Week 1: Lab #1 Introduction Lab

Adapted from Biochemistry, BIO187 and BIO188 Labs

In order to effectively work in a modern laboratory, each student must be familiar with both a system of standardized measurements and the basic skill set necessary for taking measurements and observations in the laboratory. Scientific investigators use the metric system in the laboratory. The metric system has two distinct advantages over other measuring systems.
The first is that the measuring system is based on physical constants (one calorie of energy will heat exactly one milliliter of water exactly one degree Celsius); the second is that the different units are based on multiples of ten (one kilometer is one thousand meters, one meter is one hundred centimeters, as opposed to one foot is twelve inches and one yard is three feet). This laboratory exercise will familiarize you with part of the metric system and others tools of laboratory measurement and observations.

A Note about Significant Figures:
When reporting measurements, it is possible to estimate only one digit beyond the precision of the measuring instrument. For example, if a ruler is marked in centimeters, you can estimate tenths of a centimeter [millimeters] by estimating the amount of the distance between the two numbers marked on the ruler that the object being measured covers. This last digit is considered an "uncertain digit," since it was estimated. All the certain digits and the uncertain digit in a measurement together are called the **SIGNIFICANT FIGURES** of the measurement. It is proper to report only to one uncertain digit in a measurement. There are many rules regarding significant figures and their use in reporting measurements and in calculations that would be covered in detail if you were a laboratory science major. As it is, just remember that use of significant figures keeps measurement numbers from getting out of hand [it's easier to read 2.52 cm than 2.51965702 cm, and the first number is likely at the edge of your instrument's accuracy anyway.]
See your lab instructor if you have further interest in significant figures.

PreLab Preparation

Before each lab, please read over its handout(s) in their entirety. YOU MUST WAIT UNTIL LAB TO ANSWER ANY QUESTIONS WRITTEN IN ANY HANDOUT. You will learn how to write the proper type of pre-lab and notetaking in lab using this handout during lab this week [this will take at least 30 minutes.] Be sure to purchase a bound lab notebook and a pair of lab safety glasses or goggles; bring them to the lab along with this handout and your printout of the lab syllabus.

Remember to wear proper attire, including closed shoes.

Purpose and Objectives:

The purpose of this laboratory session is to review some of the basic skills needed for this semester's lab work. As part of this lab, you will explore the metric system; learn to use liquid volume measurement tools such as graduated cylinders, plastic pipets, and micropipettors correctly; learn to read laboratory balances accurately.

- To become familiar with the metric system of measurements and scientific instruments of measurement.

Materials:

calculators (at least 2 per group)
inch/centimeter rulers (at least 2 per group)
200 ml dH2O colored w/ red food coloring
50 and 100 or 150 beakers
25 mL, 50 mL, &100 ml graduated cylinders
1 mL, 5 mL, & 10 mL disposable pipets
green pipet aids
micropipettors (P-10, P-100 and P-1000)
pipet tips
plastic weigh boats
balances
table salt (NaCl)
weigh spatulas
plastic transfer pipets
beakers of distilled water

Part I. Syllabus and Safety

Your instructor will highlight important parts of the lab syllabus, including proper lab attire and the schedule of laboratory exercises. You are responsible for everything in both the overall class syllabus and the laboratory syllabus whether or not the instructor goes over any particular portion. The lab instructor will also point out the safety equipment available in the laboratory such as safety showers, emergency eye wash stations, and Material Safety Data Sheets. The lab instructor will also discuss highlights of emergency procedures.

Part II. Pre-lab Preparation

Pre-Lab: Before you come to lab each week, you must complete a pre-lab of the week's activities in your lab notebook with objectives, materials, & procedures (see specific instructions below.) **Pre-labs MUST be handwritten**, with the exception of the materials list. Your Pre-lab will be checked quickly and initialed by the instructor at the beginning of lab, then more thoroughly graded at the time of the notebook check. **Students MUST have completed the pre-lab assignment before the start of the laboratory period. Students missing their completed pre-lab at the door of the lab may be turned away from lab and miss all points for the day's laboratory exercises.**

Your pre-lab must include:
The laboratory exercise # & title, the date (including the year), and page numbers.

- **PURPOSE AND OBJECTIVES** [*handwritten*]: Briefly explain the purpose of the lab exercise. This is often found in the first part of the lab protocol or listed in the objectives for that particular exercise.

- **MATERIALS:** List materials needed for the exercise. Bulleted lists work best, or you may neatly cut and tape the materials list from your protocol in this space.

