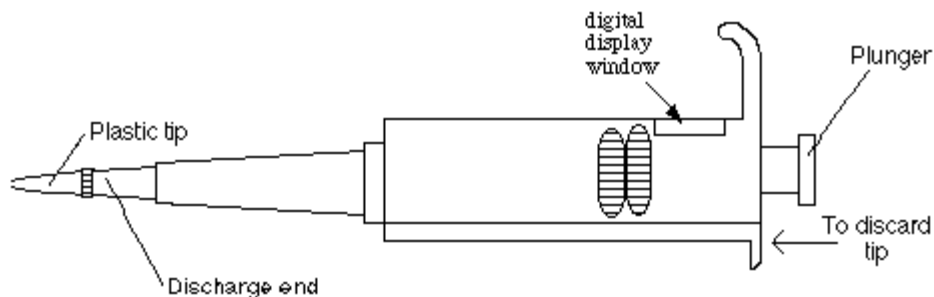


How to Use a Micropipettor

Sample Delivery with Variable Automatic Micropipets:

The micropipettor is used to transfer small amounts (< 1 ml) of liquids. The scales on micropipettors are in microliters ($1000\mu\text{l} = 1$ ml). The brand of micropipettors we will be using is made by Rainin and called a "Pipetman". These are very expensive, delicate instruments costing \$250-300 apiece. Pipetmen come in three sizes which are capable of pipetting three ranges of volumes: **P20** = 0.5- 20 μl , **P200** = 20-200 μl , and **P1000** = 200-1000 μl . They are used in conjunction with disposable (often sterile) plastic tips. The following is an illustration of a micropipettor:



The Pipetman

Using a Micropipettor:

1. **Never exceed the upper or lower limits of these pipettors.**



The limits are:

P20: 0.5 to 20.0 μl

P200: 20 to 200 μl

P1000: 200 to 1000 μl

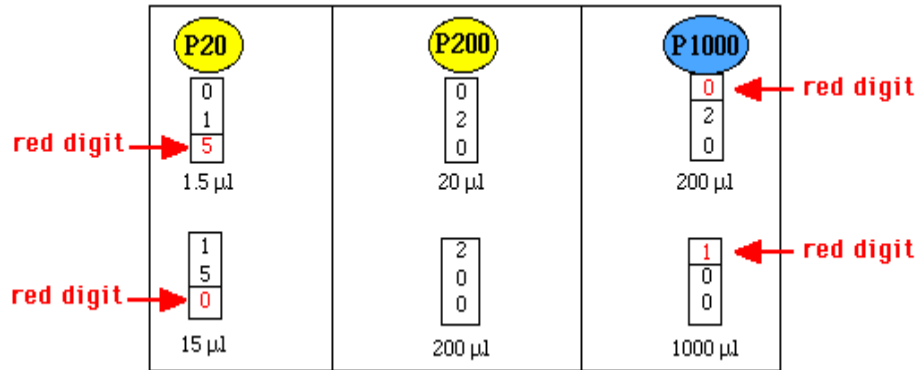
2. Set the desired volume by turning the centrally located rings clockwise to increase volume or counterclockwise to decrease volume.

P20: Maximum volume 20 μl . Accurate between .5 μl and 20 μl . Numbers on the micropipettor (typically black-black-red) are read as XX.X μl . The change in color indicates the position of the decimal point.

P200: Maximum volume 200 μl . Accurate between 20 μl and 200 μl . Numbers on the micropipettor (one color) are read as XXX μl .

P1000: Maximum volume 1000 μl (= 1 ml). Accurate between 200 μl and 1000 μl . Numbers on the micropipettor (typically red-black-black) are read X.XX ml. Note that this micropipettor reads milliliters while the other two read microliters.

