Lab 2: Measurement of Volume & Mass in Biotechnology Lab

The ability to accurately measure specified amounts of liquids and solids is an essential skill for a biotechnology lab worker. Today, you will become familiar with the instruments used to measure volume and mass in a biotechnology lab and learn how to measure accurately. These activities allow you to check your accuracy so that you may improve your skills.

In this lab, you will be introduced to laboratory instruments that are used to measure volume and mass in biotechnology labs. At first, you may find that it takes considerable time to use these instruments precisely—this is part of the learning process! This lab is for learning how to use these instruments. Eventually, with enough practice, you should become proficient, efficient, and accurate in your measurement skills.

Before using the instruments, let's learn their names and what they are used to measure:

Volume measurements:

• **Graduated cylinders**
  - For measuring volumes from 10 mL – 5 L
  - Common sizes: 10 mL, 25 mL, 50 mL, 100 mL, 250 mL, 500 ml, 1 L
  - How to use: before using, make sure you know the total volume the graduated cylinder could hold and the value of each of the graduations. Measuring with a graduated cylinder is complicated somewhat by a meniscus. A meniscus is the curvature of the surface of the water. Water "sticks" to the walls of the graduated cylinder, but only on the sides and not the middle. When you look at the surface from the side, the liquid level is not straight. Measurement should be at the lowest point of the meniscus (see figure to the right). Read the meniscus at eye level in order to get an accurate reading.

Graduated cylinder
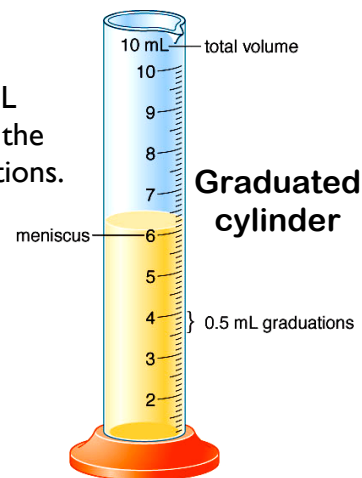
• **Pipets**
  - For measuring volumes from 1 mL – 50 mL
  - Common sizes: 1 mL, 2 mL, 5 mL, 10 mL, 25 mL
  - See more information on using pipets in Activity 2a below.

pipets

• **Micropipets**
  - For measuring volumes from 0.1 μl – 1 mL
  - Common sizes:
    - P-1000: measures from 100 μl – 1000 μl (1 mL) ★
    - P-200: measures from 20 μl – 200 μl
    - P-100: measures from 10 μl – 100 μl ★
    - P-20: measures from 1 μl – 20 μl
    - P-10: measures from 0.5 μl – 10 μl ★
    - P-2: measures from 0.1 μl – 2 μl

★*: present in our biotech lab*
  - See more information on using pipets in Activity 2b below.


micropipet

Volume measurements, continued:

• Choosing the correct tool for measuring volume
   When measuring volume, always use the smallest size pipet or graduated cylinder for the job in order to minimize error. For example, if you have to measure out 4.5 mL of liquid, a 5 mL pipet would be more accurate than a 10 mL pipet. This is because smaller sized pipets have smaller diameters and more precise graduations. Therefore, there will be less error when judging the meniscus of the sample when using a smaller sized pipet.

• A note about flasks and beakers
   Flasks and beakers are to hold liquids; they are <u>NOT</u> for measuring volume because they are not very accurate.
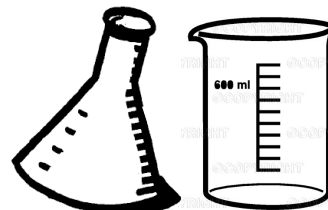
Flasks & beakers aren't accurate for measuring volume

Mass measurements:

   Biotechnology reagents often need to be weighed in very small amounts, or masses. Measurements must be made using precision instruments. In a biotechnology laboratory, mass measurements are performed on electronic balances or scales. There are two basic types of electronic balances in most biotechnology labs:

Tabletop balance

• Tabletop balance (less precise)
   - for measuring masses of ~ 1 g – 1000 g (1 kg)

• Analytical balance (more precise)
   - for measuring masses of ~ 10 mg – 1000 mg (1 g)

Analytical balance

   Regardless of which type of balance you are using, you will follow a similar weighing protocol. This ensures that the balance is used properly and is not damaged.

