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# On the microvolume measurement from 0.1 µL up to 100 mL using a microsyringe and micropipette

**Leandro S. Sampaio[1,2], Mila R. Avelino[1,3], Luis V. G. Tarelho[1]**

[1] *National Institute of Metrology, Standardization and Industrial Quality (INMETRO), Duque de Caxias-RJ, 25250-020, Brazil*
[2] *National Service of Industrial Learning (SENAI CIMATEC). 41650-010, Salvador-BA, Brazil*
[3] *Mechanical Engineering Department, State University od Rio de Janeiro (UERJ), Rio de Janeiro-RJ, 20950-000, Brazil*

ABSTRACT
This work focuses on the measurement of small liquid volumes, comparing results obtained using the gravimetric method with different resolutions. The main objective of this study is to characterize the behaviour of microsyringes and micropipettes during the measurement of microvolumes in the range between 0.1 and 1 µL using the gravimetric method, with metrological traceability, and compare the results in the range up to 100 µL. The methodology addresses the challenges associated with measurements below 1 µL by establishing metrological traceability through mass measurement using the gravimetric method. Given that the smallest standard weight is nominally 1 mg, relying solely on the dispensed liquid volume would result in a lack of metrological traceability for volumes under 1 µL. To enhance reliability, eleven independent measurements are taken at each volume point to calculate an average. The results are presented, to conclude the analysis, and simulations were conducted to explore potential improvements by employing a scale with a resolution of 0.001 mg, along with its associated measurement uncertainty.

**Section:** RESEARCH PAPER

**Keywords:** micropipettes; microsyringes; gravimetric method; metrological traceability; measurement uncertainty

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**Corresponding author:** Mila Rosendahl Avelino, e-mail: mrosendahl@inmetro.gov.br

## 1. INTRODUCTION

The gravimetric method is the procedure predominantly used in laboratories and national metrology institutes for volume calibration. This method covers a wide range of measurement scales, from microvolumes to large-capacity vessels, making it accessible to a diverse user base that relies on calibrated instruments. Its widespread adoption can be attributed to the method's familiarity and relative ease of application. Furthermore, the instruments used for these measurements are typically integrated into daily laboratory operations, allowing for shared use, rather than requiring dedicated volume measurement equipment. This characteristic presents a significant cost-effective advantage in measurement practices [1], [2].

Advances in nanotechnology research have increased the demand for metrologically reliable results, particularly for calibration of microvolumes in the range from 0.1 µL to 1 µL. Micropipettes, including piston pipettes, are predominantly used for volume measurements in various fields, such as healthcare, chemistry, biology, pharmacy, and genetics [3], [4]. According to the ISO 8655-2 standard [5], these instruments must meet specific metrological requirements, including maximum permissible error values and labeling specifications. For calibration with the gravimetric method, the ISO 8655-6 standard [6] serves as the reference standard.

Micropipettes and microsyringes are the main instruments used for measuring microvolumes. Micropipettes are widely used in laboratories, and operate through a mechanical or electronic piston system. In most cases, the aspirated liquid does not come into contact with the piston mechanism, as it is contained in a disposable external tip. This design helps prevent sample contamination and particle carryover. Micropipettes can be single-channel or multichannel (typically 8 or 12 channels).

For laboratory tasks that require repeated dispensing operations, multichannel micropipettes are convenient and commonly used, improving workflow efficiency.

This study investigates the measurement of small volumes of liquids, specifically in the range of 0.1 μL to 100 μL, through a comparative analysis of the results obtained with the gravimetric method, using balances with different resolutions.

The main objective of this investigation is to characterize, with metrological rigor, the behavior of microsyringes and micropipettes in measuring microvolumes between 0.1 and 1 μL, using the gravimetric method, under established traceability conditions. This characterization includes analyzing the repeatability, reproducibility, and sensitivity of both instruments when operating at their lower performance limits, where the effects of surface tension, evaporation, and operator influence become dominant factors in uncertainty.

Furthermore, the study examines the effect of balance resolution and weighing system stability on overall measurement uncertainty, seeking to determine the minimum volume that can be measured with reliable metrological traceability using standard laboratory equipment. Particular attention is paid to the calibration steps related to the tare procedure and environmental corrections, as these have been identified as crucial for achieving consistent mass-to-volume conversions.

Furthermore, the results obtained for microvolumes are compared with measurements of larger nominal volumes, extending up to 100 μL, in order to assess the scalability of the proposed gravimetric approach and verify its applicability across different measurement ranges. This comparative evaluation provides experimental evidence to define practical transferability limits between microsyringe and micropipette calibration processes [7].

## 2. STATE OF ART

The techniques used to construct the first micropipettes resemble those of a model currently under development. As technology evolved, new features were added to improve repeatability and ergonomics, increase the range of use, electronic capabilities, and the use of multichannels [7]. For micropipettes, the measurement range can be from 0.1 μL to 50 mL.

Microvolume measurement with a micropipette allows for significant time savings and requires no special operator skills, compared to the same measurement using a microsyringe. Even with these advantages, there are details in the measurement that cannot be ignored. The ISO 8655-6 standard [6] specifies the measurement procedure using the gravimetric method to determine the volume of piston-operated volumetric instruments. However, several experimental parameters can influence micropipette calibration [8] and [9]. These parameters, such as the time to aspirate and dispense the volume, the variation in the angle of aspiration and dispensing of the liquid, the immersion depth of the tip, the type of tip used, and the altitude, can lead to inconsistent and inaccurate results and are not explained in detail in the ISO 8655-6 standard.

