

EXERCISE 1 - Lab Procedures

I. Familiarize yourself with the equipment on display in the lab

Several items that you will be using during the semester are on display in the lab:

Pasteur pipet and bulb	test tube	beaker
blow-out pipet	Erlenmeyer flask	Spec 20 cuvette
delivery pipet	top loading balance	disposable cuvette
transfer pipet	centrifuge tube	Petri dish
pipet filler	Eppendorf tube	squeeze bottle liquid dispenser
micropipettor	Eppendorf tube holder	Parafilm
graduated cylinder	magnetic stir bar	weigh boats

- ◆ Examine each item and make sure you are able to recognize and name it.
- ◆ Make a simple sketch of any unfamiliar items in your laboratory notebook for study purposes.

II. Compare the accuracy and precision of volumetric measuring devices

In this part of the lab, you will use a 100 mL beaker, a 100 mL graduated cylinder, a 10 mL graduated cylinder, a 5 mL pipet, and two different micropipettors (20-200 μL and 100-1000 μL) to measure specified volumes of deionized water. After measuring each volume, you will weigh the water and record the results. Later, you will carry out some statistical tests to compare the accuracy and precision of your measurements.

- ⇒ **IMPORTANT: Before you begin, copy the following table into your lab notebook so that you can use it to record your results. Data collected during lab should always be recorded directly into your lab notebook, NOT into your lab manual or on loose sheets of paper. Recording data into your lab manual or on loose sheets of paper and then copying it into your notebook increases the chance that the data will be lost or that errors will be made during the transfer.**

Weight of Water Measured by Various Volumetric Measuring Devices

<u>Trial</u>	<u>100 mL beaker (g)</u>	<u>100 mL graduated cylinder (g)</u>	<u>10 mL graduated cylinder (g)</u>	<u>5 mL pipet (g)</u>	<u>20-200 μL pipettor (g)</u>	<u>100-1000 μL pipettor (g)</u>
1	_____	_____	_____	_____	_____	_____
2	_____	_____	_____	_____	_____	_____
3	_____	_____	_____	_____	_____	_____
4	_____	_____	_____	_____	_____	_____
5	_____	_____	_____	_____	_____	_____
6	_____	_____	_____	_____	_____	_____
7	_____	_____	_____	_____	_____	_____
8	_____	_____	_____	_____	_____	_____

⇒ **IMPORTANT: Before you make any measurements, make sure you calibrate your electronic balance. In addition, as you carry out your experiments, make sure every member of your group has a chance to practice using each measuring device.**

A. How accurately and precisely do beakers measure volume?

1. Measure 60 mL of dH₂O using a 100 mL beaker. When making your measurement, be sure the lowest point of the meniscus just touches the 60 mL calibration mark on the beaker. You can use a Pasteur pipette with a small bulb to add or remove water, adjusting the volume as close to 60 mL as possible.
2. Place a large (at least 10 cm x 10 cm) plastic weigh boat on your electronic balance and tare the balance to zero. Carefully pour all of the dH₂O from the beaker into the weigh boat, and record the weight, to the nearest 0.01 g, in your data table. Finally, discard the dH₂O into the waste receptacle provided, place the weigh boat back on the balance, and re-tare the balance.
3. Repeat this procedure seven more times using the same beaker, for a total of eight measurements.

B. How accurately and precisely do graduated cylinders measure volume?

1. Measure out 60 mL of dH₂O using a 100 mL graduated cylinder. When making your measurement, make sure the lowest point of the meniscus just touches the 60 mL calibration mark on the graduated cylinder. You can use a Pasteur pipette with a small bulb to add or remove water, adjusting the volume as close to 60 mL as possible.
2. Determine the weight of the dH₂O measured with the 100 mL graduated cylinder. Follow the same procedure you used to weigh the dH₂O from the beaker. Record the weight, to the nearest 0.01 g, in your data table. Finally, discard the dH₂O into the waste receptacle provided, place the weigh boat back on the balance, and re-tare the balance.
3. Using the same graduated cylinder, measure and record the weights of seven more 60 mL volumes of dH₂O, for a total of eight measurements.
4. Now, repeat the procedure eight more times using a 10 mL graduated cylinder to measure 3 mL volumes of dH₂O. Record the results in your data table.

C. How accurately and precisely do pipets measure volume?

1. Pipet fillers are designed to draw liquid into a pipet and accurately deliver specific volumes. Before you make any measurements, learn how to use the pipet filler by carefully reading the instruction sheet provided. Ask your instructor for help if you do not understand the instructions.
2. Insert the top (blunt) end of a 5 mL blow-out pipet into the pipet filler. The pipette should be attached firmly, but do not push it in too far or it will be difficult to remove when you are done.
3. Immerse the tip of the pipet into a container of dH₂O and practice using the pipette filler to slowly fill and empty the pipet. If you are having difficulty, ask your instructor for help.