<u>Weighing protocol:</u>
   1. Turn the balance on if it is not already on by pressing the "on" button.
   2. Make sure that the weigh pan is clean.
   3. Add a weigh boat or piece of weigh paper. *Never place chemicals directly on the weigh pan*.
   4. Press the "tare" or "zero" button to zero the balance.
   5. Using a clean scoop, add the chemical to be weighed to the weigh boat very carefully until the desired mass is obtained.
   6. Clean up any spilled chemicals.

Calculating percent error:

Most of the time, there is some error in a measurement, meaning that the amount that was actually measured isn't exactly the same as the amount that was expected (what should have been measured). As you practice, and in your future work in labs, it is important to know how to calculate percent error. This tells you how "off" your measurement was from what was expected. Ideally, you would want your percent error to be below 5%; of course, the closer the percent error is to 0 %, the better!

To calculate percent error, use the following equation:

$$\frac{|\,\text{actual amount measured} - \text{expected amount}\,|}{\text{expected amount}} \times 100$$

For example, let's say you wanted to measure 5 mL, and after checking your measurement, you found that you actually had measured 4.5 mL. Your "expected amount" is 5 mL, and your "actual amount measured" is 4.5 mL. Therefore, to calculate percent error:

$$\frac{|\,\text{actual} - \text{expected}\,|}{\text{expected}} \times 100 \ \ = \ \ \frac{|\,4.5\text{ mL} - 5\text{ mL}\,|}{5\text{ mL}} \times 100 \ = \ 10\ \%\ \text{error}$$

Activity 2a
Measuring Small Volumes in a Biotechnology Lab

Purpose & Background

The purpose of this activity is to learn how to accurately measure small volumes using pipets. In addition, you will learn which pipets are best used to measure specific volumes.

We have 1 mL, 2 mL, 5 mL, and 10 mL pipets, and electronic pipettors that create suction to draw liquid into the pipets.

Basic instructions for using pipets:

1. Insert the top end of the pipet into the electronic pipettor.

2. Once the pipet is in the electronic pipettor, insert the bottom tip of the pipet into the liquid that you want to measure.

3. Use the top button to draw liquid up and into the pipet; use the bottom button to release liquid. The harder you press these buttons, the faster the liquid will move in or out of the pipet. It takes some practice to get used to controlling the flow of liquid, so start slowly by pushing the buttons gently.

Some guidelines to follow:

• Always keep the pipet in an almost vertical position when there is fluid in it. To avoid contaminating the electronic pipettor, do not allow liquid to go past the top of the pipet into the pipettor. If this happens, <u>let me know immediately</u> so I can clean out the pipettor.

• For maximum accuracy in measuring volume, pipet at eye level by ducking down so your eye is at the level of the meniscus in the pipet.
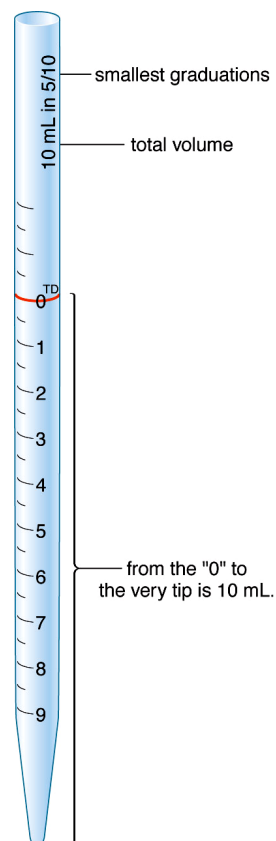
Procedure
1. Using a permanent marker (Sharpie), label four empty 15 mL plastic tubes with A, B, C, and D, and your group's initials.
2. Carefully study each pipet available for use. What is the maximum amount each pipet can measure? What is the value of the smallest graduation on each pipet? For each pipet, randomly put your thumbnail somewhere on the graduations. If you drew up liquid to that height, what volume would be measured? Take some notes in your lab notebook so you are sure you can interpret volume measurements later. Check with your lab colleagues and instructor to ensure that you are reading the pipet correctly.
3. Using the smallest pipet possible, measure the following dye solutions into each tube according to Table 2.1 below. Add each volume to the inside of the tube, allowing the liquid to flow down to the bottom. Finger flick the tube to mix the liquids.

