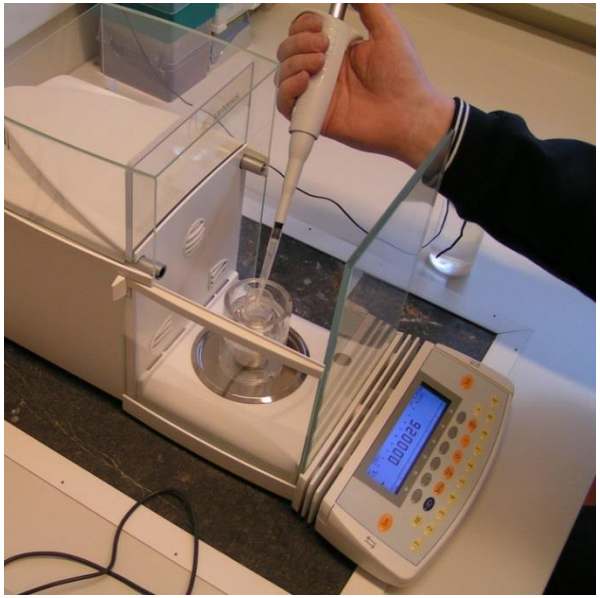
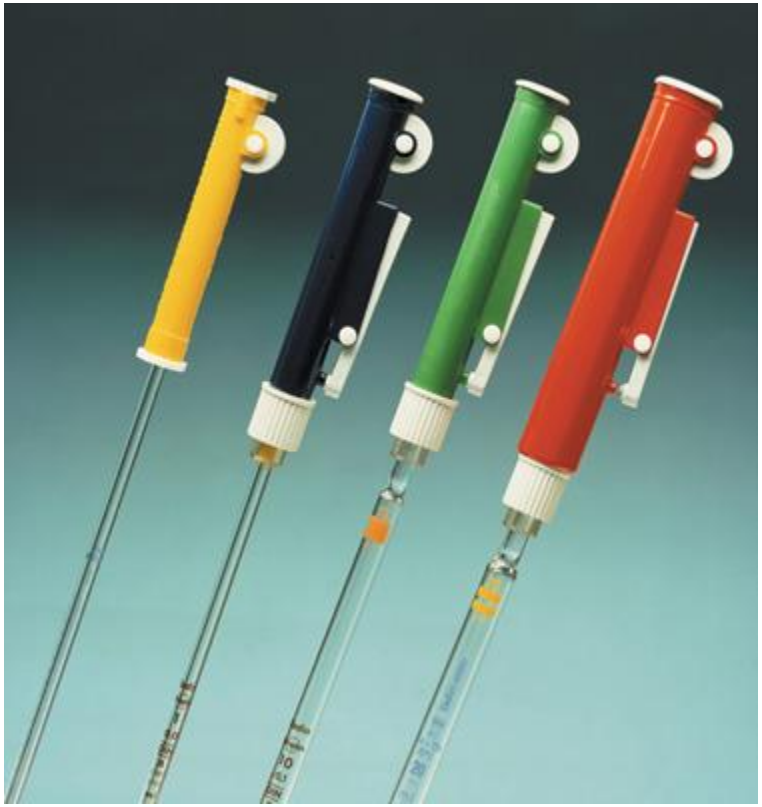
Pipetting Small Volumes

