Experiment 1
Use of Micropipettes: measuring volume accurately

Prior to your hands-on training, you should understand:

- The function of micropipettes in the laboratory
- Basic parts of micropipette
- What volumes are measured with P10, P100 and P1000 micropipettes
- How to read the volume indicator on a P10, P100 and P1000

I. Objective:

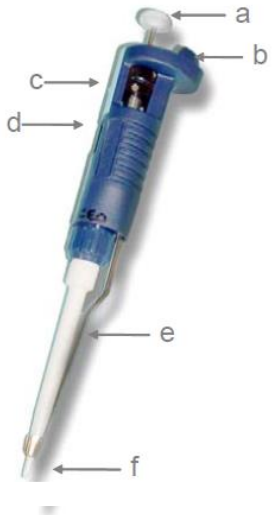
- **Learn how to use micropipettes**

II. Background:

Micropipettes are the standard laboratory equipment used to measure and transfer small volumes of liquids. The liter is the metric volume standard, and one microliter (µl) is one millionth of a liter. Inaccurate pipetting is a chief contributor to poor laboratory results. Micropipettes are used for accurately transferring small volumes of liquid in the milliliter and microliter range. You will use them throughout this semester and in advanced courses that you take in the future. It is essential that you master their use if you are to be successful in your experiments.

A. Parts of a micropipette

a. Plunger button
b. Tip ejector button
c. Volume adjustment dial
d. Digital volume indicator
e. Shaft
f. Attachment point for a disposable tip



B. Three sizes of micropipettes

The micropipettors in this laboratory come in three different sizes each of which measures a different range of volumes. The three sizes are P20, P200 and P1000. These sizes are noted on the top of the plunger button.

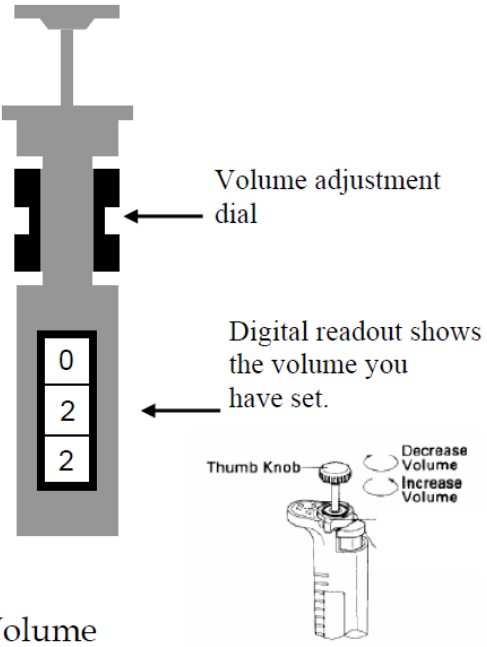
Size Micropipette	Range of volumes measured
P10	0.5-10 µl
P100	10-100 µl
P1000	100-1000 µl

C. Adjusting Volume on micropipettes

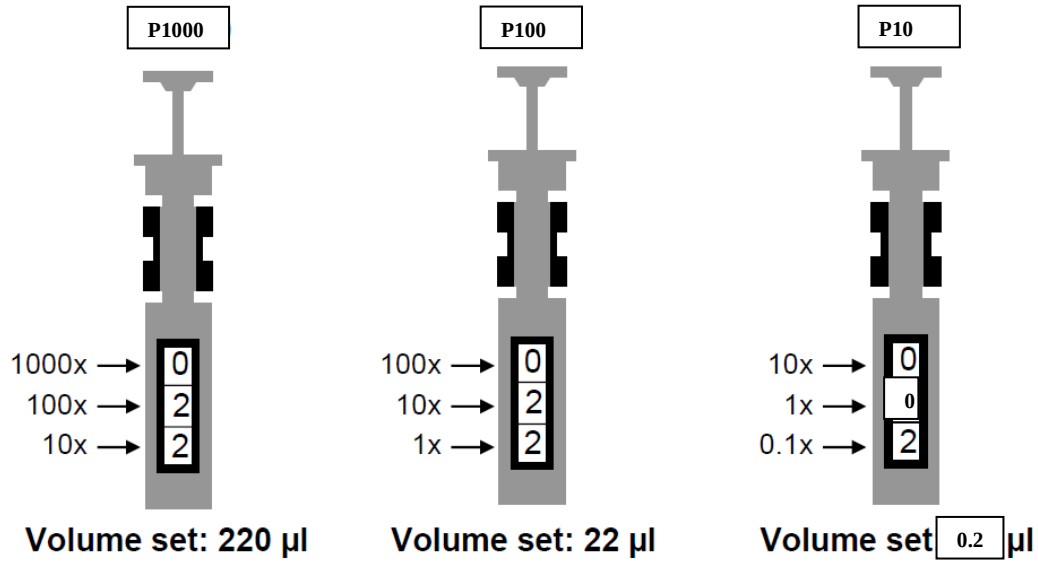
The black volume adjustment dial near the top of the micropipette allows you to adjust the volume that is measured. It can be dialed to the left or right to increase or decrease the volume.