Table 2.1 Pipetting Practice Table

Tube	Red Dye Vol. (mL)	Blue Dye Vol. (mL)	Yellow Dye Vol. (mL)	Total Volume (mL)
A	1.1	1.7	0.33	
B	1.27	0.85	2.9	
C	0.7	2.8	1.8	
D	1.6	1.9	0.66	

4. When you have finished, compare your tubes to the "key" tubes prepared by the instructor. If the volume of any tube is not within one meniscus of the "key" tube, it should be remade.

Activity 2b
Measuring Very Small Volumes in a Biotechnology Lab

Purpose & Background
   The purpose of this activity is to learn how to accurately measure very small volumes (microliter, µl, amounts) using micropipets. This activity introduces micropipetting technique. As with all fine motor skills, learning how to use a micropipet takes practice and determination. You must be able to measure these tiny volumes with accuracy.

Basic instructions for using micropipets:
1. Check that you have the correct micropipet for the job. We have three micropipet sizes: a P-10 (for 0.5 – 10 µl), a P-100 (for 10 – 100 µl), and a P-1000 (for 100 – 1000 µl).
2. Dial the desired volume. Make sure that you understand how to read the scale. *Hint:* By knowing the maximum volume of the micropipet (usually written on the micropipet), you can figure out what each of the digits on the readout means.
3. Push the end of the pipet into the proper size tip. The small tips (some are yellow, some are white) are for the P-10 and the P-100 micropipets,
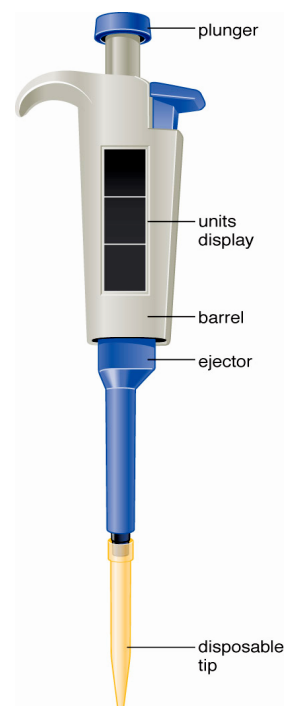
and the large, blue tips are for the P-1000 micropipet. The tips are disposable and usually intended for one use.

How to take up a sample with a micropipet:
4. Before picking up the micropipet, open the cap or lid of the tube from which you are taking fluid (or, have your lab partner do this).
5. Hold the micropipet in one hand, at a 45° angle from vertical. In this way, contaminants from your hands or the micropipet will not fall into the tube.
6. **Depress** the plunger of the micropipet to the **first** stop, and **hold** it in this position.
7. Place tip into the solution to be pipetted.
8. Draw fluid into the tip by *slowly* releasing the plunger.

How to expel a sample from the micropipet:
9. With your other hand, open the cap or lid of the tube you are filling.
10. Gently touch the micropipet tip to the inside wall of the tube into which you want to expel the sample. This creates a tiny surface-tension effect that helps draw the fluid out of the tip.
11. Slowly, depress the plunger of the micropipet to the first stop, and then the second stop to expel the last bit of fluid, and **hold** the plunger in this position.
12. Slowly, remove the pipet from the tube, *keeping the plunger depressed to avoid drawing any liquid back into the tip*.
13. Always change tips for each new reagent you pipet. To eject a tip, depress the ejector button on the top of the micropipet.