⇒ **IMPORTANT: Never allow liquid to be drawn into a pipet filler since that could damage the filler and certainly would contaminate the liquid being delivered!**

4. Use the pipet filler to slowly fill the pipet with slightly more than 3 mL of dH₂O. (Note that pipets are usually calibrated in reverse, so that the zero mark is near the top and the calibration marks increase towards the tip. In this case, the calibration marks show how much liquid has been delivered, assuming the pipet was originally filled to the zero line, not the amount the pipet contains. Therefore, if a 5 mL pipet is filled slightly above the 2 mL line, it will contain slightly more than 3 mL.)

- Raise the tip of the pipet out of the water and use the pipet filler to slowly release dH₂O back into the container until the bottom of the meniscus exactly reaches the 2 mL line on the pipet (i.e. the pipet contains exactly 3 mL of dH₂O). Touch the tip of the pipet to the inside of the container to remove any water on the outside of the tip. You will next transfer this measured volume to a weigh boat. Be sure the pipet remains vertical and no water escapes during this transfer.
- Place a weigh boat on an electronic balance, tare the balance to zero, and release all 3 mL of dH₂O from the pipet into the weigh boat. **[IMPORTANT: After the water drains out of the pipet, follow the instructions on the instruction sheet to expel the small amount of water remaining in the tip. If necessary, ask your instructor for help.]** After all water has been expelled from the pipet, touch the tip of the pipet to the weigh boat to remove any water suspended from the tip. Record the weight in your data chart and then re-tare the balance.
- Using the same pipet, measure and record the weights of seven more 3 mL volumes of dH₂O, for a total of eight measurements.

D. How accurately and precisely do micropipettors measure volume?

Three different sized micropipettors are available in lab. The button color and the numbers written on the button indicate their measuring range. Examine each micropipettor, and then fill in the table below:

<u>Button Color</u>	<u>Measuring Range of Micropipettor</u>
_____	_____
_____	_____
_____	_____

When making measurements, you must use the correct size micropipettor. In this exercise, you will be measuring 0.17 mL of dH₂O. Which size micropipettor should you use to measure this volume?

_____ or _____

The following descriptions are for Pipetman micropipettors. Other brands may work differently. Ask your instructor if you are not clear on how to use your micropipettor. To adjust a micropipettor to the desired volume, turn the wheel in the handle until the desired volume, in microliters, is displayed. Although most micropipettors have a 3-digit display, these digits represent different values in different sized micropipettors. For example, if the digits 093 appear in the micropipettor display, this represents different volumes:

<u>2 – 20 μL micropipettor</u>		<u>20 – 200 μL micropipettor</u>		<u>100 – 1,000 μL micropipettor</u>	
<u>display</u>	<u>value in μL</u>	<u>display</u>	<u>value in μL</u>	<u>display</u>	<u>value in μL</u>
0	tens	0	hundreds	0	thousands
9	ones	9	tens	9	hundreds
3	tenths	3	ones	3	tens

If the three different sized micropipettors were adjusted as shown in the table above,

What volume will the 2 – 20 μL micropipettor deliver? _____

What volume will the 20 – 200 μL micropipettor deliver? _____

What volume will the 100 – 1,000 μL micropipettor deliver? _____

⇒ NOTE: Have your instructor check your answers before you continue with this exercise!

To help you remember how to read the displays, the 2 – 20 μL micropipettor has a red line between the second and third digits. This line represents a decimal point in the microliters displayed. For example, “200” means 20.0 μL , and this is the largest number that should be displayed because it is the maximum this micropipettor can measure. On the other hand, the 100 - 1000 μL micropipettor has a red line between the first and second digits. This line represents a comma in the microliters displayed. For example, “100” means 1,000 μL , and this is the largest number that should be displayed because it is the maximum this micropipettor can measure.

⇒ **IMPORTANT: Never try to set an automatic micropipettor beyond the range it was designed to measure. If the dial resists turning, STOP TURNING IT! At best, you will get an inaccurate measurement, and you may permanently damage the micropipettor!**

To measure 0.17 mL (170 μL), you can use either the 20 – 200 μL micropipettor or the 100 – 1,000 μL micropipettor. Let’s try both sizes and then compare how accurately and precisely they measure 170 μL of dH_2O . We’ll begin by making measurements with the 20 – 200 μL micropipettor.