- **PROCEDURE** [*handwritten*]: The procedures will be listed in your protocol, and, unless told ahead of time, assume that you will be doing all of it. You will practice now [during lab] using the pages of this handout.

Follow the following steps to complete a practice prelab in lab:

Step 1. If you do not have your notebook with you today, Get out a few clean sheets of paper.

Step 2. Write your full name, lab section [including date and time,] and lab instructor's name on the cover of your lab notebook. Also write the names of your lab partners on the inside cover (you may wait to do this until next week and your lab group is set).

Step 3. Skip the first page of your notebook to leave for a Table of Contents. As a main heading on the first line of the next page, write the lab number, lab title and the date of that particular lab. Number your pages at the top corner (keep it consistent on each page). You can choose to write on every other page (i.e. just the "front") or you can write front-to-back, just be sure to keep it consistent and number the pages accordingly.

Step 4. Write "Purpose" or "Objectives" as a new subheading on the left side of the page. Find the Purpose and Objectives section on Page 2 of this handout. Neatly handwrite any and all bullet point statements found in this section onto your sheet under your "Purpose and Objectives" subheading.

Step 5. Write "Materials" as a new subheading on the left side of the page. Find the Materials list on Page 2 of this handout. Cut out the entire section and neatly tape it on your sheet below your "Materials" subheading. [Or, you may handwrite the Materials list if you prefer.]

Step 6. Read over the "Metric System" and "Measurement of Liquids…" background material found on pages 5-7.

Step 7. Write "Procedures" as a new subheading on the left of your sheet under the Materials section. You will start your procedures flowchart here. The purpose of this flowchart is to summarize the steps in the lab procedure with enough detail to show the instructor that you have read the handout and are prepared for lab.
PLEASE NOTE THAT DURING LAB YOU WILL ALWAYS BE ABLE TO HAVE AND FOLLOW THE ACTUAL FULL LABORATORY HANDOUTS THAT YOU HAVE PRINTED OUT.

Step 8. Turn to Page 8. DO NOT ACTUALLY PERFORM ANY OF THESE ACTIVITIES YET, INCLUDING ANSWERING QUESTIONS. Starting with *Step 1* and proceeding to *Step 6*, summarize each step (and only the steps, no background information or questions) and write each summary down. It's ok if you don't understand what each procedure actually is. What you are looking for is to write down the important information such as verbs, amounts of time, etc. The instructor will come around the room to help as you write these down.
NOTE: Just as you would not be able to actually perform any laboratory procedures at home while you are preparing your weekly flowchart, you are not to perform these laboratory procedures while you are summarizing them now.

Step 9. The instructor will be coming around to check your work.

Step 10. Move on to the next section "About Micropipettors" on page 9. Read the background information and then summarize *Steps 1-10* for micropipettors on pages 9 and 10. The instructor will come around again to help as you proceed.

Step 11. Move on to the next section "About Balances" on page 10. Read the background information and then summarize *Steps 1-3* on page 11.

Step 12. Wait for the instructor to introduce the lab.

Part III. Notebooks

Your lab instructor will highlight portions of the syllabus about proper note taking. You will start taking notes in your bound lab notebook DURING LAB with Part IV below. REMEMBER THAT YOUR LABORATORY NOTES ARE ALWAYS IN THE **PAST TENSE**, BECAUSE THEY ARE A RECORD OF WHAT YOU **DID** IN THE LAB. See Lab Syllabus for more details.

Part IV. Laboratory Procedures

A. Some Metric System Basics and Unit Conversion

The Metric System

The prefixes used in the metric system indicate the fraction or multiple of the base unit being used [See Tables below].

What you need to know for this class

The most important pieces of information you need to know for this class are how to convert between microLiters (µL), milliliters (mL) and Liters (L). You may also convert between millimeters (mm), centimeters (cm) and meters (m). You will also need to weigh things in grams (g) or milligrams (mg). Also, one thousand grams is a kilogram [kg, a “kilo,” get it?]

Let’s look at measurements of volume first:

Your instructor will measure out 1 microLiter [µL] of liquid and show it to the class.
Your instructor will measure out 1 milliLiter [mL] of liquid and show it to the class.
Your instructor will measure out 1 Liter [L] of liquid and show it to the class.

· **Question 1 [answer in notebook]:** Which of the above volumes is the largest? Which is smallest?

