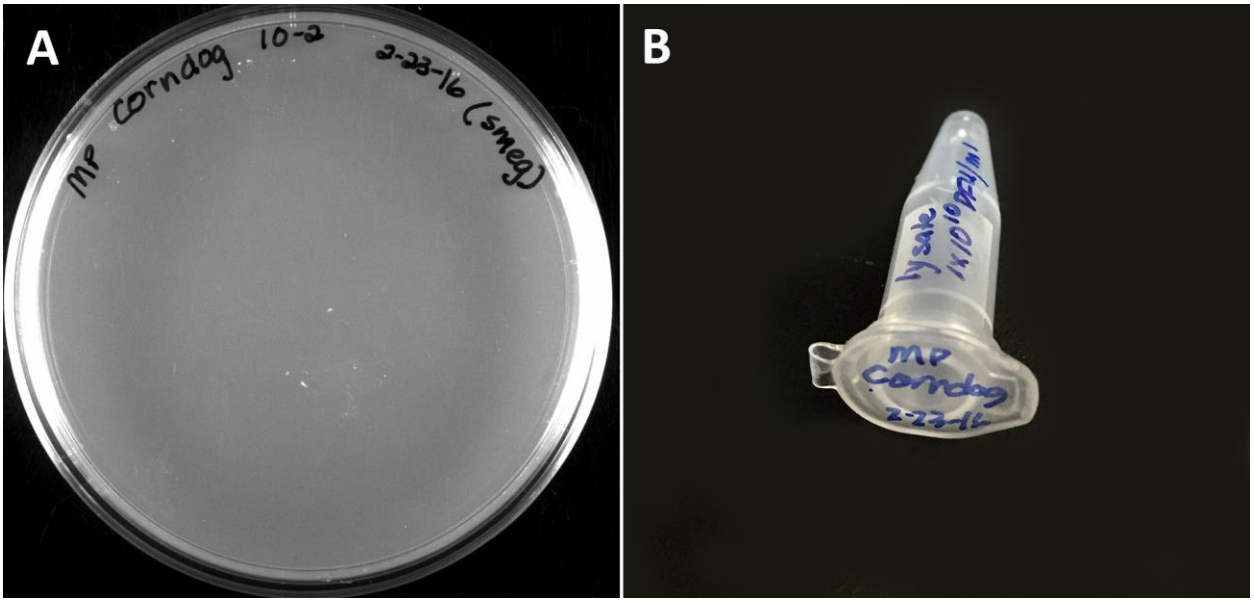

Figure 1. Examples of proper labeling. (A) Petri plates are labeled on the bottom (agar-side) to ensure that the label accompanies the experiment even if the lid is lost, displaced, or swapped with another plate. By labeling around the edge of the plate you can see the results without looking through the label. (B) Microcentrifuge tubes are labeled with an identifier on the top of the tube so it can be located when it is in a tube rack. Additional information should be written on the side of the tube, in the designated area, where the label will not rub off.

Preventing Contamination

Microbes are everywhere: on lab surfaces and equipment, on human skin and hair, and in the air. In a microbiology laboratory we use materials and procedures to ensure that we grow only the bacteria and phages we are interested in. If we pick up *other* bacteria, fungi, or phages from our surrounding environment we say that we have "contamination." Sterilization and aseptic technique help avoid contamination. "Sterile" materials have been rendered free of biological organisms—including phages, bacteria, and fungi. The most common method for sterilizing common lab materials and reagents, such as phage buffer, bacterial growth media, plastic tips, and glass tubes, is to use an autoclave, a machine that uses pressure and steam to heat materials to 121 °C and 18 psi. Materials that have been autoclaved are frequently marked with special tape that appears white before autoclaving, and striped afterward. Alternatively, some liquids that do not tolerate extreme heat can be passed through a filter ("filter-sterilized") to physically remove cellular organisms.

Sterilization of larger or sensitive surfaces (such as you!) requires alternative methods. Lab benches and equipment are routinely treated with "disinfectants"—chemicals that kill or inhibit growth of contaminants on surfaces. Common lab disinfectants used to remove bacteria are 70 % alcohol, 10 % bleach, or a phenolic like CiDecon™. Many phages are not sensitive to 70 % ethanol, so for disinfection of phage-contaminated materials bleach and phenolics are better choices. In addition, you should wash your hands thoroughly with hot water and soap before and after each lab class since *you* are probably the biggest source of contamination in the lab!

Aseptic technique refers to the best lab practices that minimize contamination of your sterile working materials and experiments. Many of these practices are just common sense: for example, make sure you declutter and disinfect your working space before you start an experiment.

