

Biology 3A Laboratory

Laboratory Safety and Measurement of Liquid Volumes: Beakers, Volumetric Flasks, Graduated Cylinders, Pipettes, and Burettes

Objectives

- To understand the importance of laboratory safety
- To understand how liquid volumes are measured
- To investigate the accuracy of beakers, volumetric flasks, graduated cylinders, pipettes and burettes
- To learn basic types of pipettes and their functions
- To understand the use and choice of pipettes for specific measurements
- To demonstrate repeatable, accurate pipette techniques

Introduction

Department of Biological Sciences Laboratory Safety Policy

Many of the reagents (chemicals) and some of the equipment in a biology laboratory are potentially dangerous. Following rules of laboratory safety and using common sense throughout the course are important to the enhancement of a student's laboratory experience.

The following safety rules apply to all biology department laboratory areas.

- Wear safety glasses or goggles when directed by your instructor, or during exercises in which glassware and solutions are heated, or during exercises in which dangerous fumes may be present, creating a possible hazard to eyes or contact lenses.
- Wear closed-toe shoes at all times in the laboratory.
- Assume that all reagents are poisonous and act accordingly.
 - Read the labels on reagent containers for safety precautions, and understand the nature of the chemical that you are using.
 - Stopper or cap all reagent bottles when not in use.
 - If you should get any reagent in your mouth, rinse your mouth thoroughly with water and immediately inform the laboratory instructor. If reagents come into contact with your skin or eyes, wash the area immediately with water and immediately inform the laboratory instructor.
 - Do not pipette anything by mouth.
 - Discard chemicals as instructed; if you have a question, ask your instructor.
 - Do not pour chemicals back into containers.
 - Do not taste or ingest any reagents.
- Exercise great caution when using heat, especially when heating chemicals.
 - Do not leave a Bunsen burner or other flame source unattended.

- Do not light a Bunsen burner near flammable materials.
- Do not move a lit Bunsen burner.
- Keep long hair and loose clothing restricted and well away from any flame.
- Turn the gas valve off when the Bunsen burner is not in use.
- Use proper ventilation and hoods when instructed.
- Do not leave stopper in place when heating test tubes.
- Handle hot glassware or lab ware with a test tube clamp or tongs.
- Do not eat or drink in the laboratory, unless so directed by the instructor.
- Do not smoke within 25 feet of the laboratory door.
- Do not operate any laboratory equipment until you have been instructed in its use.
- Do not engage in horseplay, pranks or other acts of mischief or disrespect for others.
- Do not perform unauthorized experiments.
- Keep your work area neat, clean, and organized.
- Use extra care when working with scalpels, knives, glass tubing or other sharp-edged objects.
- Wear clothing that, if damaged, would not be a serious loss, or use the provided aprons or laboratory coats, since some chemicals may damage fabrics.
- Read and understand the experiments you will be doing before coming to the laboratory.
 - Follow the procedures set forth by the laboratory manual.
- Know the location of emergency equipment
 - first aid kit
 - eyewash bottle
 - fire extinguisher
 - showers
 - fire blanket
 - telephone
- Report all accidents to your laboratory instructor immediately.
- Discard needles, razor blades, scalpel blades and other such items only in "Sharps" containers.
- Discard cracked or broken microscope slides and other glass only in the broken glass containers.
- Report all unsafe conditions to your instructor.
- Clean your work area and glassware and wash your hands before leaving the laboratory.

Volumetrics

Typical volumetric containers used in chemistry and biology labs include beakers, flasks, cylinders, pipettes and burettes. In most cases, these are made of glass and calibrated at a specific temperature. However, for very small volume measurements, micropipettes, constructed out of plastic, with disposable tips, are used.

A beaker is a simple container for liquids, very commonly used in laboratories. Beakers are generally cylindrical in shape, with a flat bottom. Beakers are available in a wide range of sizes, from 10 mL up to several liters. They may be made of glass (typically Pyrex®) or of plastic. Beakers may be covered, perhaps by a watch glass, to prevent contamination or loss of the contents. Beakers are often graduated, or marked on the side with lines indicating the volume contained. For instance, a 250 mL beaker might be marked with lines to indicate 50, 100, 150, 200, and 250 mL volumes. The accuracy of these marks can vary from one beaker to another. A beaker is distinguished from a flask by having sides which are vertical rather than sloping. In the lab, beakers are used more often than flasks.

