



Forward Pipetting Technique with a Manual Micropipette

Pipetting is an important skill, and good laboratory techniques can help ensure the accuracy and precision of your results. The ramifications of improper pipetting can lead to inaccurate results, cross-contamination, and fatigue in the operator. Therefore, using the micropipette with intention and understanding the mechanics is paramount. By the end of this video, you should be able to recall the steps involved in forward pipetting. Before working with specimens or reagents, consider practicing with water.

In a clean workspace, don the required personal protective equipment and ensure that your workspace supports good posture, reducing the physical stress and fatigue of repetitive motion.

Your workstation probably contains several sizes of micropipettes. It is essential to select a micropipette that has been calibrated to include your desired volume.

From the pipette stand, identify a clean micropipette that is calibrated within the required range.

To transfer 500 μl , we will select the 100 to 1000 μl range micropipette. Use the pipette stand when you are not actively pipetting to reduce prolonged heat exposure from your hand.

Changing the set volume on the micropipette from its last use may be necessary.

While holding the micropipette, slowly adjust the volume display to 500 μL by rotating the dial.

Some micropipettes have a volume lock control that will need to be disengaged before adjusting the volume and reengaged before attaching the pipette tip.

Do not set the micropipette to aspirate a volume outside the range designated by the pipette manufacturer.

It is essential to select the correct size and shape tip that fits your micropipette and seat the tip securely on the micropipette to prevent leakage. Pipette tips are single-use items.

Select a pipette tip that fits your micropipette's specifications. For instance, pipette tips may contain a filter that helps mitigate the risk of contamination.

Holding the micropipette vertically, wrap your four fingers around the micropipette's body with your thumb free to maneuver the micropipette's plunger.

Be sure to attach the tip to the micropipette fully but firmly by pushing it down vertically. Avoid wobbling or forcefully jamming the micropipette into the pipette tip.

Lift away from the pipette box and visually confirm that the tip is seated correctly.

Before we transfer Buffer A into Buffer B, it is recommended that the pipette tip be pre-wet.

Pre-wetting is a technique that improves pipetting accuracy by balancing the environment between the tip's air column and the liquid to be transferred. This can be achieved by aspirating and dispensing the liquid to be moved three times. With your non-dominant hand, open the container containing Buffer A. Gently depress the top circular plunger button with your dominant thumb to the first stop.

Holding the micropipette vertically, immerse the pipette tip just under the surface of the liquid.

Slowly and smoothly, release the downward pressure from your thumb until you have entirely released the button and your thumb is no longer pressing down.

Again, depress your thumb to the first stop and expel the liquid back into the tube.

Repeat the aspiration and dispensing of the liquid using the same pipette tip at least three times before proceeding with the final aspiration of your desired volume.

Aspirating means drawing the liquid up into the tip. The micropipette's angle and tip's immersion depth can affect your pipetting's accuracy. Avoid immersing too deeply, as the excess liquid can stick to the outside of the tip, and too shallow will draw in air, both affecting the accuracy. Additionally, aspirate while holding the micropipette at 90 degrees.

After pre-wetting, slowly aspirate the required volume while maintaining a vertical position.

Wait until the entire volume has been aspirated.

Viscous liquids must be aspirated at a much slower rate than thinner liquids.

Pause for one second.

It's essential to keep the pauses of aspirating and dispensing consistent.

The plunger should be in the neutral position.

Remove the micropipette and tip from your liquid sample, touching the tip to the inside of the original tube to remove excess liquid on the outside of the tip.

Visually inspect the pipette tip to ensure a full draw and that there are no air bubbles present.

If there is evidence of air, dispense the liquid back into the container by pressing the plunger down to the first stop, eject the liquid, and try again.

Close Buffer A with your non-dominant hand.

When moving the micropipette containing liquid in the tip, it is essential to minimize cross-contamination. Avoid fast movements or hovering over objects in your workspace. It is also recommended that only one container be opened at a time. With your non-dominant hand, open Buffer B. Ensure that your hand holding the micropipette remains stable.

With your dominant hand still holding the micropipette, thoughtfully move the micropipette into Buffer B to be dispensed.

Place the tip of the pipette tip into Buffer B's tube with the end gently touching the inside of the container at a 20-45-degree angle.

Depress the plunger slowly and smoothly with your thumb until you reach the first 'soft' stop.

Wait one second.

Then proceed to the second 'hard' stop or 'blow-out' where your thumb is pressed down as far as the micropipette allows to ensure the liquid is entirely dispensed from the pipette tip.

Maintaining your thumb at the second stop position, slide the tip against the side of the container and lift the pipette tip entirely out of the final container.

Release the pressure from your thumb on the plunger to the neutral position. Close the top of Buffer B's container.

Carefully move the micropipette over to the waste container.

Using your thumb, press the tip eject lever and eject the pipette tip from the micropipette.

Place the micropipette upright on the pipette stand for decontamination.

Avoid resting or storing the micropipette on its side to protect the pipette's internal mechanisms.