Calibration of multi-channel pipettes using gravimetric method in accordance with the ISO 8655-6

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Abstract. A calibration system was set up for calibrating multi-channel pipettes using the gravimetric method in accordance with the ISO 8655-6. The calibration system consisted of a display unit, a motor control unit and a measuring unit. The measuring unit included a single weighing cell, twelve individual containers for receiving the dispensed volume from multi-channel pipettes and transport rails to automatically convey the containers to the weighing cell one by one. This paper described the measurement traceability for volume measurements to the SI unit of mass by using the calibration system and the validation of the calibration system for volume measurements. The calibration system was shown to be capable of measuring the dispensed volume from 30 \( \mu l \) to 1000 \( \mu l \) with the measurement uncertainty of 0.7 \( \mu l \) to 2.9 \( \mu l \).

1. Introduction
Pipettes are widely used for measuring the volume in various fields such as health, chemistry, biology, pharmaceuticals and genetics. In order to ensure that the measurement results are reliable, laboratories would send their pipettes for calibration [1]. The gravimetric method according to the international standard, ISO 8655-6 [2] is commonly used for determination of the measurement errors for pipettes owing to the simplicity, accuracy and traceability. Multichannel pipettes can increase the throughput by eliminating the time-consuming and repetitive pipetting. Hence, the applications of multi-channel pipettes are ever increasing, as they can increase the output and productivity instantly. According to the ISO 8655-6, the measurement method for multi-channel pipettes is similar to that for single-channel pipettes in that they comprise a set of single-volume measuring and delivering units that are all operated simultaneously by a single piston operating mechanism. During the measurements, each channel is regarded as a single channel to be tested and reported as such. Ideally, all channels of the multi-channel pipette should be simultaneously dispensed to the corresponding containers and then the weighing instrument simultaneously weighs the dispensed fluid volume, but that demands the weighing instrument with a number of weighing cells, that might be costly. The multi-channel pipette calibration system set up in the SCL had up to 12 containers but only had one weighing cell. Hence, the objectives of this study were to verify the repeatability and the linearity of the calibration system by using standard weights, and validate the calibration system by comparing the volume measurement results for six selected multi-channel pipettes with that of FORCE Technology.

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2. Details of the multi-channel pipette calibration system
The setup of the multi-channel calibration system was shown in Figure 1. Twelve glass containers housed in twelve brackets where the multi-channel pipette was dispensing the test liquid were orderly placed in the rack, as shown in Figure 2. Guide rail was provided on both side of the measuring unit for conveying the containers to the weighing position one by one.

2.1. Performing the measurement
The distilled water from the multi-channel pipette were respectively dispensed into the corresponding containers. The containers were then conveyed onto the weighing cell as simulated in Figures 3 and 4. The orientation of the pipette was not recommended to be changed during the measuring cycle.

2.2. Calculation of the dispensed volume
The delivered volume was determined by using the gravimetric method. That was achieved by dividing the mass by the density of the distilled water, calculated from equation (1)

\[ V_{20} = \frac{m}{\rho_m} \times \left( \frac{\rho_m - \rho_a}{\rho_w - \rho_a} \right) \times \left[ 1 - \alpha_t \times (t_{dw} - t_{20}) \right] \]  

Where \( V_{20} \) = volume, at temperature of 20 °C (in milliliters, ml),  
\( m \) = \((m_L - m_E)\) the net balance reading (in grams, g); where \( m_L \) the weighing result of the balance after pipetting, \( m_E \) the weighing result of that before pipetting,  
\( \rho_m \) = reference density of weights used to adjust the balance (i.e. 8 g/ml),  
\( \rho_w \) = density of water at calculation temperature \( t \) °C, in g/ml; \( \rho_a \) = density of air, in grams per milliliter, g/ml,  
\( \alpha_t \) = thermal expansion coefficient of the pipette (in litre per degree Celsius, l/°C),  
\( t_{dw} \) = temperature of the distilled water (in degree Celsius, °C).