The digital readout shows the volume that will be measured. As you turn the volume adjustment dial, the numbers in the digital readout will change.

On each of the three sizes of micropipettes (P10 P100 P1000) the digital readout has three numbers. These three numbers correspond to different volumes on the different size pipettes. See the figure below for instructions on interpreting digital readout.



Micropipettor: Reading the Volume



In P1000 the top number refers to 1000's of µl, the middle number refers to 100'sµl and the bottom number refers to 10's of µl's.

In P100, the top number refers to 100's of µl, the middle number refers to 10's µl and the bottom number refers to µ'ls.

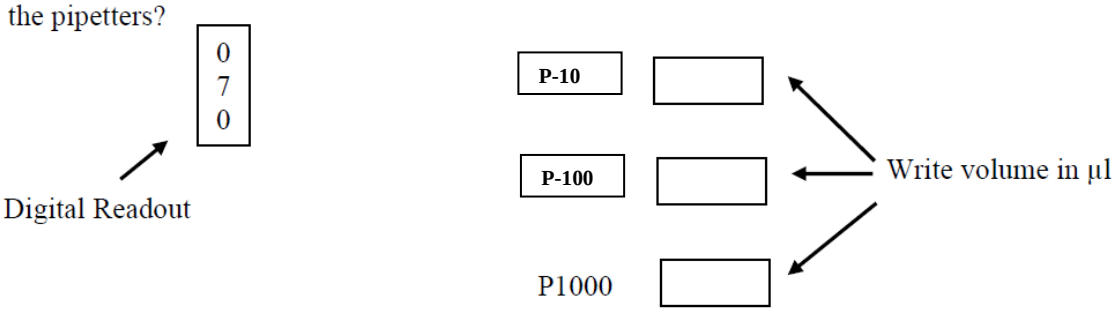
In P10, the top number refers to 10's of µl, the middle number refers to µl's and the bottom number refers to $1/10^{ths}$ of µl.

Micropipette and Tip Guide

Size	Range	Top view and Color	Example Setting	Tip size and color	Tip sample
P-10	0.5-10 µl	0.5 ~ 10 white	0 6 5 0 → 6.5 µl	micro white	
P-20	2-20 µl	2 ~ 20 yellow	1 7 8 → 17.8 µl	medium white or yellow	
P-100	10-100 µl	20 ~ 200 yellow	1 0 0 → 150 µl		
P-1000	100-1000 µl	200 ~ 1000 blue	0 6 7 0 → 670 µl or 0.67 ml	large white or blue	

Practice

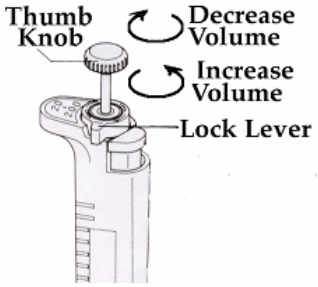
In the boxes below, write how many µls the following digital readout correspond to in each of the pipetters?



Which micropipettor would be appropriate to measure 250µl? _____ Fill in the numbers that should appear in the digital display if that pipettor were to measure 250µl.

How to set volume:

The volume is set by turning the dial. When decreasing the volume, slowly turn the dial clockwise to reach the required volume. Be careful not to overshoot. When increasing the volume, slowly turn the dial anticlockwise to reach the required volume. Be careful not to overshoot.



Operating the micropipette:

D. Pipette Tips

Liquids **are never** drawn directly into the shaft of the pipette. Instead, disposable plastic tips are are attached to the shaft. There are two sizes of tips. The larger blue tips are used for the P1000. The smaller clear tips are used for the P20 and P200.

The tips are racked in plastic boxes with covers. When you receive a box, it will be sterile. Please be careful when touching box or tips not to contaminate them. The box should be closed when not in use to prevent airborn contamination.

Inserting the Tip

1. Select the correct size tips.
2. Open the box without touching the tips with your hands.
3. Insert the micropipette shaft into the tip and press down firmly. This will attach the tip to the shaft.
4. Remove the micropipettor with the tip attached.
5. Close the box without touching the tips with your hands.

E. Punger Settings

The plunger will stop at two different positions when it is depressed. The first of these stopping points is the point of initial resistance and is the level of depression that will result in the desired volume of solution being transferred. The second stopping point is when the plunger is depressed beyond the initial resistance until it is in contact with the body of the pipettor. At this point, the plunger cannot be depressed further. This second stopping point is only used for the complete discharging of solutions from the plastic tip.