Laboratory flasks come in a number of shapes and a wide range of sizes, but a common distinguishing aspect is a wider vessel "body" and one (or sometimes more) narrower tubular sections at the top called necks which have an opening at the top. Like beakers, laboratory flask sizes are specified by the volume they can hold. Laboratory flasks have traditionally been made of glass, but can also be made of plastic. Volumetric flasks come with a stopper or cap for capping the opening at the top of the neck. Stoppers can be made of glass, plastic, or rubber. In general, flasks can be used for making, holding, containing, collecting, or volumetrically measuring solutions.

A graduated cylinder is used to accurately measure out volumes of liquid chemicals. They are more accurate and precise for this purpose than beakers or flasks. Often, the largest graduated cylinders are made of polyethylene or other rigid plastic, making them lighter and less fragile than glass.

A pipette is a laboratory instrument used to transport an accurately measured volume of liquid. Pipettes are commonly used in chemistry and biology. Pipettes come in several designs for various purposes with differing levels of accuracy and precision, from single piece flexible plastic transfer pipettes to more complex adjustable or electronic pipettes. A pipette works by creating a vacuum above the liquid-holding chamber and selectively altering this vacuum to draw up and dispense liquid. Pipettes that dispense between 1 and 1000 μL (1 mL) are termed *micropipettes*, while *macropipettes* dispense greater volumes of liquid.

A burette is a graduated glass cylinder with a valve on the bottom end used to deliver solution in precisely-measured, variable volumes. Burettes are used primarily for titration, to deliver one reactant until the precise end-point of the reaction is reached.

Procedure 1. The accuracy of 100 mL beakers

In this procedure we will determine the accuracy of typical lab beakers.

1. Using a 200 gram top-loading balance, tare a dry 150 mL beaker.
2. Using a 100 mL beaker, as carefully as possible, measure 20 mL of de-ionized (DI) water
3. Pour this measured amount of water into the 150 mL beaker. Flick any remaining water in the 100 ml beaker into the 150 mL beaker.
4. Record the weight of the water to the nearest 0.01 g in Table 1.
5. Again, using the 100 mL beaker, measure 20 mL of water and add it to the 150 mL beaker.
6. Record the new weight in Table 1.
7. Repeat this procedure until you have 100 mL of water in the beaker on the balance.
8. Calculate the errors in Table 1.

Table 1. Weight of water volumes measured using a glass beaker

Total volume of water (mL)	Total calculated weight of water (g)	Total measured weight of water (g)	Calculated weight of Water added in each step (g)	Measured weight of water in each step (g)	Error (Column 5 – Column 4)	% Error $100 \times (\text{Column 6} / \text{Column 4})$
20						
40						
60						
80						
100						

Procedure 2. The accuracy of 25 mL graduated cylinders

1. Using a 200 gram top-loading balance, tare a dry 150 mL beaker.
2. Using a 25 mL graduated cylinder, as carefully as possible, measure 20 mL of de-ionized (DI) water
3. Pour this measured amount of water into the 150 mL beaker.
4. Record the weight of the water to the nearest 0.01 g in Table 2.
5. Tare the balance.
6. Again, using the 25 mL graduated cylinder, measure 20 mL of water and add it to the 150 mL beaker.
7. Record the new weight in Table 2.
8. Repeat this procedure until you have 100 mL of water in the beaker on the balance.
9. Calculate the error and percentage error in Table 2.

Table 2. Weight of water volumes measured using a 25 mL graduated cylinder

Total volume of water (mL)	Calculated weight of water (g)	Measured weight of water (g)	Error (Column 3 – Column 2)	Percentage Error 100 x (Column 4 / Column 2)
20				
20				
20				
20				
20				

Procedure 3. The accuracy of 100 mL graduated cylinders

1. Using a 200 gram top-loading balance, tare a dry 150 mL beaker.
2. Using a 100 mL graduated cylinder, as carefully as possible, measure 20 mL of de-ionized (DI) water
3. Pour this measured amount of water into the 150 mL beaker.
4. Record the weight of the water to the nearest 0.01 g in Table 3.
5. Tare the balance.
6. Again, using the 100 mL graduated cylinder, measure 20 mL of water and add it to the 150 mL beaker.
7. Record the new weight in Table 3.
8. Repeat this procedure until you have a total of 100 mL of water in the beaker on the balance.
9. Calculate the error and percentage error in Table 3.