3. Verification of the repeatability and linearity of the calibration system
According to the ISO 8655-6, the weighing instrument used to calibrate pipette with nominal capacity above 10 µl shall be of resolution of 0.01 mg. The repeatability and linearity shall be of 0.02 mg and the standard uncertainty shall not be more than two to three times the resolution. The specification of
the weighing instrument meets requirements of the ISO 8655-6. However, in real application, due to various environment and human factors, the performance of weighing instrument might be different. Also, the measurement results of the weighing instrument shall be metrologically traceable to the SI of mass. Hence, the weighing instrument’s performance was verified using standard weights.

3.1. Verification method

Ten stainless steel mass standards ranging in mass from 2 mg to 10 000 mg were selected. To simulate the actual operation of the calibration system, the standard weights were placed onto the containers. If the repeatability and linearity of each channel were verified one by one, a large number of standard weights and a number of steps might be involved. To streamline the calibration process, a comprehensive calibration procedure was proposed, as indicated in the table 1.

| Channel | Steps | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---------|-------|---|---|---|---|---|---|---|---|---|----|
| 1       | 2     | 10000| 5 | 000| 2 000| 1 000| 200| 100| 20 |
| 2       | 5     | 2 000| 1000| 5 | 000| 2 000| 1 000| 200| 100|
| 3       | 10    | 5 | 2 000| 1000| 5 | 000| 2 000| 1 000| 200|
| 4       | 20    | 10 000| 2 000| 1000| 5 | 000| 2 000| 1 000| 200|
| 5       | 100   | 20 000| 1000| 200  | 1 000| 5 | 000| 2 000| 1 000|
| 6       | 200   | 10 000| 200  | 1 000| 2 000| 1 000| 5 | 000| 2 000| 1 000|
| 7       | 1 000 | 200  | 100  | 20  | 10 | 5 | 2  | 10  | 000 | 2 000|
| 8       | 2 000 | 1 000| 200  | 100  | 20 | 10 | 5 | 2  | 10  | 000 | 2 000|
| 9       | 5 000 | 2 000| 1 000| 200  | 100 | 20 | 10 | 5 | 2  | 10  | 000 | 2 000|
| 10      | 10 000| 5 000| 2 000| 1 000| 200 | 100 | 20 | 10 | 5 | 2  | 10  | 000 | 2 000|
| 11      | 10 000| 5 000| 2 000| 1 000| 200 | 100 | 20 | 10 | 5 | 2  | 10  | 000 | 2 000|
| 12      | 10 000| 5 000| 2 000| 1 000| 200 | 100 | 20 | 10 | 5 | 2  | 10  | 000 | 2 000|

3.2. Verification results

The repeatability (standard deviation of 10 measurement results) and linearity (the mass value minus the reading of the weighing instrument) measurement results were shown in table 2. Based on the standard deviation and zero offset of measurement; resolution of the system and the uncertainty due to reference weights, the overall expanded uncertainty of measurement was estimated of 0.06 mg.

| Nominal mass value | 0.002 | 0.005 | 0.01 | 0.02 | 0.1 | 0.2 | 1 | 2 | 5 | 10 |
|-------------------|-------|-------|------|------|-----|-----|---|---|---|----|
| Repeatability     | 0.0003| 0.0003| 0.0003| 0.0003| 0.0003| 0.0003| 0.0003| 0.0003| 0.0003| 0.0003|
| Linearity         | 0.0001| 0.0000| 0.0000| 0.0000| 0.0000| 0.0000| 0.0000| 0.0000| 0.0000| 0.0000|

4. Comparison of the results for six multi-channel pipettes at SCL and at FORCE Technology

Six selected multi-channel pipettes ranged from 30 l to 1000 l were selected as unit under tests for comparisons with that of FORCE Technology. The calibration system used for FORCE Technology was different, which calibrated one channel at a time using precision balances of finer resolution.

