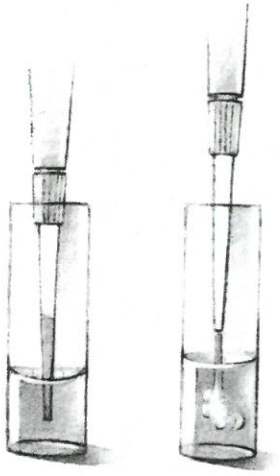
10 tips to improve your pipetting technique

Operator technique has a major effect on pipetting performance.

OBTAIN MORE accurate and precise laboratory results by following ARTEL's "10 Tips To Improve Your Pipetting Technique".

Using the PCS®, ARTEL has determined the impact on accuracy and precision of 10 commonly practiced pipetting techniques. Starting with the most important, these are listed in the order of their effect on pipetting performance.

1. Prewet the Pipette Tip



Aspirate and expel an amount of the sample liquid at least 3 times before aspirating a sample for delivery.

Evaporation within the tip can cause a significant loss of sample before delivery. Prewetting increases the humidity within the tip thus reducing both the amount of, and variation in, sample evaporation. Using the same tip (without prewetting) to deliver multiple samples results in lower volume for the first few samples.

2. 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 the variation.

3. 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 wicks sample from the tip if it contacts the tip opening. Unnecessary tip wiping increases the possibility of sample loss.

4. 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.

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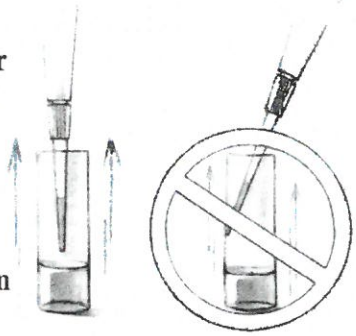
5. Pause Consistently after Aspiration

Pause with the tip in the liquid for one or two seconds after aspirating the sample.

The amount of liquid in the tip "bounces" slightly when the plunger stops. Slow, even plunger release and a consistent, brief pause after aspiration minimize errors resulting from this phenomenon.

6. 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 (<50µL). Surface tension effects cause the sample volumes to vary if the exit angle varies. Touching the tip against the sides results in loss of sample.

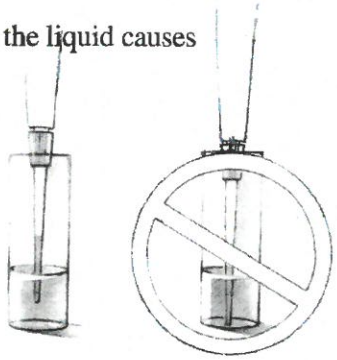
7. Minimize Handling of the Pipette and Tip

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 #2, the volume of sample delivered varies with temperature.

8. Immerse the Tip to the Proper Depth

Immerse the tip 2 to 5mm below the meniscus and well clear of the container walls and bottom during sample aspiration.



Inserting the tip too far into the liquid causes excess droplets of liquid to cling to the outside of the tip. Pressing or resting the tip against the container walls or bottom restricts entry of the sample.

9. Use the Correct Pipette Tip

Securely attach a tip designed for use with the pipette.

Mismatching a tip and pipette or using poor quality tips can result in an inadequate seal between the pipette and the tip. Quality tips are flexible and have thin walls, providing an airtight seal and more dependable delivery of the sample.

10. Use Consistent Plunger Pressure and Speed

Depress and release the plunger smoothly and with consistent pressure and speed for each sample.

Pipettes, like all precision instruments, give more reproducible results when operated with attention to detail and with proper technique.

The ARTEL PCS® Pipette Calibration System is an effective tool for training operators in correct pipetting technique and provides a rapid, accurate alternative to complex gravimetric pipette calibration methods. Call ARTEL toll-free 888-406-3463 for more information about the PCS® or to request a reprint of "Effects of Common Techniques on Accuracy and Precision of Pipetting Results".

References

Lochner KH, Ballweg T, Fahrenkrog H-H. "Factors influencing the measuring accuracy of piston pipettes with air interface." (German). *J Lab Med* 1996; 29 (7/8): 430-40.

