Application Guide

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Techniques for Pipetting Challenging Liquids

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Introduction

Pipetting volatile, viscous, foaming, and oily liquids reproducibly with high accuracy and precision can be challenging. These liquids can stick to the plastic or drip as a result of their inherent characteristics. These challenges can be overcome by following best pipetting practices and accounting for the specific properties of the liquid. In this practical guide, we provide advice for selecting an appropriate pipette with compatible pipette tips and correct pipetting technique for difficult liquids.

Which Pipette Should I Choose?

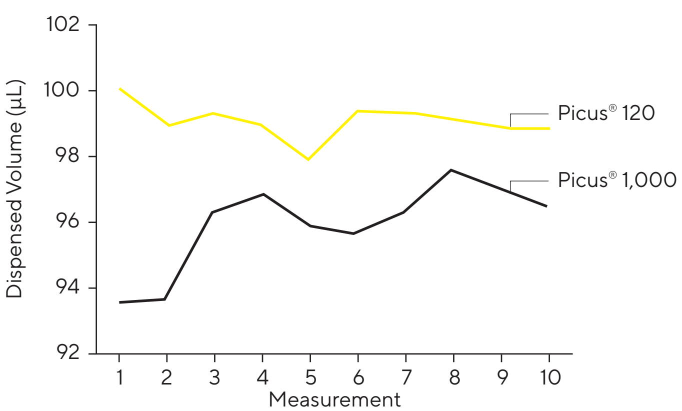
To achieve the most accurate and reproducible results possible, choose a pipette with a nominal volume (maximum volume) that is closest to the volume to be pipetted. For instance, when pipetting 100 µL of a reagent, instead of using a 1,000 µL pipette, choose a smaller nominal volume pipette—even though it might be possible to pipette 100 µL with both. To illustrate this, Figure 1 shows two dispensing series of 100 µL ethanol, using an electronic Sartorius Picus® 50–1,000 µL pipette and a 5–120 µL pipette. With the Picus® 5–120 µL pipette, better accuracy and reproducibility were obtained when the user, pipetting mode (reverse pipetting), and pipetting technique (number of pre-wetting cycles) remained constant.

In reverse pipetting, the target volume is aspirated with an excess amount. Upon pressing the plunger to the first stop, the total target volume is dispensed, and the excess amount is left in the tip. The excess amount is either returned or discarded by pressing the plunger to the second stop. The presence of the excess volume has significant benefits for pipetting performance in certain circumstances, such as when pipetting volatile or viscous liquids. The difference between these pipetting techniques is described in Figure 2.

Electronic pipettes offer additional pipetting techniques that help reduce variation and time spent pipetting. These include multi-dispensing, multi-aspiration, dilution, and titration among others.

Figure 1

Pipetting Accuracy and Reproducibility Are Best at Maximum Volume

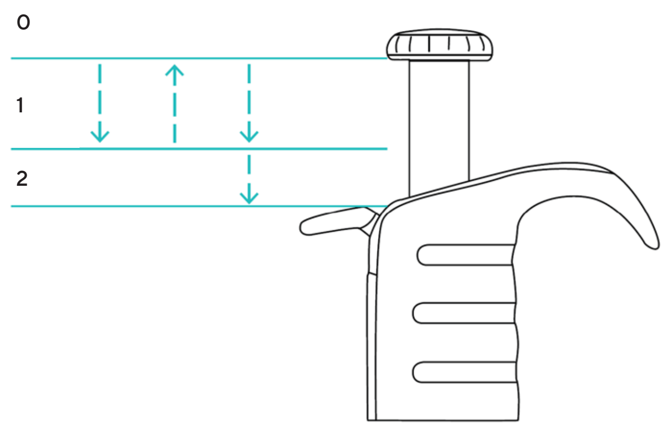


Note. Using a pipette with a nominal volume as close as possible to the volume being pipetted helps accuracy and reproducibility. A series of 10 measurements of 100 µL ethanol was completed using an electronic Sartorius Picus® 5–120 µL pipette (CV% 0.69) and a Sartorius Picus® 50–1,000 µL pipette (CV% 1.70). Reverse pipetting with preceding pre-wetting of the tip was used. Sartorius Optifit 5–200 µL and 100–1,000 µL pipette tips were employed, and tips were changed between each measurement. Accuracy and reproducibility were better with the Picus® 5–120 µL pipette.

