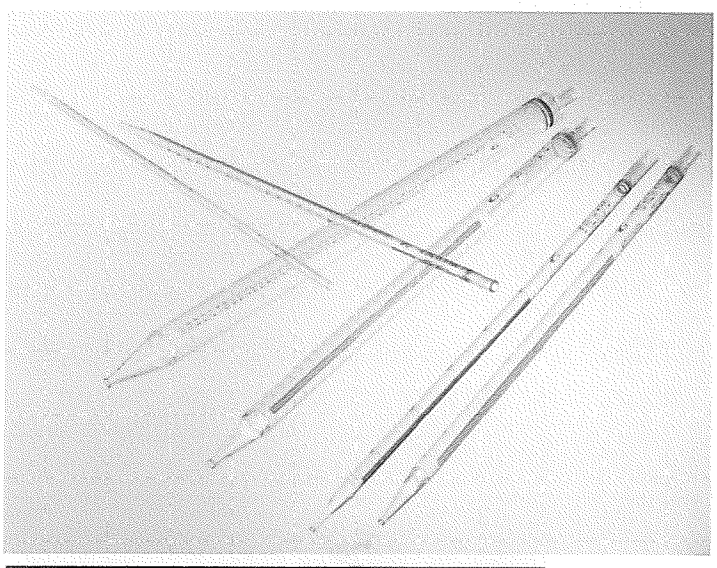
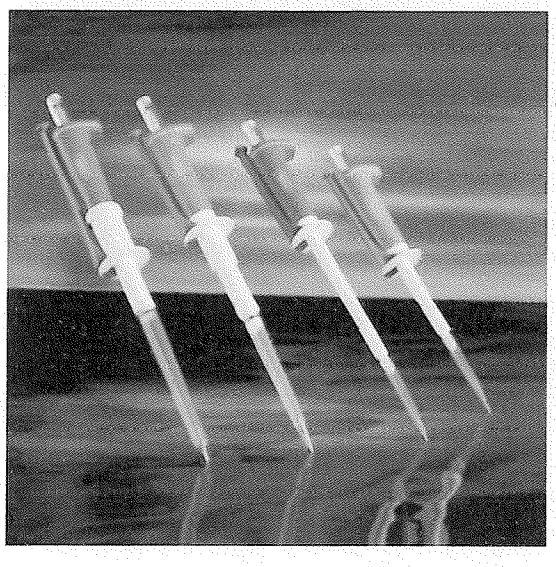
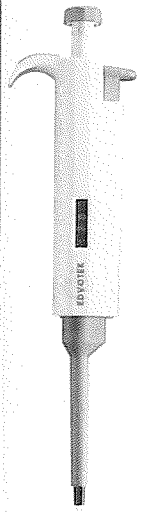
Pipetting: Measuring Small Volumes in a Biotechnology Lab

Lab 3A: Measuring Small Volumes in a Biotechnology Lab
Lab 3B: Measuring Very Small Volumes in a Biotechnology Lab
Lab 3D: Checking Accuracy of Micropipets Using a Balance







Learning to Pipet
Table of Contents

Requirements

Read Lab Manual
Take Notes on Lecture (10 Points)
In your Lab Notebook (100 Points):

- Procedures & safety precautions
- Flow Chart
- Reflection/Analysis
- Conclusion
- Thinking Like Biotechnician (skip #2 for 3b, skip #3 for 3a & 3b)

Read 3 Articles and complete comprehension questions (30 points)

Laboratory 3a Measuring Small Volumes in a Biotechnology Lab

Background

Serological pipets are used to measure volumes from 0.5 mL (500 µL) to 100 mL. The most commonly used pipets are 10-, 5-, 2-, and 1-mL pipets. Pipets are named by the maximum volume they measure. Therefore, a 5-mL pipet measures volumes up to 5 mL.

This activity introduces pipeting technique (see Figure 3.1). As with all fine motor skills, learning how to accurately use a pipet takes practice and determination, but it is essential that you are able to measure volumes with accuracy. The standard practice is to allow for *no more than 10% deviation from the intended value.* In many applications, much less deviation is acceptable. You must also be precise in measurement; that is, you must be able to replicate your measurements repeatedly.

Figure 3.1. A pipet is used to measure milliliter (mL) volumes. Pipet at eye level for more accuracy.
Photo by author.

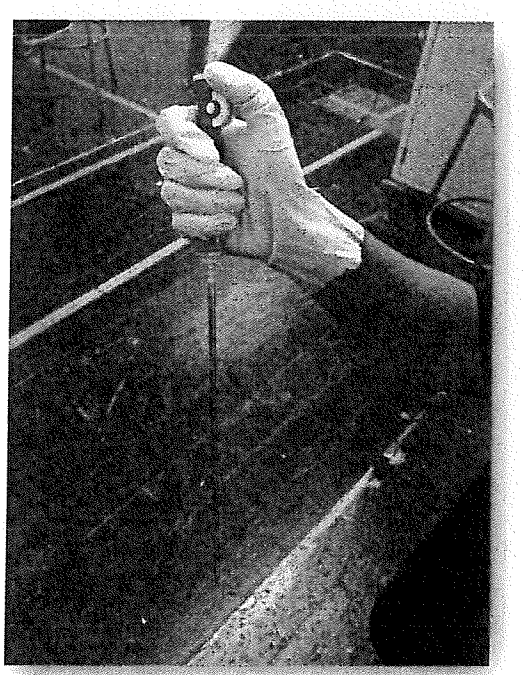


Figure 3.2. Use blue pipet pumps with 1- and 2-mL pipets. Use green pumps with 5- and 10-mL pipets.
Photo by author.

To measure and deliver samples, technicians most commonly use a plastic pipet pump. A blue pump is appropriate for a 1- or 2-mL pipet (see Figure 3.2), and a green pump is appropriate for a 5- or 10-mL pipet (see Figure 3.3). To ensure accurate measuring, operate the pipet pump slowly, and watch carefully as volumes are taken up and dispensed by the pipet. **Mouth pipeting is never allowed.** Other pipet aids may be available, including pipet bulbs or electronic pumps.

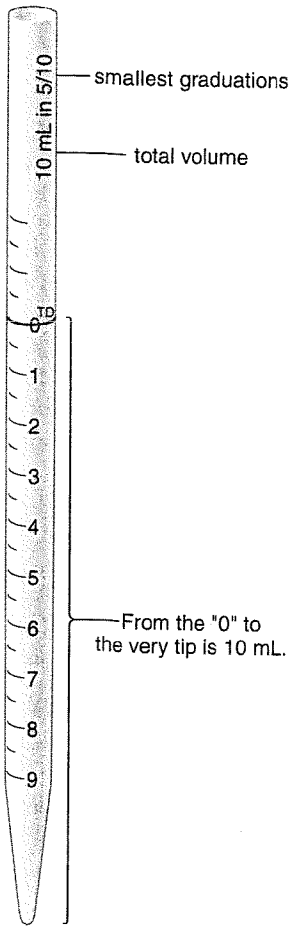


Figure 3.3. 10 mL Pipet A 10-mL pipet measures 1 to 10 mL in 0.5-mL increments. Use a green pipet pump with a 10-mL pipet.

