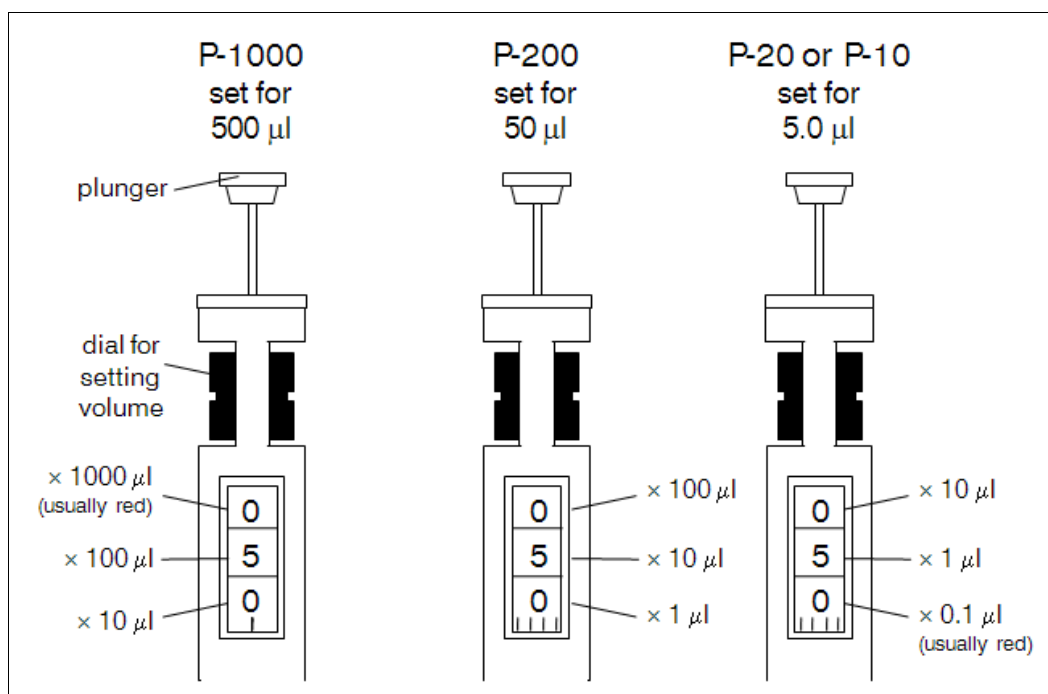


# Using Micropipettors

For biology experiments, you will often need to measure volumes much less than 1 ml—in fact, as little as 1 **microliter** ( $\mu\text{l}$ ), which is 1/1000 ml or  $10^{-6}$  liter. This kind of precision requires a special instrument: a **micropipettor**, often called a “Pipetman™” because that was the first brand-name in use. Micropipettors only work accurately if you use them correctly!

Each micropipettor is designed for accurate handling of a specific range of volumes. Our Pipetmen are labeled on top of the plunger button: **P-20** (for pipetting 2–20  $\mu\text{l}$ ), **P-200** (20–200  $\mu\text{l}$ ) or **P-1000** (200–1000  $\mu\text{l}$ ). We also have **P-10** pipettors which can pipette from 0.5 to 10  $\mu\text{l}$  but are usually only used for very small volumes (2  $\mu\text{l}$  or less). The volume is set using a dial which changes a digital readout on the front to tell you what volume you’ve set. The readout is different for each size of pipettor, so Figure 77 and be sure you know how to read the pipettor you’re using.



**Figure 1.** Micropipettors (viewed from the front), showing how to read the volume settings.

**Testing pipettor accuracy.** How can you figure out if you are actually pipetting such small volumes accurately? There are two concerns: (1) is the pipettor functioning correctly? and (2) are you using it correctly? Micropipettors have seals that need to be replaced periodically and can get out of adjustment over time. As you use these instruments, you will become very good at measuring accurately, but you may not be so accurate to begin with. Fortunately, there is a simple test procedure that will let you test your pipettor as well as your ability to use it.

Remember that water has a density of 1 g/ml (one ml weighs 1 gram). This means that you can simply pipette a specific volume of water onto a piece of weighing paper and see if it has the correct weight! It’s a good idea to do this when you first start using micropipettors to test your technique, and you should also do it anytime it seems like your pipettor might not be giving the right volume. It only takes a second and can make the difference between a successful experiment and a flop.

To test your pipettor, set it to the volume indicated in the table below. Measure the desired volume of water at least three times (six is better), weigh the water and determine the actual volume. Then determine the **accuracy** of your pipettor by averaging the three volumes and the **precision** by taking the standard deviation. The table below shows the expected accuracy and allowable error range for each type of micropipettor we use. If you find that your pipettor is not measuring accurately, *tell your instructor*, who can replace parts or re-calibrate it (or help you see what you might be doing wrong). Do not just go on measuring inaccurately!

