Transferability of Pipette Calibrations – a Prerequisite for Method Validation and Method Transfer

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Introduction

One of the most obvious ways to maintain quality in a laboratory setting is the regular calibration of instruments. Regardless of the measuring instrument, a traceable calibration is achieved by comparison to a standard. If performed outside the laboratory, then the calibration must also be "transferable". A transferable calibration is one that is still valid when the instrument is returned from the calibration lab into the users laboratory[1].

Some calibrated items are very stable and the calibrations are easily transferred (e.g., weight sets and reference materials). Other calibrations (e.g., calibrations of balances) are less transferable, so must be performed in the users lab. In addition, calibrated instruments can be used improperly, so it is crucial that users be properly trained to use all calibrated instruments.

Handheld pipettes are particularly susceptible to operator misuse and changes in environmental conditions (air temperature, barometric pressure, and relative humidity).[2-3] Because of these properties, pipette calibrations are poorly transferable and are best performed in the users environment. Ideally, the pipette should also be calibrated using the same tips and same technique as the user employs in their ordinary work.

Thus the resulting overall uncertainty of the volume measurement made using handheld pipettes may vary from location to location. Reducing uncertainties depends on a variety of parameters. Ideally, a calibrated pipette in San Francisco, CA should perform with the same measurement uncertainty as one in Denver, CO, or anywhere else in the world. Calibration strategies for achieving this ideal are presented in this poster.

References:
[1] Rodrigues, G.W. Proceedings of NCSL International Meeting, June 2003
[2] Vaccaro, W.J. *American Laboratory News* **Sept. 2007**, 16-17
[3] Carle, A.B. *American Laboratory News* **Jan. 2008**, 8-10

Factors in Pipette Calibration

Piston-operated air-displacement pipettes are ubiquitous in laboratories around the world. The accuracy and precision of pipettes, and hence their total uncertainty of measurement, is susceptible to a variety of variables. So are the measurements during the pipette calibration process (calibration method, calibration transfer standard, etc.)

In general, the cumulative uncertainty pertaining to the use of any calibrated pipette depends on the following parameters:

a. Environmental Parameters (T_S, T_A, P, RH)
b. Pipette Tips
b. Calibration Transfer Standards
c. Chain of Traceability (*who* is calibrating *where*?)
d. Method of Calibration
e. Operator Training and Demonstrated Skills (in handling pipette *and* calibration instrumentation)

The following guidelines address pertinent variables of pipette calibrations, and the errors which may be introduced by each factor (selective samples listed):

Numerical Estimations of Error – ISO 8655-2 Annex B
a. Error due to Environment
b. Error due to Equipment
c. Error due to Technique
d. Equipment Failure Error

Tip Quality / Consumables that Affect the Quality of Tests
ISO 17025 Section 4.6.2
ISO 8655-2 Annex B: Tip Design and Quality
ASTM E1154 Section 11.2.1

Operator Qualification: Education, Training, Experience, Demonstrated Skills
ISO 17025 Section 5.2.1

Sources of Uncertainty

A. Gravimetric Method

Exact gravimetric measurements need to account for the following parameters:

a. Liquid density (T)
b. Air buoyancy (T, P)
c. Evaporation (T, RH)
d. Balance resolution (ISO 8655-6)
e. Air currents (settling, evaporation)
f. Electrostatic forces (glass, plastic vessels)

Delivered volume is described by the following equation

$$V_G = Z(T_W, T_A, P_A) \times (W_1 - W_0 + e)$$

B. Photometric Method

Photometric measurements are not sensitive to environmental conditions, provided the temperature factor is considered in the calculation of the absorbance value. Then, only the following parameters are crucial:

a. Accurate Photometer
b. Accurate Reagents

Trail of Traceability in Pipette Calibrations

International Measurement System (SI)

International treaties govern maintenance of the SI system, while National Agencies such as NIST (USA), PTB (Germany) and NPL (Great Britain) are responsible for maintaining the standards of the International Measurement System (SI) and disseminating them to individual calibration institutions within their jurisdiction.
The choice of suitable calibration transfer standards is imperative to keep measurement uncertainties minimal throughout the calibration chain. Calibrated masses for balances are a commonly used transfer standard due to the ruggedness of the material (easy to ship), and the stability towards environmental conditions. Balances do not transfer calibrations well as measurements are influenced by environmental conditions, local gravity, etc. Pipettes are not reliable as a calibration transfer standard due to a large impact of environmental conditions, operator skills and consumables (tips) on the measured results. Ideally, they are calibrated right where they are being used.