Follow these guidelines for correct pipeting technique:

Pipeting Technique Using a Pipet Pump

1. Use a blue pipet pump for 1- or 2-mL pipets. Use a green pipet pump for 5- or 10-mL pipets. Use a red pipet pump for 25- or 50-mL pipets. Make sure you can understand the values of the graduations on the pipet.
2. Insert the pipet into a pipet pump. Gently twist and push the top of the pipet (end with label) into the pump *just until it is held securely.* Do not push too far as this may damage the inside of the pump.
3. Put the pipet tip into the solution. Be careful the sample does not overflow the container due to displacement.
4. Keeping the tip under the surface and holding the container at eye level, roll the pump wheel up to pull solution into the pipet. Pull the solution up until the bottom of the meniscus (the concave surface of the liquid in the pipet) is at the volume value desired.
5. Move the pipet into the recipient container. Roll the pump wheel down (all the way) to dispense solution from the pipet. Touch the tip to the side of the container so that adhesion pulls off any liquid on the side of the pipet. Allow the solution to leave the pipet, but do not force out the last tiny bit. The pipets labeled "TD" measure "to delivery." If the last remaining drop is blown out, a mismeasurement will occur.
6. Remove the pipet from the container. Holding the pipet pump bottom, gently twist and pull the pipet out of the pump. Discard the pipet if it is disposable. Clean it if it is reusable.

Precautions for Using Pipets

- **Use a pipet pump to withdraw and dispense liquids** (see Figure 3.4). Do not pipet by mouth.
- **Hold the bottom of the pipet pump when inserting and removing the pipet.** The bottom part of the pipet pump sometimes sticks to the pipet, and can accidentally be pulled out and thrown away. The pipet pump is useless if this happens.
- **Always keep the pipet in an almost vertical position when there is fluid in the tip.** To avoid contamination, do not allow liquid to accidentally run back into the pipet pump.
- **Use your thumb to roll the pipeting gear up and down. Do not pull or push on top of pipet pump.**

Figure 3.4. Always use a pipet pump or pipet aid with a pipet. Never pipet by mouth! Keep the pipet almost vertical when pipeting.
Photo by author.

- **Do not pipet a liquid sample into another liquid, unless directed to do so.** Instead, pipet a sample onto the inside of the recipient vessel and allow it to flow down the vessel. This practice minimizes cross contamination of samples. Gently mix by finger flicking, vortexing, or inverting the container. Avoid bubbling.

Purpose

Which pipets are best used to measure specific volumes?
How precisely can you measure using each pipet (1, 2, 5, and 10 mL)?
How accurately can you prepare samples using each pipet (1, 2, 5, and 10 mL)?

Materials

Tubes, glass, 13×100 mm
Plug caps for 13×100 mm tubes
Permanent lab marker pens
Red dye (1 mL dye: 499 mL dH_2O)
Blue dye (1 mL dye: 499 mL dH_2O)
Green dye (1 mL dye: 499 mL dH_2O)
Yellow dye (1 mL dye: 499 mL dH_2O)
Peg racks for 10–13 mm tubes
Pipets, 1 mL
Pipets, 2 mL
Pipets, 5 mL
Pipets, 10 mL
Pipet pump, blue
Pipet pump, green
Tubes, 50 mL, sterile
Tube racks for 50 mL tubes
Plastic beaker, 1000 1L

Procedure

1. Using a permanent marker, label four empty 13- × 100-mm tubes with I, II, III, and IV, your initials, and the date.
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? Record all of this information. Check with your lab colleagues to ensure that you are reading the pipet correctly.
   Also, check to see if there is a "TD" label on the pipet. If there is, that means that the pipet is accurate "to delivery" and that the tiny drop left in the bottom of the pipet after dispensing should not be forced out.
3. Using the smallest pipet possible, measure the following dye solutions into each tube according to the matrix shown in Table 3.1. Unless told otherwise, add the smallest volume first. Add the next volume to the inside of the tube, allowing it to flow down to the bottom. Finger flick the tube to mix the volumes.
4. Compare the No. 1 through 5 tubes with the "key" tubes provided by the instructor. These are the standards for comparison. For each sample tube, check the level of the final volume, the final color (indicative of accurate measurement and thorough mixing), and proper labeling.

Table 3.1. Practicing with Pipets Matrix

Tubes	Solution I	Solution II	Solution III	Solution IV	Solution V
1	6.3 mL	0.5 mL	0.25 mL	- - -	- - -
2	2.4 mL	1.08 mL	- - -	0.19 mL	0.73 mL
3	4.0 mL	1.5 mL	0.5 mL	- - -	- - -
4	3.5 mL	2.0 mL	- - -	0.25 mL	0.2 mL

5. If the volume of any tube is not within one meniscus of the "key" tubes, it should be remade. Keep track of your attempts to prepare the sample tubes. Make a data table to record your evaluation of your tube preparation with all the observations from Procedure steps 3 through 4.
6. Make a table to collect data on how many students in the class had their first Tube 1 samples fall within the one meniscus range of acceptable variation.

Reflection/Analysis

For volumes between 0.1 mL and 10 mL, explain which pipet and pump are appropriate to use for measuring and dispensing. Make three suggestions that other technicians can use to improve their pipeting accuracy. Based on the Tube 1 class data, describe how much precision the students in the class demonstrated in preparing these samples.

Thinking Like a Biotechnician

1. A 250-µL sample is needed from a sterile vessel that is too thin to use with anything but a sterile pipet. Which pipet and pipet pump could be used to withdraw the mL equivalent to 250 µL from the vessel?
2. 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?
3. Practice pipeting dye-solution samples to create the mixtures specified in Table 3.2.
   a. Measure all solutions into 13×100-mm tubes. Label each tube with the tube letter, your initials, and the date.
   b. Although you may help each other, pipeting the samples as independently as possible is better practice.

Table 3.2. Pipeting Practice Matrix

Tube Letter	Red Dye Volume (mL)	Blue Dye Volume (mL)	Green Dye Volume (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	

- Use the smallest instrument possible for each measurement.
- More than one pipet may be necessary to measure these amounts.
- Evaluation criteria: labels, final volume, and final color/mixing.

aboratory 3b Measuring Very Small Volumes in a Biotechnology Lab

Inspired by a Gene Connection Lab. Gene Connection is a San Mateo County, California, biotechnology education organization.

Background

Very tiny amounts of chemicals and biological reagents are used in many biotechnology experiments. To measure these minute volumes, technicians use micropipets that measure microliter (µL) amounts.

This activity introduces micropipeting technique. As with all fine motor skills, learning how to use a micropipet takes practice and determination. You must be able to measure these very tiny volumes with accuracy. Operate the micropipet slowly and carefully.

Picking and Setting the Micropipet

1. Check that you have the correct micropipet for the job. Most labs have three sizes of micropipets: a P-10 (for 0.5 to 10 µL), a P-100 (for 10 to 100 µL), and a P-1000 (for 100 to 1000 µL (see Figure 3.5).
   Note: Some laboratories have a P-20 (for 2 to 20 µL) instead of a P-10, or a P-200 (for 20 to 200 µL) instead of a P-100.
2. Dial the desired volume. Do you understand how to read the scale? If not, ask your instructor or review the background information in the text. **Hint:** By knowing the maximum volume of 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, white tips are for the P-10; the medium-size, yellow tips are for the P-20, P-100, and P-200; the larger blue tips are for the P-1000. The tips are disposable and usually intended for one use.