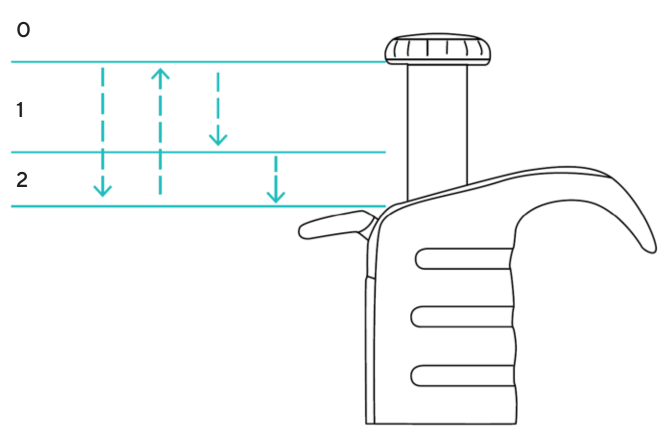
Figure 2

Schemas for Fundamental Pipetting Modes

A. Forward Pipetting



B. Reverse Pipetting



Note. The downward arrow signifies pushing the plunger and the arrow pointing up means releasing the plunger. Level 0 is the resting position for the plunger; level 1 is the first stop, which is easily felt to achieve consistent technique; level 2 is the second stop, which is used to completely empty the tip.

Which Pipetting Technique Should I Use?

Forward and reverse pipetting are two basic pipetting modes that can be used with a mechanical pipette. In forward pipetting, the target volume is aspirated and dispensed, and a separate blowout step is used to completely empty the tip by pressing the plunger to the second stop.

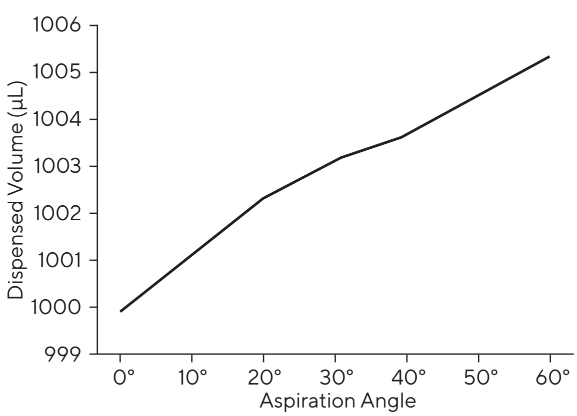
While the optimal pipetting technique depends on the liquid type, some approaches are common for every situation. Fundamental best practices include keeping the pipette upright (at a 0° angle) when aspirating liquid into the tip and slightly tilting it (at a 30°– 45° angle) when dispensing liquid into the target vessel.

Figure 3A shows the impact of the aspiration angle on accuracy when pipetting 1,000 µL of water. The actual dispensed volume increases almost linearly with increasing aspiration angle. Figure 3B shows the negative impact on accuracy and precision when maintaining the pipette at a 0° angle instead of a 45° angle when dispensing with reverse pipetting technique. Holding the pipette at a 45° angle leads to more accurate and precise results when reverse pipetting 1,000 µL of water.

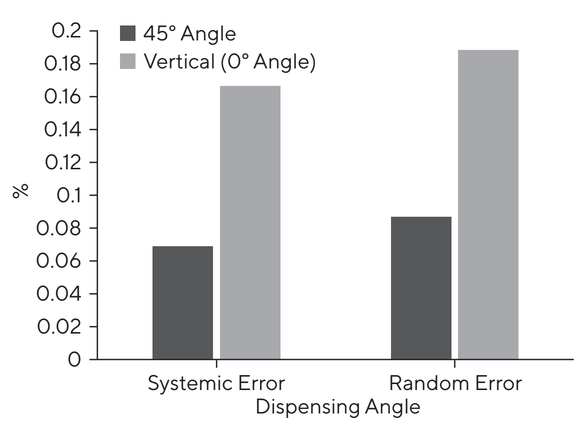
Figure 3

Effect of Aspiration and Dispensing Angles on Accuracy and Precision

A.



B.