Ylätupa S. "Choosing a Pipetting Technique Affects the Results of Your Analysis." *European Clinical Laboratory* 1996. 10:14.

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MLA pipette™

Operator's Manual

MLA Precision, D-Tipper and Selectable Pipettes

Introduction

This manual provides information on the use and care of MLA Precision, D-Tipper and Selectable Pipettes.

Features

All MLA Precision, D-Tipper and Selectable Pipettes are of the air displacement type and are "to deliver" instruments, i.e. they have a fixed stroke and consistently deliver the stated or calibrated volume when the plunger is fully depressed. Except for MLA Selectable Pipettes, each pipette may be adjusted or calibrated above or below its stated volume. The range of adjustment is approximately ±10%. This calibration feature is useful when working with solutions whose viscosity and specific gravity differ from distilled water. Calibration is accomplished readily by inserting a small key into the plunger and turning the key.

MLA Precision and D-Tipper Pipettes

The MLA Precision Pipette has manual de-tipping and the MLA D-Tipper Pipette has mechanical de-tipping. Each model is available in 30 standard sizes ranging from 5 to 1,000 microliters and is available in special volume sizes to meet specific customer requirements. The stated volume is engraved on the bonnet/piston assembly. Pipettes are color coded according to volume size. (See Pipette Information Table.)

MLA Selectable Pipettes

The Selectable Pipette is available in triple range models containing three pipette sizes, such as 50/100/200 microliters. Select the volume by setting the applicable value, engraved on the plunger, adjacent to the line on the bonnet.

Pipette Tips

It is recommended that MLA Pipettes be used with MLA Pipette Tips. The use of tips from other sources may degrade the pipette performance. For information on MLA Pipette Tips, refer to the Pipette Information Table.

Pipetting Procedure

a. Using MLA Pipette Tips, press the pipette nozzle firmly into a fresh tip.

b. Fully depress the pipette plunger and then immerse the tip into the solution (approximately 1/8 inch - 3mm deep).

c. Smoothly release the plunger and allow the solution to enter the pipette tip.

d. Remove the tip from the solution and touch the tip against the side of the vessel to remove any solution that may have adhered to the outside of the tip.

e. Place the tip against the side of the receiving vessel as close to the bottom as possible or, if the vessel contains liquid, as close to the liquid as possible. Smoothly depress the plunger.

f. While holding the plunger depressed, slowly withdraw the tip keeping it against the wall of the container.

g. Release the plunger and remove the tip.

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Hints

a. When pipetting serum or other biological fluids, a liquid film may be retained in the tip that may change the pipetted volume. Pre-wetting the tip with the liquid to be pipetted can reduce this effect.
b. Smoothly depress and release the plunger maintaining the same speed of action for all samples. Do not let the plunger snap back.
c. Fully depress the plunger before inserting the pipette tip into a solution. This will prevent an air bubble from forming in the solution.
d. Hold the pipette as vertically as possible at all times. Insert the tip to the same depth into the sample each time.
e. If an air bubble forms in the tip during intake, return the sample, discard the tip, and apply a fresh tip.
f. Remove and clean the nozzle insert daily. Replace the nozzle insert if necessary.
g. Check that the nozzle assembly is screwed tightly into the pipette body.

Tip Removal Procedure using the D-Tipper Pipette

a. Grasp the pipette as shown in Figure 1.
b. With thumb and forefinger, apply slight upward pressure to the pipette bonnet. This action will eject the tip.

Calibration

Calibratable pipettes are supplied with a key. The pipette is factory calibrated to deliver the volume engraved on the pipette bonnet. Factory tests and calibration are performed at 21.5 ± 2°C using distilled water. To change volume, proceed as follows:

a. Determine the pipette delivered volume by testing the pipette.
   NOTE: Gravimetric or colorimetric techniques may be used to determine the pipette delivered volume. A procedure for the gravimetric method, or information about a MLA Pipette Calibration Kit using a color dilution principle, can be obtained from the Technical Service Department.
b. Insert the key into the plunger. (See Figure 2.)
c. To increase volume, turn the key clockwise. To decrease volume, turn the key counter clockwise. Hold the plunger button while turning the key.
   NOTE: Do not turn the key more than 4 complete revolutions in the clockwise direction.
d. Test the pipette again to determine the delivered volume.