Let’s convert between units:

What you need to know:

1,000 µL = 1 mL	**10 mm = 1 cm**	**1,000 mg = 1 g**
1,000 mL = 1 L	**100 cm = 1 m**	**1,000 g = 1 kg**

Your instructor will go over how to convert between units.

·**Question 2:** A piece of evidence is 75 cm away from the murder victim. How far away is it in meters?

·**Question 3:** If you have 2 mL of a liquid, how many µL do you have?

·**Question 4:** If you’re asked to measure 1.5 L, how many mL would you be measuring?

·**Question 5:** If you need to measure 0.5 mL, how many µL would you be measuring?

·**Question 6:** How many mg is 0.8 grams?

Table 2.1 The Metric System: Base units in bold and italic

Length	Mass	Volume	Temperature
nanometer (nm)	nanogram (ng)	nanoliter (nL)	Celsius (°C)
micrometer (µm)	microgram (µg)	microliter (µL)	(AKA Centigrade)
millimeter (mm)	milligram (mg)	milliliter (mL)	
centimeter (cm)			
meter (m)	***gram (g)****	***Liter (L)***	
kilometer (km)	***kilogram (kg)****		

*While prefixes are added to "gram" as they are to meter and Liter, and a kilogram is a 1000 grams, technically the kilogram is the base unit in the SI system (the scientific version of the metric system.)

Table 2.2 Prefixes Used in the Metric System

The prefixes used in the metric system indicate the fraction or multiple of the base unit being used.

Prefix Symbol Meaning size relative to
base unit Length Mass Volume
giga- Billion 1,000,000,000
mega- Million 1,000,000
kilo- k Thousand 1,000 kilometer (km) kilogram (kg)*
hecto- Hundred 100
deca- Ten 10

Base Units 1 meter gram* Liter
deci- tenth 0.1
centi- c hundredth 0.01 centimeter (cm)
milli- m thousandth 0.001 millimeter (mm) milligram (mg) milliliter (mL)
micro- µ millionth 0.000001 micrometer (µm) microgram (µg) microliter (µL)
nano- n billionth 0.000000001 nanometer (nm) nanogram (ng) nanoliter (nL)
*See note for Table 2.1

B. Measurement of Liquids using Graduated Cylinders, Plastic Pipets, and Micropipettors

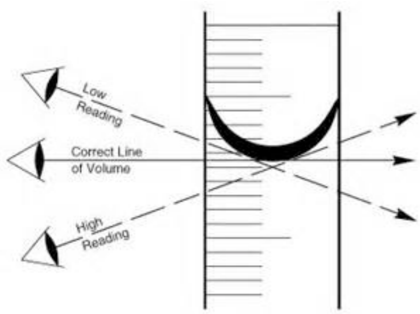
A way to measure milliliter amounts of liquids is to use a graduated cylinder. These come in several different sizes, usually from 10 mL up to 2 Liters. Amounts of liquids 25 mL to 1 mL are often more easily measured using pipets. You will learn about micropipettors, used for very tiny amounts of liquid (under 1 mL), later in this laboratory exercise.

1. About Graduated Cylinders

For relatively large volumes (greater than 10 ml), graduated cylinders are most often used. A graduated cylinder has markings on the side of the glass or plastic which indicate the volume. Depending on the total capacity of the cylinder, the meaning of the markings will vary.

For a 10 ml, 25 ml, or 50 ml cylinder, they are most often 1 ml apart. For a 250 ml or 500 ml cylinder, they are most often 10 ml apart.

One of the problems with most cylinders is that an aqueous solution (that is, one made up in water) often shows a pronounced meniscus or curvature when it is placed in the cylinder. This is due to the binding of water molecules to one another and to the sides of the glass. The volume in a cylinder is read most accurately by looking at it straight on and reading the volume at the bottom of the meniscus. A graduated cylinder can be used either to measure the volume of unknown solution or to measure out a certain volume of solution and then to transfer it to another container.



2. About Pipets
For intermediate volumes (between 1 ml and 25 ml), pipets are most often used. A pipet is a calibrated piece of glass or plastic tubing with markings along the side. Again, depending on the total capacity of the pipet, the meaning of the markings will vary. For a 1 mL pipet, the markings may be either 0.1 mL or 0.01 mL apart. For a 5 mL or 10 mL pipet, the markings are usually 0.1 mL apart. Each pipet usually is marked at the top so that the gradations are clear: it will say 5 in 1/10 ml or 1 in 1/100 ml. As with graduated cylinders, there is often a pronounced meniscus with aqueous solutions.