TO SUM UP:
Taking up sample: Down to first stop and hold → Into liquid → Slowly release
Releasing sample: Down to second stop and hold → Lift micropipet out of tube → Eject tip

Some guidelines to follow:
   • Set pipet volume *only* within the range specified for that micropipet. Don not attempt to set a volume beyond the pipet's minimum or maximum values.
   • Never forget to apply a tip when using a micropipet. Failure to do this will cause liquid to enter into the nose cone. Since a micropipet works by air displacement, its internal mechanism must remain dry.
   • Always keep a micropipet in a vertical position when there is fluid in the tip. Do not allow liquid to accidentally run back into the nose cone.
   • Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid. Releasing the plunger too abruptly will cause leakage or bubbles that will trap air and make the measurement inaccurate.

Procedure
1. Using a permanent marker (Sharpie), label four empty microcentrifuge tubes with A, B, C, and D, and your group's initials.
2. Add the specified amounts of each solution to tube A or B, as listed in Table 2.2. Add each volume to the inside of the tube—the drop will stick because of adhesion.
3. Spin the four tubes in the microcentrifuge (make sure you know how to use it first!) for 1 to 2 seconds to bring the mixture of liquids to the bottom of the tubes.

Table 2.2 Micropipetting Practice Table

Tube	Yellow Dye Vol.	Green Dye Vol.	Blue Dye Vol.	Red Dye Vol.
A	4.3 µl	5.5 µl	2.2 µl	0 µl
B	6.3 µl	1.3 µl	7.6 µl	8.8 µl
C	41 µl	12 µl	33 µl	14 µl
D	255 µl	110 µl	340 µl	195 µl

4. Compare your tubes with the "key" tubes prepared by the instructor to get an idea of your level of accuracy.
5. Calculate how much liquid you expect to have in each tube, A, B, C, and D. Then use your micropipet to measure how much liquid you have actually pipetted. To do this, set your micropipet to volume that you expected based on your calculation. Then, attempt to remove the liquid from the tube. If there is liquid remaining in the tube, wind the dial up until all the liquid has been taken up and then read the volume off of the pipet to see what you actually measured. If you find that you have an air bubble at the bottom of your tip, wind the dial down until the air is gone and then read the volume off of the pipet to see what you actually measured. This is somewhat complicated to learn from a description, so ask your instructor to demonstrate when you reach this step in the procedure.
6. Now that you have the values for your expected volumes and your actual volumes, calculate percent error for your measurements for tube A, B, C, and D using the equation on p. 13. Record your results in a data table like the one below (Table 2.3).

Table 2.3 Percent error for micropipetting practice

Tube	Expected volume	Actual volume	% Error
A			
B			
C			
D			

Activity 2c
Measuring Mass

Purpose & Background

In this activity, you will measure small amounts of glucose on an appropriate balance and make glucose solutions with a give mass of glucose in a specified volume. You will verify your mass and volume measurements using glucose test strips.

Before you start, you may want to refer back to the weighing protocol described in the introduction on page 13.

Procedure

1. Using the <u>analytical</u> <u>balance</u>, measure the required mass of glucose to prepare the solutions listed in Table 2.4.

2. Prepare each solution in a 15 mL plastic conical tube. Label each tube with the name and concentration in mg/dL of the sample, and your group's initials. Add the glucose that you have weighed carefully to the tube. Use your squirt bottle to wash any residual glucose down into the tube. Mix to dissolve the glucose using the vortex on your bench. Add more water (can use your squirt bottle) to bring the final volume up to 10 mL. Mix once more using the vortex.
3. Obtain four Diastix glucose test strips. Dip a test strip into each solution for 1 to 2 seconds, then lay the strips test-side up on a paper towel. Let the color develop for 90 seconds. Then, compare the color of the test strips to the key on the Diastix package. Record the Diastix data value for each sample in a table similar to Table 2.4 below.