Another instrument frequently used to measure microvolumes is the microsyringe. Microsyringes consist of a plunger that operates within a calibrated tube, facilitating the aspiration and dispensing of fluids, which can include liquids and gases [8]. Their applications span a variety of fields, including chromatography, drug administration, and the precise measurement of liquid volumes.

Microsyringes are valued for their precision and reliability in handling minute quantities of substances [9] and [10]. The use of syringes dates back several centuries; however, significant advances in microvolume measurement emerged in the late 1940s. The microsyringe developed by Clark Hamilton incorporated lead shielding and was originally designed for the manipulation of radioactive isotopes [9]. In the 1950s, Hamilton redirected his focus to microvolume measurement, particularly emphasizing its applications in chromatography. This shift marked a fundamental development in the accuracy and reliability of microvolume handling in various scientific fields [11].

Figure 1. Illustration of a microsyringe, commonly used for precise measurement and dispensing of microvolumes in laboratory applications. This device provides accurate volume control, particularly in the range of 0.1 to 1 μL.

The microsyringe is an instrument characterized by its high precision, consisting of very fine channels that facilitate the precise manipulation of liquid volumes. Using microsyringes requires careful handling, as many models are constructed of glass, which increases the likelihood of damage [12]. Furthermore, heat exchange between the user's hand and the liquid can affect measurement accuracy, due to the delicate and closely spaced nature of the graduation markings [13]. Measurement errors related to parallax are also common, as graduation markings are often very fine, requiring significant skill and experience on the part of the user. Figure 1 illustrates a microsyringe with a maximum volume of 5 μL and a resolution of 0.05 μL.

### 2.1. Gravimetric method

The gravimetric method is the most widely used approach for volume calibration in laboratories and national metrology institutes. This versatile technique covers all measurement ranges, from microvolumes to large-capacity vessels, making it accessible to users with varying levels of expertise. Its popularity is due to its simplicity, ease of implementation, and extensive adoption in metrological practice, as noted in [14]. Furthermore, gravimetric measurement often employs standard laboratory equipment, such as analytical balances and temperature-controlled enclosures, eliminating the need for dedicated volumetric systems. This characteristic not only enhances operational efficiency but also significantly reduces costs associated with calibration activities.

Beyond its practicality, the gravimetric method ensures direct traceability to the International System of Units (SI) through mass, which strengthens the reliability and comparability of results among laboratories. However, to achieve such consistency, it is essential to control influencing factors, such as temperature, air buoyancy, and evaporation during the measurement process. The selection of calibration points for the scale must be made according to the intended application of the equipment, and the information contained in the calibration certificate should be critically analyzed and correctly implemented to guarantee the appropriate use of its values [15]. In the context of laboratory glassware calibration, where a high degree of precision is required, even small systematic deviations

Table 1. Physical properties of fluids and correction parameters.

| Parameter | Symbol | Typical value | Unit | Source / Method | Expanded uncertainty ($k$ = 2) |
|---|---|---|---|---|---|
| Density of water at 20 °C | $\rho_w$ | 0.998203 | g mL$^{-1}$ | ISO 8655-6 / IAPWS-95 | 2 × 10$^{-6}$ g mL$^{-1}$ |
| Air density | $\rho_a$ | 1.2041 × 10$^{-3}$ | g mL$^{-1}$ | CIPM 2007 formula | 5 × 10$^{-6}$ g mL$^{-1}$ |
| Density of standard weight | $\rho_p$ | 8.0 | g cm$^{-3}$ | OIML R111-1 | 0.01 g cm$^{-3}$ |
| Volumetric expansion coefficient (glass) | $\alpha$ | 2.5 × 10$^{-5}$ | °C$^{-1}$ | Manufacturer / literature | 1 × 10$^{-6}$ °C$^{-1}$ |
| Temperature | $T$ | 20.0 ± 0.3 | °C | Calibrated thermometer | U(T) = 0.1 °C |

can produce significant errors in the final volume determination. Therefore, adherence to the best weighing practices and continuous monitoring of environmental stability are crucial for maintaining the integrity of gravimetric volume calibration.


Figure 2. Air saturation system of the Sartorius Genius ME215P. This system has an external acrylic body and a stainless steel interior, with an acrylic weighing container. Operating temperature (20 ± 1) °C; scale resolution 0.01 mg.


Figure 3. Air saturation system of the Mettler Toledo scale model XP26PC. Operating temperature (20 ± 1) °C; scale resolution 0.001 mg; measurements in µL.

## 3. METHODOLOGY

The methodology employed in this study involves a measurement routine using microsyringes and micropipettes. This procedure involves determining the mass difference between a full and an empty container, followed by converting the mass value to volume. A critical and essential aspect of this approach is the need to carefully adjust the liquid meniscus to the designated measurement point when using a microsyringe for measurement, before dispensing the volume [16]. This attention to detail is essential to ensure accurate and repeatable measurements.

The method developed for this study is based on the procedures described in the ISO 8655-6 standard, in which the total measurement value (the mass of the container plus the mass of the liquid) is considered. This approach not only ensures the correct use of the measured values, but also addresses the challenges associated with measuring volumes below 1 µL. The metrological traceability of volume measurements by the gravimetric method is fundamentally linked to mass; specifically, 1 µL is approximately equivalent to 1 mg. According to OIML R111-1 [17], the smallest available standard weight has a nominal value of 1 mg. Therefore, if only the volume of the dispensed liquid were considered, metrological traceability for measurements involving volumes less than 1 µL would not be achievable.

