

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

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Use of Micropipets

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On-Line Resources for Biology

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A. Gilson Pipetmen Autopipets (and similar models)

We primarily use the Gilson autopipets in the core course labs. We have four sizes identified by the number on the round button on the plunger. The value is the maximum volume in microliters that can be transferred with that size pipet.



Other autopipet sizes we have on hand.

P1000 **P200** **P100** **P20**

Size indicator button on a Gilson P1000.

What size of autopipet is right for the job?

RULE OF THUMB: Always select the SMALLEST size pipet that will handle the volume you wish to move to achieve the greatest accuracy. Accuracy *decreases* as you use unnecessarily large pipets for small volumes.

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The following table shows the useful volume ranges for each pipet type. Make special note of the min and max values.

SIZE	Useful Range	Max Volume	Min Volume
P1000	200-1000 ul	1000 ul	200 ul
P200	20-200 ul	200 ul	20 ul
P100	10-100 ul	100 ul	10 ul
P20	0.5-20 ul	20 ul	0.5 ul

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B. How to Read the Volume on the Autopipet

Look at the front face of the pipet and you will see a window with 3 (three) digits inside. The diagram below shows the MAXIMUM value that can or should be dialed in on each size pipet. **To exceed these values will put the pipet out of calibration.** Beside each "window" below is the numbers place it represents. Please take the time to learn how to read them so as to avoid damaging them by dialing values out of their range.



Gilson P1000 scale window.

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

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C. How to change the load volume:

Hold the pipetor horizontally with the plunger to your left. Change or set the *load volume* by turning the knurled plastic knob at the top of the hand grip. Roll it towards you to decrease the values and away from you to increase. **WATCH CAREFULLY** as you change the values to make sure you don't take the numbers out of range. If in doubt, review the [allowable volumes](#) for each size pipetor, and then ask for help.

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D. Technique

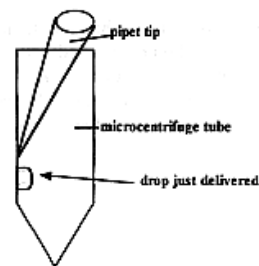
- **GRIP:** Hold the pipetor like a knife in a horror flick with your *index finger* on the plunger. Your index finger gives you much finer control over the plunger action.
- **LOADING:** Load a sterile tip (**blue** for P1000; **yellow** for P200 and all smaller sizes including **VWR autopipets**) and then reclose the tip box to maintain sterility.
 - Push the plunger down slowly to the point of *first resistance*: this sets the load volume.
 - While holding the plunger at the load volume set point, put the tip into the solution so that it is immersed just enough to cover the end (3-4 mm).
 - Slowly release the plunger to draw up the liquid making sure to keep the tip immersed. Visually

inspect the load to make sure it is correct.

- **DELIVERY:** To deliver the volume, place the tip into the receiving vessel and press the plunger *all the way to the bottom* - this expells all the liquid and gives a little extra volume to get the last droplet out - **AND THEN, WITHOUT RELEASING THE PLUNGER**, withdraw the tip.
- **CHANGE TIP?** Repeat as necessary using the same tip if NOT changing solutions. Use a fresh tip for every change of solution, or whenever it is prudent to maintain sterile conditions and avoid cross contamination of solutions or cultures.
- **TIP DISCHARGE:** While holding the tip over an appropriate waste receptacle, press the white tip discharge slider on the back of the grip.

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E. Small Volumes Technique: With small volumes, especially the 1-10 ul range used in molecular biology protocols, you must keep track of the droplets you pipet. Carefully expell the liquid droplet *on the side wall* of the tube so that you can see it.



If adding to a larger volume, flush the tip with the solvent liquid after expelling the droplet to make sure you get all the delivery liquid. With small volumes you'll usually need to centrifuge and then vortex the tube to get a good mixing of the reagents.