Table 2.4 Glucose Mass Testing

Solution to be Prepared (mg/dL)	Equivalent concentration (g/mL)	Final volume (mL)	Mass of glucose (g)	Diastix Test Result
250.0	0.0025	10	0.025	
100.0	0.001	10	0.01	
50.0	0.0005	10	0.005	
0	0	10	0	

4. Determine how closely the measured concentration is to the expected concentration. What do the data say about your mass measuring technique? Identify two technical errors that could be made that would result in inaccurate concentration readings.

Activity 2d
Checking the Accuracy of Micropipets using a Balance

Purpose and Background

A balance can be used to determine if a micropipet is measuring within an acceptable range. Since 1 mL of water weighs 1.0 g (because the density of pure water is 1 g/mL), you can estimate the expected mass for any volume of water. For example,

1.5 mL of water should weigh 1.5 g.
0.25 mL of water should weigh 0.25 g.
150 µl (= 0.15 mL) of water should weigh 0.15 g.

Water dispensed by a micropipet can be weighed on a balance. By comparing the actual observed mass to the expected mass, you can make an error determination and calculate percent error as you have done in Activity 2b. In this activity you will measure small volumes of water on a balance to check the accuracy of your micropipet measurements.

$$\frac{|\text{actual mass} - \text{expected mass}|}{\text{expected mass}} \times 100$$

Procedure
1. Place a weigh boat on the appropriate balance and tare (zero) the balance.
2. Using the proper micropipet, measure the specified volume of water onto the weigh boat. In a table similar to Table 2.5, record the mass of the volume of water measured and the type of balance used.
3. Calculate percent error for each measurement and record your result in the data table also.
4. For any error that is outside of the acceptable range, remeasure. Make sure that you are using the balances and micropipets correctly. Get help if necessary. If you are still getting measurements outside the range of acceptable error, the pipet may be out of calibration. Make the measurement with another student's pipet in order to check the calibration of your pipet. Notify your instructor of any equipment that does not appear to function correctly.

Table 2.5 Micropipetting Precision: Mass versus Volume

Micropipet	Volume (µL)	Volume (mL)	Expected Mass (g)	Actual Mass (g)	Percent Error	Acceptable Error (%)	Type of balance used
P-1000	1000					3	
P-1000	255					3	
P-100	100					5	
P-100	17					5	
P-10	10					10	
P-10	2.4					10	

Lab 2 Homework

Name: _____

1. A 1.75 mL sample is needed for an experiment. Both a 2- and a 5-mL pipet will measure this amount. Which pipet is best to use and why?

2. What do you think would happen to the accuracy of your measurement if you depressed the plunger of a micropipet to the <u>second</u> stop instead of only the first stop when taking up a sample?

3. In Activity 2c, what was the purpose of making the "0 mg/dL" glucose tube?

4. For each volume or mass measurement below, convert each unit and select the appropriate instrument for its measurement.

Instrument choices:

Graduated cylinder	*1 mL pipet*	*tabletop balance*
10 mL pipet	*P-1000*	*analytical balance*
5 mL pipet	*P-100*	
2 mL pipet	*P-10*	

		new unit:	instrument to measure with:
a)	75.34 mg =	_____ g .	_____.
b)	6.29 mL =	_____ L .	_____.
c)	8.925 g =	_____ mg .	_____.
d)	0.115 mL =	_____ μL .	_____.
e)	335 mL =	_____ L .	_____.
f)	4.67 μL =	_____ mL .	_____.
g)	34 mg =	_____ μg .	_____.
h)	0.0734 mL =	_____ μL .	_____.
i)	1800 μL =	_____ mL .	_____.
j)	805 g =	_____ kg .	_____.
k)	4.1 mL =	_____ μL .	_____.