

Use of Micropipettes

Prior to lab you should understand:

- The function of micropipettes in the laboratory
- Basic parts of micropipette
- What volumes are measured with P20, P200 and P1000 micropipettors
- How to read the volume indicator on a P20, P200 and P1000
- How much each micropipettor costs and how they are paid for

I. Objective:

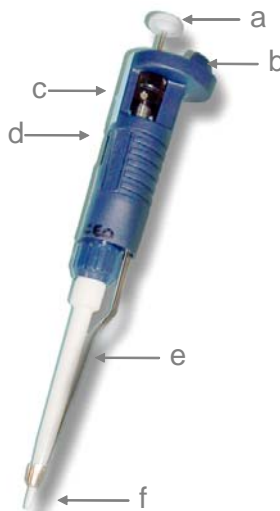
- Learn how to use a micropipettor

II. Background:

Micropipettors are the standard laboratory equipment used to measure and transfer small volumes of liquids. 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

- Plunger button
- Tip ejector button
- Volume adjustment dial
- Digital volume indicator
- Shaft
- 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.

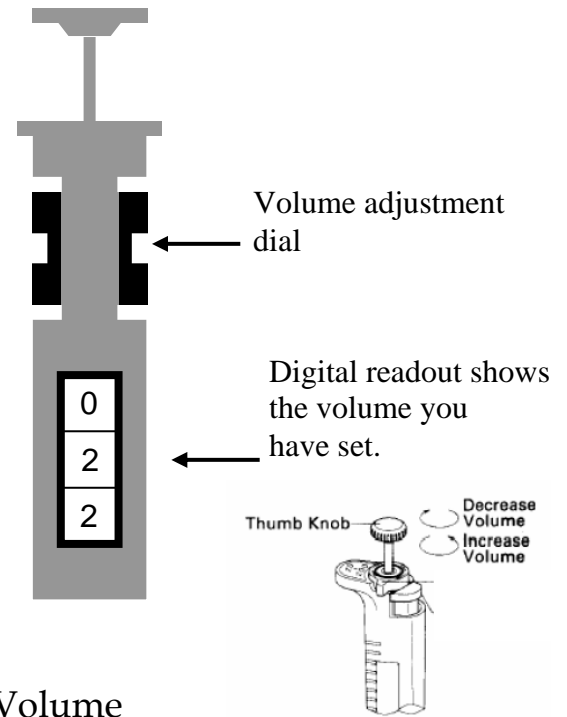
Size Micropipette	Range of volumes measured
P20	0.5-20 μ l
P200	20-200 μ l
P1000	100-1000 μ l

C. Adjusting Volume on micropipettes

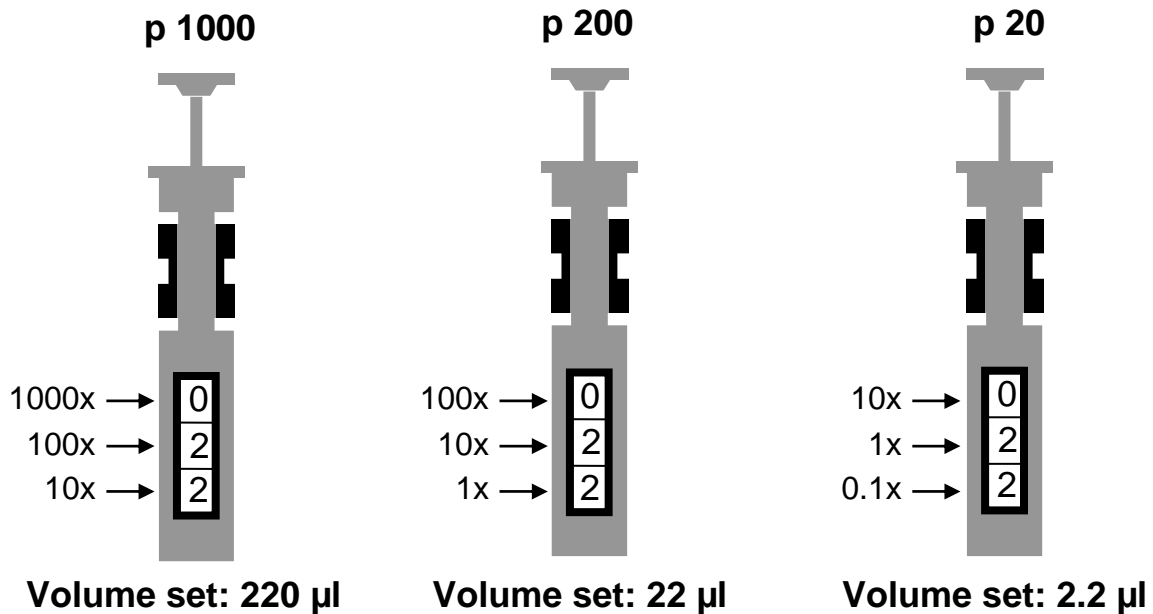
The black volume adjustment dial near the top of the micropipette allows you to adjust the volume that is measured. It can be dialed to the left or right to increase or decrease the volume.

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 (P20, P200, 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



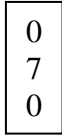
In a P100, the top number refers to 1000's of μ l, the middle number refers to 100's μ l and the bottom number refers to 10's of μ l's.