F. A Simple Check for Proper Calibration

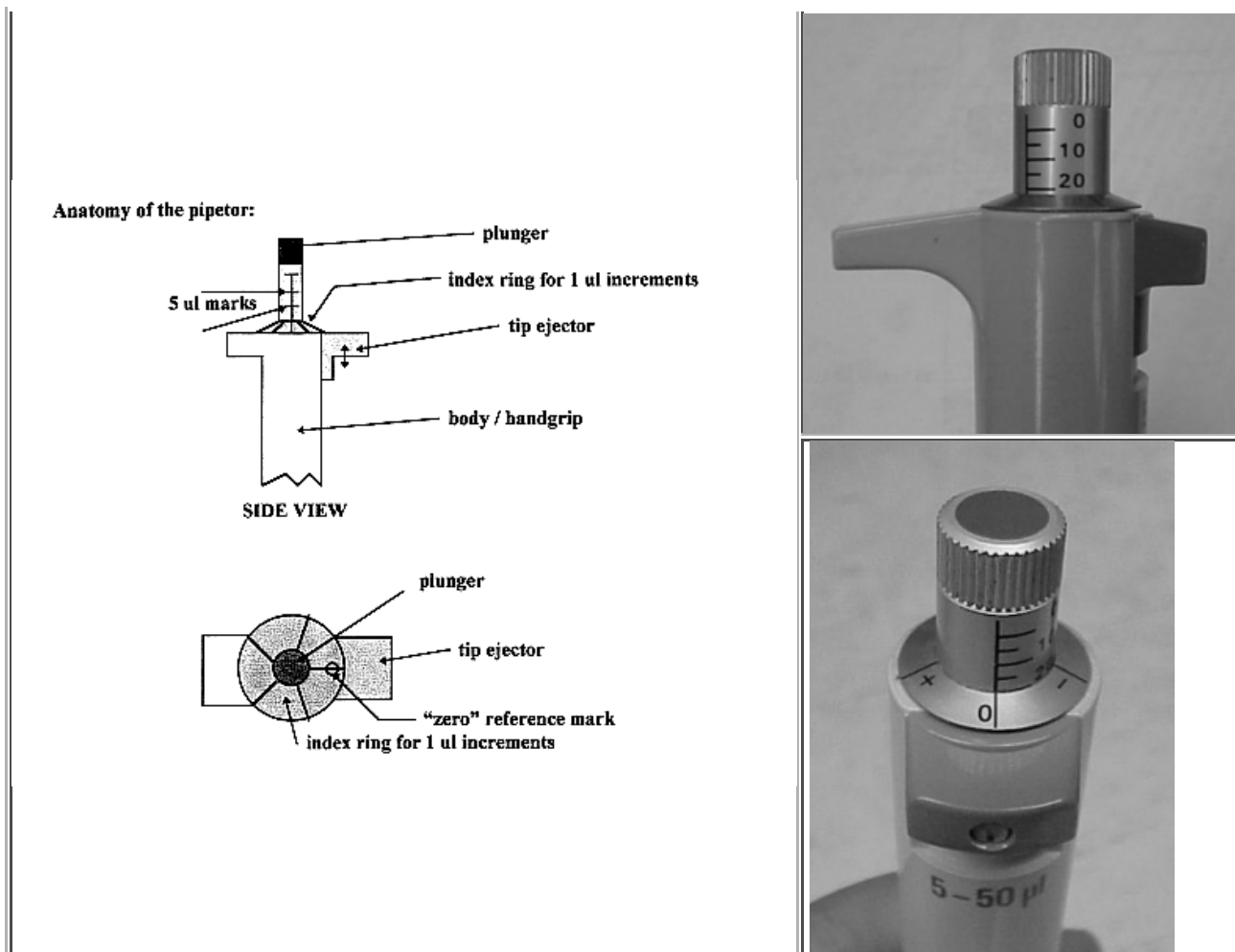
Check the calibration of your micropipet by using the fact that 1 ul of deionized (or distilled) water has a mass of 1 ug. Pipet a range of volumes spanning the micropipet's usable range and mass them on a top loading balance having at least 3 decimal place accuracy. Pipets having greater than 0.1 % error should be recalibrated.

G. Instructions for Using the VWR 5-50 ul Auto Pipets

The VWR 5-50 ul autopipets function similarly to the Gilson Pipetman autopipets. The major difference lies in how to set the load volume. This is further complicated by the loss of the 1 ul **index ring** (which surrounds the plunger) from several of the pipets.

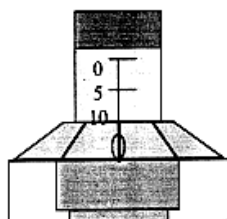
The **plunger** has two stops, the first being the load volume, the second for discharge volume. These pipetors use the same small yellow tips (yellow or orange boxes) as used for P200 and smaller Gilson autopipets. The **tip ejector** is a dark gray button near the top of the hand grip (see diagram below).





How to set the load volume:

The plunger has a vertical line demarcated by horizontal ticks at each 5 ul increment from 0 ul to 50 ul (top to bottom –see diagram at right). When the plunger is rotated to align the vertical mark with the "zero" reference mark on the index ring, the load volume is that of the horizontal line just showing above the index ring. In the diagram to the right that volume is 10 ul.



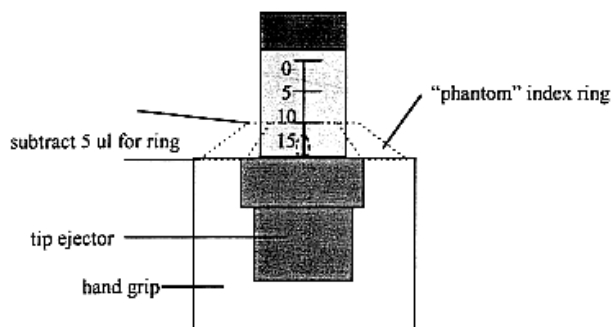
Above: Side view of VWR 5-50 ul autopipet set to 10 ul.

Increments of 1 ul are achieved by turning the plunger CW toward the "+" symbol to increase volume or CCW toward the "-" symbol to decrease volume. Each click aligns the vertical line with a new reference mark on the index ring. To set 13 ul, go to 10 ul and then 3 clicks CW.



Above: Pipet set to 20 ul.

On some of the pipetors the index ring is missing. To maintain their utility, we have marked the 1 ul increments and the zero reference point with a felt tip on the top of the hand grip. The diagram below shows the pipetor properly set for 10 ul. The index ring is equivalent in depth to the 5 ul intervals on the plunger. The absence of the index ring therefore lets the 15 ul mark show just at the top of the hand grip. To read the actual volume you'll have to subtract 5 ul from the apparent volume (15 ul in the example) indicated at the top of the hand grip (see diagram). Ask for help if you're having difficulty reading or setting the correct load volume.

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