### 3.1. Determination of water density and correction parameters

The density of the purified water used in all measurements was determined in accordance with ISO 8655-6 (2022) and the IAPWS-95 formulation for the thermodynamic properties of ordinary water. The reference temperature for calibration was (20.0 ± 0.3) °C, corresponding to a water density of $\rho_w$ = 0.998203 g mL$^{-1}$. The expanded uncertainty of $\rho_w$ was calculated as described in Euramet CG-19 (2018), with a value of $U(\rho_w)$ = 2 × 10$^{-6}$ g mL$^{-1}$ ($k$ = 2). Corrections for air buoyancy and vessel expansion were also applied using the air density $\rho_a$ = 1.2041 × 10$^{-3}$ g mL$^{-1}$ and the volumetric expansion coefficient $\alpha$ = 2.5 × 10$^{-5}$ °C$^{-1}$ for borosilicate glass. The corresponding uncertainties $U(\rho_a)$ = 5 × 10$^{-6}$ g mL$^{-1}$ and $U(\alpha)$ = 1 × 10$^{-6}$ °C$^{-1}$ were included in the overall uncertainty budget.

All measurements were corrected for air buoyancy and water density according to the parameters listed in Table 1.

The scales used in this study are shown in Figure 2 and Figure 3, which illustrate the air saturation systems and weighing configurations employed for the two resolutions evaluated. All measurements were conducted without using the tare function, ensuring that each mass reading corresponded directly to the

total load (container + liquid), as recommended for gravimetric determination of volume [18], [19].

The instruments used for volume determination are:

- Rainin micropipette model: L2, nominal value: 2 μL, value of one division: 0.002 μL;
- Micropipette Brand model: Transferpette, nominal value: 10 μL, value of one division: 0.01 μL;
- Micropipette Brand model: Transferpette, nominal value: 100 μL, value of one division: 0.1 μL;
- Hamilton Microsyringe, nominal capacity: 5 μL, resolution: 0.05 μL;
- Hamilton Microsyringe, nominal capacity: 100 μL, resolution: 1 μL.

A methodology was implemented to minimize the effects of liquid evaporation during the calibration process. According to ISO 8655 [6], when measuring volumes with a nominal value of less than 50 μL, it is essential to consider strategies to mitigate mass loss due to evaporation, as this loss can contribute to uncertainty in the volume calculation.

Eleven measurements were taken at each measurement point to calculate an average value. Each of these measurements is independent, and its value is obtained by subtracting the value after dispensing the liquid (final or full) from the value of the measurement before dispensing the liquid (initial or empty). In the first measurement, the container is empty, and the value to be recorded is only its mass. In the subsequent measurements (until the eleven measurements per point are completed), the initial value to be considered will not be the last full value of the previous measurement, but rather the value read on the scale display before dispensing the liquid. This approach was designed to minimize the effects of liquid evaporation. This procedure also reduces the influence of cumulative evaporation effects between successive measurements.

Although the contribution of evaporation to the overall uncertainty persists, its impact is reduced compared to calculations performed as described in Euramet CG-19 (2015). This approach increases the robustness of the uncertainty assessment while maintaining the integrity of the measurement process [20].

To ensure reliable results, the temperature of the purified water used for calibration must be maintained at 20 °C ± 1 °C, with no deviation greater than ± 0.3 °C from the temperature at which the water density was determined. Furthermore, the maximum water temperature at the time of calibration must not exceed this range. Environmental conditions within the laboratory must be closely monitored during the calibration process, with the ambient temperature maintained at 20 °C ± 1 °C and the relative humidity maintained within the range of 55 % to 70 %. Before any measurement, microsyringes and micropipettes must be acclimated in the laboratory for at least 1 hour to achieve thermal stabilization, thus minimizing problems related to variations in the dispensed volume.

Gravimetric measurements were performed at the Inmetro Fluids Laboratory using a balance with a resolution of 0.01 mg. Another set of measurements was performed at the Mettler Toledo facility using a balance with a resolution of 0.001 mg.

A table is presented for each calibrated instrument. The measurement results and their respective uncertainties are expressed in volume units (μL) and as a percentage (%), so that the true impact of uncertainty on the calibration result is more clearly understood. The materials required for each measurement range, as well as the methods, are described above for both micropipette and microsyringe calibrations.

### 3.2. Volume determination

Volume determination involves converting the measured mass value to the volume of liquid dispensed by the microsyringe, as articulated in equation (1). This conversion is essential for accurately quantifying the dispensed liquid volume based on the relationship between mass and density.

$$V = \frac{M}{\rho_{\mathrm{w}} - \rho_{\mathrm{a}}} \cdot \left(1 - \frac{\rho_{\mathrm{a}}}{\rho_{\mathrm{p}}}\right) \cdot [1 - \alpha\,(T - 20\,°\mathrm{C})]\,, \tag{1}$$

where, $V$ is the volume in mL; $M$ is the mass of liquid in the 2 container in g, where $M = M_{\mathrm{full}} - M_{\mathrm{empty}}$; $\rho_{\mathrm{w}}$ is the density of the liquid used in the calibration, in g/mL; $\rho_{\mathrm{a}}$ is the density of air during calibration, in g/mL; $\rho_{\mathrm{p}}$ is the density of the weight used in the scale calibration, in g/mL; $\alpha$ is the volumetric expansion coefficient of the container material in [°C](-1); $T$ is the temperature of the liquid in [°C]. This model includes corrections for air buoyancy and thermal expansion effects, which become significant in microvolume measurements. Figure 4 illustrates the experimental apparatus of the volume calibration laboratory.

An uncertainty budget was constructed based on the law of propagation of uncertainties, considering both instrumental and environmental contributions.

The standard uncertainties that are multiplied by the sensitivity coefficients for determining the volume uncertainty were obtained from Euramet-CG19 (2018) and are present in equations (2) to (10). Each uncertainty contribution was evaluated independently according to the recommendations of the Guide to the Expression of Uncertainty in Measurement (GUM).