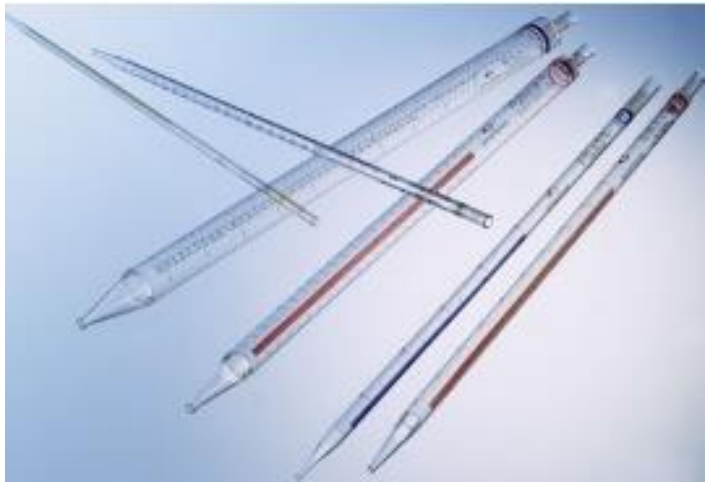


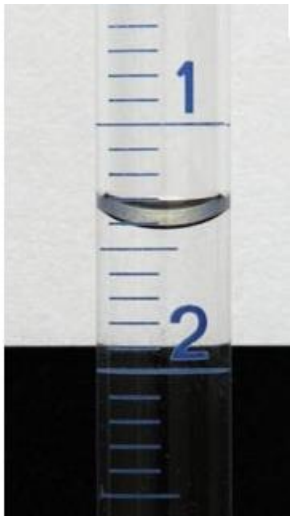
Introduction

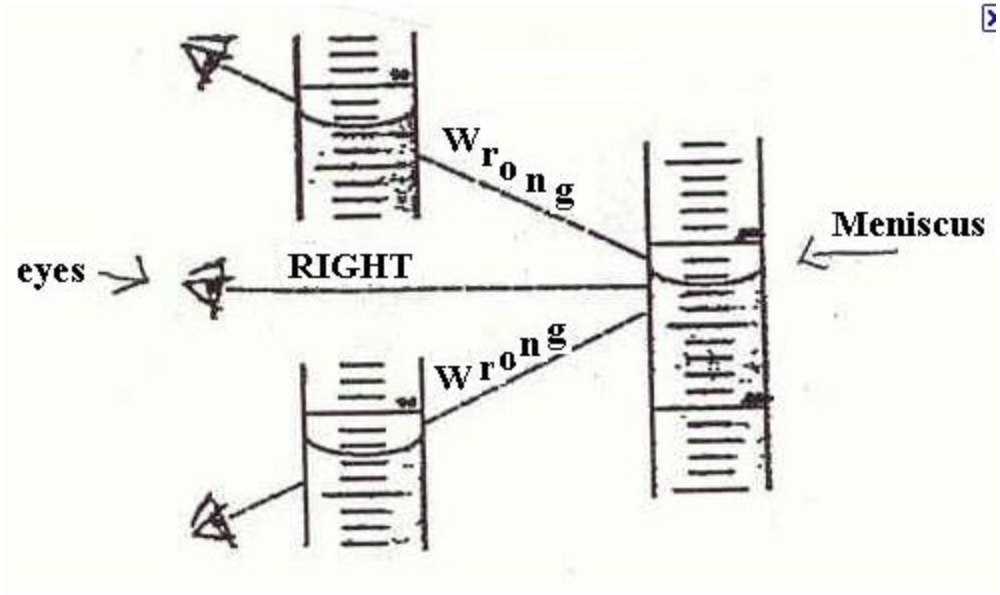
- Serological and micropipettes are used to accurately transfer small liquid volumes (micro-liter to milli-liter) accurately and precisely.
  - Continuously adjustable
  - Can be set to any transfer volume within its range, which is from 10% of the marked volume up to but not beyond the marked volume.
- Glass and plastic serological pipettes are used for volumes from 1ml – 25ml, using a pipette pump.



