Errors Associated with Pipetting of Warm and Cold Liquids

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Introduction

Many common laboratory procedures require the handling and quantitative dispensing of reagents at various temperatures. Mechanical action micropipettes are most often used for this routine task. The construction of these pipettes, however, makes their performance susceptible to variations in temperatures of the samples dispensed. This 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 ºC) during pipette calibration, and also requiring that all materials, including the liquids, be thermally equilibrated prior to the calibration.

However, many common assay protocols 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 ºC, or assays with nucleic acid-based reagents at 4 ºC or lower.

The work presented herein investigates the accuracy of micropipettes from three different manufacturers, in the most commonly used range of 2 µL to 1000 µL, when used to pipet aqueous samples at various temperatures.

Experimental

Representing real-life laboratory situations, aqueous solutions to be pipetted were equilibrated and kept at the desired temperature (4 ºC, 22 ºC, 37 ºC, and 60 ºC), while pipettes and tips were kept at ambient temperature.

Adjustable volume pipettes from three different manufacturers were examined, covering the commonly used volume ranges of 2-20 µL, 50-200 µL, and 200-1000 µL. Each pipette was tested at volume settings close to its specified minimum and maximum volume, using tips from the respective pipette manufacturer. At each volume setting, aliquots of the different temperatures were pipetted in alternating order, until ten data points were acquired for each thermostatted sample. Systematic warming or cooling of the air cushion within the pipette shaft and tip is minimized by this regular alternation.

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. During sample aspiration, the tip was immersed approximately 5-7 mm below the liquid surface into the sample vial. After sample aspiration, the tip remained in the sample for one second before removal. The sample was dispensed against the side wall of the analysis cuvette located in an Artel PCS® Pipette Calibration System.

The analytical procedure to measure the delivered sample volume is based on the principle of ratiometric photometry. This highly precise method is based on the delivery of a calibrated red dye solution into a cuvette containing calibrated blue dye solution.

Results

A plot of acquired raw data points for one volume setting (2.0 µL) at alternating temperatures is shown in **Figure 1**. Re-plotting the 10 replicates for each temperature sample in a continuous trace (**Figure 2**) clearly shows the thermal effect on the dispensed volume. The average of the 10 data points acquired at 22 ºC is used as ambient temperature reference for the following data analyses.

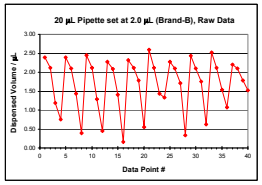

Figure 1. A 20 µL pipette, set at 2.0 µL, was employed to successively deliver reagents at temperature intervals of: 4 ºC, 22 ºC, 37 ºC, 60 ºC. Ten replicates of this interval were performed.

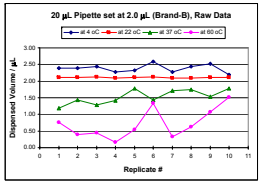

Figure 2. Data of Figure 1 were re-plotted to show the dispensed volume as a function of sample temperature.

Acquired data of each volume/temperature combination were averaged, and the dispensed volume calculated as bias versus the ambient temperature data. The results of this data processing are shown in the following graphs.

Low-temperature samples were consistently delivered in excess of the set volume by all pipettes at any volume setting, as is shown in **Figure 3**.
Samples thermostatted at higher temperatures than ambient were under-delivered, as is shown in **Figure 4** (37 ºC samples) and **Figure 5** (60 ºC samples).

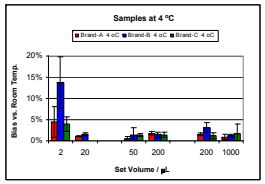

Figure 3

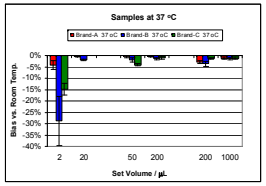

Figure 4

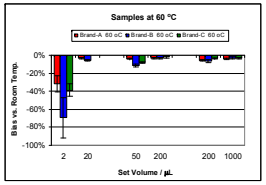

Figure 5

Discussion

Thermal effects on sample delivery volume are significantly more pronounced at smaller volumes. Using pipettes at or close to the minimum specified volume setting seems to result in less accurate sample delivery compared to using the pipettes at or close to the nominal volume settings.

These results are consistent with the thermodynamic model of the pipetting process in an air displacement micropipette. A piston inside of the pipette shaft is moved to displace air of the desired volume out of the shaft and pipette tip. The correlation between temperature, volume and pressure is described by the ideal gas law [PV = nRT]. Once the tip is immersed 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. During aspiration, 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: the captive air inside the tip is exposed to increased temperature and expands, resulting in the aspiration of a decreased liquid sample volume.

Adjustable 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. This thermodynamic model assumes that the majority of the heat transfer between sample solution and air inside of the tip occurs across the relatively large area of the exposed thin plastic tip, rather than at the small area of the direct liquid/air interface encountered during the aspiration process.

Experimental results seem to be in good accordance with this model, showing a larger discrepancy in delivered volume when pipetting volumes near the minimum specified range, as compared to the nominal volume of these pipettes.

Furthermore, the study demonstrates clearly that pipettes for small volumes (*i.e.* in the 2-20 µL range) are more susceptible to thermal effects than pipettes designed to handle larger volumes. Considering the larger surface to volume ratios of the smallest tips, and the larger ratio of air to liquid within the tips and shafts of these small-volume pipettes, the results are consistent with the thermodynamic model described above.

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. Cold liquids tend to be delivered in excess quantity, while warm liquids tend to be under-delivered. Depending on pipette manufacturer, volume set point and temperature of the sample, these errors can exceed 65%, with small volumes to be impacted most.

Whenever possible, it is recommended to pipet liquids that are equilibrated to room temperature. When using protocols necessitating the handling of cold or warm liquids with an air displacement pipette, it is recommended that the researcher determine the pipette inaccuracy of the used pipette/tip/temperature combination prior to the experiment. Since it is not always feasible to determine the precise aberration from the calibrated volume at any given temperature, volume, and tip combination, everyone interpreting the data should be aware of the potentially very significant error introduced by pipetting warm and cold liquids.

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