Scale calibration uncertainty $(u(M_1))$:

$$u(M_1) = \sqrt{\left(\frac{U(M)}{k}\right)^2}\,, \tag{2}$$

where $U(M)$ is the expanded uncertainty of the scale calibration at the measurement point; $k$ is the coverage factor expressed in the scale calibration certificate.

Uncertainty of scale resolution $(u(M_2))$:

$$u(M_2) = \sqrt{\left(\frac{r}{2 \cdot \sqrt{3}}\right)^2}\,, \tag{3}$$

where $r$ is the resolution of the scale.

Evaporation rate uncertainty $(u(M_3))$:

$$u(M_3) = \sqrt{\left(\frac{m_{\mathrm{P}}}{\sqrt{3}}\right)^2}\,, \tag{4}$$

where $m_{\mathrm{P}}$ is the lost mass for the calculation of evaporation.

Uncertainty of the density of water $u(\rho_{\mathrm{w}})$:

$$u(\rho_{\mathrm{w}}) = \frac{U(\rho_{\mathrm{w}})}{k}, \tag{5}$$

where $U(\rho_{\mathrm{w}})$ is the expanded uncertainty of the density of water; $k$ is the coverage factor obtained in the calculation of the uncertainty of the density of water.

Figure 4. Photograph of the Volume Calibration Laboratory facilities with the bench where the experimental apparatus for measuring microvolume is located, including the scale used for measurements and the instrumentation for monitoring environmental conditions. Temperature (°C) and Humidity (%) monitors visible in setup.

Uncertainty of the density of air $u(\rho_a)$:

$$u(\rho_a) = \frac{U(\rho_a)}{k}, \tag{6}$$

where $U(\rho_a)$ is the expanded uncertainty of the density of air; $k$ is the coverage factor obtained in the calculation of the uncertainty of the density of air.

Uncertainty of the reference standard weight's density $u(\rho_p)$:

$$u(\rho_p) = \frac{U(\rho_p)}{\sqrt{3}}, \tag{7}$$

where $U(\rho_p)$ is the uncertainty of the density of the standard weight used in the calibration of the scale. The value of this uncertainty is available in OIML R111-1.

Uncertainty of the volumetric expansion coefficient $u(\alpha)$:

$$u(\alpha) = \frac{U(\alpha)}{\sqrt{3}} \tag{8}$$

where $U(\alpha)$ is the uncertainty of the volumetric expansion coefficient of the container.

Temperature gauge uncertainty $u(T)$:

$$u(T) = \sqrt{\left(\frac{U_{\text{thermometer}}}{k}\right)^2 + \left(\frac{r}{2 \cdot \sqrt{3}}\right)^2}, \tag{9}$$

where, $U_{\text{thermometer}}$ is the expanded uncertainty of the calibration of the thermometer; $k$ is the coverage factor expressed in the calibration certificate of the thermometer; $r$ is the resolution of the thermometer.

Uncertainty of repeatability $u(S_V)$:

$$u(S_V) = \frac{S_V}{\sqrt{n}}, \tag{10}$$

where $S_V$ is the standard deviation to volume measurements; $n$ is the number of measurements. Repeatability was assessed under the same operating conditions and by the same operator over a short time interval.

Operator Uncertainty $u_{op}$: For this source of uncertainty, a variation can be noticed, depending on the instrument to be calibrated. For microsyringe calibration, equation (11) is used. For micropipette calibration, equation (12) will be used.

$$u_{op} = \frac{\Delta i}{\sqrt{24}}, \tag{11}$$

or

$$u(\Delta m) = 0.0001\ (\nu_n), \tag{12}$$

where $\Delta i$ is the difference between the highest and lowest value obtained among the measurements carried out with the equipment at the point being calibrated, and $(\nu_n)$ is the nominal value of the point being calibrated. The coefficient was adopted based on tolerance limits recommended in ISO 8655 for piston-operated pipettes.

All these individual components were then combined following the GUM methodology, as detailed in the following section.

The source of uncertainty related to operator influence ($u_{op}$) was explicitly considered in the calculations, since its contribution becomes significant at very small nominal volumes. This component represents the variability introduced by manual handling—such as dispensing rate, immersion depth, and tip wetting—and was estimated from repeated measurements performed by the same operator under controlled conditions. Although no statistical comparisons between operators were performed, the observed dispersion between independent series justified the inclusion of this effect in the uncertainty budget. For nominal volumes below 10 µL, the operator contribution was one of the most significant Type A components.

### 3.3. Uncertainty evaluation model

The combined standard uncertainty of the measured volume was obtained by grouping the individual sources according to the GUM (2008) approach.

Type A components include the repeatability of measurements (10) and the operator influence (11) and (12), both evaluated statistically from repeated series.

Type B components correspond to the scale calibration (2), the scale resolution (3), the density of water, air, and weights (5)–(7), the volumetric expansion coefficient (8), and the thermometer calibration (9).

All components were expressed as standard uncertainties, multiplied by their sensitivity coefficients and combined by the root-sum-of-squares method to obtain the combined standard uncertainty $u_c$. The expanded uncertainty $U$ was then calculated as equation (13).

$$U = k \cdot u_c\,, \tag{13}$$

with a coverage factor $k = 2$, corresponding to a confidence level of approximately 95 %.

Corrections for air buoyancy, density, and volumetric expansion were applied as described in Table 1, and their residual uncertainties were included in the total budget. The relative contribution of each component (operator, temperature, balance, resolution, evaporation, and correction factors) was analyzed for every volume point, allowing the identification of dominant uncertainty sources in Table 2 - Table 13.