Maintenance

During factory assembly, the internal parts of the pipette are lubricated with a specified grease. Unless the pipette is used with corrosive chemicals or solvents, routine cleaning and lubrication should only be necessary at 6 month intervals. Lubrication is necessary if the plunger is not moving smoothly or does not return to the "up" position.

The nozzle and nozzle insert in particular should be cleaned regularly. In case of accidental sample aspiration, especially corrosive chemicals or solvents, the nozzle insert and nozzle assembly should be cleaned immediately. Cleaning should be done with a lint-free cloth dampened with alcohol. Refer to Figure 3 for removing the nozzle insert.

Should the pipette fail to aspirate or dispense, or if delivered volume is low, the seals should be checked for wear and replaced, if necessary.

To disassemble the pipette for lubrication or to replace internal seals, see instructions in the appropriate seal kit or call Technical Service for assistance.

Figure 1: D-Tipping

Figure 2: Calibration

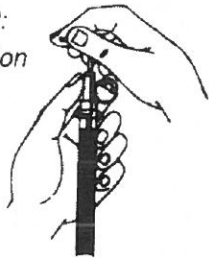
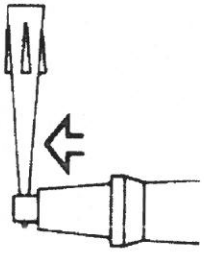
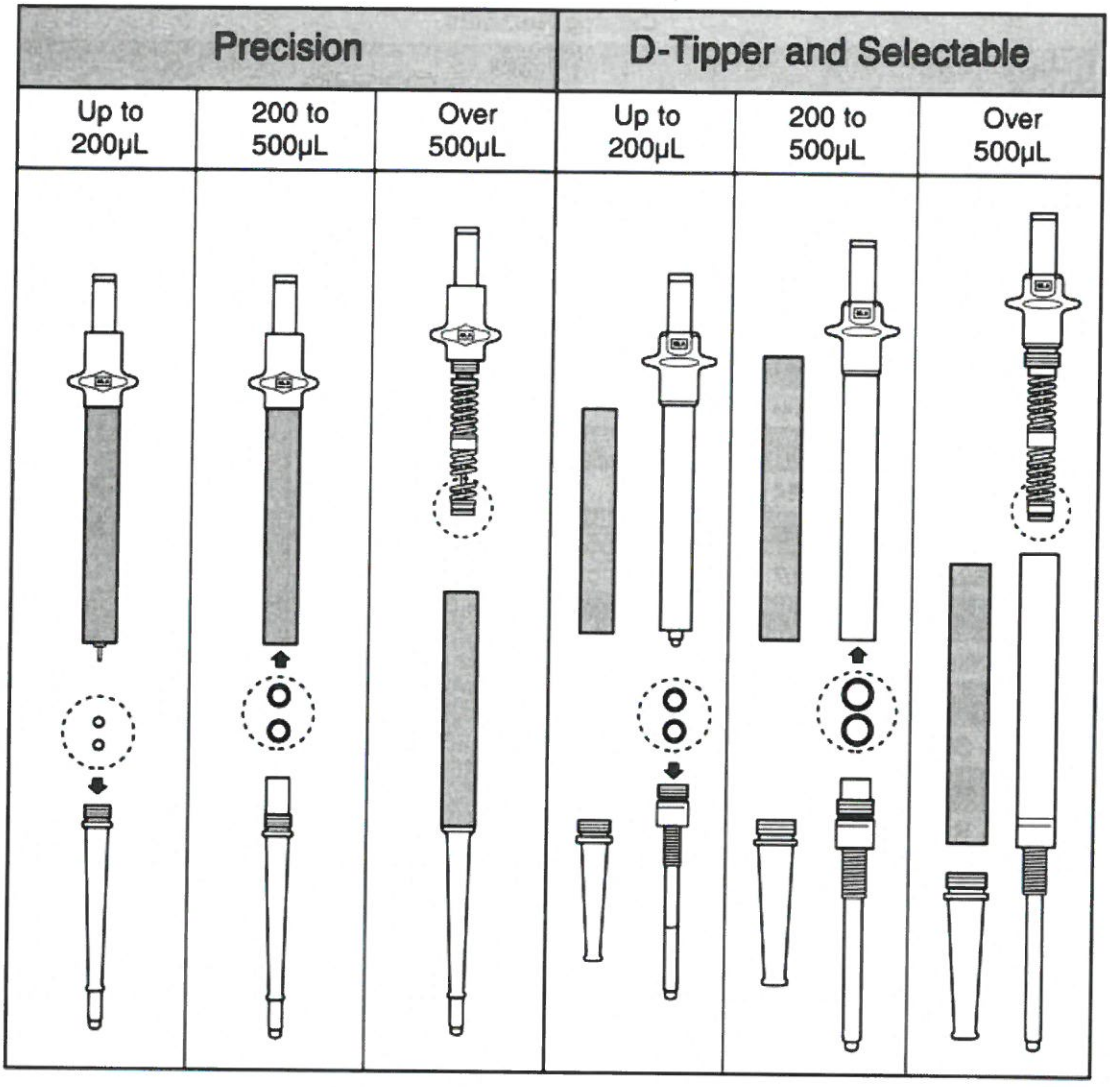