A certain volume of liquid may be transferred from one container to another by drawing the liquid up into the pipet and then allowing a volume of liquid between two specific markings to flow out. Mohr or measuring pipets are calibrated only to a point near the bottom of the glass tube. A specific volume is delivered by measuring the amount of liquid between two markings. Serological pipets are calibrated all the way to the tip and so must be "blown out" in order to deliver all of their volume. To use a pipet, it is necessary to draw liquid up into it. In the past, this was often done by sucking on the end of the tube with your mouth (as with a straw) and covering the end with your index finger. However, this type of mouth pipeting is no longer considered safe and various types of bulbs, pipet aids, or propipets are used instead. **MOUTH PIPETTING IS NOT ALLOWED IN THE COURSE.**

In this class, plastic pipet pumps will be used. A green pump should be used with 5 ml or 10 ml pipets. To use this type of pump, insert the top end of the pipet into the pump and twist it so that it seals against the plastic. Then rotate the plastic wheel to draw the liquid up into the pipet. To let liquid out of the pipet, rotate the wheel in the opposite direction. Pipets are most often used to transfer a certain volume of liquid from one container to another, although with care they also can be used to measure the volume of a solution. BE CAREFUL TO LEAVE THE WHITE RUBBER BOTTOM IN THE PUMP WHEN REMOVING THE PIPET.

3. Measuring Volumes using Graduated Cylinders and Pipets

Step 1. Take a 100 or 150 ml beaker and fill it about half full with the red colored liquid from a bottle at your station. The liquid again is just water with some food coloring added to it so you can see the liquid easily. Note that while the "stock" flask or the beaker may have markings on their sides, these values are only approximate and are never to be used for measurement.

Step 2. Pour the liquid from the beaker into a 100 mL graduated cylinder. Look at the meniscus, measure the volume, and record the results (remembering to report to the correct significant figure) in your lab notebook.

<u>Record in your notebook</u>: volume of red liquid: _______ mL

Step 3. Empty the 100 mL graduated cylinder back into the beaker.

Step 4. Notice that sometimes a smaller cylinder will both give you a more accurate reading and will be easier to handle when measuring smaller volumes. **Use the best cylinder to measure the following volumes:**

a. 78 mL **b. 12 mL** **c. 55 mL** **d. 32 mL**

<u>Record the size of the cylinder and the measurements in your lab notebook (remember significant figures)</u>.

·**Question 7:** Were you able to accurately measure each of the amounts above? Why or Why Not?

Step 5. Now fit a 10 ml serological pipet with a green pipet pump. Holding onto the beaker with your free hand, draw some of the red liquid from the beaker up into the pipet and look at it carefully. Rotate the wheel & then transfer 10.0 ml of this liquid back into the beaker.

Step 6. Repeat this process with volumes of 6.7 mL, 4.2 mL, and 3.6 mL. Note that you use the markings anywhere along the pipet to transfer a certain volume.

<u>Record the measured amounts into your lab notebook</u>

4. About Micropipettors
Micropipettors are used to measure microLiters [µL] of liquids, which are often too small to measure accurately using other instruments. In this part you will get a feel for just how small the volumes micropipettors can measure might be. There are different sizes of micropipettor, each with its own volume limit. NEVER TRY TO SET A MICROPIPETTOR ABOVE OR BELOW ITS VOLUME LIMIT – YOU WILL BREAK A VERY EXPENSIVE PIECE OF LABORATORY EQUIPMENT. For example, a "P-100" micropipettor is for volumes from about 10 µL to 100 µL. This range of volumes the micropipettor can measure is written right on the side of the pipettor. You can think of the micropipettors themselves as a very advanced form of pipet pump. Just like the green pump you used in Part B, these pumps have attachments into which the liquid is actually drawn. You will notice boxes of these plastic "pipet tips" that have been put out with your equipment. **Always use a pipet tip on micropipettors!** This keeps you from contaminating samples and allows you to see your liquid. The tips are color-coded: in general, use yellow tips for micropipettors with yellow knobs; use the larger blue tips for micropipettors with blue knobs.

5. Let's try out a micropipettor – make sure that you try each size of micropipettor yourself. You may need to share with the other members of your group. Notice that Steps 9 & 10 below have you repeating this exercise with different micropipettors at different volumes; if someone else in your group is doing the P-100 exercise, you could be trying the P-1000 or P-10 exercise and vice versa.

**Because these instruments are shared by many different classes using many different substances, you may want to put on a pair of disposable gloves for this exercise.