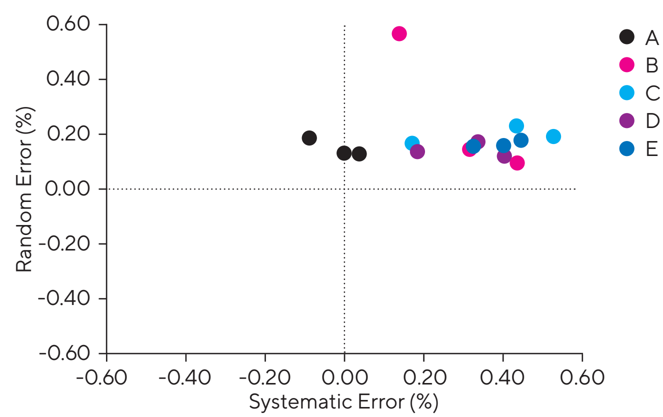
Note. The aspiration angle has an impact on the accuracy of the dispensed volume. While the pipette was set to 1,000 µL, the dispensed volume increased with increasing aspiration angle (A). Impact of the dispensing angle on the random and systemic errors when reverse pipetting 1,000 µL water (B). Data are based on 10 measurements per condition with the Sartorius Tacta® 1,000 µL pipette.

Liquid Is Dripping from the Pipette Tip, What Should I Do?

If liquid is dripping from the pipette tip, first make sure that the tip is properly sealed to the tip cone of the pipette. Keep in mind that it is sometimes impossible to visually detect improper sealing, which is why tip compatibility should be verified on an analytical balance. For the tips recommended by the manufacturer of the pipette testing the compatibility is not necessary, as it is guaranteed. Figure 4 shows the difference in pipetting performance when pipette tips from different manufacturers are used.

Figure 4

Tips and Pipettes: Compatibility Matters



Note. Different pipette tips were used in combination with a Tacta® 10 µL pipette to measure 10 µL. Each data point represents 10 measurements with the same pipette tip. Each color corresponds to one pipette tip brand, with "A" being Sartorius pipette tips and "B-E" tips from other manufacturers. The spread between data points within a brand shows the variation between individual tips within the same tip box.

In case of gross leaks of other than volatile liquids, check whether the tip cone of the pipette is clean; use a lint-free cloth and a suitable cleaning agent (e.g., ethanol) to wipe it clean. Check for damage to the tip cone or to the seal between piston and cylinder.

Volatile Liquids

Dripping from the pipette tip is common when pipetting volatile liquids and solvents such as ethanol, acetone, or acetonitrile. It is caused by the high vapor pressure of these solvents, which leads to evaporation and expansion of the air column above the liquid. To avoid dripping, pre-wet the tip, rinsing it a minimum of five times by aspirating and dispensing the desired volume. This saturates the air column, reducing evaporation. If pipetting very small volumes, the number of pre-wetting cycles can be increased or a larger volume can be used for pre-wetting.

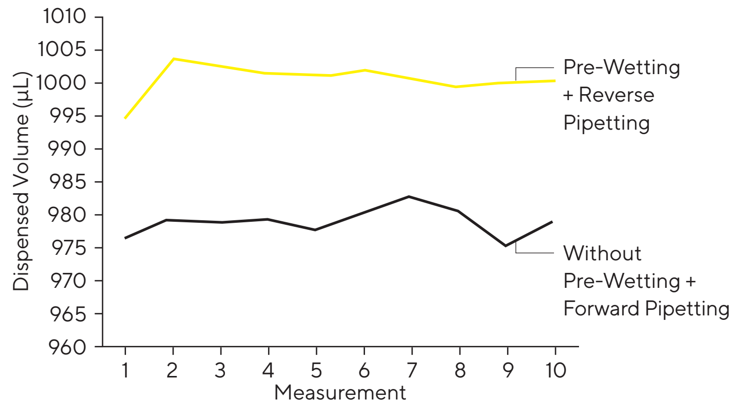
Reverse pipetting further helps with the dripping. It eliminates the impact of any remaining evaporation on dispensing the total target volume. Any additional evaporating liquid escapes from the excess and won't impact pipetting performance. In forward pipetting, any further evaporation reduces the total liquid volume and directly decreases accuracy.

Figure 5A shows the difference between forward pipetting without pre-wetting and reverse pipetting with the appropriate pre-wetting when handling acetonitrile. The value of proper pipetting technique is clear. The same recommendation applies regardless of volume, as shown in Figure 5B.