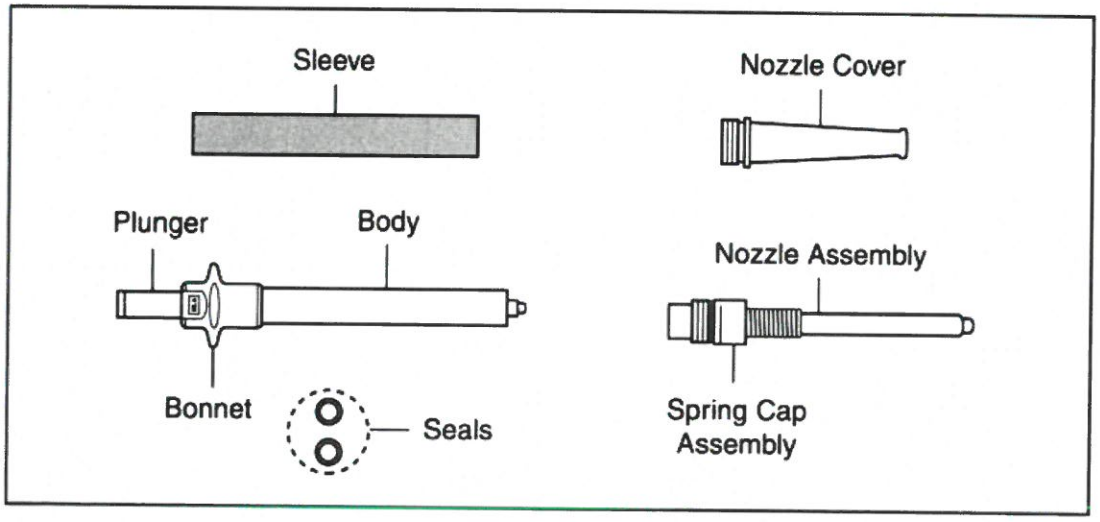
Figure 3: Nozzle Insert Removal



MLA Pipette Assembly



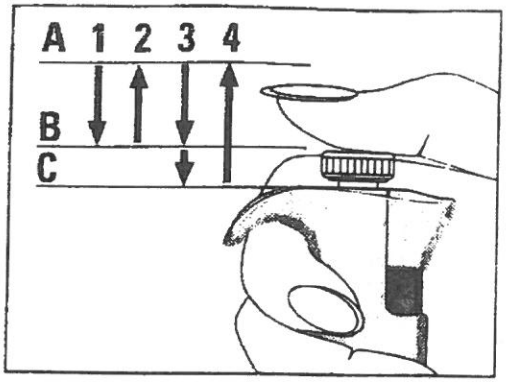
Key to Pipette Assembly



PIPETTING TECHNIQUES, Contd.