Operating the micropipette:

F. Measuring and transferring a volume of liquid

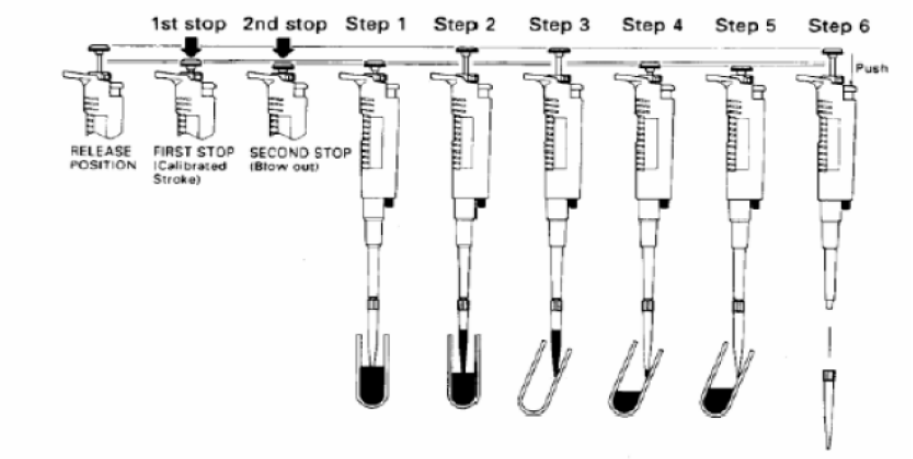
Before measuring and transferring liquid:

- Choose the appropriate size micropipettor
- Adjust to the correct volume
- Insert tip on the shaft.

Measuring and transferring liquid

The figure below shows the correct operation of the pipetteman. Important: note the first plunger stop is used in steps 1 through 4. The second plunger stop is only used in step 5.

- Depress the thumb knob to the first stop.
- Immerse the tip approximately 3 mm into the sample solution (step 1).
- Slowly release the thumb knob to the initial position (step 2). Watch as the solution is drawn up slowly into the tip. Do not release the plunger too quickly. Rapid release might draw bubbles in the solution and might splash solution on the non-sterile shaft.
- Withdraw the tip from the sample solution. Place the tip against the side wall of the receiving container (step 3).
- Smoothly depress the thumb knob to the first stop (step 4), pause, then depress the knob to the second stop (step 5).
- Remove the tip from the receiving container, and return knob to the initial position. Do not let the knob snap back.
- Remove the disposable tip by firmly depressing the tip ejector knob (step 6).
- Add as new tip and continue.



F. Micropipette Rules

Each micropipette cost $200 and is paid for by your technology fee. To keep these pipettors functioning properly it is important that they be handled with care. Please follow these rules to keep from breaking the micropipetters

1. Never adjust the volume beyond the range of the micropipettor. No micropipette should be adjusted below zero µl. The P20 should never be adjusted above 20µ, the P200 over 200 ul and the P1000 over 1ml.
2. Never force the volume adjustor dial. If the knob becomes difficult to adjust it probably means that you are exceeding the limits for the pipette or the pipette is damaged. Please report the problem to the instructor or TA.
3. Do not drop pipetters.
4. Always use a smooth motion when using the pipetters. This will help give you accurate measurements and also prevent breakage of pipettes. There should be not "snapping" noises.
5. Always keep pipettes upright. Store the micropipettes on the mounted rack on your bench when not in use. Never lay a pipette on the benchtop.
6. Always choose to appropriate size pipette for the volume you are measuring.
7. Always dispose of tips in appropriate waste containing. Never leave tips in glassware.

Experiment: Practice with Large-Volume Micropipette

This exercise will aid you to achieve accuracy and help you to be successful in your Biochemical, Immunological, Nutritional, Microbiological as well as Molecular Biology experiments for which a 100-1000µl micropipette is used. It is far easier to mis-measure when using large-volume micropipettes. If the plunger is not released slowly, an air bubble may form or solution may be drawn into the piston.

1. Use a permanent marker to label two 1.5 micro centrifuge tubes A and B.
2. Use the matrix below as a checklist while adding solutions to each tube.

Tube	Sol. I	Sol. II	Sol. III	Sol. IV
A	100µl	200 µl	150 µl	550 µl
B	150 µl	250 µl	350 µl	250 µl

3. Set the micropipette to add appropriate volumes of solutions I to tubes A and B.
4. Use a fresh tip to add the appropriate volume of Solution II on tubes A and B.
5. Use a fresh tip to add the appropriate volume of Solution III on tubes A and B.
6. Use a fresh tip to add the appropriate volume of Solution IV on tubes A and B.
7. Close the tops of the tubes.
8. Pool and mix the reagents by placing the tubes in a centrifuge and apply a short pulse of several seconds. Make sure that the tubes are placed in a balanced configuration in the microcentifuge rotor. Spinning tubes in an unbalanced position will damage the microcentifuge rotor.
9. A total of 1,000µl of reactants was added to each tube.

10. To check the accuracy of your measurements, set the micropipette to 1000µl and carefully withdraw the solution from each tube.

a. Is the tip barely filled?

OR

b. Does a small volume of fluid remain in the tube?

OR

c. After withdrawing all fluid, is an air space left in the end of the tip?

If your measurements were inaccurate, repeat the exercise to obtain nearly perfect results.