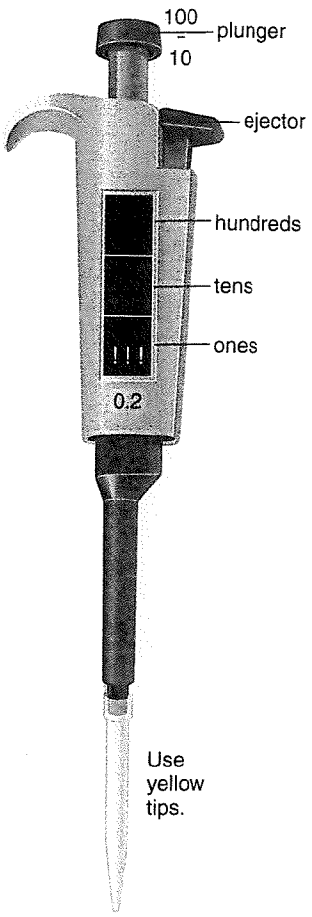


Figure 3.5. P-100 Micropipet. Different models of micropipets are operated slightly differently. Make sure you know how to operate and read the micropipet before using it.

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 45º angle from vertical. In this way, contaminants from your hands or the micropipet will not fall into the tube. Hold the test tube in your other hand. Both should be almost at eye level.
6. **Depress** the plunger of the micropipet to the **first** stop, and **hold** it in this position.
7. Place the tip into the solution to be pipeted.
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. Hold the micropipet in one hand, at about a 45º angle from vertical. Hold the tube in your other hand. Both should be at about eye level.
11. Gently touch the micropipet tip to the inside wall of the reaction 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.
12. Slowly, depress the plunger of the micropipet to the first stop. Then, continue to the second stop to expel the last bit of fluid, and hold the plunger in this position (see Figure 3.6).
13. Slowly, remove the pipet from the tube, keeping the plunger depressed to avoid drawing any liquid back into the tip.
14. Always change tips for each new reagent you pipet. To eject a tip, depress the ejector button on the top of the micropipet.

Precautions for Using Micropipets

- Set pipet volume *only* within the range specified for that micropipet. Do not attempt to set a volume beyond the pipet's minimum or maximum values.
- When using a micropipet, first apply a tip. 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.

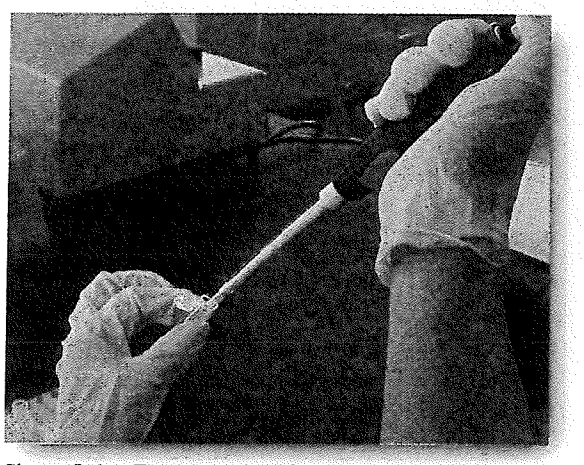


Figure 3.6. To ensure that the entire sample is released, push the plunger all the way down to the second and final stop as you withdraw the tip from the collection tube.
Photo by author.

Purpose

Which micropipets are best used to measure specific volumes?
How precisely can you measure using each micropipet?
How accurately can you prepare samples using each micropipet?

Materials

Tube rack for 1.7 mL tubes
Reaction tubes, 1.7 mL
Permanent lab marker pens
Pack of 4 colors, package of 4
Red dye (1 mL dye:499 mL dH_2O)
Blue dye (1 mL dye:499 mL dH_2O)
Green dye (1 mL dye:499 mL dH_2O)
Yellow dye (1 mL dye:499 mL dH_2O)
Micropipet, P-10
Micropipet, P-100
Micropipet, P-1000
Micropipet tips for P-10

Micropipet tips for P-100
Micropipet tips for P-1000
Tubes, 50 mL, sterile
Tube racks for 50 mL tubes
Plastic beaker, 1000 1L
Microcentrifuge
Wax paper

Procedure

Practicing with a P-10 or P-20

1. Using a permanent marker, label two empty reaction tubes with A and B, and your initials.
2. Add the specified amounts of each solution to tube A or B, as listed in Table 3.3. Unless told otherwise, add the smallest volume first. Add each volume to the inside of the tube without letting the drops touch. The drop will stick because of adhesion. When all volumes have been added, bring the drops to the bottom of the tube with a quick wrist flick.

Table 3.3. P-10 or P-20 Practice Matrix

Reaction Tubes	Solution I	Solution II	Solution III	Solution IV	Solution V
A	4.0 µL	5.0 µL	2.0 µL	- - -	- - -
B	6.5 µL	2.5 µL	- - -	- - -	2.0 µL

3. Spin tubes A and B in the microcentrifuge for 1 to 2 seconds to pool the solutions. See the centrifuge instructions at the end of the procedures.
4. After centrifuging, compare your tubes with the standard "key" tubes and other A and B tubes in the class. Both tubes A and B should contain 11 µL. Check the tube volume, color/mixing, and labeling.
5. As an additional check of accuracy, set the micropipet to 11 µL and carefully withdraw all of the fluid in tube A. The contents should *just* fill the tip, with no air space at the bottom of the tip and no leftover fluid in the tube. Repeat with tube B.
6. Is there any liquid left in the microtest tube? If so, determine its volume. What *percent error* did you have in your pipeting of this small volume? Use the equation below to determine the percent error in pipeting this sample:

$$\frac{\text{amount left in test tube}}{\text{total amount pipeted}} \times 100 = \%\ \text{error in measurement}$$

Practicing with a P-200 or P-100

1. Label an empty reaction tube C.
2. To tube C, add the solution volumes shown in Table 3.4. Unless told otherwise, add the smallest volume first. Add each volume to the inside of the tube without letting the drops touch. The drop will stick because of adhesion. When all volumes have been added, bring the drops to the bottom of the tube with a quick wrist flick.

Table 3.4. P-100 or P-200 Practice Matrix

Reaction Tube	Solution I	Solution II	Solution III	Solution IV	Solution V
C	22.3 µL	31.6 µL	- - -	44.4 µL	- - -

3. Spin tube C for 1 to 2 seconds. Make sure that you balance the microcentrifuge with another tube that contains the same volume.
4. Check the accuracy of your technique with the P-100 or P-200. Set the micropipet to 98.3 µL and withdraw the contents of tube C. Also, compare your tube with the standard "key" tubes and other "C" tubes. The contents should *just* fill the tip, with no air space at the

bottom of the tip, and no leftover fluid in the tube. Check the tube volume, color/mixing, and labeling.

5. Is there any liquid left in the microtest tube? If so, determine its volume. What percent error did you have in your preparation of this sample?

