

Session 1

Laboratory Orientation and Safety

Learning Objective

Learn how to work safely in a biological laboratory and become oriented with some of the basic equipment, techniques, and measurements used in molecular biology.

Introduction

Today you will be introduced to the world of biological laboratories. As with all laboratories, safety is the most important aspect of working in a biological laboratory. The key to safety is recognizing the risks and hazards associated with an activity and taking the appropriate precautions. The laboratory exercise will include a safety training to orient you with working in a biological laboratory and give you some practice with some of the fundamental laboratory equipment used in the laboratory during the course. In addition, we will review the metric system and units of concentration and quantitative data analysis. We will also include a brief discussion of good lab notebook habits.

Background

Working in a Biohazard Level 1 (BL1) Lab

No matter what type of laboratory you are working in, safety is the most important consideration. Working in a biological laboratory involves special considerations to avoid risks of exposure and contamination. In this laboratory will be working with microorganisms and procedures which fall into the Biohazard Level 1 (BL1) category. This is lowest risk level associated with biological laboratories, and the microorganism we will be using is a ***non-pathogenic*** strain of *Escherichia coli*. Even though there the organism poses no immanent health risk, it is still important to use proper techniques to avoid exposure and spreading the organism outside the laboratory.

There are numerous ways in which exposure in a laboratory can occur. Our skin provides a good boundary to biological agents, but is susceptible to irritation from contact with chemicals and solutions. If the skin barrier is broken by cuts or injection caused by a sharp object, biological agents can enter the body. Exposure can also occur through the ingestion of liquids, the inhalation of aerosols, or transport across mucous membranes in the eyes, nose, or mouth.

Personal protective equipment (PPE) and proper laboratory clothing are an essential defense against exposure. In this laboratory, you will be required to wear long pants (covering the entire legs), and closed-toe, flat-bottom shoes. In addition, long hair should be pulled back to be kept away from equipment and materials. While in laboratory, everyone is required to wear a long-sleeve laboratory coat, safety glasses with side-

shield, and gloves. The use of certain equipment requires additional PPE which your instructors will explain at the time of use.

Good hygiene is very important when working in a biological laboratory. Remember to wash hands thoroughly when leaving lab. Never put lab equipment (e.g. serological pipettes) in your mouth. Keep all food and drink items outside of the lab! Clean up spills appropriately depending on the type of spill (see below). Wipe down bench tops every day with ethanol to sterilize the work area. Keep coats and backpacks outside of the lab area to avoid clutter and tripping hazards.

Proper handling of laboratory waste is essential to the safety of laboratory workers as well as people and animals outside the lab and the environment. There are many different types of waste generated in a biological laboratory. Each type of waste is handled in a different manner. In characterizing types of waste, the important questions are: 1) Does the waste contain hazardous materials? 2) Is the waste a sharp? 3) Does the waste contain biological agents? 4) Is the waste solid or liquid?

Most of the reagents used in this laboratory are non-hazardous. Your instructor will inform you which solutions contain hazardous materials and will directly specifically where to collect waste for disposal. Do not use any reagents or chemicals except those given to you by the instructor. All forms of sharp waste including: pipette tips, cuvettes, razor blades, tooth picks, etc, are disposed of in a single bin in the laboratory regardless of whether or not they were in contact with biological material. Non-hazardous and non-sharp waste is treated in one of four ways depending on the type of waste. If the waste is non-biological and liquid, it can be poured down the drain. If liquid waste contains biological agents, it must be bleached for 20 minutes prior to being poured down the drain. Check with your instructor before pouring any solutions down the drain! For solid waste, there are separate receptacles for non-biological waste (normal trash bins) and biological solid waste. The biological solid waste bags must be tagged and sterilized by your instructors prior to being disposed.

Equipment items in the laboratory pose specific hazards. Your instructor will explain hazards of specific equipment before you use it. Rotors in centrifuges spin at extremely high speeds and possess high amounts of kinetic energy. Proper balancing of centrifuges, by placing identical weight tubes on opposite sides of the rotor, is critical to safe operation. Gel electrophoresis rigs run on high direct current voltages and must be operated with the lid in place. Gel illumination tables use high intensity UV light and must be used with special glasses or face shields to protect the eyes. Several equipment items in the lab run at extreme temperatures; care must be taken to avoid burns.

Laboratory Notebooks

Good laboratory notebook practices are important for success in research. A notebook allows a researcher to record and lookup procedures used, observations about an experiment, results from experiments, and ideas or thoughts about improvements or additional experiments. A properly maintained notebook is not only useful for the author, it is also useful for people who are trying to understand and repeat experiments.

In addition to the information described above, entries should include the date and time of the experiment. Entries into a laboratory notebook should be sequential and should not leave excessive amounts of blank space between entries.