Micropipettor specifications

Pipettor	Volume to Set	Accuracy (avg. volume)	Precision (std. dev.)
P-1000	500	$500 \mu\text{l} \pm 4 \mu\text{l}$	$\leq 1 \mu\text{l}$
P-200	100	$100 \mu\text{l} \pm 0.8 \mu\text{l}$	$\leq 0.12 \mu\text{l}$
P-20	10	$10 \mu\text{l} \pm 0.15 \mu\text{l}$	$\leq 0.05 \mu\text{l}$
P-10	5	$5 \mu\text{l} \pm 0.08 \mu\text{l}$	$\leq 0.03 \mu\text{l}$

**Using the micropipettor.** To use the micropipettor, you must first insert the barrel into a disposable plastic tip. *Never let the barrel contact liquid directly! Tell your instructor if liquid gets inside the barrel somehow.* There are three sizes of tips: large ones for the P-1000, medium ones for the P-200 and P-20, and very fine ones for the P-10. Be sure you are using the right tip with the right pipettor. The tips are usually sterile, so keep their boxes closed and don't let them touch any surfaces or your skin.

The micropipettor works basically like a syringe: you press down on the spring-loaded plunger to displace air from the tip and release the plunger to draw liquid in. As you push down on the plunger, you should be able to feel two "stops." You will feel the first stop as you press the plunger about  $\frac{2}{3}$  of the way down, but you can push past this stop and push the plunger to the very bottom. The **first** stop gives you the measured volume and is used to draw the liquid into the tip. The **second** stop is used only to "blow out" the very last of the liquid from the tip. Figure 78 shows how the two stops are used.

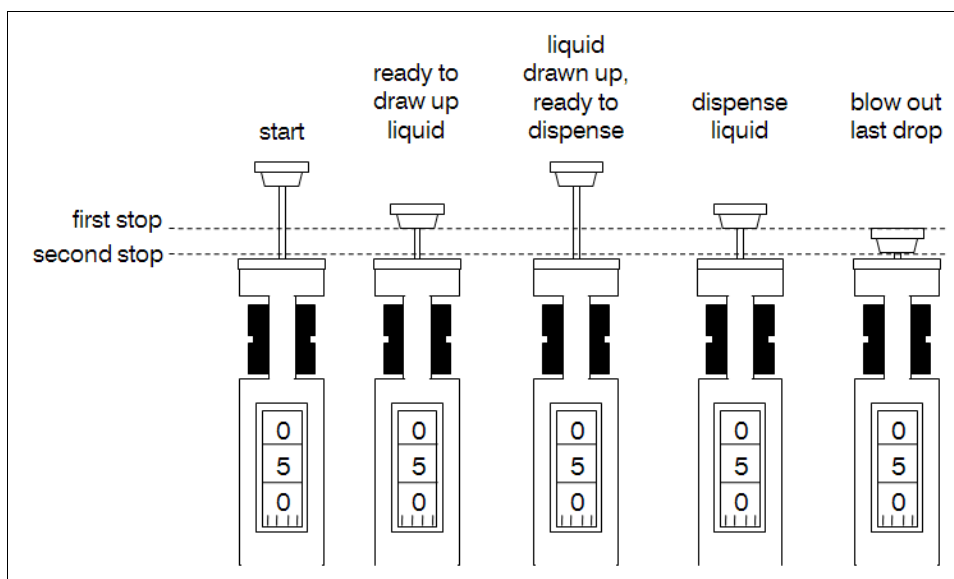


Figure 2. Using the two plunger stops to pipette liquid accurately.

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⚠ Be sure you can feel both "stops" and know their purpose. The most common reason for poor pipetting accuracy is that a student doesn't recognize the first stop.

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The directions below will help you get started with the micropipettors. Follow them carefully for best accuracy—even small details like how deeply the tip goes into the liquid can make a difference with these small volumes. If something goes wrong or your pipettor doesn't seem to be working, *tell your instructor.* Do *not* just throw it back in the drawer so that it affects someone else's experiment.

1. Set the dial for the desired volume by turning the black ring. See Figure 77 if you're not sure how to read the dials.

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⚠ *Be very careful not to turn the ring too far. It should not move upward beyond the opening for your fingers. If you turn it too far, the adjusting screw will come loose, and the pipettor will have to be recalibrated. If this happens, be honest and inform your instructor—don't just leave the faulty pipettor to screw up another student's experiments!*

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2. Insert the end of the pipettor into a disposable plastic tip. Don't touch the tips with your hands: you should be able to insert the pipettor into the tip while it's still in the box by pressing down gently but firmly.
3. Press the plunger down until you feel it stop (at the first stop: *not* all the way down!). Do this before you put the tip in the liquid, rather than by blowing bubbles in your solution (it's more accurate that way due to viscosity of the solution).
4. **Holding the pipettor vertically**, Insert the tip *just below the surface of the solution* (only 1-3 mm deep).

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⚠ *If you press the plunger after putting the tip in liquid, or if you put the tip too deeply into the liquid, or if you tilt the pipettor, you won't get good accuracy. Also, note that micropipettors are not accurate in thick or viscous liquids.*

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5. Release the plunger *s-l-o-w-l-y*. If you let it up too quickly, you'll get air bubbles that will affect your accuracy, and you might even suck liquid into the innards of the pipettor.

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⚠ *If by any chance you accidentally suck liquid into the pipettor, tell your instructor and ask for cleaning instructions. If you just pretend it didn't happen, the liquid can contaminate later samples and corrode the guts of the pipettor.*

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6. Take a look at the tip and see if the volume looks right. After a while, you'll have a good idea of what different volumes look like, and you'll recognize when there's a problem.
7. To dispense the liquid, press the plunger slowly back down to the first stop, *pause*, then press it all the way to the second stop to blow out the last drop.
8. Eject the tip into a tip bucket or the trash by pressing the white button behind the plunger.