Practicing with a P-1000

1. Label an empty reaction tube as tube D.
2. To tube D, add the solution volumes shown in Table 3.5.

Table 3.5. P-1000 Practice Matrix

Reaction Tube	Solution I	Solution II	Solution III	Solution IV	Solution V
D	253 µL	- - -	- - -	- - -	557 µL

3. Along with a balance tube, spin tube D in the microcentrifuge for 1 to 2 seconds (see Figure 3.7).
4. Check the accuracy of your technique with the P-1000. Set the pipet to 810 µL and withdraw the contents of tube D. Also, compare your tube with the standard "key" tube. Check the tube volume, color/mixing, and labeling.
5. Is there any liquid left in the microtest tube? If so, determine its volume. What percent error did you have in preparing this sample?

Centrifuge Instructions

- Tightly close the caps on all of the tubes to be placed in the microcentrifuge (also called microfuge).

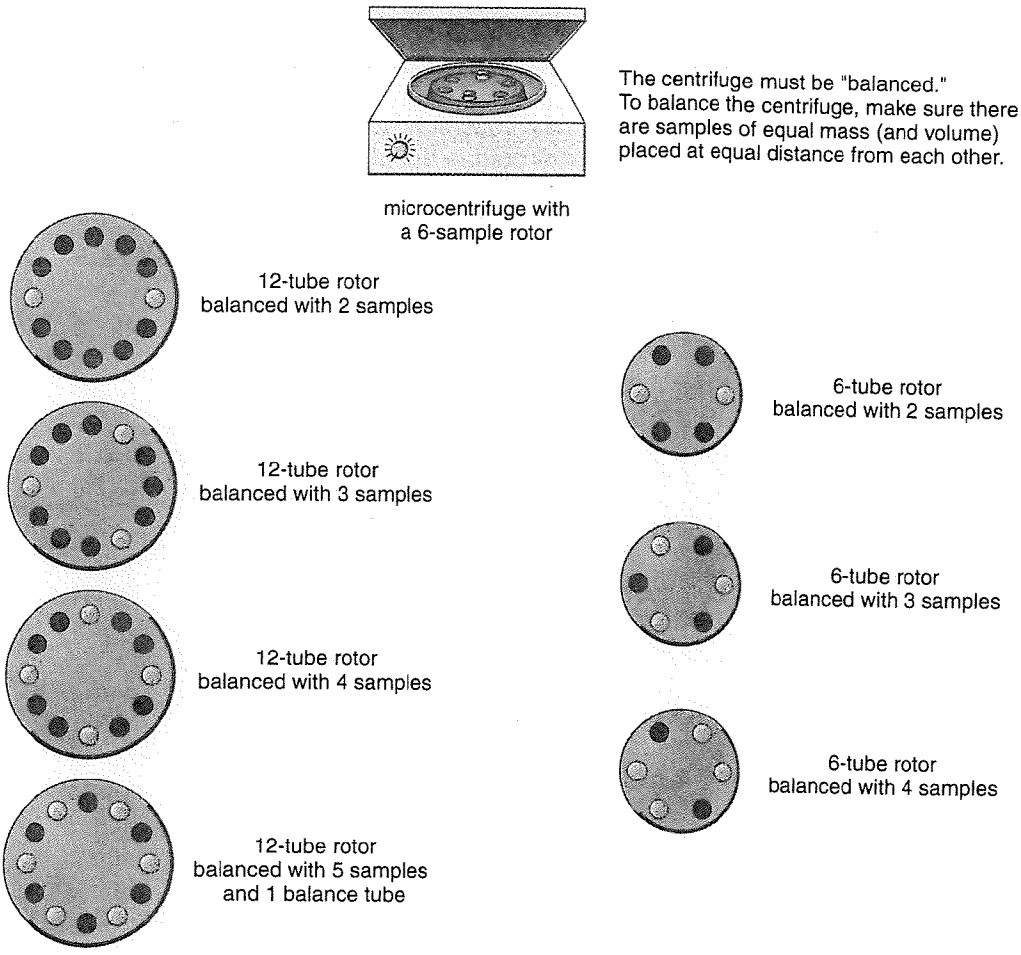


Figure 3.7. Centrifuge Basics.

- The microfuge (sample holder) rotor must always be balanced. You cannot, for example, spin *one* tube in a microfuge. **Spinning in an unbalanced arrangement like this would damage the motor and ruin the centrifuge.**
- The volume and mass of sample in the tubes should be the same. Otherwise, the rotor will spin unevenly (like wet towels spinning out of balance in a washing machine). You can always prepare a "blank" tube with the same volume of liquid to balance a single tube.
- After you have replaced the metal top (if your type of microfuge has a rotor top) and secured the lid of the microfuge, give the tubes a 1- to 2-second pulse. This will mix and pool all the reagents into a droplet in the bottom of each tube.

Reflection/Analysis

For volumes between 1 and 1000 µL, explain which micropipet is appropriate to use for measuring and dispensing. Make three suggestions that other technicians can use to improve their micropipeting accuracy.

Thinking Like a Biotechnician

1. For most experiments, several reagents must be added to the same tube. Propose a method to keep track of the samples that have been added to a reaction tube.
2. Demonstrate the effect of micropipeting incorrectly by doing the following:
   a. Set a P-20 or P-10 to 2 µL.
   b. Purposely, misuse the P-20 or P-10 pipet, and depress the plunger to the second stop.
   c. Suck up this apparent 2-µL volume and release onto a piece of wax paper.
   d. Now, correctly collect a 2-µL volume using the P-20 or P-10.
   e. Release it onto the wax paper next to the other drop. Are the drops noticeably different in size?
   f. How much more is there in the "misused" volume? (Use the pipet to suck up the misused volume in 2-µL increments.)
   g. If a balance or scale is available, and you have been trained to use it, make these measurements on it. Determine the percentage error that would occur if you were to accidentally misuse the pipet in this fashion.
3. Practice micropipeting samples to create mixtures by producing the four tubes in Table 3.6.
   a. Measure all solutions into 1.7-mL microtubes. Label all tubes with the tube number, your initials, and the date.
   b. Although you may help each other, micropipeting as independently as possible is better practice.

Table 3.6. Micropipeting Practice Matrix

Tube No.	Red Dye Volume (µL)	Blue Dye Volume (µL)	Green Dye Volume (µL)	Total Volume (mL)
1	27.2	313.0	59.3	
2	555.0	222.0	7.8	
3	133.3	19.8	235.0	
4	9.4	4.1	2.25	

- Use the smallest instrument possible for all measurements.
- Change tips every time.
- More than one pipet may be necessary to measure amounts.
- Total volumes may be checked by using a P-1000.
- Evaluation criteria: labels, final volume, and final color/mixing.

Laboratory 3c Measuring Mass

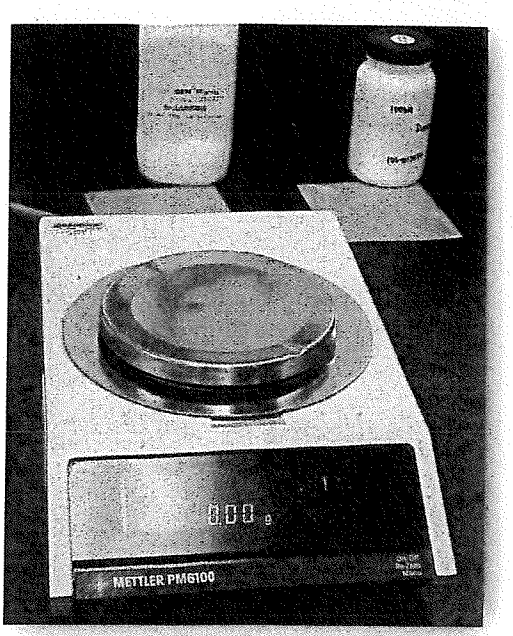


Figure 3.8. This electronic tabletop balance reads to 0.01 g.
Photo by author.