Table 3. Weight of water volumes measured using a 100 mL graduated cylinder

Total volume of water (mL)	Calculated weight of water (g)	Measured weight of water (g)	Error (Column 3 – Column 2)	Percentage Error 100 x (Column 4 / Column 2)
20				
20				
20				
20				
20				

Accuracy and Use of Burettes

A burette is used to deliver solution in precisely-measured, variable volumes. Burettes are used primarily for titrations. In a titration one reactant is delivered until the precise end point of the reaction is reached. To fill a burette, close the stopcock at the bottom and use a funnel. You may need to lift up on the funnel slightly, to allow the solution to flow in freely. Before using a recently filled burette, condition the burette with titrant solution and check that the burette is flowing freely. To condition a piece of glassware, rinse it so that all surfaces are coated with solution, then drain. Conditioning two or three times will insure that the concentration of titrant is not changed by a stray drop of water. Before use always check the tip of the burette for an air bubble. To remove an air bubble, whack the side of the burette tip while solution is flowing. If an air bubble is present during a titration, volume readings may be in error. Just before use, check for solution on the tip to see if your burette is leaking. The tip should be clean and dry before you take an initial volume reading.

Reading the Burette

Take an initial volume reading. Using a burette reading card, a black rectangle on a white card, may help you to take a more accurate reading. Read the *bottom* of the meniscus. Be sure your eye is at the level of meniscus, not above or below. Reading from an angle, rather than straight on, may result in a parallax error.

Procedure 4. Accuracy of Burette Measurements

1. Using a small funnel fill a 50 mL burette with de-ionized (DI) water.
 - a. Be sure to run water through the burette tip and wash out any bubbles
 - b. The burette should not be “full;” it should be set at some number beyond the 0.00 line. This is the “initial reading.”
 - c. It is critical that you read the burette to the nearest 0.01 mL.
2. Place a 200 gram top-loading balance under the burette tip. Place a dry 150 mL beaker on the balance, beneath the burette tip. Tare the 150 mL beaker.
3. Record the initial reading in Table 4.
4. Ask your instructor to dispense approximately 10-15 mL of water from the burette into the 150 mL beaker.
5. Record the weight of this water in Table 4.
6. Tare the balance.
7. Repeat steps 3 through 6 four more times until you have approximately 50-75 mL of water in the beaker on the balance.
8. Calculate the error and percentage error in Table 4.

Table 4. Weight of water volumes delivered by a burette

Initial Burette Reading	Final Burette Reading	Burette mLs dispensed	Calculated weight of water (g)	Measured weight of water (g)	Error (Column 5 – Column 4)	Percentage Error $\frac{100 \times \text{Error}}{\text{Column 4}}$

Accuracy and Usage of Pipettes

In this section we will use both glass macro-pipettes and single channel micropipettes. The glass pipettes come in several sizes. We will use a 10 mL pipette. In addition, these glass pipettes are clearly marked TD or TC. TD stands for “to deliver,” while TC stands for “to contain.” TD pipettes, such as the one you will use today, are meant to be blown out “to deliver” the entire volume. Thus, to deliver 10 mL, you will load the pipette to 10 mL and then force all of the liquid out.

Procedure 5. Accuracy of Glass Pipette Measurements

1. Using a 200 gram top-loading balance, tare a dry 150 mL beaker.
2. Using a 10 mL glass pipette and green pipette pump draw 10 mL of DI water into the pipette tube.
3. Transfer this measured amount of water into the 150 mL beaker.
4. Record the weight of the water to the nearest 0.01 g in Table 5.
5. Tare the balance.
6. Again, using the 10 mL pipette, transfer 10 mL of DI water into the beaker.
7. Record the new weight in Table 5.
8. Repeat this procedure until you have 50 mL of water in the beaker on the balance.
9. Calculate the error and percentage error in Table 5.