Basic Laboratory Tools, Equipment, and Measurements

In lab exercise, you will become familiar with the most important piece of equipment in a biological laboratory, pipettes. Pipettes are used for accurately and quickly measuring, aspirating, and dispensing liquid volumes. You will be primarily using piston-driven air displacement pipettes of three sizes. These pipettes use disposable tips to hold the liquid. The smallest size (2-20 microliters) is represented on the piston and tip box by a red color, the medium size (20-200 microliters) by green, and the large size (200-1000 microliters) by blue. You should become very familiar with the amounts used by each of these pipettes.

To use a pipette, first select the proper pipette for the volume needed and set the volume of liquid using the dial. Then, pickup a tip by firmly pressing the bottom of the pipette shaft into the top of the pipette tip. Press the plunger down the first stop position and then submerge the tip in the liquid to be collected. Next, slowly release the plunger making sure to keep the tip below the surface of the liquid. Move the pipette with tip full of liquid to the container into which the liquid is to be dispensed. If there is already liquid in this container, submerge the tip below the surface of the liquid. To dispense, slowly and smoothly depress the plunger to the second stop and hold. Remove the tip from the liquid before releasing the plunger. Eject the tip into a sharps collection bin using the release button. Practice to perfect the technique, and ask your instructor to watch if you are using the pipette correctly. Errors in pipetting will result in incorrect volumes and are one of the easiest ways to spoil an experiment.

After you have measured and dispensed several different liquids, they will need to be mixed. Proper mixing is essential to ensuring that the small liquid volumes are uniform. There are several different mixing techniques that can be used based on the properties of the liquid in the tube. First, the capped tube may simply be turned upside repeatedly to mix solutions. This is the gentlest way to mix and is suitable for larger volumes and mixtures which are susceptible to damage from shear. The second way to mix is to hold the tube by the top with one hand while brushing or flicking the bottom with the other hand. This method is suitable for smaller volumes where inverting the tube may not result in adequate mixing and is still very gentle. The last method involves using a vortexer. This method provides the best mixing quickly, but causes high amounts of shear which may not be acceptable for some solutions.

After a solution has been mixed it may need to be centrifuged to collect all of the liquid droplets at the bottom of the tube. This can be done by placing the tubes in the centrifuge (properly balanced), and pressing and holding a short spin button. This type of spin only needs to reach a few thousand rpm and only needs to be held for several seconds. Centrifuging can also be used to separate insoluble solid material from liquid, which requires longer times and higher rotational speeds.

Many measurements made in biological laboratory require the use of a spectrophotometer. A spectrophotometer measures the amount of light at a specific wavelength which is transmitted or absorbed by a sample. The amount of light absorbed by the sample corresponds to the concentration of the solution. This is described by the Beer-Lambert expression:

$$(1) \quad A = \epsilon \cdot l \cdot c$$

Where A is the absorbance of the sample, l is the path-length of light through the sample, ϵ is the molar absorptivity of the substance, and c is the concentration. So, for a single material measured in the same cuvette (same path length) the absorption increases linearly with concentration. To get an accurate reading, a spectrophotometer must be “zeroed” or “blanked” using just the solvent in which the substance is dissolved to adjust for any background absorbance that occurs at the selected wavelength of light.

Your success in this laboratory class depends on your ability to use a metric system of units. A more thorough review of metric and SI units and prefixes is presented in Appendix A. We will be working primarily with units for mass (grams), volume (liters), molecular weight (Dalton), and quantity of molecules (moles). Because we work with very small quantities in biology, we frequently use the prefixes milli (10^{-3}), micro (10^{-6}), nano (10^{-9}), and pico (10^{-12}). We also frequently use combined units for concentration including mass concentration (g/L), molar concentration or molarity (mol/L or M), solution weight percent (wt% = g/mL x 100%), solution volume percent (vol% = mL/mL x 100%), and concentration factors. You should become very familiar with these values and be able to convert between them quickly.

Session 1: Pre-Laboratory Exercises

Name: _____

Date: _____

1) What should you be wearing when you come into lab? Are you wearing it?

2) What is the policy on food, beverages, coats, and backpacks in lab?

3) What information should you record in your laboratory notebook?

4) Performing the following conversions (write answers in scientific notation):

a. 200 mL water _____ L water

b. 1 mL water _____ μ L water

c. 0.75 mg water _____ μ g water

d. 2 mol water (MW = 18 g/ mol) _____ g water

e. 100 mL of 5 M NaCl _____ mol NaCl

f. 300 ng DNA _____ μ L of 150 ng/ μ L DNA

Laboratory Protocol

The exercise: You will become familiar with the use of your pipettes, vortexer, microcentrifuge, and spectrophotometer in this lab while making serial dilutions of a colored solution and measuring the absorbance.

Materials: Food coloring solution
Deionized water
Microcentrifuge tubes
Cuvettes
Pipette tips
Lab pen
Waste beaker

Equipment: Pipettes
Vortexer
Microcentrifuge
Spectrophotometer

Protocol: Part I – Pipette Practice

1. Take out 12 microcentrifuge tubes and place them in the tube rack
2. To 5 of the tubes, have one person add the following amounts of water: 1 uL, 10 uL, 100 uL, 500 uL, and 1mL.
3. To another tube, use the incorrect technique by pressing the plunger to the second stop before aspirating liquid to add “100 uL”.
4. Repeat steps 2 and 3 for the other person in the group.
5. For each group member, show your results to an instructor and demonstrate your pipetting technique by moving 50 uL of liquid from the 500 uL tube to the 1uL tube.