FORWARD TECHNIQUE

A = Ready position
B = First stop
C = Second stop

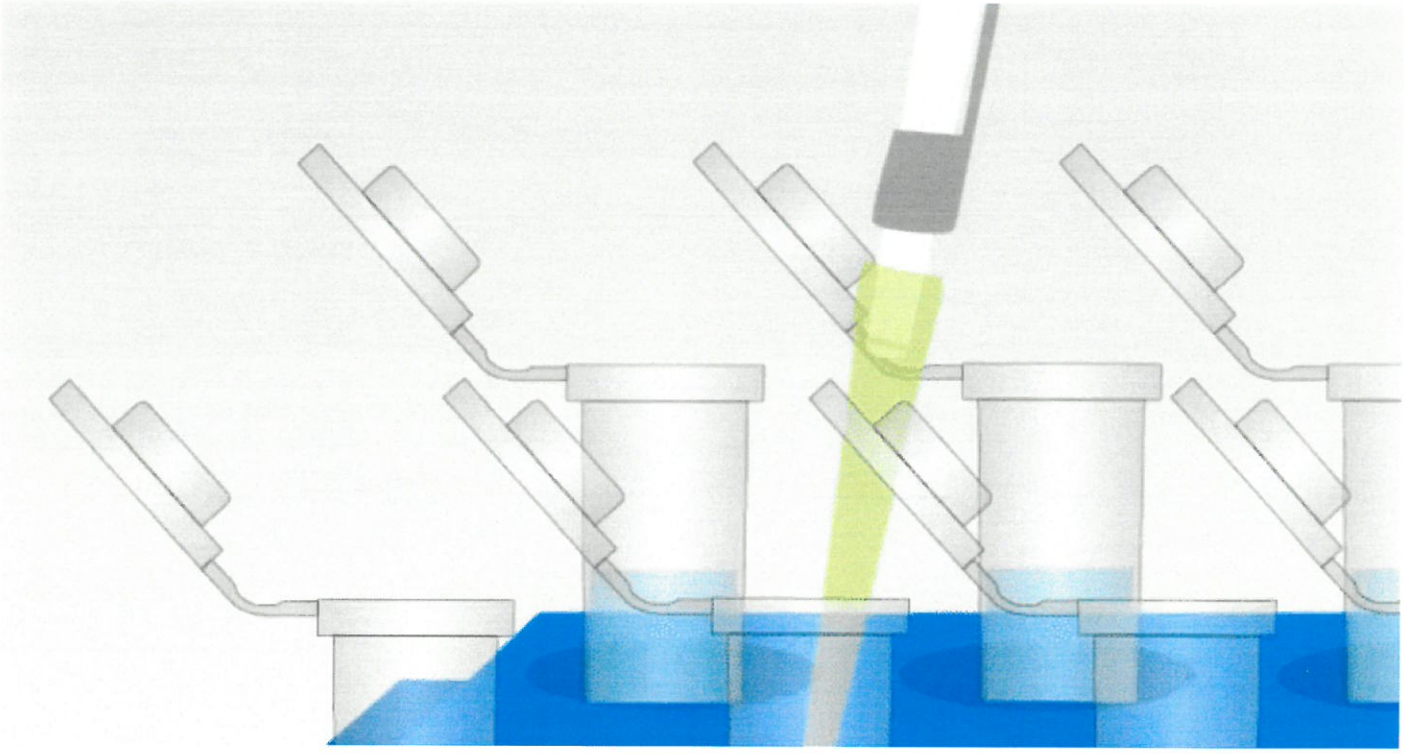


Fill a clean reagent reservoir with the liquid to be dispensed.

