Errors in Liquid Delivery – The Importance of Laboratory Conditions on Pipetting

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Introduction

Many common laboratory procedures require quantitative pipetting and mechanical action air-displacement micro-pipettes are frequently used for this purpose. The design of these pipettes, however, makes their performance susceptible to sample temperature, as well as to environmental relative humidity and barometric pressure. The susceptibility to environmental effects is reflected in pipette calibration standards (*e.g.*, ISO 8655-6 and ASTM E1154), which stipulate control and reporting of these environmental factors during pipette calibration.

Real-world usage of pipettes, however, often differs from calibration ideals. The work presented herein investigates the accuracy of micropipettes from three different manufacturers, ranging from 2 µL to 1000 µL. The first study investigated the accuracy of dispensed sample volumes when pipetting aqueous samples of different temperatures than the pipettes and tips. The second study investigated the effects of low humidity on pipetting accuracy, and the third study examined the impact of barometric pressure at high altitude.

Experimental

Adjustable volume pipettes from three leading manufacturers were examined, covering the volume ranges of 0.2-2 µL, 2-20 µL, 50-200 µL, and 200-1000 µL. Each pipette was tested at volume settings close to its specified minimum and maximum volumes, using tips from the respective pipette manufacturer. Ten replicates were averaged for each data point shown here. An Artel PCS® Pipette Calibration System, based on the principle of ratiometric photometry, was used to precisely determine the dispensed sample volumes.

A. Thermal Disequilibrium

Representing real-life laboratory situations, aqueous solutions to be pipetted were equilibrated and kept at the desired temperature (4 °C, 22 °C, 37 °C, and 60 °C), while pipettes and tips were kept at ambient temperature. At each volume setting, aliquots of the different temperatures were pipetted in alternating order to minimize systematic warming or cooling of the air cushion within the pipette shaft and tip. A new pipette tip was used for every sample delivery, and the tips were not pre-wetted, so that immediately prior to the aspiration, each tip was in thermal equilibrium with the ambient laboratory air.

B. Low Humidity and High Temperature

The accuracy of the pipettes described above was investigated in an environment of hot aridness. The combination of low relative humidity (7%) and high ambient temperature (44 °C) results in high evaporation potentials. Hot and dry conditions may be encountered by laboratories operating in some regions of the US, and other regions around the world, sometimes only seasonally.

Sample volumes were determined when tips were changed after each dispense and were compared to a second study in which each tip was pre-wetted 5 times prior to delivering the aspirated sample.

C. High Altitude – Low Barometric Pressure

Low pressure testing was performed at the weather observatory atop Mt. Washington in New Hampshire, USA (elev. 6,288 feet, 1917 m). Due to access restrictions and limited time at the mountain top only a few pipettes were tested. The accuracy at high elevation was compared to accuracy during calibration near sea level.

Results

A. Thermal Disequilibrium

Acquired data of each volume/temperature combination were averaged, and the dispensed volume calculated as bias versus the ambient temperature data (22 °C).

Low-temperature samples were consistently delivered in excess of the set volume by all pipettes at any volume setting, as is shown in **Figure 1**.

Samples at higher temperatures than ambient were consistently under-delivered, as is shown in **Figure 2** (37 °C samples) and **Figure 3** (60 °C samples).

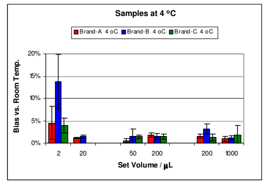

Figure 1

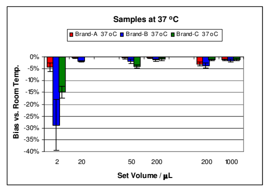

Figure 2

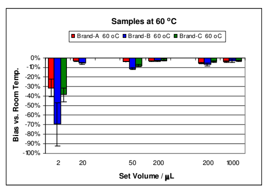

Figure 3

B. Low Humidity and High Temperature

Acquired data for each volume were averaged, and the dispensed volume calculated as bias versus the set volume of the pipette. These volume deviations are compared to the manufacturer's inaccuracy specifications for each pipette model.