In a P200, the top number refers to 100's of μ l, the middle number refers to 10's μ l and the bottom number refers to μ 'ls.

In a P20, the top number refers to 10's of μ l, the middle number refers to μ l's and the bottom number refers to 1/10th of μ l.

Practice

In the boxes below, write how many μl s the following digital readout correspond to in each of the pipettors?

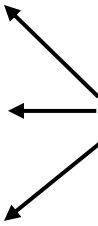
Digital Readout 

P20

P200

P1000

Write volume in μl



Which micropipettor would be appropriate to measure $250\mu\text{l}$? _____ Fill in the numbers that should appear in the digital display if that pipettor were to measure $250\mu\text{l}$.

D. Pipette Tips

Liquids **are never** drawn directly into the shaft of the pipette. Instead, disposable plastic tips 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 airborne 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.

F. Measuring and transferring a volume of liquid

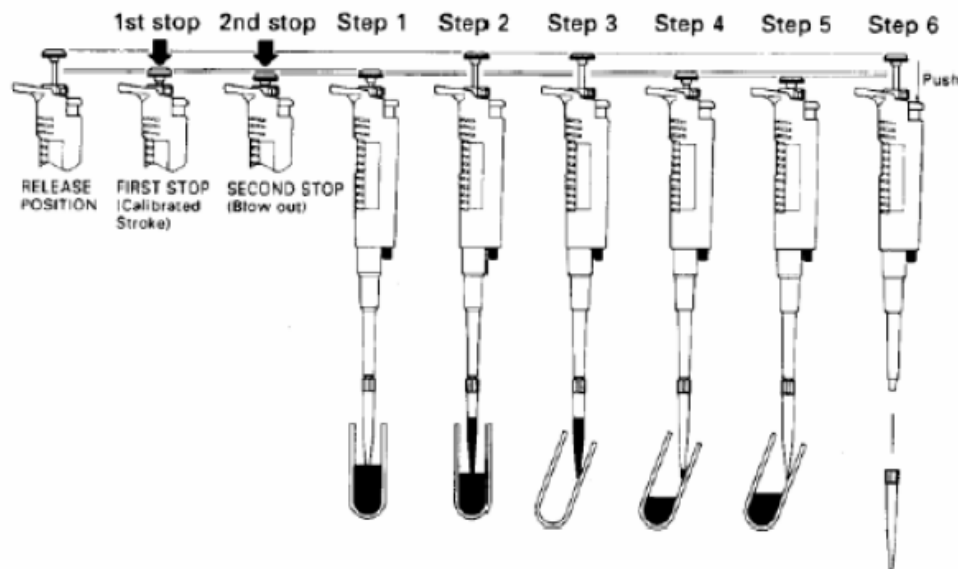
Before measuring and transferring liquid:

- 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 pipette. 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 micropipettors

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 μl 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 pipettors.
4. Always use a smooth motion when using the pipettors. 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.

III. Materials:

Each Station should have:

P20, P200, and P1000
Small and Large tips
Microcentrifuge tubes
Disposable Cuvettes
Calibrated Spectrophotometer
Bottle of blue dye
Beaker of water
Liquid waste beaker
Container for discarded pipette tips
Water rinse bottle
Kimwipes

Note: We will be using a spectrophotometer to measure the amount of blue dye in each sample. We will discuss the how spectrophotometers work in future laboratory exercises. In brief they measure how much light a sample absorbs. The spectrophotometer passes a beam of light through a special sample holder called a cuvette. It then measures how much of this light is absorbed by the sample. The absorbance of light is displayed on the spectrophotometer. The more dye in a sample, the greater the absorbance.

IV. Procedure:

A. Preparing a dilution of Blue dye

1. Choose appropriate micropipettor to measure 75 μ l.
2. Transfer 75 μ l of blue dye to empty microfuge tube.
3. Use a P1000 to transfer 1ml of water to microfuge tube.
4. Mix tube vigorously

B. Spectrophotometer reading

1. Pour the blue dye you diluted in the microfuge tube into a cuvette
2. Insert cuvette into spectrophotometer
3. Observe absorbance reading from digital display on spectrophotometer
4. Record measurement in data sheet below

C. Comparison to instructor value

1. Obtain correct absorbance value for 75 μ l dilution from instructor
2. Divide your value by the instructor's value to determine whether you were within 10% of the instructor's value. If you are outside the 10% range you are making an error in your pipetting.

D. Independent trials

1. Repeat 75 μ l dilution two more times as outline in boxes A-C.
2. Rinse and tap dry cuvette between each measurement.
3. Use a new microfuge tube for each dilution
4. Record values in data collection sheet.
5. Compare with instructors value for same dilution

Repeating with other dilutions

1. Repeat procedures as outlined in boxes A-D except as outlined below.
2. Prepare new dilutions using the following quantities of blue dye
 - a. 30 μ l
 - b. 15 μ l
 - c. 5 μ l
3. Record your data on the table collection sheet attached.
4. Conduct three trials for each dilution

V. Short Report

Name: _____

Due at end of class session

Data Collection Sheet

Dilutions	Trials	Student Absorbance	Instructor Absorbance	Ratio (S/I)
75 μ l	1			
	2			
	3			
30 μ l	1			
	2			
	3			
15 μ l	1			
	2			
	3			
5 μ l	1			
	2			
	3			