

Pipetting Learning Module

I. Pipetting Technique

Proper technique involves drawing up (aspirating) and dispensing (ejecting) the liquid in a **smooth** motion. Putting the pipette tip in the water and quickly releasing the plunger will give you inaccurate results—even if your pipette is properly calibrated.

Reverse Pipetting

Use this technique when pipetting viscous liquids or volatile solvents. Reverse pipetting also helps when pipetting ultra-micro samples of 0.5 μL or less. Push the piston down to the purge position (the second stop), then draw the liquid up. There is too much liquid in the tip at this point. However, when the liquid is dispensed by pushing the piston to the aspirate position (the first stop), the extra liquid is left inside the tip. Using this method, the tip is "automatically" pre-wetted. The extra liquid also helps when pipetting volatile solvents, because some of the solvent will tend to evaporate into the air cushion.

Use a Sensible Pipette for the Volume You Want to Dispense

The accuracy of your pipette decreases as the dispensed volume approaches the minimum the pipette can handle. For example, if you are dispensing 15 μL , then a 1 mL pipette would be terrible, a 200 μL pipette not so good, and 20 μL pipette ideal.

Use the Largest Volume Possible

Large volumes are easier to pipette accurately than small. Say you are performing an assay where you have to accurately pipette 5 μL . Pipetting that small amount accurately is not easy and will likely contribute greatly to the statistical error in your results. On the other hand, you could dilute the stock solution 10 times and pipette 50 μL of the solution. You could easily and accurately pipette this amount, which would yield much tighter error bars.

II. Practice Pipetting

1. Make 50% Glycerol (1 volume ddH₂O and 1 volume Glycerol)
2. Pipette the same volume of 50% Glycerol ten times onto a tared balance (adjust to zero everytime), close the door of balance, wait until the weight become stable, record the weight each time

Pipettor	Volume	Repeat
P1000	500ul	10 times
P200	100ul	10 times
P20	10ul	10 times
P10	5ul	10 times

3. Calculate the Standard Deviation (SD) in Microsoft Excel. Try to make sure the standard deviation is as small as possible.

Example: 200 ul volume

200ul weight (g)
0.2205
0.2179
0.2184
0.219
0.2216
0.2165
0.2176
0.2173
0.2175
0.2189
Standard Deviation:
0.001550484

4. Repeat practice for many days until the standard deviation becomes very small and stable. Then continue to do real experiments.
5. Example of pipetting SD acceptable range