Serological pipettes





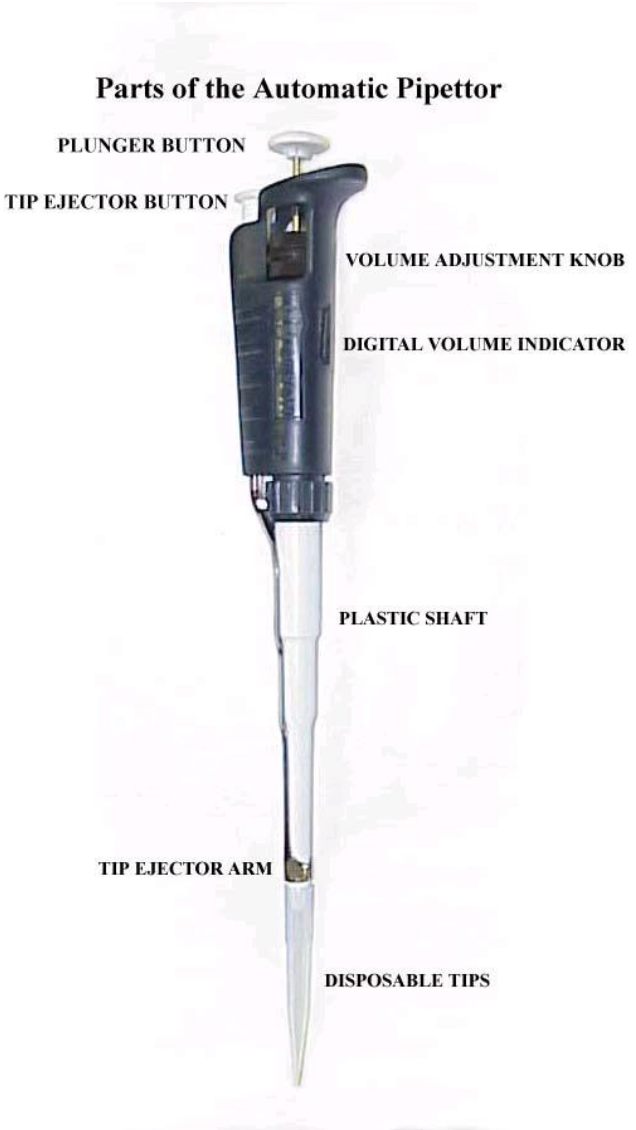


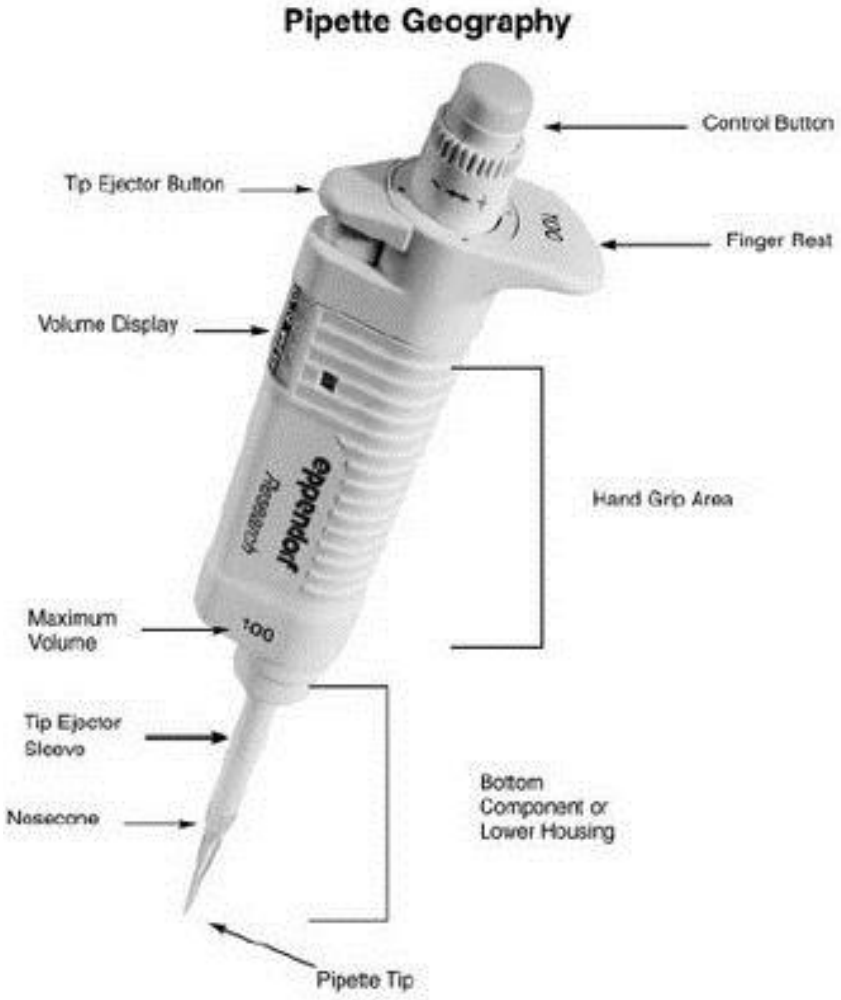
The MicroPipette

Make sure you know how to

- Select the proper micropipetter to transfer a specified volume of sample
- Set a specified volume on the pipette volume indicator using the volume adjustment knob
- Read the volume setting in correct units
- Select the correct tips and properly seat them
- Demonstrate the correct technique to accurately transfer a sample of a stock solution to another container (usually a microfuge tube).
- Properly eject the tip into a waste container.

Parts of the Pipette





Operating the Micropipette

Step 1: Select the correct pipetter and set the volume

Operating the Micropipette

Step 2: Read the volume

(a): P-20 Model
6.86 m l = 0.00686
or 6.86×10^{-3} ml

(b): P-200 Model
132.4 m l = 0.1324
or 1.324×10^{-1} ml

(c): P-1000 Model
262 m l= 0.262
or 2.62×10^{-1} ml

Performance