### 3.4. Minimum weight methodology

The operating principle of an electronic scale obtains the result of the measured value based on the displacement of the coil inside a magnetic core, and subsequent conversion of the electrical voltage into a unit of mass. For small values, such as microliters, it is necessary to evaluate the scale's ability to measure reliably from the starting point, i.e., 0.1 mg, considering that the mass of the dispensed volume of 0.1 μL is approximately 0.1 mg.

The minimum weight methodology is already described in a specific chapter in the Euramet CG 18 scale calibration guide, which is a European calibration guide for mass metrology, and in the USP (United States Pharmacopeia). Most scale users use this instrument only for measuring mass. They are technicians who work in laboratories in the areas of health, environment, biotechnology, among other areas, and need to measure values below 1 mg. Regarding the guarantee of the validity of the results, the traceability of volume measurements is based on the gravimetric method, that is, using a scale that has been previously calibrated with standard weights. According to OIML R111 and the Technical Regulation for Metrology and Conformity Assessment (RTAC) referring to Inmetro Ordinance 289 of July 5, 2021, the smallest standard weight is 1 mg. Therefore, metrological traceability in the measurement of 0.1 mg directly linked to the standard weight would not be possible.

Good measurement practices suggest the importance of evaluating whether the instrument used can achieve the required accuracy and provide valid results. For measuring mass on scales, one methodology used is that of minimum weight. According to Euramet CG-18, the minimum weight is the smallest quantity required for a weighing to achieve a specified relative accuracy.

Since microvolume measurement using the gravimetric method is performed by measuring mass and then calculating the equivalent volume using the minimum weight, the technique used here is adopted to ensure reliability in measurements below 1 mg.

In fact, volume measurements that have a mass value of 1 mg or more continue to have direct traceability to the standard weight. On the other hand, volume measurements in the range of 0.1 to 1 μL, which have a mass value lower than 1 mg, will have their reliability guaranteed by calculating the minimum weight for the scale used in the measurement.

Equation (13) describes how to calculate the minimum weight:

$$R_{\min} = \frac{U_{\text{certificate}}}{R_{\text{eq}}}, \tag{13}$$

where $R_{\min}$ is the desired minimum weight and $U_{\text{certificate}}$ is the expanded uncertainty of the calibration certificate of the weighing instrument at the measurement point, considering the total load that the weighing instrument is measuring, and $R_{\text{eq}}$ is the tolerance defined for the process.

The measurement uncertainty of the calibration certificate of the scale at the point where the weighing is performed ($U_{\text{certificate}}$) must consider the entire mass load that is on the scale at the time of weighing. For example, if the scale is used for measuring microvolumes, the liquid will be dispensed into a weighing container that may or may not be coupled to an air saturation system. Since this container is deposited on the scale plate, it must be considered as the measured mass to select the uncertainty of the scale calibration point for calculating the minimum weight.

This significantly impacts the calculated value of the minimum weight, since in most cases the mass value of the weighing container is greater than the mass value of the measured volume. Even if the scale's Tare function is used, which discounts the mass value of the object placed on the plate, making the indication 0 g, the value for calculating the minimum weight must be the total mass composed of the weighing container plus the dispensed volume. The Tare function serves only to facilitate the indication of what is measured, excluding the value of the container where the liquid is dispensed.

To define the tolerance ($R_{\text{eq}}$), the technical specifications of the main manufacturers of micropipettes that have instruments in the measurement range below 1 μL were analysed, such as Brand, Gilson, Eppendorf, Sartorius, and Rainin. For this thesis, a tolerance of 12 % was assigned based on the values that each manufacturer provides in its catalogue; in this way, it was possible to cover all manufacturers and obtain a value consistent with the reality of the micropipettes and scales used. Since the minimum weight is linked to the uncertainty stated in the scale's calibration certificate, the result obtained is exclusive to the respective scale and, with each recalibration, the minimum weight must be calculated again to check if it continues to meet the intended requirements.

## 4. RESULTS

Using the new gravimetric method, based on a change in the ISO 8655-6 procedure in the scale tare stage, and the minimum weight, valid results were obtained for microvolume calibration in the range of 0.1 μL up to 1 μL, with appropriate metrological traceability.

Measurements were conducted at the Inmetro Fluids Laboratory, using a scale with a resolution of 0.01 mg (Sartorius), as depicted in Figure 2. Additional measurements were

performed at Mettler Toledo Facilities, in a scale with a 0.001 mg resolution. Figure 3 presents the apparatus.

### 4.1. Microsyringe measurements

In both laboratories, the environmental conditions during the calibration process adhered to the specifications outlined in the section on the gravimetric method. Table 2 and Table 3 present the results of measurements taken with microsyringes using the 0.01 mg resolution scale for volumes of 5 µL (Table 2) and 100 µL (Table 3). Table 4 and Table 5 present the results of measurements taken with microsyringes using the 0.001 mg resolution scale for volumes of 5 µL (Table 4) and 100 µL (Table 5).

For each calibrated instrument, a table is provided and presents the measurement results along with the corresponding uncertainties expressed in both unit volume (µL) and percentage value (%). This format facilitates a clearer assessment of the real impact of uncertainty on the calibration results, enabling users to better understand the reliability and precision of the measurements obtained.

In all tables of measurement results, it is possible to observe that the relative values of the expanded uncertainty ($U_{\%}$) decrease coherently as the nominal value is increased.

