The pipette is a reliable precision instrument that has been 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. As your laboratory's demand for accuracy and precision increases, the importance of understanding and developing optimal pipetting technique becomes imperative.

Let's review the results of a study conducted at ARTEL to determine these differences and assess their impact upon accuracy and precision.

Method & Procedure

The reference pipetting method used in each of these experiments was as follows: The pipette mechanism was “warmed up” by gently depressing and releasing the plunger 15 to 20 times. The pipette tip was prewet by aspirating and dispensing an aliquot of the sample solution three times. With the plunger depressed to the first stop, the tip was immersed approximately 1 millimeter into the sample solution and held there for a half second. The aliquot was aspirated from the sample solution by gently releasing the plunger, keeping the tip in the sample solution for two seconds before removal. The aliquot was delivered into the blank solution vial by placing the pipette tip on the side of the glass vial at a 45° angle just above the meniscus and slowly depressing the plunger past the first stop to deliver the entire aliquot.

Each experiment consisted of two runs of ten data points each, using an adjustable 20 μL Eppendorf manual action air displacement pipette set at 5.00 μL and Eppendorf disposable pipette tips, on the ARTEL PCS® Pipette Calibration System, an automated instrument/reagent system which verifies the volumetric delivery of pipettes. Each experiment was performed as a comparison of one pipetting technique versus another. The results for each pair of techniques were compared concerning precision and accuracy. The measurement used for rating the precision of each technique was the coefficient of variance (%CV). Accuracy was defined as the percent difference in mean delivery volumes between the two pipetting techniques.

Eliminating sources of error

- Don't leave your tips high and dry.

The experiment comparing dry and prewetted pipette tips revealed the greatest discrepancies observed during this study. Dry pipette tips consistently delivered significantly lower volumes than did the prewetted tips, a fact which other researchers have noted. Though no difficulty with precision was observed using either method exclusively in a single run, differences in accuracy of up to 7% between runs using dry and prewetted tips were noted while using the 20 μL pipette set at 5 μL. Additional experiments using a 250 μL pipette set at 25, 50, 100 and 250 μL consistently showed differences in the accuracy of the volume pipetted of up to 2%.

Prewetting the pipette tip influenced accuracy by increasing the humidity within the tip, thus minimizing evaporation of the solution. Similarly, increased ambient humidity minimized evaporation. The beneficial effect of prewetting was less significant with constant or high ambient humidity. Ambient humidity for these runs was 50%.

- Not too hot... not too cold...

Variation in the temperature of the solution being pipetted was observed to be the second largest cause of pipetting error in this study. Three sample solutions were...
What are other techniques which can affect accuracy and precision?

Other error causing techniques noted in this study included:

a. prolonged delay between aspiration and removal of the tip from the sample
b. dragging the tip along the side of the container when the tip is exiting the sample
c. variations in the size and shape of the sample container, and
d. rate of plunger depression and release.

What impact will these techniques have upon my results?

Individually none of these factors resulted in an error greater than 2%. Cumulatively, however, two or more of these sources of error (e.g., prolonged delay and rate of plunger depression) could affect delivered volume significantly.

What factors other than technique differences can affect my results?

Component failure (e.g., a plunger seal or corroded piston), incorrect pipette tip, or incorrect installation of the tip can also affect your results.

References:

7. Ylätupa Dr. S. “Choosing a Pipetting Technique Affects the Results of Your Analysis”, European Clinical Laboratory 1996. 10:14.

Effects of Sample Temperature on Pipetting Accuracy

Experiment showing the effect of sample temperature on the accuracy of pipetting results, using a 20 μL adjustable air displacement pipette set at 5.00 μL. Ambient temperature 25.0 °C.