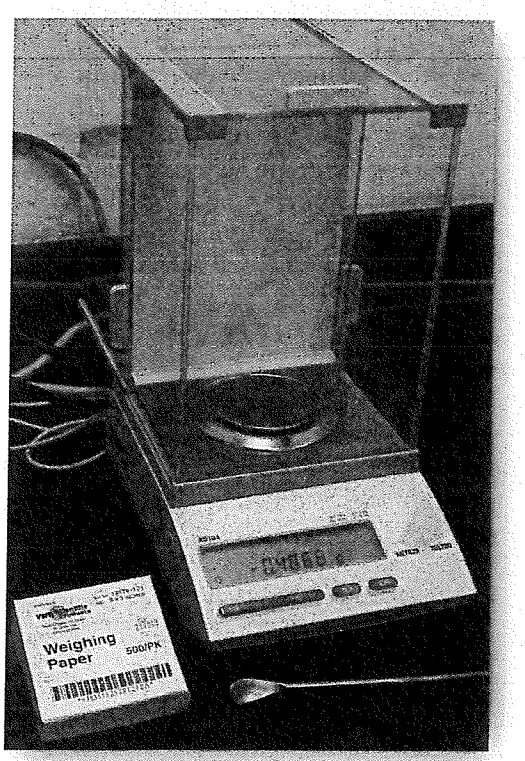


Figure 3.9. This electronic tabletop balance reads to 0.0001 g.
Photo by author.

Background

Using DNA, enzymes, and other reagents in the laboratory requires weighing small amounts, or masses, of these chemicals. Measurements must be made using precision instruments. In a biotechnology laboratory, mass measurements are performed on electronic balances or scales. There are several different kinds of balances with different features, depending on the manufacturer and the cost.

Basically, electronic balances come in two forms: 1) tabletop/portable (see Figure 3.8) ranging from $300 to $1500 each, and 2) analytical balances, beginning in price at about $1500 each (see Figure 3.9).

The tabletop (top-loading) balances vary in the precision they measure, and each balance has a maximum mass that may be measured. Some measure to within 1 g, some measure to within 0.1 g, and some measure to 0.01 g. The last decimal place is an approximation.

Each balance is used in a similar fashion. The weighing protocol that follows ensures that the balance is used properly and is not damaged.

Weighing Protocol

Note: Wear goggles and gloves when using chemicals.

1. Set the balance on a clean, dry, flat surface.
2. If there is a leveling apparatus, level the balance.
3. Check that the power cord is plugged in properly. Press the "ON" button. The balance will undergo a series of self-checks.
4. Check to make sure that the balance is displaying a "g," to show that it will be measuring in grams. If it is not displaying the "g," press the "MODE" button until it does.
5. Make sure that the weigh pan is clean.
6. Press the "TARE" or "zero" button to zero the balance.
7. Add a weigh boat or a piece of weigh paper. **Never place chemicals directly on the weighing pan. Weigh boats and weigh paper are single-use-only items.**
8. Press the "TARE" button again to zero the balance.
9. Using a clean scoop, add the chemical to be weighed to the weigh boat until the desired mass is obtained. Keep the stock bottle directly over the weigh boat to minimize spills.
10. Remove the weigh boat/paper. Close bottles and return them to the chemical stock area. Clean any spilled chemicals.

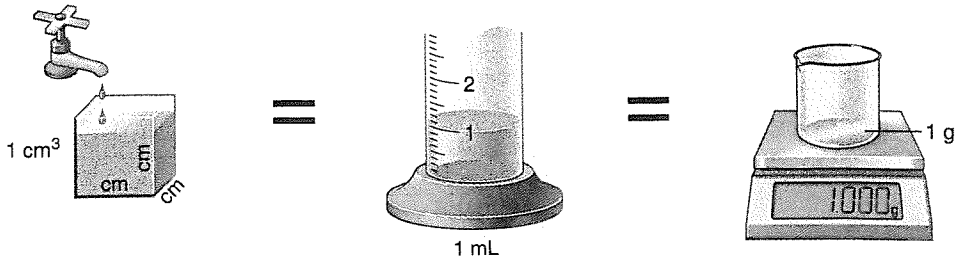
In this activity, you will prepare and test glucose solutions using Diastix® (Bayer Diagnostics) brand glucose test strips. The test strips, which detect the presence of glucose in urine and other solutions, measure in mg/dL. One dL, or deciliter, is equal to 0.1 L, or 100 mL. Using the B ← → S Rule, one can convert between dL and mL.

Laboratory 3d Checking the Accuracy of Micropipets Using a Balance

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, you can estimate the expected mass for any volume of water (see Figure 3.11). 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.



1 milliliter of water takes up a cubic centimeter of space and weighs 1 gram.

Figure 3.11. Mass/Volume Equivalents.

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. For any measurement, calculate the % error using the following equation:

$$\frac{(\text{observed mass} - \text{expected mass})}{\text{expected mass}} \times 100$$

Purpose

To measure small volumes of water on an appropriate balance.
To check the accuracy of a micropipet measurement.

Materials

Balance, analytical
Balance, tabletop milligram
Weigh paper, 7.6×7.6 cm
Micropipet, P-10
Micropipet, P-100
Micropipet, P-1000
Micropipet tips for P-10
Micropipet tips for P-100
Micropipet tips for P-1000
Tap water

Procedure

1. Review the use of pipets, pipet pumps, micropipets, and electronic and analytical balances. Specifically, check the following:
   a. Make sure you can read and set the micropipets.
   b. Make sure that you are withdrawing and dispensing volumes properly. Remember to check the feel of the first and second stops on the micropipets.

c. Review the proper way to set up (zero/tare) and read the electronic and analytical balances.
d. Review the proper way to convert between metric units, specifically mL and µL (ie, use the B ←→ S Rule).

2. For each micropipet, measure the specified volume of tap water onto a piece of weigh paper on a "tared" balance. In a data table similar to Table 3.8, record the mass of the volume of water measured. Record the type of balance used.

Table 3.8. Pipeting Precision: Mass versus Volume

Micropipet	Volume (µL)	Volume (mL)	Expected Mass (g)	Actual Mass (g)	% Error	Acceptable Error (%)	Type of Balance Used
P-1000	1000.0					3	
P-1000	500.0					3	
P-1000	257.0					3	
P-100	100.0					5	
P-100	53.0					5	
P-100	20.0					5	
P-10	20.0					10	
P-10	13.7					10	
P-10	2.0					10	

3. Determine if the micropipet is measuring within the acceptable range of error. Calculate and record the percentage error for each micropipet delivery using the % error equation:

$$\frac{\text{amount left in test tube}}{\text{total amount pipeted}} \times 100 = \%\text{ error in measurement}$$

Data Analysis

For any error that is outside of the acceptable range, remeasure. Make sure that you are using the instruments (balances and micropipets) correctly. Get help if necessary. If you 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. Record comments. Notify your instructor or lab supervisor of any equipment that does not appear to function correctly.