Volume range	Inaccuracy (E%) Min. vol.	Max. vol.	Imprecision (CV%) Min. vol.	Max. vol.
Micropipettes				
0.1 - 2 µL	< +/- 6.0 %*	< +/- 2.0 %	< 5.0 %*	< 1.5 %
0.5 - 10 µL	< +/- 2.5 %**	< +/- 1.0 %	< 1.8 %**	< 0.5 %
1 - 10 µL	< +/- 2.5 %	< +/- 1.0 %	< 2.5 %	< 0.7 %
2 - 20 µL	< +/- 2.5 %	< +/- 1.0 %	< 1.7 %	< 0.5 %
5 - 50 µL	< +/- 1.5 %	< +/- 1.0 %	< 1.0 %	< 0.5 %
10 - 100 µL	< +/- 1.5 %	< +/- 0.8 %	< 1.0 %	< 0.2 %
20 - 200 µL	< +/- 1.5 %	< +/- 0.8 %	< 0.6 %	< 0.2 %
100 - 1000 µL	< +/- 1.5 %	< +/- 0.5 %	< 0.5 %	< 0.2 %

* At 0.5 µL. Indicative data at 0.2 µL: E <+/- 12 %, CV < 8 %

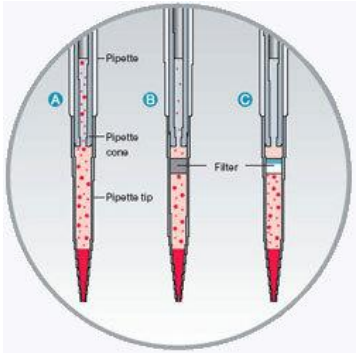
Operating the Micropipette

Step 3: Attach the disposable tip

Attaching the disposable tip

Tip boxes are labeled for the range of barrel they accommodate (there are some manufacturer-specific types so check if you are mixing and matching).
Center the end of the barrel in the tip and tap straight down, gently, twice.

Some tips include a filter barrier – mostly used for PCR reactions.