Table 2 presents the results for microsyringe calibration within the range of 5 µL, measured in the scale with 0.01 mg resolution. Although the microsyringe is a measuring instrument that typically provides lower uncertainties compared to results obtained with a micropipette (Table 7), the uncertainty values are notably high at measurement points below 1 µL. This observation can be attributed to the influence of the scale used in the measurements, which affects the overall precision at these lower volume levels.

Table 3 presents the results of the microsyringe calibration for a volume of 100 µL, measured in the scale with 0.01 mg resolution. While the uncertainties are smaller compared to those obtained with a micropipette (Table 8), the primary source of uncertainty impacting these measurements is related to the handling of the microsyringe by the operator ($U_{\mathrm{op}}$). Additionally, two secondary sources of uncertainty include factors related to the scale used and the repeatability of the measurements. These influences highlight the importance of proper technique and instrument consistency in achieving reliable calibration results.

Table 4 presents the results of the calibration for the 5 µL microsyringe, demonstrating improved outcomes, with uncertainty values falling reasonably within the expected range. The primary influence on these measurements is the handling of the microsyringe by the operator ($U_{\mathrm{op}}$), followed by the repeatability of the measurements. It is evident that the uncertainty in this volume range could be reduced by enhancing measurement accuracy. Specifically, improving the scale calibration uncertainty, either by using standard weights with accuracy class E1 or by increasing the resolution to 0.0001 mg, would yield only a marginal reduction in uncertainty. This

Table 2. Results for the 5 µL Microsyringe measured in the 0.01 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 0.1 | 0.100 | 0.04 | 41.2% | 2.00 | 1323 |
| 0.2 | 0.200 | 0.04 | 21.3% | 2.00 | 1323 |
| 0.5 | 0.500 | 0.04 | 8.8% | 2.00 | 1441 |
| 1 | 0.990 | 0.04 | 4.5% | 2.00 | 1420 |
| 2 | 1.980 | 0.04 | 2.2% | 2.00 | 1426 |
| 5 | 5.000 | 0.05 | 0.9% | 2.00 | 1422 |

Table 3. Results for the 100 µL Microsyringe measured in the 0.01 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 10 | 10.290 | 0.10 | 0.97% | 2.01 | 484 |
| 20 | 20.350 | 0.11 | 0.55% | 2.00 | 560 |
| 50 | 50.880 | 0.08 | 0.17% | 2.01 | 451 |
| 100 | 101.510 | 0.08 | 0.07% | 2.00 | 1081 |

Table 4. Results for the 5 µL Microsyringe measured in the 0.001 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 0.1 | 0.106 | 0.01 | 11.7% | 2.01 | 374 |
| 0.2 | 0.204 | 0.01 | 6.0% | 2.01 | 449 |
| 0.5 | 0.507 | 0.02 | 3.0% | 2.01 | 456 |
| 1 | 1.009 | 0.02 | 1.8% | 2.01 | 287 |
| 2 | 2.005 | 0.02 | 1.0% | 2.01 | 252 |
| 5 | 5.013 | 0.04 | 0.8% | 2.02 | 143 |

Table 5. Results for the 100 µL Microsyringe measured in the 0.001 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 10 | 10.291 | 0.03 | 0.25% | 2.01 | 231 |
| 20 | 20.327 | 0.05 | 0.24% | 2.00 | 238 |
| 50 | 50.793 | 0.07 | 0.14% | 2.01 | 413 |
| 100 | 101.531 | 0.07 | 0.07% | 2.01 | 403 |

Table 6. Comparison of microsyringe measurements on scales with different resolutions (uncertainty in %). All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | U scale resolution 0.01 mg | U scale resolution 0.001 mg |
|---|---|---|
| 0.1 | 39.27 % | 11.73 % |
| 0.2 | 20.64 % | 6.03 % |
| 0.5 | 8.37 % | 3.01 % |
| 1 | 4.47 % | 1.82 % |
| 2 | 2.18 % | 0.97 % |
| 5 | 0.92 % | 0.80 % |
| 10 | 0.97 % | 0.25 % |
| 20 | 0.55 % | 0.24 % |
| 50 | 0.17 % | 0.14 % |
| 100 | 0.07 % | 0.07 % |

consideration is important when evaluating the associated costs of such modifications.

Table 5 presents the results of the microsyringe calibration of 100 µL, measured in the scale with 0.001 mg resolution. Eventhough uncertainties are smaller compared to the measurement using a micropipette, the source of uncertainty that most impacts these measurements is related to the handling of the microsyringe by the operator ($U_{\mathrm{op}}$). Two others, which are at a secondary levels of influence, are related to the scale used and the repeatability of the measurements.

In Table 6, the significance of using a scale with a resolution of 0.001 mg for microsyringe calibration below 1 µL is clearly illustrated. The results demonstrate a substantial difference between the two scales at the 0.1 µL measurement point, which diminishes as the nominal volume increases. This trend aligns with observations made during the micropipette calibration, reinforcing the notion that the influence of scale resolution becomes less pronounced at higher volume measurements.