Work Area Preparation

When preparing for an experiment requiring aseptic technique, you want to organize your bench first. Remove any unnecessary clutter, and disinfect your workspace with 70 % ethanol. As the ethanol dries it dehydrates bacteria on the bench surface the same way hand sanitizer kills bacteria on your skin. Be mindful not to try to speed up the drying process by waving your hands around or blowing on the surface because this will only introduce additional contaminants. When working with certain bacteria and viruses, it is a good idea to use a second disinfectant containing phenol or quaternary ammonia compounds as well. Because the active ingredients in these supplementary disinfectants denature proteins, it is important to always wear gloves when handling them.

Bunsen Burner Use

Classic aseptic technique requires the use of a Bunsen burner. Once lit, the flame should be adjusted so that blue "cone" can be seen in the flame. The tip of this cone is the hottest part of the flame. The function of the flame is to provide an updraft by heating the air (Figure 2). Because heat rises, airborne bacteria, spores, fungi, and dust particles are forced upward and away from your work area.

Therefore, it is important to work inside this "zone," being careful not to disturb the updraft with rapid movements that disturb the air around your bench. You also need to arrange your supplies to be close enough to the Bunsen burner to take advantage of the updraft. Until you are familiar with using a Bunsen burner, it is important to constantly be mindful of the flame. Even with an apparent blue cone the flame is hard to see and can be overlooked. Never leave a flame unattended in case a lab mate or instructor doesn't see it and inadvertently reaches over or through it.

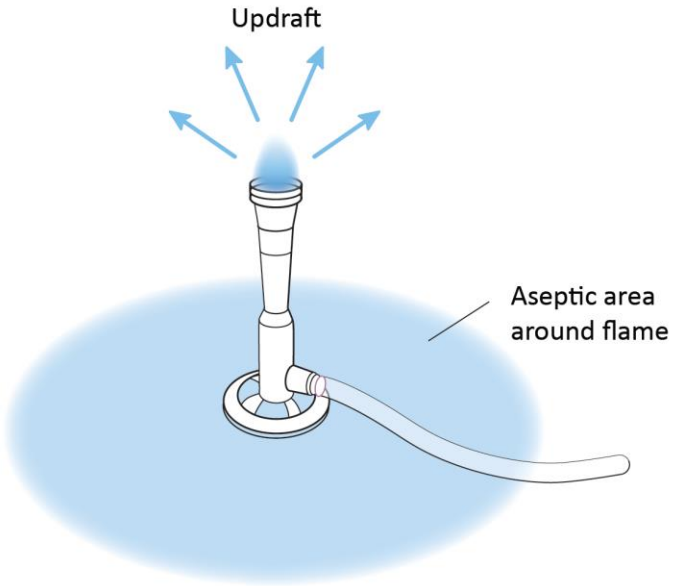

Figure 2: How a Bunsen burner creates an updraft to keep the area aseptic.

Aseptic Transfer of Materials and Reagents

When you move liquids from one vessel to another, follow three simple rules to maintain an aseptic environment. First, always flame the opening of glass bottles and test tubes every time you open them and again before you close them. The goal of flaming a glass vessel is not to heat the glass itself, but to heat the air inside the neck of the vessel. This will cause the air to rise and prevent airborne contaminants from falling inside. This requires one or two quick passes through the flame only because extended exposure to the flame can cause the glass to break. This is an example of classic aseptic technique. With today's plastic components, however, flaming is not in widespread use.

The second rule is to never lay down a lid or a cap on the bench top. Because you will most likely be holding a pipette in your dominant hand, you will have to figure out how to hold the bottle and the lid in one hand, or some other arrangement. This is a feat of dexterity that has nothing to do with the size of your hands, and it comes with practice. Finally, the

third rule to good aseptic technique is to never use a pipette for more than one transfer. After you pipette from one sterile vessel into another, ALWAYS get a new pipette or pipette tip, regardless of how sure you are that you didn't contaminate it the first time.

Bench Top Management

As noted above, you want to keep the supplies you are using within the aseptic field created by the Bunsen burner. In addition, you want to minimize clutter and keep your workspace well organized. If you have supplies you know you will need, keep them close to the aseptic zone, but out of the way until needed. Keep pipette tip boxes and microcentrifuge tubes closed at all times to prevent contamination. Wipe up any spills immediately with disinfectant.

Disposal and Cleanup

After you finish your work for the day and start cleaning your lab bench, you need to follow proper disposal protocols. Bacteria, either in liquid or on plates, should not be discarded in the sewer or garbage until treated properly. Follow the protocol outlined by your instructor for the disposal of old plates and culture tubes. When working with microorganisms, always disinfect your workstation with 70 % ethanol and a disinfectant like CiDecon, before leaving and wash your hands thoroughly.