1. Depress the push button to the first stop.
2. Dip the tip under the surface of the liquid in the reservoir to a depth of about 1cm and slowly release the push button. This action will fill the tip. Withdraw the tip from the liquid, touching it against the edge of the reservoir to remove excess liquid.
3. Deliver the liquid by gently depressing the push button to the first stop. After a delay of about one second, continue to depress the push button all the way to the second stop. This action will empty the tip.
4. Release the push button to the ready position. If necessary, change the tip and continue pipetting.

REVERSE TECHNIQUE

Top 5 Errors in Pipetting



Pipettes are not just fancy handlebars for your tips, they are essential for precisely measuring and dispensing liquids. These standard 'tools of your trade' enable you to accurately repeat experiments, validate results, make important comparisons between projects and eventually publish that outstanding paper.

But there are a few pipette pitfalls. And they don't just trap beginners! Even the experts in the lab must retrace their steps from time to time to sidestep the introduction of volumetric errors due to poor or inconsistent pipetting techniques.

Want to avoid these experimental sinkholes? Here are the top 5 pipetting errors to watch out for in your next project:

1. Not Accounting for the Viscosity of a Sample

Take, for example, a sample that contains large, sticky molecules – like glucose. The sample clings to the tip surface, traveling slowly when dispensed. Other less viscous and more volatile samples, such as ethanol, travel faster and have a tendency to evaporate. In both cases you must account for the physical properties of the sample because the dispensed volume will not be equal to the set volume. To counteract this discrepancy,

use ultra-low retention pipette tips, make an adjustment to the pipette itself, or make specific volume markings to help maintain accuracy.

2. Dispensing Liquids Too Quickly

Aliquoting bulk samples into single-use tubes is always a quick and easy task. The measured volume is set, and you are only working with a single tip, sample or mixture. But be mindful of pipetting a little too quickly: depending on the desired volume, the size of the tube, and where it is dispensed into the tube (such as at the upper wall or directed at the point at the very bottom) you can lose some of your sample onto the bench with a backsplash. Slow, steady aspiration ensures a clean draw and seamless delivery with no air bubbles or splashes.

3. Contamination By "Double Dipping"

On the bench, you might perform a variety of assays in which a reaction consists of multiple reagents – and your sample. You can introduce contamination by "double dipping:" when you use the same tip to draw and dispense a unique sample and then immediately pipette another sample without changing the tip first. Contamination like this can be tricky to pinpoint if it is not caught right away, as it will affect results in other assays downstream. Pipette tips are relatively inexpensive to replace, however redoing entire experiments is not. Make it standard practice to discard your tip and load a new one before any new reagent – or sample – is measured!

4. Cleaning Irregularly—or Not at All

Everyone knows what to do when liquid is aspirated entirely into the pipette barrel. That's right, disassemble for a deep cleaning to prevent your pipette from becoming the source of contamination. But what about pipettes that pass through many hands without incident? Heavy use will cause parts to wear out more quickly, resulting in a significant decrease in how accurately your liquid samples are measured. Repair may be as simple as occasionally replacing an o-ring, but eliminating contamination is something that takes a proactive approach.

One good habit is to wipe down the pipette each morning with an approved cleaning solution or disinfectant. Some labs even reserve one set of pipettes for "general" use and another set for project-specific uses, such as those that require an "RNAse free" environment. But if that is not something that is practiced in your lab, you can help eliminate the risk of contamination by

wearing a new, clean pair of gloves before reaching for a pipette and then wiping it down at the beginning of every project! Also, check with your lab manager to make sure that the pipettes are serviced twice per year to ensure that they are accurate and reliable for many years to come.

5. Always store pipette upright

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Never lay a pipette down on the counter, especially with any residual fluid left in the tip. Laying a pipette down with fluid in the tip, can have the potential for fluid to enter into the shaft which can cause contamination, corrosion and/or breakage. When fluid is aspirated into the shaft, the pipette should be wiped down with an alcohol pad and dried immediately by using a pipe cleaner in the shaft. Do not leave your pipette lying on the workbench where it can come into contact wih chemicals or fall off and break.

Always store pipettes vertically to prevent liquids from running inside the shaft of the pipette.