Upon concluding the analysis and consolidating the results, simulations were conducted to assess the potential improvements that could be achieved by employing a scale with

Table 7. Results for the 2 µL Micropipette measured in the 0.01 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 0.1 | 0.100 | 0.06 | 55.2% | 2.00 | 2632 |
| 0.2 | 0.170 | 0.06 | 34.9% | 2.00 | 5101 |
| 0.5 | 0.430 | 0.07 | 17.1% | 2.00 | 2799 |
| 1 | 0.920 | 0.09 | 9.3% | 2.00 | 942 |
| 2 | 1.890 | 0.09 | 4.7% | 2.00 | 3060 |

Table 8. Results for the 10 µL Micropipette measured in the 0.01 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 5 | 5.050 | 2.88 | 2.00% | 2.00 | 1705 |
| 10 | 10.060 | 1.79 | 2.00% | 2.00 | 1895 |

Table 9. Results for the 100 µL Micropipette measured in the 0.01 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 20 | 21.020 | 0.12 | 0.57% | 2.00 | 891 |
| 50 | 50.750 | 0.15 | 0.29% | 2.00 | 1142 |
| 100 | 100.960 | 0.21 | 0.20% | 2.00 | 29061 |

Table 10. Results for the 2 µL Micropipette measured in the 0.001 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 0.1 | 0.108 | 0.02 | 19.5% | 2.00 | 573 |
| 0.2 | 0.209 | 0.02 | 9.2% | 2.00 | 1238 |
| 0.5 | 0.436 | 0.05 | 11.3% | 2.00 | 549 |
| 1 | 0.952 | 0.05 | 5.9% | 2.01 | 475 |
| 2 | 1.947 | 0.07 | 3.3% | 2.01 | 327 |

Table 11. Results for the 10 µL Micropipette measured in the 0.001 mg scale resolution. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 5 | 5.180 | 0.07 | 1.25% | 2.00 | 1705 |
| 10 | 10.031 | 0.11 | 1.11% | 2.00 | 1895 |

Table 12. Results for the 100 µL Micropipette measured in the 0.001 mg scale resolution. Results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal Value (µL) | Average Value (µL) | *U* (µL) | *U* (%) | *k* | $\nu_{\mathrm{eff}}$ |
|---|---|---|---|---|---|
| 20 | 20.903 | 0.07 | 0.31% | 2.00 | 1341 |
| 50 | 50.780 | 0.11 | 0.21% | 2.00 | 1094 |
| 100 | 101.313 | 0.21 | 0.20% | 2.00 | 2287 |

a resolution of 0.0001 mg and its corresponding measurement uncertainty. The findings indicated that at all measurement points, the simulated values did not yield an improvement exceeding 1 % of the expanded uncertainty. Consequently, it is concluded that investing in a scale with an accuracy higher than 0.001 mg for microvolume calibration of up to 0.1 µL is unnecessary. If the laboratory aims to enhance its results, it should focus on addressing other sources of uncertainty, which are often linked to the operation of the instrument (micropipette or microsyringe) and improvements in the repeatability of measurements.

### 4.2. Micropipette measurements

Results from measurements performed with micropipettes are presented in Table 7 to Table 12. Both scales have an air saturation system, which minimizes the evaporation of the volume dispensed by the micropipette.

Table 7 presents the results of the calibration of the 2 µL micropipette measured in the scale with 0.01 mg resolution. It shows that the uncertainty values are significantly high in the values below 1 µL. This is justified by the influence of the scale used, with the source of uncertainty related to the uncertainty of the scale calibration. In the first analysis of the result, it seems that it would be sufficient to improve only the uncertainty of the scale calibration (expanded uncertainty provided in the scale calibration certificate), and then this problem would be solved. However, improving this result is not so simple, since the source of uncertainty that contributes significantly to the result of the expanded uncertainty of the scale calibration was its own resolution. In other words, having a scale with a resolution of 0.001 mg, the uncertainty of its certificate would be better when compared to the uncertainty of the calibration of the scale with a resolution of 0.01 mg, and consequently would not affect the result of the microvolume calibration in the range shown in Table 13. Thus, it is possible to conclude that in this calibration, the source of uncertainty related to the scale resolution does not seem to have much impact, with values below 2 %, but it does have an indirect impact and can be a decisive factor in improving the Calibration Measurement Capability (CMC) of the laboratory.

Another significant influence for this measurement range is related to the handling of the micropipette by the operator and is closely linked to the construction system of the equipment and its use.

Therefore, the relative influence of the uncertainty provided in the scale calibration certificate and the uncertainty of the operator are very significant. Since the uncertainty value of the scale calibration certificate is predefined, its influence in percentage values on the combined uncertainty will vary depending on better measurements by the technician performing the measurements. If they are more homogeneous, the source of uncertainty related to the operator decreases, which results in a greater contribution to the uncertainty of the scale.

Table 8 presents the results of the calibration of the 10 µL micropipette measured in the scale with 0.01 mg resolution. In this measurement range, the handling of the micropipette by the operator is the source of uncertainty that has the greatest influence. The mechanical micropipette has a system for aspirating and dispensing the liquid using a piston and spring, which provides greater variability of the results even with the necessary care for its use.

In these measurements, the influence of the scale decreases in relation to the measurements of the 2 µL micropipette (Table 7), but it is still somewhat significant and subject to improvement if the user wants better uncertainties.

Table 9 presents the results of the calibration of the 100 µL micropipette measured in the scale with 0.01 mg resolution, which was calibrated only up to 100 µL. In this calibration, the scale used is not the main source of uncertainty either. The handling of the micropipette by the operator ($U_{\mathrm{op}}$) continues to be the source of uncertainty that has the greatest influence.

Table 10 presents the results of the calibration of the 2 µL micropipette measured in the scale with 0.001 mg resolution. It is possible to verify a significant decrease in the expanded uncertainty when compared with the results of the same micropipette, but performing the measurement on the scale with a resolution of 0.01 mg. Even so, the uncertainty values are still slightly high in the values below 1 µL, which is justified by the influence of the micropipette handling by the operator, and is closely linked to the construction system of the equipment and its use, as previously explained.

Table 11 presents the results of the calibration of the 10 µL micropipette measured in the scale with 0.001 mg resolution. In these measurements, the handling of the micropipette by the operator was what most influenced the expanded uncertainty. The repeatability of the measurements also had a slight influence on the source of uncertainty, as it is related to the standard deviation of the measurements.