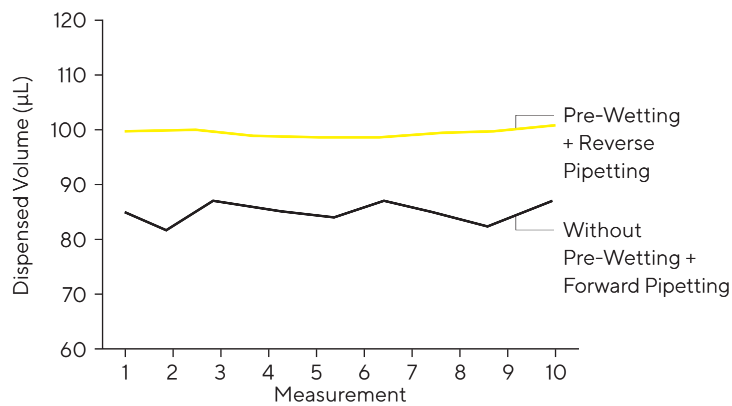
Figure 5

Pipetting Volatile Liquids

A.



B.



Note. When pipetting volatile liquids, the pipette tip should be pre-wetted (rinsed) at least five times and the liquid should be reverse pipetted. A series with 10 dispensings of 1,000 µL acetonitrile was conducted with the mechanical Sartorius Tacta® 1,000 µL pipette, either by pre-wetting the tip and reverse pipetting or without pre-wetting and pipetting with the forward technique (A). Sartorius Optifit 100–1,000 µL pipette tips were used, and the pipette tip was changed between measurements. The same experimental approach was used but with 100 µL acetone and a mechanical Sartorius Tacta® 100 µL pipette (B).

Viscous Liquids

Pipetting viscous liquids accurately is sometimes considered to be practically impossible. Cutting the pipette tip so the viscous liquid flows more quickly should be avoided, as it will have a catastrophic effect on accuracy and repeatability. Reliable dispensing of viscous liquids is, however, just a matter of proper technique and patience as slow aspiration and dispensing speeds are critical.

Accuracy is improved with reverse pipetting. Viscous liquid, such as glycerol, adheres to the inside surface of the tip and in forward pipetting reduces the accuracy of dispensing. With reverse pipetting the adhering amount is reduced from the excess amount present in the tip and the complete target volume is dispensed.

It is also important to not immerse the tip too deep into the liquid; 2–3 mm is typically sufficient. This prevents liquid from adhering to the outside of the tip and transferring to the target vessel during dispensing, which causes extra volume to be transferred inadvertently. If liquid does stick to the outside, touch the wall of the reagent vessel gently to wipe it off.

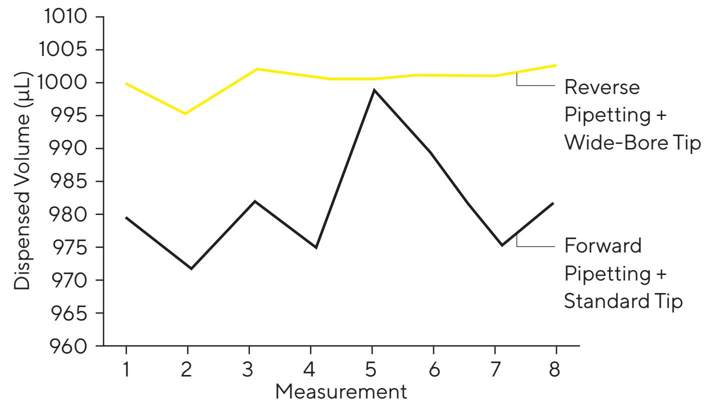
Maintaining a slow flow speed is also critical as this avoids aspiration or dispensing of air instead of the desired liquid. Additionally, the pipette tip should remain immersed in the liquid for several seconds longer when aspirating as this allows the liquid to settle into the tip. If the tip is removed from the liquid too early, air may be aspirated instead. When dispensing, it is recommended to hold the tip against the target vessel wall, so the liquid adheres to the target vessel. Wide-bore tips can also be used for facilitating the flow of the liquid especially in cases where there are particles in the solution.

Figure 6 shows the difference between pipetting 1,000 µL of glycerol with reverse pipetting and an Optifit wide-bore tip compared to forward pipetting and a standard Optifit tip.

Picus® NxT pipette allows repeated blowout with forward pipetting, in cases where the user feels it may be helpful.