Without pre-wetting the tips, only two pipettes delivered samples within the manufacturer's specifications, as indicated by the red asterisks in **Figure 4**.

Pre-wetting reduced the inaccuracy at most volume settings, and allowed more pipettes to deliver samples within specifications, as shown in **Figure 5** and noted by the red asterisks.

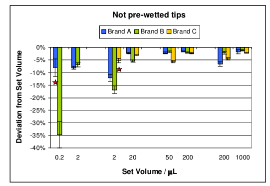

Figure 4

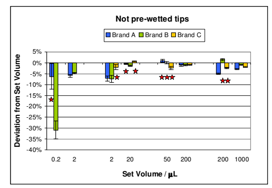

Figure 5

C. High Altitude – Low Barometric Pressure

Data acquired at each volume under high elevation conditions (805 Mbar) were averaged and the dispensed volume was calculated as bias versus the calibration lab data at sea level (1013 mbar).

All volumes tested delivered less at high elevation as is shown in **Figure 6**.

Pipettes set to deliver 100% of nominal volume were less effected by barometric pressure than when the same pipettes were set to deliver 10% of nominal volume.

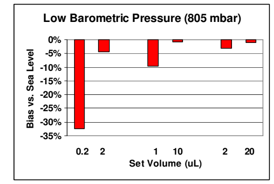

Figure 6

Discussion

Using pipettes at or close to the minimum specified volume setting results in less accurate sample delivery compared to using the pipettes at or close to the nominal volume settings, as evidenced in all three studies.

The thermal disequilibrium results are consistent with a thermodynamic model of the pipetting process in air displacement micropipettes. Upon immersion of the tip into a cold liquid, thermal conduction begins to cool the captive air inside of the pipette, leading to a reduction of air volume inside the pipette. This volume discrepancy is balanced out by aspirating more liquid sample into the tip, hence resulting in an over-delivery of sample. The opposite effect is encountered when immersing the tip into a warm liquid, resulting in the aspiration of less sample volume.

Pipettes set at their minimum operating volume contain the same captive air volume as those set at their maximum operating volume, but less liquid is handled. Thus, the ratio of air to liquid is increased, and an identical change of volume on the air side has a larger proportionate impact on the liquid.

When pipetting in conditions of low humidity, evaporation inside of the pipette tip increases the volume of captive gas (also called "dead air volume") by converting water to water vapor. The resulting volume change reduces the amount of liquid sample that is aspirated and available for dispensing.

In the studied case, elevated ambient temperature and low relative humidity increases this Evaporation Potential, leading to consistent under-delivery of sample. Pre-wetting the tips thoroughly prior to aspirating the sample, increases the vapor pressure of water inside of the pipette, thus decreasing the Evaporation Potential (ΔE_p).

Pipettes at minimum set volumes experience a larger effect by the change in ΔE_p, as the ratio of dead air volume to liquid aliquot is larger than at the pipette's nominal volumes (*vide supra*).

Conclusions

Researchers who are pipetting warm or cold liquids need to be aware that this technique is prone to introduce significant errors into common laboratory procedures.

Whenever possible, it is recommended to pipet liquids that are equilibrated to room temperature. Whenever this is not feasible, it is recommended that the researcher determine the pipette inaccuracy of the used pipette/tip/temperature combination prior to the experiment.

Pipette users need to be aware that low humidity may induce significant errors in delivered sample volumes, especially in laboratories which are heated, air-conditioned, or located in dry environments. These errors are compounded by elevated ambient temperatures.

Pipettes of the air displacement design should be calibrated at a barometric pressure (or altitude) where they will be used. Pipettes that have been calibrated by the manufacturer or a third party calibration service should be checked or re-calibrated before use in a laboratory at a significantly different altitude.

Air displacement micropipettes are capable of excellent accuracy and precision under ideal environmental conditions. However, real world situations often require pipetting under less optimal conditions. The data presented in this poster provide evidence for the importance of calibrating or verifying pipettes under the environmental conditions where pipettes are actually used.

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