4.1. Comparison results

The measurement results for the dispensed volume of the six multi-channel pipettes were compared with that obtained from FORCE Technology. For comparison measurements, the $E_n$ value [3] is calculated from equation (2).

$$E_n = \frac{X_{Lab} - X_{Ref}}{\sqrt{U_{Lab}^2 + U_{Ref}^2}}$$

The calculation results were shown in table 3. The results showed the comparability of the calibration system between the SCL and Force Technology, despite the calibration system was different.
Table 3. Results of $E_n$ calculation based on the maximum deviation obtained from the twelve channels per each calibration point

| Nominal Volume (Unit $= \mu l$) | FORCE Technology | Expanded measurement uncertainty | Measured delivered volume | Expanded measurement uncertainty | SCL |
|--------------------------------|------------------|----------------------------------|---------------------------|---------------------------------|-----|
| 30                             | 30.19            | 0.38                             | 29.85                     | 0.4                             | 0.6 |
| 50                             | 50.15            | 0.18                             | 49.75                     | 0.7                             | 0.6 |
| 100                            | 100.00           | 0.33                             | 98.8                      | 1.3                             | 0.9 |
| 300                            | 298.64           | 0.24                             | 299.2                     | 1.7                             | 0.3 |
| 500                            | 499.96           | 0.43                             | 500.7                     | 2.0                             | 0.4 |
| 1000                           | 999.37           | 0.80                             | 1000.5                    | 2.9                             | 0.4 |

4.2. Uncertainty of measurements

The uncertainty of measurement for the measurement of the volume dispensed by the multi-channel pipette had been carried out in accordance with principles in the Evaluation of Measurement Data – Guide to the Expression of Uncertainty in Measurement, JCGM 100:2008. The uncertainty of measurement in each measured delivered volume was calculated by combining in quadrature a number of uncertainty components, in which the following four uncertainty components were the dominant components for the calibration system at SCL:

- Uncertainty due to the repeatability of measurement,
- Uncertainty due to the effect of evaporation of the dispensed water,
- Uncertainty due to the effect of heat transferred from the operator.

The uncertainty of measurement for measuring the delivered volume ranged from $30 \mu l$ to $1000 \mu l$ by using the calibration system was summarized in table 4.

Table 4. The uncertainty of measurement for various dispensed volume ranges.

| Volume dispensed ($\mu l$) | Uncertainty of measurement ($\mu l$) |
|---------------------------|-----------------------------------|
| 30 to 50                  | 0.70                              |
| Above 50 to 100           | 1.3                               |
| Above 100 to 300          | 1.7                               |
| Above 300 to 500          | 2.0                               |
| Above 500 to 1000         | 2.9                               |

5. Conclusions

An assessment of the repeatability and linearity errors of the newly set up calibration system for calibrating multi-channel pipettes was performed using ten mass standards in value from $2 \text{ mg}$ to $10 \text{ g}$. The repeatability and linearity of the calibration system were found in compliance with the stringent criteria as indicated in the ISO 8655-6. The comparison results between the SCL and Force Technology for the measured volume results of six selected multi-channel pipettes ranging from $30 \mu l$ to $1000 \mu l$ further confirmed that the multi-channel pipette calibration system was suitable for the intended use. This meets the aim of providing multi-channel pipette calibration services for the local chemical / medical testing and certification laboratories in accordance with the international standard of ISO 8655-5.

6. References

[1] Ismail Z., Hayu R., Sutanto H. and Hafid XXI IMEKO World Congress “Measurement in Research and Industry” p 1059

[2] ISO 8655-6 2002 Piston-operated volumetric apparatus – Part 6 : Gravimetric methods for the determination of measurement error

[3] W. Wöger, Remarks on the $E_n$-Criterion Used in Measurem. Comp.: PTB-Mitteilungen 109 (1999) 24