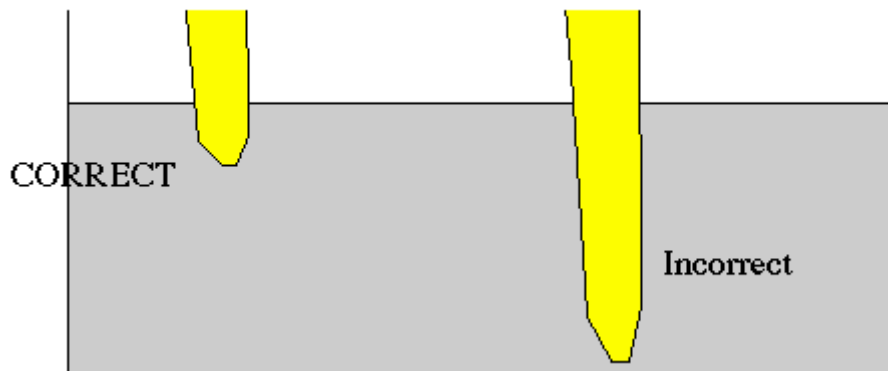
The examples below show how to read the micropipettor:



<http://abacus.bates.edu/~ganderso/biology/resources/pipet.html>

P1000		P200,P100		P20	
1	1000's	2,1	100's	2	10's
0	100's	0	10's	0	1's
0	10's	0	1's	0	1 decimal

- Place a tip on the discharge end of the pipettor. NOTE: If sterile conditions are necessary do not allow the pipet tip to touch any object (including your hands).
- 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. Because this first stopping point is dependent on the volume that is being transferred, the distance you have to push the plunger to reach the point of initial resistance will change depending on the volume being pipetted. The second stopping point can be found 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 further depressed. This second stopping point is used for the complete discharging of solutions from the plastic tip. You should not reach this second stop when drawing liquid into the pipettor, only when expelling the last drop. Before continuing, practice depressing the plunger to each of these stopping points until you can easily distinguish between these points.
- Depress the plunger until you feel the initial resistance and insert tip into the solution, just barely below the surface of the liquid and not as deep as possible.



6. Carefully and **slowly** release plunger. NOTE: If the solution you are pipetting is viscous, allow the pipet tip to fill to final volume before removing it from solution to avoid the presence of bubbles in the plastic tip which will result in an inaccurate volume.
7. Discharge the solution into the appropriate container by depressing plunger. This time, depress the plunger to the point of initial resistance, wait one second, and then continue pressing the plunger as far as it will go in order to discharge the entire volume of solution.
8. Remove tip by pressing down on the tip discarder.

NOTES:

- Never point a pipettor up. This may cause liquid to run down into the pipettor destroying it.
- When withdrawing liquids with the pipettor, always release the plunger slowly. This prevents liquid from rushing into the end of the pipette and clogging it up. This is especially important with large volume pipettors (200-1000 μ l).
- Be sure you use the proper size tip for each pipettor.
- Always use a new tip for each different liquid.
- Use the correct pipettor for the volume that is to be dispensed. Never use the 200-1000 μ l pipette to dispense volumes below 200 μ l. going below or above the range of the micropipettor may damage the instrument.

Adapted from: <http://www.bio.davidson.edu/Courses/Bio111/Bio111LabMan/Preface%20D.html>

The Fixed Volume Micropipet:

Fixed volume micropipets are pre-set to measure only a specific volume. Although the volume delivered by a give pipet cannot be changed, the pipet is operated analogously to the variable micropipette. Fixed volume micropipets often do not have an tip ejector button, so the tips are removed manually.

The Experiment

Procedure:

You will use a variable micropipette to prepare seven different dye mixtures in the wells of a microtiter plate. You will then dispense 5 μl of each diluted dye solution onto a pipet card in triplicate.

1. Label a microtiter plate with your initials and number seven of the wells as 1-7. Prepare solutions 1-7 using the ingredients shown in the following table.

Tube	Red (μl)	Blue (μl)	Yellow (μl)	Glycerol (μl)	Alcohol (μl)	Buffer (μl)	Total Volume (μl)
1	5	-	-	-	-	40	45
2	-	-	10	10	10	15	45
3	-	10	10	-	-	25	45
4	5	15	-	-	-	25	45
5	13	-	-	-	10	22	45
6	-	10	-	10	-	20	45
7	6	-	6	13	-	20	45

2. After preparing the dye mixtures, pipet 10 μl of each mixture onto the corresponding numbered row of circles on the Pipet Card. Pipet the dye mixture onto the center of each circle.
3. Compare the colors of your circles to those of your classmates. Tape your cards into your laboratory notebook.