Table 5. Weight of water volumes measured using a 10 mL glass pipette

Total volume of water (mL)	Calculated weight of water (g)	Measured weight of water (g)	Error (Column 3 - Column 2)	Percentage Error $100 \times (\text{Column 4} / \text{Column 2})$
10				
10				
10				
10				
10				

Procedure 6. Accuracy of Micropipette Measurements

Micropipettes come in several sizes and either fixed volume or adjustable volume. In our lab we have three sizes of micropipettes, 1000 μL (P1000), 200 μL (P200), and 20 μL (P20). These are the maximum volumes that can be transferred with each pipette. However, these pipettes are adjustable, and lesser volumes are also possible. These pipettes are very accurate if used properly.

1. Using a 200 gram top-loading balance, tare a dry 150 mL beaker.
2. Obtain a P1000 micropipette. Note the mechanism that allows you to set the volume you wish to deliver. Set the pipette for 1000. This is 1000 μL , or 1 mL.
3. Install a blue pipette tip on the micropipette body.
4. Press the button on the top of the pipette. Notice that there are two stops. The first stop is for withdrawing (sucking up) your sample, while the second stop is for dispensing.
5. Place the blue tip about 1-3 mm into a beaker of DI water.
6. Press down to the first stop and then release the plunger slowly. One mL of water will be drawn into the blue tip.
7. Place the blue tip into the 150 mL beaker and press down to the second stop. The 1 mL of water will be transferred into the beaker.
8. Record the weight of the water to the nearest 0.01 g in Table 6.
9. Tare the balance.
10. Again, using the P1000 micropipette, transfer 1 mL of DI water into the beaker.
11. Record the new weight in Table 6.
12. Repeat this procedure until you have 5 mL of water in the beaker on the balance.
13. Calculate the average error from the third column of Table 6.

Table 6. Weight of water volumes measured using a 1000 μL micropipette

Total volume of water (mL)	Calculated weight of water (g)	Measured weight of water (g)	Error (Column 3 - Column 2)	Percentage Error $100 \times (\text{Column 4} / \text{Column 2})$
1.00				
1.00				
1.00				
1.00				
1.00				

Procedure 7. Further Analysis of Micropipette Measurements using a Volumetric Flask

Since these micropipettes transfer very small volumes, we cannot use the balances to examine their accuracy. We will use the pipettes to transfer known amounts of a colored substance (potassium permanganate). We will use a volumetric flask to dilute this amount to a known volume. The concentration of the diluted sample will then be measured using a spectrophotometer.

1. Set the wavelength (top knob) to 540 nm.
2. Turn the spectrophotometer on by rotating the left knob labeled 0%T. Allow the instrument to warm up at least 10 minutes.
3. Decant about 10 ml of the 0.01M KMnO_4 solution into a small, dry beaker.
4. Using a P1000 pipette and new tip, transfer 1000 μL of the KMnO_4 solution into a 500 mL volumetric flask.
5. Add DI water to this flask carefully until you have filled it to the etched line.
 - a. Remember to read the bottom of the meniscus
 - b. You can fill very fast up to the neck of the flask. Then you should use a wash bottle to complete the filling.
6. Place the appropriate sized rubber stopper into the flask and invert several times.
7. Label with a grease pencil and place this flask aside.
8. Using a P1000 pipette and the same tip, transfer 1000 μL of the KMnO_4 solution into a 100 mL volumetric flask.
9. Follow steps 4 through 6.
10. Using a P1000 pipette and same tip, transfer 1000 μL of the KMnO_4 solution into a 50 mL volumetric flask.
11. Follow steps 4 through 6.
12. Using a P200 pipette and new tip, transfer 200 μL of the KMnO_4 solution into a 50 mL volumetric flask.
13. Follow steps 4 through 6.
14. Set-up five clean, dry cuvettes in a test tube rack.