Thinking Like a Biotechnician

Suppose another lab technician in your group is dispensing 100 µL volumes into 10 1.7-mL tubes. On inspection of the samples, several are visibly different from the others and not within the acceptable range of error.

1. Suggest one thing that the technician could do in his operation of the micropipet to improve his pipeting technique.
2. Suggest something that the technician could do to ensure that the micropipet is measuring correctly.

PIPETTING

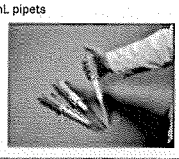
MEASURING SMALL VOLUMES IN A BIOTECHNOLOGY LAB

TIMELINE

Monday—Lecture, per quiz
Tuesday—Lab 3A & Lab 3B
Thursday—3A individual skills quiz
Monday—No School
Tuesday—3B individual skills quiz
Thursday—Lab 3D, post quiz

LAB 3A: MEASURING SMALL VOLUMES IN A BIOTECHNOLOGY LAB

- Concept
  - Learn how to use serological pipets to measure volumes between 1mL - 10mL
  - Introduce pipeting techniques
- Background
  - The most commonly used pipets are 10-, 5-, 2-, and 1-mL pipets
  - They are named by the maximum volume they can hold
  - A blue pump represents a 1- or 2-mL pipet
  - A green pump represents a 5- or 10-mL pipet
  - A red pump represents a 25- or 50-mL pipet



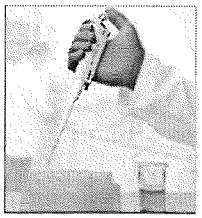
LAB 3A CONTINUED

- Using a pipet pump
  - Make sure to use the correct pump when pipeting
  - Insert the pipet into a pipet pump
  - Put the pipet tip into the solution
  - Keeping the tip under the surface and holding the pipet at eye level, roll the pump wheel to pull up the solution
  - The meniscus should be at the volume desired
  - Move the pipet into the new container and roll the pump wheel down to expel the contents
  - Touch the tip to the side of the container to remove any liquid on the side
  - Remove the pipet from the container then gently twist and pull the pump off

LAB 3A TIPS & CHANGES

Label tubes A-D
Solution I (red) II (yellow) III (green) IV (blue) V (clear)
Skip Procedure 6
Work in pairs
Meniscus—measure from the bottom of the meniscus
Add smallest volume first
Label the tops of the tubes (sample, initials, date)
Use the smallest pipet possible
Rinse pipet between colors, wrist flick remaining drops
At the end of class, rinse out test tubes and dry them on your rack

LAB 3B: MEASURING VERY SMALL VOLUMES IN A BIOTECHNOLOGY LAB



- Concepts
  - Introduce micropipeting techniques
  - Measure very tiny volumes with accuracy
- Background
  - Micropipets measure volumes in microliters
  - You will be using a P-10, P-100, or P-1000
  - You should use the smallest micropipet possible
- How to Use a Micropipet

http://www.youtube.com/watch?v=wlO4zJLR8R8&feature=relmfu

TIPS FOR 3B

Work as a group of 4, take turns

Solution I (red) II (blue) III (green) IV (yellow) V (clear)

Add smallest volume first

10—biggest # is tens place, 100-biggest # is hundreds place, 1000—biggest # is thousands place (but this depends on the style of pipet)

Change tips—put in beaker and then in trash

Have recipient tube ready

Label the tops of the tubes (sample, initials, date)

LAB 3D: CHECKING THE ACCURACY OF MICROPIPETS USING A BALANCE

- Concepts
  - Learn how to use a balance
  - Learn how to check the accuracy on a pipet
  - Calculate % error and % error in measurement
- Background
  - A balance can be used to determine if a micropipet is measuring within an acceptable range
  - 1 mL of water weighs 1 gram
  - Pipet a certain amount of water and expel onto a zeroed balance
  - Calculate the % error using: $\frac{\text{observed mass} - \text{expected mass}}{\text{expected mass}} \times 100 =$ % error
  - Calculate the % error in measurement using: $\frac{\text{amount left in test tube}}{\text{total amount pipeted}} \times 100 =$ % error

LAB 3D TIPS & CHANGES

1ml water=1 gram, so 1uL=1mg

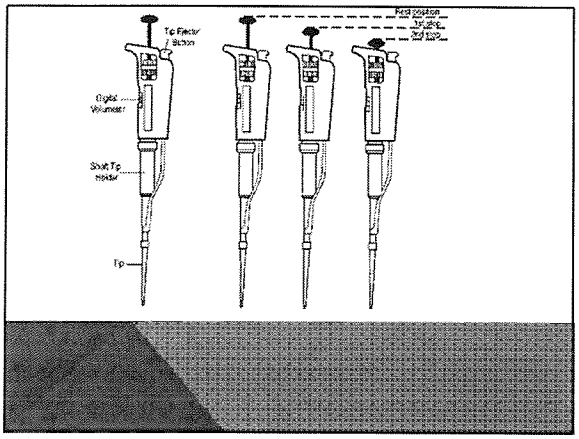
Check pipets monthly

Larger pipets have larger errors

When delivering water from the micro-pipet onto the weigh paper, use a 45 degree angle to prevent splattering

GENERAL TIPS

- Hold pipet at eye level
- Keep pipet in vertical to almost vertical position
- Open caps or lids of containers before picking up a pipet
- Never leave pipet tip box open
- Make sure to use the smallest pipet possible
- More then one pipet may be necessary
- Microcentrifuge in large groups so it goes quicker
- Make sure the balance is zeroed



TUTORIALS

Using a pipet demo (tutorial 2)
Micropipet (tutorial 3)

Measurements and Micropipetting

This laboratory introduces micropipetting techniques that are used in many of the experiments you will perform during this workshop. Mastery of these techniques is important for good results in the experiments that will follow. Most molecular biology and microbiology laboratories are based on microchemical protocols that use very small volumes of DNA and reagents. These require the use of an adjustable micropipettor that measures as little as one microliter (ml), one millionth of a liter!

Become familiar with metric units of measurement and their conversions. We will concentrate on liquid measurements based on the liter, but the same prefixes (milli and micro) also apply to dry measurements based on the gram. The two most useful units of liquid measurement in molecular biology are the milliliter (mL) and the microliter (ml).

1 mL = 0.001 liter 1,000 mL = 1 liter

1 ml = 0.000001 liter 1,000,000 ml = 1 liter

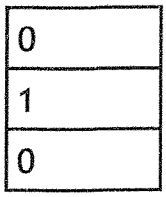
A digital micropipettor is essentially a precision pump fitted with a disposable tip. The volume of air space in the barrel is adjusted by screwing the plunger farther in or out of the piston, and the volume is displayed on a digital readout. Depressing the plunger displaces the specified volume of air from the piston; releasing the plunger creates a vacuum, which draws an equal volume of fluid into the tip. The withdrawn fluid is then expelled by depressing the plunger again.

The volume range of a digital micropipettor varies from one manufacturer to another. A small volume micropipettor (with a range of 0.5–10 ml or 1-20 ml) and a large volume micropipettor (100-1,000 ml) are used most frequently. A mid range micropipettor (10-100 ml or 20–100 ml) is used less frequently.

