Good Pipetting Technique

Taken from Mettler Toledo, who makes Rainin pipettes, and BiteSize Bio.

> Quick videos on optimal pipetting technique - Mettler Toledo
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> In depth video on optimal pipetting technique - Mettler Toledo

Pipette Selection

Use the pipette on which the desired volume is the closest to the pipetting maximum. The accuracy of the pipette decreases as the dispensed volume approaches the minimum the pipette can handle. So for dispensing 15 µL, for example, a P1000 would be terrible, a P200 not so good and a P20 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 5 µL accurately is not easy and will likely contribute greatly to the statistical error in your results. On the other hand, if you diluted the stock solution tenfold and pipetted 50 µL, this would be much more accurate, giving you much tighter error bars.

Volume Setting

When setting the micrometer on a pipette, always "dial down" to the desired volume. By doing so, you ensure better accuracy by reducing the effects of mechanical backlash or slippage within the micrometer's gears. When dialing a lower volume, simply arrive there without overshooting the slightest. When dialing up, rotate the knob a third of a turn higher, and then dial down.

Pre-rinsing

Pre-rinsing is a fast, easy way to increase accuracy by up to 0.2%. It helps neutralize the capillary effect in micro-volume pipettes, and for larger volume tips, equalizes the temperature of the air inside the tip with the temperature of the sample.

Aspiration

Maintaining consistency during aspiration can improve accuracy by up to 5%. To ensure that you are achieving the highest accuracy, use consistent pipetting rhythm from sample to sample. Maintain your speed and smoothness while pressing and releasing the plunger, and keep consistent pressure on the plunger when at a stop. Problems with these can cause fountaining, sample splash up, and bubbles. For pipettes 1 mL (P1000/P5000), improve accuracy by pausing 1 second after pickup to allow the sample to fully aspirate.

Immersion Depth and Angle

Immersing the tip to the correct depth will improve your accuracy by up to 5%, 1–2 mm for P20s, 2–3 mm for P200s, 3–6 mM for P1000s, and 6–10 mM for P5000. Immersing the tip too deeply can cause too much liquid to be aspirated and create bubbles. Conversely, positioning the tip too close to the surface can aspirate air.

Dispensing

Improve your pipetting accuracy by up to 1% with good dispensing technique. For the highest consistency, touch the vessel wall with the tip while dispensing. Then at the first stop, without purge, **"touch off"** by sliding the tip up the wall to remove any liquid clinging to the orifice. (No purge at second stop required.) With aqueous liquids, two other techniques also work well. You can dispense above the surface of the liquid instead of while touching the wall and then touch off. If concerned about cross-contamination, don't touch off. Alternatively, you can dispense the liquid and purge while the tip is immersed below the liquid surface. Then touch off as before.

Reverse Pipetting

Reverse pipetting is a good technique for pipetting viscous liquids or volatile solvents. 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 but 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, improving accuracy. The extra liquid also helps when pipetting volatile solvents because some of the solvent will tend to evaporate into the air cushion.

Sample and Ambient Temperature

The sample, pipettes, and tips should be allowed to equilibrate to ambient temperature whenever possible (e.g. cold room). Take the sample temperature into account. In a recent Nature Methods publication, it was observed that when repeatedly pipetting cold samples, the first dispensed volume is always larger than expected, but subsequent pipetting with the same tip gave the correct volume. The same was true for hot samples, except that the first dispensed volume was smaller than expected. The error was 0.1–0.2% per degree of difference between the sample temperature and 20 °C ambient temperature.

Their solution was simple: dispense one volume back into the original vessel before pipetting each instance. The first volume equilibrates the tip and air column temperature to the sample's temperature, preventing the temperature difference from expanding/contracting the air volume whose displacement measures the sample.