



TROEMNER

Technical Paper

Proper Pipetting Techniques & Tips

PROPER PIPETTING TECHNIQUES & TIPS

• Optimize Pipette Performance

• Increase Reproducibility of Your Results

- **Technique** - Most end users have a tendency to believe that the volume delivery is completely dependent on the setting of the micrometer dial. Obviously, this is not the case, since many factors associated with pipettes come into play.
- **Position** - Pipettes should be held vertical during the aspiration of liquids, however, some end users often hold pipettes at many different angles during a pipetting interval. Holding a pipette 30° off vertical can cause as much as 0.7% more liquid to be aspirated due to the impact of hydrostatic pressure. Always store pipettes in an upright position when not in use.
- **Tips** - Use Manufacturers Tips
- **Pre-Wetting/Pre-Rinsing Tips** – Failing to pre-wet tips can cause inconsistency between samples since liquid in the initial samples adhere to the inside surfaces of the pipette tip, but liquid from later samples does not. Also, if a new volume is dialed in on the pipette's micrometer, you will receive better results at the new volume by taking the old tip off and placing a new one on the shaft before you commence pipetting.
- **Release of Plunger** - Releasing the plunger abruptly can cause liquid to be “bumped” inside the pipette during a liquid transfer application. This can cause liquid to accumulate inside the instrument which in turn can be transferred to other samples causing variability in sample volume and the potential for cross contamination. It is recommended that a smooth, consistent pipetting rhythm be employed since it helps to increase both accuracy and precision. After the liquid has been aspirated into the tip, the pipette should be placed against the wall of the receiving vessel and the plunger slowly depressed. This will help all of the liquid in the tip to be dispensed. After a pause of about 1 second, depress the plunger to the bottom or blowout position (if equipped) and remove the pipette from the sidewall by utilizing either a sliding action up the wall or a brief movement away from the wall (called “touching off”).
- **Immersion Depth** - The pipette tip should only be inserted into the vessel containing the liquid to be transferred about 1-3mm. If the tip is immersed beyond this, the results could be erroneously high. This is due to the fact that liquid could adhere to the tip and be transferred along with the aliquot in the tip. If the tip is not immersed far enough then air could be drawn into the tip which could yield results that are incorrect on the low end.
- **Equilibration Time** – Troemner recommends that the tip, the pipette, the liquid being transferred, and the transfer container itself all be allowed to equilibrate to the same temperature. This is done to lessen the effects of thermal expansion which can dramatically impact the delivered volume.
- **Thermal Conductance** – Thermal energy can be transferred from the operator's hand to the air within the pipette (dead air) or even to the internal components themselves. This can have a dramatic impact on the amount of liquid dispensed due to the effects of expansion and/or contraction. To lessen this effect, it is recommended that some type of thermally insulated gloves like latex or cloth be worn.
- **Temperature** – The volume delivery performance specifications of pipettes have been referenced by most manufacturers at room temperature which is defined as 20-25°C. Any deviation from this specification can affect the amount of liquid dispensed due to the expansion or contraction of the internal components. Temperature is probably the most important factor that influences pipette performance. In fact, the density of water in a gravimetric analysis is calculated as a function of temperature.