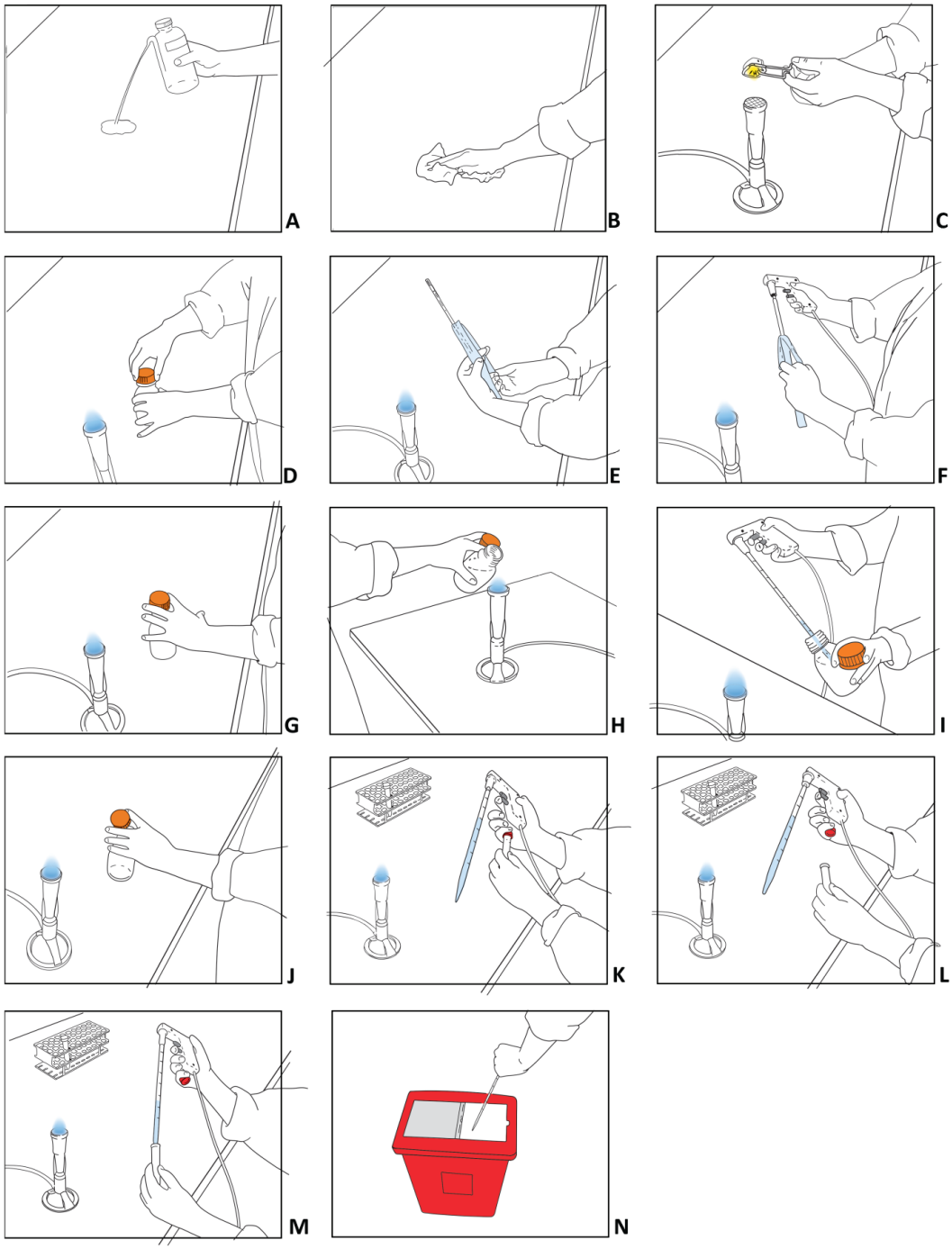

Figure 3. Transferring liquid samples using aseptic technique. Before beginning, dispense ethanol or other disinfectant onto the surface of your bench (A) and wipe from back to front (B). Light your Bunsen burner (C). Prepare your materials, and loosen all bottle and tube caps (D). Peel down both sides of the pipette package (E) and hold both flaps while attaching the pipettor (F). Using your first and second fingers, remove the bottle top (G) and pick up the bottle with the same hand and "flame" (H). Remove your sample (I) and reflame and recap the bottle (J). Using the smallest finger of your pipettor hand, remove the top of the tube (K and L) and add your sample to the tube (M). Recap the tube before discarding the pipette (N). When finished, turn off your Bunsen burner, tidy up, and wipe down your bench.