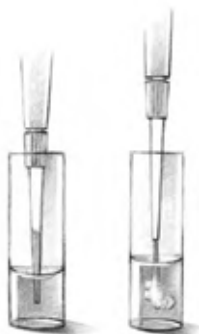


Ten tips to improve your pipetting technique

By Dr. Rodrigues

The pipette is a reliable precision instrument used and trusted for many years. However, as with many forms of instrumentation, a pipette will perform only as well as the operator's technique allows. Differences in technique--some more than others--can alter delivery volumes and impact test results and data integrity. As your laboratory's demand for accuracy and precision increases, so too does the importance of understanding and developing optimal pipetting technique.

The following tips are presented as a guide to proper pipetting technique to produce more accurate and precise laboratory results.



Pre-wet the pipette tip. Aspirate and expel sample liquid at least three times before aspirating a sample for delivery. Evaporation within the tip can cause a significant loss of sample before delivery. Pre-wetting increases the humidity within the tip, thus reducing both the amount of and variation in sample evaporation. Using the same tip (without pre-wetting) to deliver multiple samples results in lower volume for the first few samples. The need to pre-wet increases when working with volatile samples such as organic solvents.

Work at temperature equilibrium. Allow liquids and equipment to equilibrate to ambient temperature. The volume of sample delivered by air displacement pipettes varies with air pressure, relative humidity and vapor pressure of the liquid, all of which are temperature dependent. Working at a single, constant temperature minimizes this variation.

Examine the tip before dispensing sample. Wipe the tip only if there is liquid on the outside of the tip, and then, very carefully. Absorbent material rapidly carries sample from the tip if it contacts the tip opening. Unnecessary tip wiping increases the possibility of sample loss.

Use standard mode pipetting. Choose standard mode pipetting rather than reverse mode for all but viscous samples, if accurate and precise results are desired. In reverse mode pipetting, the plunger is depressed completely (past the first stop) to aspirate the sample and then depressed only to the first stop to deliver the sample. If reverse mode is used with normal aqueous fluids, the pipette tends to deliver more than the calibrated volume. On the other hand, using normal mode with viscous samples, especially when liquid is retained in the tip, results in under delivery.

Pause consistently after aspiration. Pause with the tip in the liquid for about one second after aspirating the sample. It takes a moment for the liquid in the tip to finish moving after the plunger stops, so failure to pause will cause the volume to be too low. Slow and even plunger release and a consistent, brief pause after aspiration minimize this error.

Pull the pipette straight out. Pull the pipette straight out of the container after aspirating a sample. Do not touch the tip to the sides of the container. This technique is especially important when pipetting small volumes (< 50 µL). Surface tension effects cause the sample volumes to vary if the exit angles vary. Touching the tip against the container walls results in loss of sample.



Minimize handling of the pipette and tip. Set the pipette down between sample deliveries and avoid handling the tip. Body heat transferred to equipment during handling disrupts temperature equilibrium. As explained in Tip No. 2, the volume of sample delivered varies with temperature.



Immerse the tip to the proper depth. During sample aspiration, immerse the tip 2-5 mm below the meniscus and well clear of the container walls and bottom. Inserting the tip too deep into the liquid causes excess droplets to cling to the outside of the tip. Pressing or resting the tip against the container walls or bottom restricts entry of the sample.

Use the correct pipette tip. For accurate volume delivery, choose a tip that is designed for use with the type of pipette being employed and securely attach it. Mismatching a tip and pipette or using poor quality tips can result in an inadequate seal between the pipette and tip. Quality tips are flexible and have thin walls, providing airtight seals and dependable sample delivery.



Ten tips to improve your pipetting technique



Use consistent plunger pressure and speed. Depress and release the plunger smoothly and with consistent pressure and speed when dispensing each sample. Pipettes, like all precision instruments, produce more reproducible results when operated with attention to detail and proper technique.

Common Pipetting Errors

Working too quickly.

Removing the pipette tip before sample aspiration is complete.

Dragging the pipette tip along the side of the container when exiting the sample.

Releasing the plunger too rapidly.

Not pre-wetting a new tip, particularly when working with volatile samples.

Techniques among pipette users vary with background, personal preferences and training. These differences in execution can affect laboratory results' accuracy and precision. To ensure accuracy and consistency, facilities should adopt standard operating procedures for pipetting technique and ensure all operators are trained to an adequate level of proficiency. By increasing result consistency, the facility's quality and credibility will be enhanced as well.

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