Operating the Micropipette

Step 4: Depress the Plunger to the First Stop

Depress the plunger first: don't put the tip in the liquid and then depress the plunger (the pressure is different).

Step 5: Immerse Tip in Sample

The tip must be below the surface of the liquid throughout the process of pulling up the liquid.

Ideally the pipette is held vertically.

To aspirate the sample into the tip, allow the pushbutton to return *slowly and smoothly* to the fully extended starting position.

NEVER LET THE PLUNGER SNAP UP!

Leave the tip in the solution for 1-2 sec (longer for a viscous solution), then slowly withdraw.

Operating the Micropipette

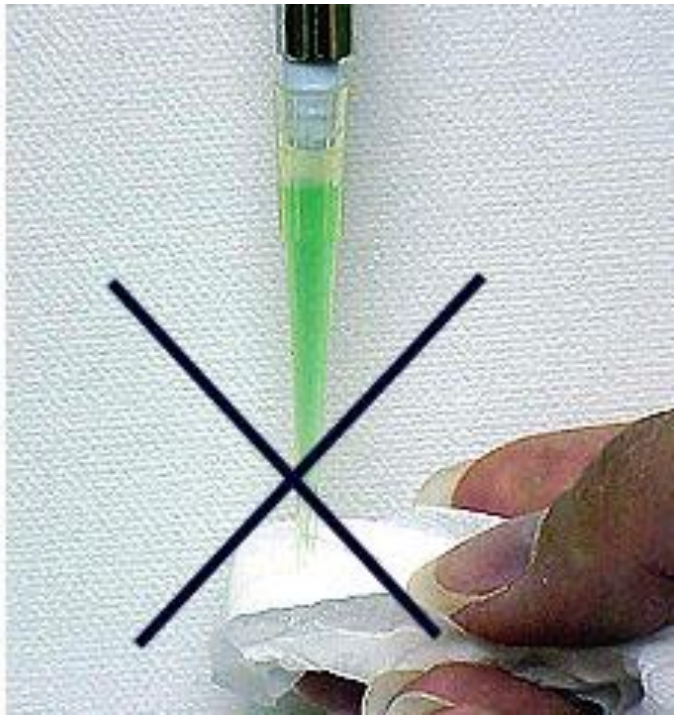
Step 6: Withdraw the tip from the solution

Withdraw the pipet from the receiving vessel carefully, touching or sliding the tip along the wall of the vessel.

Operating the Micropipette

Remove the tip from the sample liquid. There should not be liquid on the outside of the tip if you have used it correctly – generally we do NOT touch the tip with anything. Some reagent containers may not allow ideal practice – in that case wipe away any droplets on the outside of the tip with a lint-free tissue, such as KIMWIPES. Don't touch the tip opening or you will wick away some of the sample. Note on the left that the volume is not correct (air at bottom).

Step 7: Withdraw the Tip



Note – you should be wearing gloves – no bare hands!

Operating the Micropipette

Step 8: Dispensing the solution to a tube

(a) Start Dispensing	(b) 1st Stop = Dispense	(c) 2nd Stop = Expel

a) Touch the tip end to the bottom or side wall of the receiving vessel
b) Depress the plunger slowly to the FIRST STOP.
c) Pause for at least one second, longer for larger volumes or viscous liquids.
d) Press the plunger to the SECOND STOP (the second point, of greater resistance, at the bottom of the stroke) to expel any residual liquid in the tip – an air bubble should force out the last drop of liquid.