15. Fill the first cuvette $\frac{3}{4}$ full with DI water.
16. Fill each of the other cuvettes $\frac{3}{4}$ full with one of the KMnO_4 dilutions.
17. Wipe the exterior of the water filled cuvette (called the “blank”) with a KimWipe®.
18. Place the blank into the spectrophotometer well and close the lid.
19. Using the knob on the RIGHT side (labeled “100%T/0A”), adjust the absorbance to 0.0
20. Remove the blank and wipe and place one of the tubes containing the diluted KMnO_4 into the spectrophotometer well.
21. Read the absorbance.
22. Record the absorbance in Table 7.
23. Repeat steps 17 through 21 for each of the tubes.
24. Wash all glassware and rinse twice with DI water.
25. Repeat the entire dilution and absorbance readings two more times to complete Table 7.
26. Wash and rinse all glassware with DI water, turn off the spectrophotometer.

Table 7. Absorbance of KMnO_4 dilutions

Dilution	Concentration (M)	ABS 1	ABS2	ABS 3
1 mL / 500 mL				
1 mL / 100 mL				
1 mL / 50 mL				
0.2 mL/ 50 mL				

Procedure 8. Completing a Titration

In this procedure you will titrate an unknown concentration of HCl using NaOH and the indicator solution, phenolphthalein.

1. Remove the balance and beaker from beneath the burette tip.
2. Place a stirring hotplate beneath the burette tip.
3. Empty any water from your burette
4. Rinse the burette once with the titrant solution, 0.01M NaOH.
5. Condition and fill the burette with the titrant and rinse any bubbles out of the burette tip.
6. Read the initial level of NaOH and record it in Table 8.
7. Place a magnetic stirring bar into a 125 mL Erlenmeyer flask.
8. Using a 1 mL glass pipette and pump, place 1 mL of the unknown concentration acid solution (HCl) into the flask.
9. Using a wash bottle of DI water, rinse the flask walls and increase the volume to about 30 – 40 mL.
10. Using the dropper on the bottle, add 6-7 drops of Phenolphthalein indicator to the acid solution.
11. Place the flask under the burette tip on the stirring motor.

12. Turn on the stirring motor (**not** the heat!) to provide a slow movement of the solution. You are now ready to titrate the acid in the flask:
 - a. The titrant solution should be delivered quickly until you are within a couple of mL's from the endpoint.
 - b. The endpoint should be approached slowly, one drop at a time.
 - c. Use a wash bottle of DI water to rinse the tip of the burette and the sides of the flask.
 - d. When you are near the endpoint, you can deliver a partial drop of solution.
 - e. When you reach the endpoint, the solution will change color from yellow/clear to pink/reddish color
13. Read the level of NaOH at the endpoint and record it in Table 8.
14. Empty and rinse the flask well with DI water.
15. Repeat steps 7 through 13 two more times, recording your data in Table 8. Note that the final reading from one titration will become the initial reading for the next, unless you need to re-fill your burette.

Table 8. Results of titration of HCl using NaOH

Initial Burette Reading	Final Burette Reading	Burette mLs dispensed

Please complete and hand in the lab safety quiz **TODAY!**

Biology 3A Laboratory
Volumetric Exercises

NOTE: For this laboratory exercise, you will submit your figures and written answers typed

- Using Excel, create a line graph titled **Figure 1** that shows the relationship of total volume versus total measured weight of the water sample measured in Procedure 1. In Excel you should create a “scatter plot” then use “add trendline” after you have made the graph (see Appendix Two in Knisely, 2009) **Note:** This graph should have its origin at 0,0. Make sure that you label all axes, complete a reasonable figure caption, and delete the gray background and black line around the graph.
- In most of the procedures you weighed and measured the volume of water. Write a paragraph in which you answer these questions. What effect, if any, does temperature have upon these measurements? Are there any markings on your glassware related to this potential problem?
- Calculate the mean percentage error and standard error from the final columns of Tables 1 through 6 below.

Device	Mean % Error	Standard Error
Beaker		
25 mL Graduated Cylinder		
100 mL Graduated Cylinder		
Burette		
10 mL Glass pipette		
P1000 (1000 μ L) pipette		

- Using Excel, create a bar graph, titled **Figure 2**, with error bars (\pm standard error) showing the relationship between type of measuring device and the percentage error. Note: All bars should be positive in this graph.
- Write a paragraph (that means several sentences) discussing the accuracy of these measuring devices and their uses from question #4.
- Calculate the mean absorbances and 95% confidence intervals of these means from Table 7 and enter them in the table below.