REMEMBER TO ALWAYS USE A TIP ON YOUR MICROPIPETTOR – YOU WANT TO SEE YOUR LIQUID AS YOU MEASURE IT.

Step 1. Pick up a "P-100" or "P-200" micropipettor from the rack at your table.

Step 2. Take a look at the micropipettor to find the measurement setting numbers on one side.

Step 3. Carefully turn the measurement setting wheel [different for different micropipettors] to 75 µL [it might read 075].

Step 4. Put a tip on your micropipettor – watch the demonstration by the instructor. The pipet tip should remain in the box while you are putting it on the micropipettor to avoid contamination.

Step 5. Place the micropipettor with attached tip into the beaker of red water – ONLY UP TO JUST OVER THE VERY TIP OF THE YELLOW TIP. SEE THE DEMONSTRATION AT THE FRONT OF THE ROOM.

Step 6. Depress the button to the first (**soft**) stop while the pipettor tip is OUT OF ANY

LIQUID, place the tip of the yellow pipet tip into the liquid, then carefully and ***slowly*** let the button come back up to draw the 75 µl of water up into the plastic tip. **Do not "pop" the button on the pipette, as this often will result in the formation of an air bubble.**

Step 7. Place the tip into a plastic weigh boat and touch it to the inside of the plastic. Depress the button past the first stop to the second (**hard**) stop, so that all of the liquid is transferred to the boat. (This displaces the volume of liquid originally drawn up into the pipette tip, plus a volume of air so that all of the solution is ejected from the tip). Be sure to take the tip OUT of the liquid you've dispensed before you bring the button back up to the first stop [higher than the second stop.]

Step 8. Repeat Steps 5 – 7 above several times, and then pass your pipettor along to a lab mate so they can try all the steps. Try to get the same volume each time [this is harder than it sounds – you need to check the tip for air bubbles]

Step 9. Repeat Steps 1 – 7 above using a P-1000 micropipettor to measure 750 µL

Step 10. Repeat Steps 1 – 7 above using a P-10 micropipettor to measure 7.5 µL – you will notice just how small a volume this is…

·**Question 8 [answer in notebook]:**

a) Which micropipettor would you use to measure 0.7 mL?
b) What about 0.07 mL?
c) What about 1 mL?

C. Use of Balances

Weight:

Disclaimer: While pesky chemists will insist that grams actually measure the **mass** of an object (the amount of material in it) and the **weight** of an object is in fact the force mass exerts because of gravity, biologists often use grams for "mass" and "weight" interchangeably, at least when we do our experiments on the planet Earth.

Weight is another physical characteristic that is measured in the laboratory. To weigh objects, we use a balance. In the past, balances were actually designed to weigh objects by a lever and fulcrum; either the weight of two objects was compared or the weight of an unknown is balanced on the lever against a weight of known quantity. Modern scales use internal mechanical devices similar to springs to measure weight.

Weighing substances "directly" by taring a scale. When you weigh objects that must be placed in a container (such as the 50 mL of water or a powdered solid), it is useful to "tare" the scale.

Taring a scale involves setting the reading of the scale to zero with the container being used on it. For example, if you wanted to weigh out 7 grams of NaCl, you would first set a weigh boat on the scale. Then, you would set the readout to zero (this is taring the scale). Next, you add NaCl to the weigh boat until the scale reads 7 grams.

Step 1: Place an **empty** weigh boat on the scale and tare the scale to zero [press the "tare" or "zero" button or the cross bar].

Step 2: Measure out exactly 2.81 g of table salt (NaCl) by scooping it with a weigh spatula.

·**Question 9.** Record the measured amount of NaCl into your notebook. Were you able to measure exactly 2.81g? Why or Why not?

Step 3: Return the table salt (NaCl) to the container so another group member can try this exercise. Typically you would never return weighed out chemicals to their source bottle – you could contaminate the source (however today's source containers are only used for this exercise so you may return the NaCl to the source container).

Part V. Conclusion of Results and Clean-up [Performed at EVERY lab]:

Write a brief "conclusion of results" in your lab notebook based on the scientific exercises performed during today's lab. Remember to write objectively your results for each different section and then a conclusion about those results (How are these results significant to crime scene investigators?).

Clean-up your area so that everything is back where it was at the beginning of lab, making sure that all trash and chemicals are disposed of properly.

Wipe down your lab bench with paper towels and the alcohol provided [wear gloves.]

Once your area is clean, give your notebook in to the instructor for signing. You are required to bring your lab notebook with you every week.