Operating the Micropipette

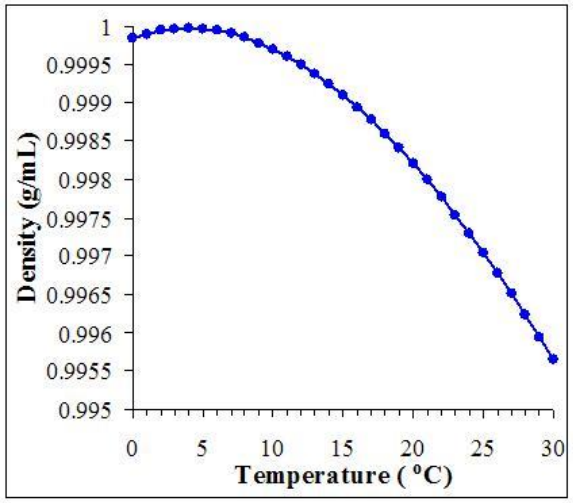
Step 9: Use the ejector button to eject the tip into a waste container (use a Biohazard can as required).

First Lab: Equipment and Supplies

- A set of micropipettes and tips
- Several capped solution containers (water, then glycerol – why?)
- Parafilm squares
- A Mettler balance

Checking the volume

- If you know the density of the liquid you are pipetting, and you have a calibrated mass balance, you can pipette a specified volume into a pre-weighed container and determine the mass of the volume



ntainer is also called Taring it.

eck you pipetting?

At 20C, H_2O has density as shown:

1ml = 0.9985 gm

1ul = 0.00099 gm = 0. 99ug

A very close approximation of ug to ul

6/10/2015

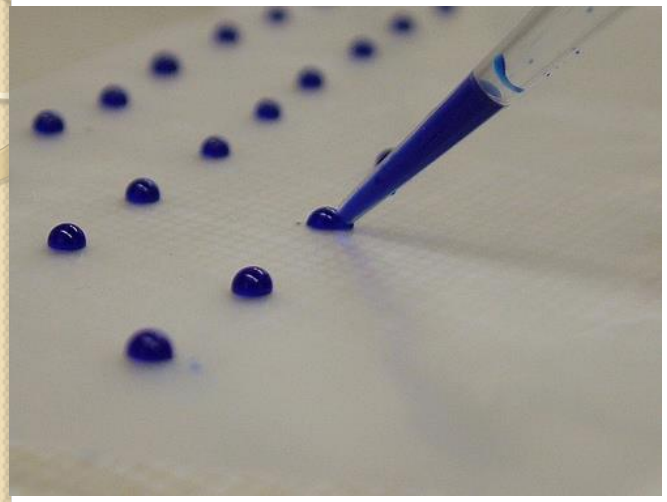
Using the Balance

Make sure the balance has sufficient sensitivity for the volume you want to measure.

Air currents can change the reading on a very sensitive balance.

Make sure you know where the Tare (Zero) control is.

Using Parafilm or weigh boats



Aqueous solutions bead up on Parafilm – you can leave the backing paper on, but pipette solution onto the waxy side not the paper side.

After weighing the volume, blot it up with a Kimwipe, re-tare and repeat the pipetting.

Accuracy and Precision

- Accuracy means the closeness with which the dispensed volume approximates the volume set on the pipette
- The level of accuracy is specified as mean error, the average deviation of *replicate measurements* from what is expected from the volume you set.
- Precision is the "scatter" or variance of individual measurements obtained from the same volume setting.
- Precision can also be expressed as standard deviation (variance divided by the mean).

Accuracy and Precision (Continued)

- Device capabilities: relative accuracies are generally about 1% or less for micropipetters within range
  - These micropipettors have recently been calibrated.
- Precision error is less than 0.5 % except when transferring the smallest *recommended* volume for a given pipette model
  - Using the pipettes to transfer volumes which are below the recommended range will introduce larger errors

Lab Practice with Pipettes

- Practice setting a few volumes
- Practice reading the digits of set volumes
- Practice seating the tip, drawing up and dispensing samples of water and of a glycerol solution (glycerol is denser *and* more viscous).
- Get the "feel" of the 1st (set volume to pull up) and 2nd (blow-out for delivery) stops
- Practice using the pipette and record how well measurements match settings (indirectly, your skill).