1. Adjust the 20 - 200 μL micropipettor to deliver 170 μL of dH_2O . Ask your instructor to check your work.
2. Next, attach an appropriate plastic disposable tip. These tips are color coded: yellow or white tips are used on micropipettors with a yellow button and blue tips are designed for micropipettors with a blue button. Make sure the tip is attached snugly so it is air-tight.

⇒ **IMPORTANT: Always use the micropipettors with a disposable tip attached, and never get any liquid into the micropipettor itself. This would cause permanent damage to the micropipettor!**

3. To get a feel for how a micropipettor handles, grasp the handle of the micropipettor with your thumb resting on the top button. Now slowly push the button down until it meets resistance. This is referred to as the “first stop”. If you push down harder past the first stop, the button will move down a little further, until it reaches the “second stop”. Now, SLOWLY release the button until it returns to its original position. Practice pressing down on the button and releasing it until you can easily feel the first and second stops.
4. The correct procedure for filling and emptying the micropipettor tips is as follows:
 - a. First, push the button down to the FIRST STOP ONLY.
 - b. Next, immerse the end of the tip of the micropipettor into the liquid you wish to measure and SLOWLY release the button, drawing the liquid up into the plastic tip.
 - c. Lift the tip just above the level of the liquid and gently touch the side of the container with the end of the tip to remove any hanging droplets from the tip.
 - d. Insert the tip into the container that will receive the liquid and SLOWLY press the button ALL THE WAY DOWN TO THE SECOND STOP to expel all the liquid from the tip.
 - e. Gently touch the side of the container with the end of the tip to remove any hanging droplets, and remove the micropipettor tip before slowly releasing the button again. You have just transferred the correct amount of liquid from the first container to the second.

⇒ **IMPORTANT: The button should be pushed down to the FIRST STOP when the tip is being filled, while it should be pushed all the way to the SECOND STOP when the tip is being emptied. This will expel any liquid that sticks to the tip through capillary action. If you use the second stop to fill the tip, your volume will be inaccurately measured!**

⇒ **IMPORTANT: Keep your micropipettor upright at all times so that none of the solution in the disposable tip slides down into the micropipettor---this could permanently damage this expensive instrument!**

5. Place a small plastic weighing boat on an electronic balance and tare the balance to zero.
6. Transfer the 170 μL of dH_2O to the weigh boat from your 20 – 200 μL micropipettor. Record the weight in the appropriate column of your table, and re-tare the balance to prepare it for the next measurement.

7. Measure, weigh, and record the weights for seven more 170 μL volumes of dH_2O in the same way, for a total of eight measurements.
8. Now set the 100 – 1000 μL micropipettor to measure 170 μL of dH_2O . The process for measuring and transferring liquid with this micropipettor is the same, except for the color of disposable tips. Make eight measurements with this micropipettor and record their weights in your table.

E. What is the best way to measure 2.7 mL of dH_2O ?

You are working in a lab where you need to put 2.7 mL of dH_2O into a test tube. One member of your group thinks it would be best to use a 5 mL pipet to measure this volume. Another member of your group thinks it would be best to use the 100 – 1000 μL micropipettor three times, measuring out 0.9 mL each time. You think it doesn't make any difference. Design and carry out an experiment to see who is correct. Make sure you repeat each method for measuring 2.7 mL at least eight times.

F. How does incorrect use of the micropipettors affect their accuracy and precision?

According to the instructions for using the micropipettors, it is very important to press the button down to the FIRST stop when getting ready to fill the tip, and all the way down to the SECOND stop when emptying the tip. (Note: detailed instructions for using the micropipettors are on the previous page.)

A lab tech working at a medical lab, who did not pay close attention during her cellular and molecular biology class in college, has been using the micropipettors incorrectly. Her procedure is to press the button all the way down to the second stop both when getting ready to fill the tip and when emptying the tip. Design and carry out an experiment to determine how her mistake affects the accuracy and precision of her measurements. Make sure you repeat each method for measuring (correct and incorrect) at least eight times.

Clean up

Wash all regular glassware:

1. Pour contents into an approved container (Ask your instructor if you are unsure of how to discard a particular chemical.)
2. Rinse with tap water.
3. Scrub with soapy water and a test tube brush.
4. Rinse 3 times with tap water.
5. Rinse 3 times with distilled water.
6. Store inverted over absorbent towels until dry.

All disposable glassware goes into the special glass disposal receptacle.

All instruments should be turned off and unplugged.

Wipe off your workspace with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Leave your work area in the same order that you found it in.

Ask your instructor to check your work area before you leave.