



Liquid Delivery Errors – The Impact of Laboratory Conditions on Volumetric Measurements

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Introduction

Many common laboratory procedures require the handling and quantitative dispensing of reagents. Mechanical action micropipettes are most often used for this routine task in laboratories around the world. The construction of these pipettes, however, makes their performance susceptible to variations in temperatures of the samples dispensed, as well as to the relative humidity of the environment in which they are used. The susceptibility to thermal effects is reflected in pipette calibration standards (i.e. ISO 8655-6 and ASTM E1154), stipulating stringent control of temperatures (20 ± 0.5 °C) during pipette calibration, and also requiring that all materials, including the sample liquids, be thermally equilibrated prior to the calibration. The same calibration standards, as well as most pipette manufacturers' guidelines, recommend pipettes to be calibrated in an environment of 45-75% relative humidity.

Real-world usage of pipettes, however, differs from these calibration ideals. Many common assay protocols, for example, require the dispensing of reagents that are not in the specified temperature equilibrium. Two common examples are tissue culture applications, which employ reagents and buffers at 37 °C, or assays with nucleic acid-based reagents at 4 °C or lower.

Levels of relative humidity in laboratories are subject to large variations, depending on the type of laboratory, on the laboratory's geographic location, on the time of the year, as well as on the facility's heating and air-conditioning equipment.

The work presented herein investigates the accuracy of micropipettes from three different manufacturers, ranging from 2 μ L to 1000 μ L. 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.

Experimental

Adjustable volume pipettes from three leading manufacturers were examined, covering the volume ranges of 0.2-2 μ L, 2-20 μ L, 50-200 μ L, and 200-1000 μ L. 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 ratio-metric 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.

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

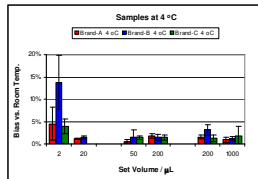


Figure 1

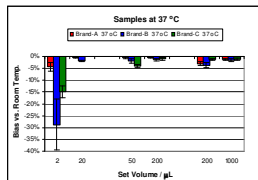


Figure 2

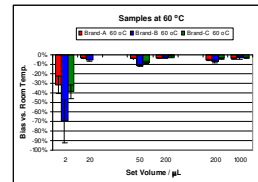


Figure 3

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.

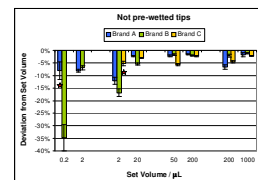


Figure 4

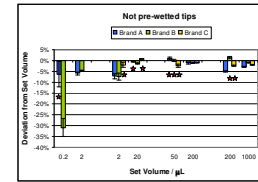


Figure 5

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 both studies.

The results are consistent with the thermodynamic model of the pipetting process in an air displacement micropipette. Upon immersion of the tip in 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 sample, resulting in the aspiration of a decreased liquid 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 "air" 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 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 pipettes' 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 in laboratories, which are heated or air-conditioned, or which are located in dry environments need to be aware that low humidity may induce significant errors in delivered sample volumes. These errors are compounded by elevated ambient temperatures.

While scientists rarely have control of the laboratory's relative humidity, it is imperative to be aware of this potential error source. It is recommended to pre-wet the pipette tip at least five times to establish a humidity equilibrium inside of the tip and shaft.

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