Traceability Trails

As shown in **Figure 1**, Tier-1 Mass Laboratories compare their weights to the ones at the appropriate National Standards Bureau, rendering them traceable to the International Measurement System. Tier-2 Mass Labs, in turn, compare their weights to Tier-1 masses and extend traceability to individual Calibration Labs by comparing their weights to the Tier-2 masses. The Pipette Calibration Lab uses its traceable weights to calibrate their balances, which are used to calibrate the pipettes sent to the lab from the various QC Labs (Route 1, mail-in calibrations).

The traceability connection between the pipette Cal Lab, and the QC Lab is shown as a dotted line, because the traceability is questionable unless it can be shown that the pipette calibration lab used the same disposable tips, technique, and environmental conditions that are found in the QC Lab.

As shown in the right hand side of Figure 1 (Route 2), using a higher level pipette calibration lab has little impact on the overall uncertainty of liquid delivery in the QC lab, because there still exists questionable transferability in the pipette calibration.

Figure 2 depicts a more consistent way of calibration: balances located in each QC Lab are calibrated with Tier-1 traceable masses. Uncertainties are reduced significantly as a stable calibration transfer standard is used (weights), and the pipettes are calibrated in the environment in which they are used, by the end user, and with the appropriate tips. Potential damage during shipment is also eliminated. Pipettes calibrated by this method in different locations may perform more similar than in the first scenario (Figure 1). All of the inherent issues pertaining to gravimetric calibrations (*vide supra*), including operator skills, still persist and will influence the total uncertainty. These variations may still be too large for some method transfer and validation purposes across multiple locations, particularly when micro-liter volumes are handled.

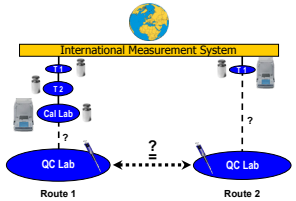

Figure 1. Questionable Transferability

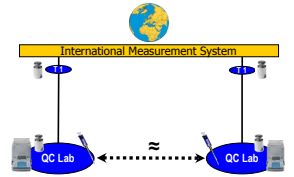

Figure 2. Better Gravimetric Traceability

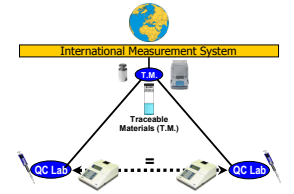

Figure 3. Photometric Traceability

In **Figure 3**, a photometric calibration approach is described. Traceability of calibrations to SI is extended to the QC Labs by Photometric Volume Transfer Standards ("traceable materials"). These standards are manufactured on equipment with traceable calibration to the International Standard (e.g., NIST), and extend traceable pipette calibration directly to the QC Lab. Highly accurate photometers are required to provide a method largely impervious to environmental influence, and to allow pipette calibrations with low total uncertainties directly in the QC Lab. Low uncertainties result as pipettes are calibrated at their location of use, with the appropriate tips, and without potential for inducing shipping damage.
Calibrations are comparable from one location to another, as the same traceable calibration standard is used in each laboratory, resulting in high confidence for method transfer and method validation tasks.

Conclusions

Confidence in the identical performance of pipettes at various locations is imperative for successful method validations and method transfers. Variations in pipetting from location to location are minimized when the entire liquid delivery system (pipette, tips, environment and operator) are calibrated together, using a reliable and traceable method, directly in the users laboratory.

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