Part II – Making 1/4X Serial Dilutions

1. Add 1050 uL of deionized water to each of 6 microcentrifuge tubes. Label the tubes with numbers 1 through 6. (Hint: you do not have a pipette large enough for this volume, try using two different pipettes once each).
2. To tube #1, add 350 uL of the food coloring solution.
3. Mix tube #1 by vortexing for several seconds.

4. Remove 350 μ L from tube #1 and add it to tube #2. Mix tube #2 by vortexing.
5. Repeat step 4 sequentially for each of the remaining tubes by adding liquid from tube #2 to #3, #3 to #4, #4 to #5, and #5 to #6. Remember to mix between each addition.
6. Notice the different color intensities of the different solutions. Save the solutions for Part III.

Part III – Absorbance Measurement

1. Remove 350 μ L of liquid from tube #6 and discard into a waste beaker.
2. Place all of the tubes into the centrifuge (Remember to balance them properly) and spin briefly to collect all of the liquid into the bottom of the tubes. Carefully remove tubes from centrifuge.
3. Label 6 cuvettes with numbers 1 through 6 and additional cuvette with a 'B' for blank.
4. To the blank cuvette, add 1 mL deionized water.
5. To each of the numbered cuvettes, add 1 mL of the corresponding numbered solution.
6. The instructors will have already setup the spectrophotometers. Use the blank solution to zero the spectrophotometer, an instructor will show you how.
7. Measure the absorbance of all of the samples #1 through #6 and the blank solution. Make sure to record the measurements in your lab notebook. (You also should have written down all of the steps you performed in Parts II and III; if you didn't do that now!). Don't lose the data, you will need it for the post-lab assignment.

Session 1: Post-Laboratory Exercises

Name: _____

Date: _____

- 1) Describe appropriate laboratory clothing?

- 2) What personal protective equipment must you wear at all time when in the laboratory?

- 3) Explain the following different waste streams that are generated in a biological laboratory and how we dispose of them:
 - a. Non-hazardous non-biological solid waste

 - b. Non-hazardous non-biological liquid waste

 - c. Non-hazardous biological solid waste

 - d. Non-hazardous biological liquid waste

 - e. Sharps (all types)

- 4) What are the different types of exposure

- 5) Describe the proper loading of a centrifuge

6) In your own words, describe the proper method for using a laboratory pipette

7) What units of measure are used on your standard bench top pipette?

8) If you make 5 successive 1/2X serial dilutions, what is the concentration of the final solution with respect to the original solution?

9) Data Analysis

a. Plot the data from the dye dilution lab in Microsoft Excel using an xy-scatter (without a line). On the X-axis, plot the concentration of the dye as a fraction of the original sample. On the Y-axis, plot the absorbance of light at 500 nm.

b. **Answer below:** Are there any data points that look unusual (Hint: look around the ends)? Why might these values be misleading? Remove these data points from the plot.

c. Add several types of trend lines including linear, logarithmic, and exponential. You can add a trend line by right-clicking on the data set and selecting "Add Trendline." For each of these trend lines, display the equation and R^2 values (options tab). **Answer below:** Which type of equation is the best fit for the data and why?

d. Print the graph and tape it into your lab notebook

10) Metric and concentration conversions (write answers in scientific notation)

- a. 0.5 mL water _____ μL water
- b. 50 μL of 1X buffer _____ μL 5X buffer
- c. 50 ng DNA _____ μL 0.1 $\mu\text{g}/\mu\text{l}$ DNA
- d. 1X "Final Concentration" of buffer _____ μL 10X buffer
(50 μL total reaction volume)
+
_____ μL water
- e. 25 μmol dNTP _____ μL 100mM dNTP

11) What does it mean to make a 1% solution (Hint: there are two conventions, one for solid and one for liquid)?

12) How much of a 10X solution and how much water must be combined to make 1 mL of a 1X solution?

References & Additional Reading

MIT Environmental Health and Safety: <http://web.mit.edu/environment/ehs/>

Wikipedia

Biosafety Level: http://en.wikipedia.org/wiki/Biosafety_level

Pipette: <http://en.wikipedia.org/wiki/Pipette>

Centrifuge: <http://en.wikipedia.org/wiki/Centrifuge>

Spectrophotometry: <http://en.wikipedia.org/wiki/Spectrophotometer>

Beer–Lambert Law: http://en.wikipedia.org/wiki/Beer%E2%80%93Lambert_law

Metric System: http://en.wikipedia.org/wiki/Metric_system

SI Units: <http://en.wikipedia.org/wiki/SI>

SI Prefixes: http://en.wikipedia.org/wiki/Metric_prefix