Concentration (M)	Mean Absorbance	95% CI

7. Using the data from number six, create a graph, titled **Figure 3**, of absorbance (on y-axis) versus concentration (on x-axis). Again, start with the scatter plot function in Excel (Appendix 2 in Knisely, 2009). After you have this graph, right click one of the points and select “add trend line.” Highlight “linear” and click “OK.” You will now have a line on your graph. Next, click a point again and select “format data series.” Click the tab labeled “y error bars.” Select the “custom” radio button. Now into the “+” and “-” field you must add the entire standard error columns. If done correctly, you will get a plot that shows the error for each point. An example is shown below.

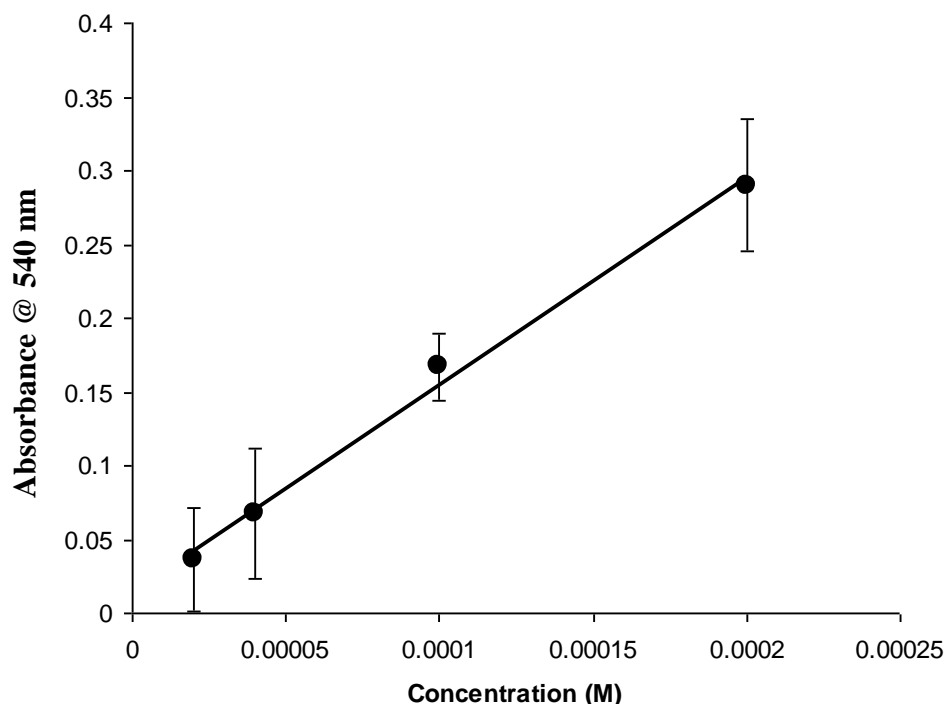
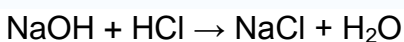


Figure 1. Example of Absorbance versus Concentration plot for dilution of KMnO_4 using micropipettes and volumetric flasks. Error bars are 95% confidence intervals.

8. The relationship between absorbance versus concentration is always linear. Using this knowledge and your figure, comment on the accuracy of your pipette measurements and your volumetric flask dilutions.

9. Calculate the molarity of the HCl solution used in the titration in Procedure 8. You should calculate the molarity for each of your three measurements, and then calculate the mean molarity and the stand error. You should present you molarity in this form: $\text{XXX} \pm \text{XXX M}$ (\pm S.E.M.) **Show your work.** *Be sure to consider significant figures in your calculations.* The equation for the reaction is:



Hint: You know the concentration of the NaOH (0.01 mole/ L) and you measured the number of mL of this solution used to neutralize the HCl.

Literature Cited

Knisely, K (2009) A Student Handbook for Writing in Biology. Sinauer Associates, Sunderland , MA