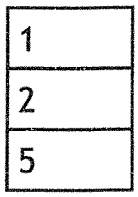
Familiarize yourself with small scale and large scale micropipettors that you will be using in this laboratory. A P20 micropipettor measures volumes between 2-20 ml. Locate the digital readouts on your micropipettors. It is located below the plunger. The digital readout has three number boxes that display the specified volume.

2
0
0

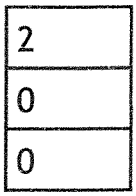
The above digital readout indicates a reading of 20 ml on a 2-20 ml volume micropipettor.



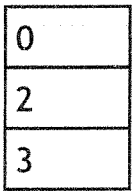
The above digital readout indicates a reading of 10 ml on a 2-20 ml volume micropipettor.



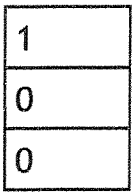
The above digital readout indicates a reading of 12.5 ml on a 2-20 ml volume micropipettor.



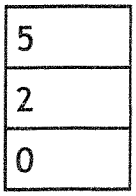
The above digital readout indicates a reading of 200 ml on a 20-200 volume micropipettor.



The above digital readout indicates a reading of 23 ml on a 20-200 volume micropipettor. .



The above digital readout indicates a reading of 1000 ml or 1 mL on a 100-1000 ml micropipettor.



The above digital readout indicates a reading of 520 ml on a 100-1000 ml micropipettor.

Take the following precautions when using a digital micropipettor:

· Never rotate the volume adjustor beyond the upper or lower range of the pipet, as stated by the manufacturer.

· Never use the micropipettor without the tip in place; this could ruin the piston.

· Never invert or lay the micropipettor down with a filled tip; fluid could run back into the piston.

· Never let the plunger snap back after withdrawing or expelling fluid; this could damage the piston.

· Never immerse the barrel of the micropipettor in fluid.

· Never flame the tip of the micropipettor.

· Never reuse a tip that has been used to measure a different reagent.

I. General Use of Digital Micropipettors

1. Rotate the volume adjustor to the desired setting. Note the change in plunger length as the volume changes. Be sure to properly locate the decimal point when reading the volume setting.

2. Firmly seat a proper-sized tip on the end of the micropipettor.

3. When withdrawing or expelling fluid, always hold the tube firmly between your thumb and forefinger. Hold the tube nearly at eye level to observe the change in the fluid level in the pipet tip. Do not pipet with the tube in the test tube rack. Do not have another person hold the tube while you are pipetting.

4. Each tube must be held in the hand during each manipulation. Open the top of the tube by flipping up the tab with your thumb. During manipulations, grasp the tube body (rather than the lid), to provide greater control and to avoid contamination of the mouth of the tube.

5. For best control, grasp the micropipettor in your palm and wrap your fingers around the barrel; work the plunger (piston) with the thumb. Hold the micropipettor almost vertical when filling it.

6. Most digital micropipettors have a two-position plunger with friction "stops". Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any solution remaining in the tip. Notice these friction stops; they can be felt with the thumb.

7. To withdraw the sample from a reagent tube:

    a. Depress the plunger to first stop and hold it in this position. Dip the tip into the solution to be pipetted, and draw fluid into the tip by gradually releasing the plunger. Be sure that the tip remains in the solution while you are releasing the plunger.

    b. Slide the pipet tip out along the inside of the reagent tube to dislodge any excess droplets adhering to the outside of the tip.

    c. Check that there is no air space at the very end of the tip. To avoid future pipetting errors, learn to recognize the approximate levels to which particular volumes fill the pipet tip.

    d. If you notice air space at the end of the tip or air bubbles within the sample in the tip, carefully expel the sample back into its supply tube. Coalesce the sample by sharply tapping the tube on the bench top or pulsing it in a microfuge.

8. To expel the sample into a reaction tube:

    a. Touch the tip of the pipet to the inside wall of the reaction tube into which the sample will be emptied. This creates a capillary effect that helps draw fluid out of the tip.

    b. Slowly depress the plunger to the first stop to expel the sample. Depress to second stop to blow out the last bit of fluid. Hold the plunger in the depressed position.

    c. Slide the pipet out of the reagent tube with the measurement plunger depressed, to avoid sucking any liquid back into the tip.

    d. Manually remove or eject the tip into a beaker kept on the lab bench for this purpose. The tip is ejected by depressing the plunger beyond the second stop.

9. To prevent cross-contamination of reagents:

    a. Always add appropriate amounts of a single reagent sequentially to all reaction tubes.

b. Release each reagent drop onto a new location on the inside wall, near the bottom of the reaction tube. In this way, the same tip can be used to pipet the reagent into each reaction tube.

c. Use a fresh tip for each new reagent to be pipetted.

d. If the tip becomes contaminated, switch to a new one.

From: http://www.cabrini.edu/sepchedna/2002%20Workshop/Labs/measurements_and_micropipetting.htm

Measurements and Micropipetting Comprehension Questions

1. How is the volume of air space in the barrel of a micropipet adjusted? Explain.

2. Explain how to read a digital readout on a miropipet.

3. State at least four precautions when using a micropipettor.

4. Explain the difference between the first stop and the second stop on a micropipet.

5. How do you prevent cross examination of reagents?

6. How do you expel a sample?

7. What is recommended for best control of the pipet?

8. Give some examples of a reading on a digital readout and the pipet used.

9. What is a digital micropipettor?

10. Convert these measurements to liters.

0.033 mL 7,594 mL 67 mL

What Is A Serological Pipette?

Often used in biological laboratories as well as in cosmetics and food production, a serological pipette typically helps transfer small amounts of liquid. It is generally a relatively narrow plastic tube with markings for volume measurements, which are usually labeled in milliliters. Liquid solutions can be transferred from one place to a different container, and a small manual pump is often used to get all of the fluid at the tip out of the device. Pipettes in which some fluid remains and is typically part of the total volume are called to contain, while to deliver variants have extra fluid at the tip that is usually discarded. Most serological pipettes are labeled according to what type they are.

Many pipettes are disposable and can be purchased as one component or a package of up to 50 or more units. The volume markings are usually derived during a temperature calibration, which can allow for accuracy in a 68° to 77°F (20° to 25°C) range. Inside the serological pipette, liquid typically forms a pattern called a meniscus in response to the interior shape. Researchers can get the correct reading by holding the device straight up and lining up the bottom of the meniscus with the nearest measurement line. Measurements can be done by emptying the entire pipette, or by draining a specific amount of fluid by noting the desired volume marking to change the level to.

Small, handheld pumps are often used to drain a serological pipette. These can feature a button to activate, a thumb roller, or a variety of other designs. Some pumps have a filter that may need to be changed periodically. Depending on the application, some pipettes are designed to be more accurate. Many are manufactured as one single part, often molded out of a plastic composite. They can also be designed without welds so there are no pieces inside the pipette that can trap liquid.

A serological pipette can have a plug, sometimes made of cotton, which helps to prevent researchers from overfilling it. In packages of many pipettes, the plug can be color-coded along with other labels to indicate specific sizes and types in the box. Serological pipettes and related scientific equipment are often used in laboratories where biological and chemical compounds are used. They are usually

non-toxic, and are sometimes used in educational settings. Hospitals and even industrial facilities can make use of serological pipette products as part of their inventory.