Figure 6

Pipetting Viscous Liquids



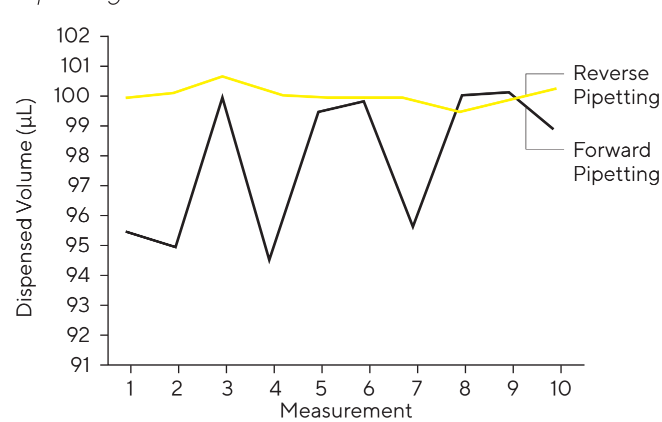
Note. For viscous liquids, use reverse pipetting, proceed slowly, and use a wide-bore tip. In the example shown here, the volume was set to 1,000 µL on a mechanical Sartorius Mline® 100–1,000 µL pipette. The dispensed volume of 1,000 µL glycerol was measured after pipetting either with reverse or forward pipetting technique, using either wide-bore or standard Optifit tips. The pipette tip was changed between measurements.

Oils

Reverse pipetting oils leads to more accurate and reproducible dispensing compared to forward pipetting, as oils form a film on plastic. Adherence of the oil reduces accuracy with forward pipetting. In reverse pipetting, the oil adhering to the plastic escapes from the excess volume and improves accuracy. In Figure 7, eugenol, an aromatic oily liquid extracted from essential oils, was pipetted using a mechanical Sartorius Tacta® 10–100 µL pipette.

Figure 7

Pipetting Oils



Note. Oils should be pipetted with reverse pipetting technique. In the example shown here, a series of 10 dispensings of 100 µL eugenol was conducted with a mechanical Sartorius Tacta® 10–100 µL pipette, using either reverse or forward pipetting techniques. The pipette tip was changed between measurements.

Foaming Liquids

Liquids containing detergent or protein tend to foam easily. When working with these liquids it is important to pipet slowly and use reverse pipetting. If forward pipetting is preferred, avoid using blowout or pressing the plunger to the second stop, as it contributes to the formation of bubbles in foaming liquids. Maximal accuracy with forward pipetting is only achieved by using blowout. The obvious advantage of reverse pipetting is that no blowout is needed to dispense the desired volume accurately and foam buildup is avoided.

How Often Should I Maintain My Pipette?

Frequent cleaning of the pipette is good practice, and the interval should be increased when working with volatile liquids and solvents. Daily wiping of the outside and the tip cone reduces the chance of cross-contamination and ensures proper tip sealing. Cleaning inside the lower end of the pipette removes dirt from building up, which helps with piston sealing and maintains low operating forces. Cleaning may also help to reveal whether the pipette is wearing out due to solvents or vapors prior to affecting results.

Remember that whenever the lower end is disassembled and reassembled, it is best to check the performance of the pipette on an analytical balance to confirm assembly was done properly and nothing is obstructing a complete seal of the piston. Detailed instructions for cleaning Sartorius pipettes can be found on: https://sartorius.com/pipette-cleaning-guide

The recommended service interval of pipettes is a maximum of one year. The selected interval should be adjusted, however, to reflect factors such as pipetting frequency, liquids that have been dispensed, and the age and model of the pipette. In cases where volatile liquids or solvents are dispensed regularly, maintenance should be done more often. Calibration should be done at least annually, or every 3–6 months depending on the frequency of use. Calibration should also be done after exchanging pipette parts, such as the cone or piston; after autoclaving on-site, a performance check can be sufficient.

Summary

Pipetting technique plays a central role in achieving the best possible results. Learning to make use of reverse pipetting is very beneficial when pipetting challenging liquids. A best practice is to always first consider the liquid and adopt pipetting practices accordingly.

Table 1 summarizes the recommended pipetting techniques for a variety of liquids.

Table 1
*Recommended Pipetting Techniques and Practices for Different Types of Liquids**

Liquid Type	Pipetting Technique	Pre-Wetting	Comment
Aqueous	Forward pipetting	3–5 times	
Viscous	Reverse pipetting	3–5 times	Slow speed, keep tip immersed in liquid longer than usual when aspirating and dispense while touching target vessel's wall
Volatile	Reverse pipetting	5+ times	
Foaming	Reverse pipetting	3–5 times	Slow speed
Oils	Reverse pipetting	3–5 times	

* Remember to always keep the pipette at a 0° angle when aspirating and at a 30°–45° angle when dispensing liquid.

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