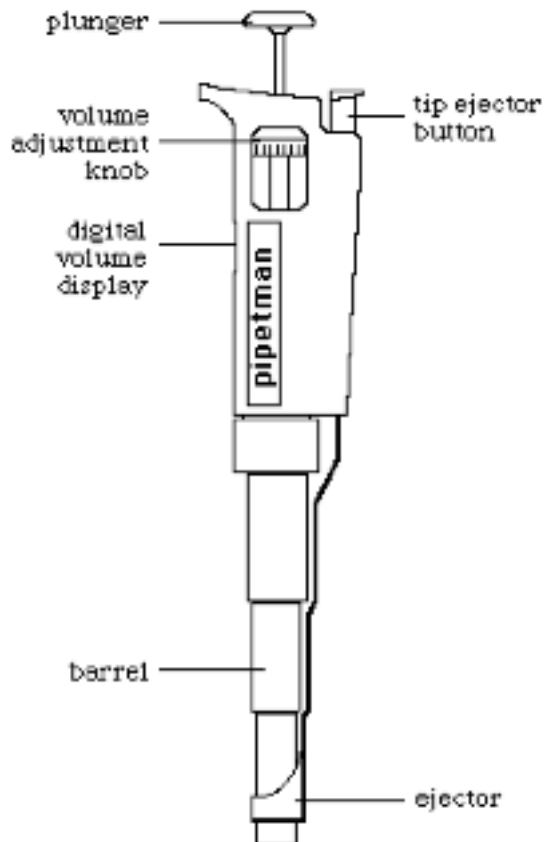


Use of Micropipettors

Micropipettors are instruments used to accurately transfer small volumes (1 μl to 1 ml) of solution. Because of their accuracy, ease of use, and convenience in sterile techniques they are a practically universal lab tool.

We will use three different micropipettors in this course. Each one is appropriate for a specific volume range. If you look at the dot on the plunger of each micropipettor you will see a number that represents the maximum volume, in microliters (μl), that can be transferred by that micropipettor. The minimum volume appropriate for each micropipettor is typically ten percent of the maximum. The dot on the plunger is also color-coded and generally matches the color of the disposable tips used with that micropipettor. The table below shows the volume range, expected accuracy, and the appropriate tips for the micropipettors that you will be using.



Micropipettor	Volume Range (μl)	Accuracy	Tip Color
P1000	100 - 1000	$\pm 10.0 \mu\text{l}$	blue
P100	10 - 100	$\pm 1.0 \mu\text{l}$	yellow
P10	1 - 10	$\pm 0.5 \mu\text{l}$	white

In most experiments, accuracy is important when transferring small volumes of liquid with a micropipettor. A researcher needs to be sure that he or she is transferring the volume desired with a reasonable degree of accuracy. The researcher can be confident of this provided two conditions are satisfied. First, the micropipettor has been calibrated and tested for accuracy (this is usually done on a yearly or semi-yearly basis). **Second, the researcher must be using the micropipettor properly.**

Proper use of the Micropipettor

Use the text below and the illustration on the previous page to become familiar with the parts of a micropipettor:

Barrel - the working end of the micropipettor; a disposable tip is seated on the lower end of the barrel before each use

Plunger - the plunger is pressed and released to withdraw and expel liquid

Tip ejector button - is used to remove a tip from the barrel without direct handling of the disposable tip

Digital volume setting - displays the volume the micropipettor is currently set to deliver

Volume adjustment knob - is rotated to change the digital volume setting

Familiarization with the micropipettor

Pick up a P100 and set the digital volume setting for 10 μl (reading down, the setting should be "010"). Push the plunger down and notice that at some point the plunger becomes more resistant and requires more effort to push further. This point is called the first stop. Notice that the plunger can be pushed well beyond the first stop until it reaches "the second stop". Be sure you can feel the first stop point and notice how far the plunger travels before reaching this point. Reset the micropipettor to 100 μl ("100"). Again push the plunger to the first stop. You should notice now that the plunger travels further to reach the first stop than it did when the micropipettor was set for 10 μl . Examine the P1000 setting window and plunger tension at various volumes as well. The table below shows how the digital volume display looks for each micropipettor when it is set at its maximum volume.

Micropipettor	Maximum Setting (in μl)	Example Setting
P1000	1 - thousands 0 - hundreds 0 - tens	0 750 μl 7 5
P100	1 - hundreds 0 - tens 0 - ones	0 75 μl 7 5
P10	1 - tens 0 - ones 0 - tenths	0 7.5 μl 7 5

To transfer solution using a micropipettor:

1. Set the digital volume setting to the desired volume by rotating the volume adjustment knob. **Note: the digital volume setting should never be adjusted above the maximum volume or below the minimum specified for a particular micropipettor.** Remember that the maximum volume is the largest volume shown on the plunger.
2. Seat a disposable tip on the micropipettor by firmly placing the end of the barrel into a tip. - Put the end of the barrel in the open end of a tip in a rack. Press down firmly to seat the tip on the barrel.
3. Depress the plunger to the first stop and immerse the end of the tip into the solution to be transferred. SLOWLY release the pressure of your thumb on the plunger to SMOOTHLY draw the solution up into the tip.
4. To expel the solution, depress the plunger ALL THE WAY TO THE SECOND STOP.
5. If expelling into a liquid solution, REMOVE the end of the tip from the solution BEFORE releasing the pressure on the plunger with your thumb.
6. You should generally use a new tip for each transfer (or whenever the tip becomes clogged, if you are repeat pipetting from the same, non-sterile solution). When you wish to eject the tip you are using, place the tip over the appropriate waste container and press the tip ejector button. If the tip is difficult to eject it is likely that you are jamming the tips onto the micropipettor harder than necessary.

TIPS for accurate pipetting:

Accurate pipetting technique is critical for accurate measurements. As always in the lab, it is important to pay attention and be careful with what you are doing.

- When drawing a solution into the tip, make sure the tip remains submerged the whole time, start to finish, until the plunger is completely released. Otherwise you will draw in air.
- Don't remove your thumb quickly when drawing up a solution. It will cause the liquid to "jump" up in the tip giving an inaccurate volume.
- Make sure to stop the plunger at the first stop when drawing up a solution.
- Make sure to push the plunger all the way to the second stop when ejecting a solution.
- Use your eyes! Look at the tip. Is there solution in it? Does it look like the appropriate amount? 500 μ l takes up less volume in the tip than 1 ml (1000 μ l).