From: http://www.wisegeek.com/what-is-a-serological-pipette.htm

What Is A Serological Pipette? Comprehension Questions

1. What does a serological pipet typically do and what is it often used for?

2. What is a meniscus and why is it important?

3. How can measurements be done?

4. What does a plug do and what can it be made out of?

5. Where are serological pipets used?

6. Where are the volume markings on the pipets derived from?

7. How are serological pipets labeled?

8. Describe a serological pipet.

9. What is a serological pipet used for?

10. What are some of the features that hand held pump on these pipets have?

Calibration of Micropipettes
Introduction:

Accuracy and Precision

Pipettes and micropipettes can deliver accurate and precise volumes of solution. Our goal is to determine how accurate and how precise our pipette and micropipette are.

Accuracy is a measure of how close a measured value is to the accepted or "true" value. It is related to the percent error between the average volume of solution measured experimentally and the volume that was expected (the accepted value). Smaller percent error reflects higher accuracy. Percent error can be negative, indicating that the measured volume was smaller than the expected volume or positive, indicating that the measured volume was larger than the expected volume. For example, we are attempting to measure two different volumes of water with our micropipette and two with our graduated pipette. Perfect accuracy would have us measure the exact volume we desire each time. However, the volume of water that we actually measure will be close but probably different from these volumes. The farther away from the correct volume, the lower the accuracy of our pipettes and/or our technique will be. The formula for percent error is in the Statistical Functions portion of the Lab Manual Introduction.

Precision measures the closeness of a set of values obtained from identical measurements of the same quantity. It is the ability to repetitively measure the same volume of solution (whether it's accurate or not). Precision is related to the standard deviation of a series of measurements of the same thing. For example, if the micropipette is set to the same volume (300 μL) and four measurements are taken at this volume, a standard deviation can be taken of these five measurements. The smaller the standard deviation, the more precise the micropipette is. We will use the standard deviation as a measure of the spread of potential errors in a given measurement. The formula for standard deviation is in the Statistical Functions portion of the Lab Manual Introduction. Standard deviation is usually reported with the average value like this:

In order to minimize the waste generated from experiments in this class, a number of the experiments involve a micropipette that can deliver between 100 and 1000 microliters (μL). The micropipettes are only to be used for this volume range. For larger volumes, a graduated pipette is in your locker (bulbs available from the stockroom or one of the side drawers).

The Equipment

1) Tips - It is advocated that only high quality tips which optimize the pipette's performance be used. A high quality tip is one that has a smooth uniform interior with straight even sides that prevents the retention of liquids and minimizes surface wetting. Also, the tip should have a clean, hydrophobic surface and a perfectly centered opening in order to ensure the

complete dispensing of the sample. These tips should always securely interface with the nosecone, because if they do not fit correctly, the amount of liquid dispensed can be dramatically influenced.

2) Liquid Viscosity - Since the pipette was originally factory calibrated using water, any liquid that has a viscosity higher or lower than water will impact the volume dispensed. Viscosity differentials should be accounted for and taken into consideration in order to enhance the accuracy of the instrument.

3) Container - The material of construction for the extraction vessel is also important, since some materials tend to force water into a convex configuration while other materials force water into a concave configuration. Obviously, this can impact the amount of liquid drawn into the tip. A glass container is recommended since it tends to force water into a concave configuration which helps to reduce or eliminate variations due to this effect.

The Operator

1) Technique - Most end users have a tendency to believe that the volume delivery is completely

dependent on the setting of the micrometer dial. Obviously, this is not the case, since many factors associated with pipettes come into play.

• **Position** - Pipettes should be held vertical during the aspiration of liquids,however, some end users often hold pipettes at many different angles during a pipetting interval. Holding a pipette 30o off vertical can cause as much as 0.7% more liquid to be aspirated due to the impact of hydrostatic pressure. Always store pipettes in an upright position when not in use.

• **Pre-Wetting/Pre-Rinsing Tips** -Failing to pre-wet tips can cause inconsistency between samples since liquid in the initial samples adhere to the inside surfaces of the pipette tip, but liquid from later samples does not. Also, if a new volume is dialed in on the pipette's micrometer, you will receive better results at the new volume by taking the old tip off and placing a new one on the shaft before you commence pipetting.

• **Release of Plunger** - Releasing the plunger abruptly can cause liquid to be "bumped"inside the pipette during a liquid transfer application. This can cause liquid to accumulate inside the instrument which in turn can be transferred to other samples causing variability in sample volume and the potential for cross contamination. It is recommended that a smooth, consistent pipetting rhythm be employed since it helps to increase both accuracy and precision. After the liquid has been aspirated into the tip, the pipette should be placed against the wall of the receiving vessel and the plunger slowly depressed. This will help all of the liquid in the tip to be dispensed. After a pause of about 1 second, depress the plunger to the bottom or blowout position (if equipped) and remove the pipette from the sidewall by utilizing either a sliding action up the wall or a brief movement away from the wall (called "touching off").

• **Immersion Depth** - The pipette tip should only be inserted into the vessel containing the liquid of be transferred about 1-3mm. If the tip is immersed beyond this, the results could be erroneously high. This is due to the fact that liquid could adhere to the tip and be transferred along with the aliquot in the tip. If the tip is not immersed far enough then air could be drawn into the tip which could yield results that are incorrect on the low end.

pipette can then be assessed by using an appropriate statistical approach. This method can be performed one of four ways: Addition, Addition-Tare, Subtraction, or Subtraction-Tare.

1) Addition is perhaps the most common mode of pipette calibration and it is performed by using the cumulative weight of a liquid to determine the volume dispensed.

2) The Addition-Tare method is performed by taring the balance each time before dispensing.

3) The Subtraction method uses the total subtracted weight of a liquid to determine the volume aspirated by the pipetting device. In this technique, you tare the balance only once, at the beginning, then you aspirate volumes of liquid from the vessel, take cumulative (negative) weights, and then calculate the volume aspirated based on the difference between the current and previous total weights.

4) The Subtraction-Tare method entails taring the balance each time before removing liquid from the vessel.

Since this method is not fool-proof, all variables must be stringently controlled and accounted for in order to produce results that are statistically accurate. The second most common type of pipette calibration process is the colorimetric or photometric method. This method involves the analysis of volumes of diluted dye in a cell of known path length. According to the Beer–Lambert Relationship, if a beam of monochromatic light passes through homogeneous solutions of equal pathlength, the absorbance measured is proportional to the dye concentration. So, with this in mind, an unknown volume of dye can be pipetted into a known volume of diluent, the resulting dye concentration can be measured photometrically, and the volume can be calculated. This method is less prone to environmental influences, but it requires the use of standardized consumables. Obviously, this means that each lot of standardized dye must be very carefully manufactured and calibrated in order to produce results of high accuracy. However, once solutions are prepared, calibrated and shown to be stable, accurate results can be obtained even at volumes less than one microliter11.

Calibration of Micropipettes Comprehension Questions

1. What is accuracy and why is it important?

2. What is precision and why is it important?

3. What is important about immersion depth?

4. What are the two most common ways to calibrate a pipet and which is more common?

5. Explain the colormetric method.

6. Explain the gravimetric method.

7. How do you prepare a balance for calibration?

8. Why should you never release a plunger quickly?

9. What should the position of the pipet be when in use as well as when not in use?

10. What is considered a high quality tip?