Table 12 presents the results of the calibration of the 100 µL micropipette measured in the scale with 0.001 mg resolution. In this calibration, the predominance of the influence related to the handling of the micropipette by the operator and a slight influence related to the repeatability of the measurements in a discrete manner were observed again.

After several measurements and analysis of the results, it is possible to conclude that for the calibration of the micropipette, the 100 µL point is where the scale used no longer influences the expanded uncertainty of the measurement, when comparing the results using the scale with a resolution of 0.01 mg and with a resolution of 0.001 mg.

## 5. RESULTS AND DISCUSSION

The measurement results presented in the tables clearly show that the relative values of the expanded uncertainty ($U_{\%}$) decrease consistently as the nominal volume increases. This trend is observed for both the microsyringe and the micropipette, reflecting the expected behavior of gravimetric calibration, in which the relative influence of individual uncertainty sources diminishes with increasing volume.

The results presented in Table 13 demonstrate that the resolution of the balance plays a decisive role in improving measurement uncertainty for the micropipette, up to approximately 50 µL. As highlighted in ISO 8655 [6] and discussed in Section 2, other sources of uncertainty also contribute significantly. Nevertheless, the balance acts as both a direct source of uncertainty—through its calibration and resolution—and an indirect stabilizing factor, since the higher-resolution balance provided better repeatability and reduced data dispersion.

For the 100 µL microsyringe calibration, the analysis indicates that for volumes of 50 µL and above, the effect of balance resolution on the expanded uncertainty becomes negligible. From this point onward, the results obtained with balances of 0.001 mg and 0.01 mg resolution show high consistency, confirming the robustness of the gravimetric method for larger

Table 13. Micropipette results comparison between measurements from scales with different resolutions. All results at (20 ± 1) °C; expanded uncertainty *U* calculated with $k$ = 2.

| Nominal value (µL) | U scale resolution 0.01 mg | U scale resolution 0.001 mg |
|---|---|---|
| 0.1 | 55.19 | 19.47 |
| 0.2 | 34.86 | 9.24 |
| 0.5 | 117.13 | 11.35 |
| 1 | 9.30 | 5.95 |
| 2 | 4.70 | 3.33 |
| 5 | 2.88 | 1.25 |
| 10 | 1.79 | 1.11 |
| 20 | 0.57 | 0.31 |
| 50 | 0.29 | 0.21 |
| 100 | 0.20 | 0.20 |

nominal volumes where the influence of scale resolution is minimized.

After applying the density and buoyancy corrections described in Section 2, the results confirmed the effectiveness of these adjustments in maintaining the metrological coherence of the measurements. The influences of air buoyancy, container expansion, and temperature variation were fully compensated within the uncertainty budget, resulting in consistent mass-to-volume conversion across the studied range.

The weighing systems used in this study—shown in Figure 2 and Figure 3—exhibited stable performance throughout the calibration sequence, with no significant drift or instability observed. These findings validate both the correction parameters and the overall experimental setup adopted for gravimetric microvolume measurements.

The experimental implementation of the proposed gravimetric method enabled the acquisition of valid and traceable results for microvolume measurements in the range of 0.1 µL to 1 µL. The method demonstrated full metrological traceability at these extremely low volumes, evidencing its flexibility and reproducibility across repeated trials. One of the main innovations verified in this study concerns the treatment of the balance tare procedure, which was critically analyzed and optimized relative to ISO 8655-6. This adjustment proved essential for achieving consistent mass readings and minimizing systematic deviations in the calculated volume.

The minimum weight was adopted in an innovative way for volume measurement, serving as a methodology to assess whether the balance could accurately measure the very small volumes proposed in this work—specifically those below 1 µL. The application of this concept allowed for verifying the lower operating limits of the balance while maintaining traceability and control over the uncertainty components associated with mass determination.

During the experiments, a specific strategy was also applied to minimize the effects of liquid evaporation during calibration. The results confirmed that this phenomenon becomes particularly relevant for nominal volumes below 50 µL, where even small mass losses can significantly increase measurement uncertainty. By maintaining controlled environmental conditions and performing immediate weighings, the contribution of evaporation was significantly reduced, improving both repeatability and reliability.

Results from measurements performed with micropipettes are presented in Table 7 to Table 12. Both balances were equipped with air-saturation systems, which minimized the evaporation of the volume dispensed by the micropipette and consequently improved the stability of the results.

As illustrated in Figure 5, the comparison between microsyringe and micropipette measurements confirms that for both devices the balance resolution strongly affects the uncertainty at the lowest volumes (~0.1 µL), but becomes progressively less influential as the nominal volume increases. Around 100 µL, the differences between the balances of 0.01 mg and 0.001 mg resolutions are negligible, indicating that other factors—such as operator handling ($U_{op}$) and repeatability—become the dominant sources of uncertainty. These trends are consistently reflected in Table 6 to Table 13.

## 6. CONCLUSIONS

The study confirmed that the proposed gravimetric approach allows traceable measurements of liquid volumes down to 0.1 µL, provided that corrections for air buoyancy, temperature, and density are properly applied. Within the evaluated range, the results showed that the relative expanded uncertainty ($U_{\%}$) decreases consistently as the nominal volume increases, demonstrating the expected behaviour of improved precision at higher volumes.

The experiments also indicated that scale resolution plays a decisive role in the uncertainty budget only up to approximately 50 µL for the micropipette and 1 µL for the microsyringe. Beyond these points, other factors—particularly operator handling and environmental stability—become more significant.

Although the observed dispersion among repeated measurements suggests that operator handling influences repeatability, this effect was only qualitatively assessed, and

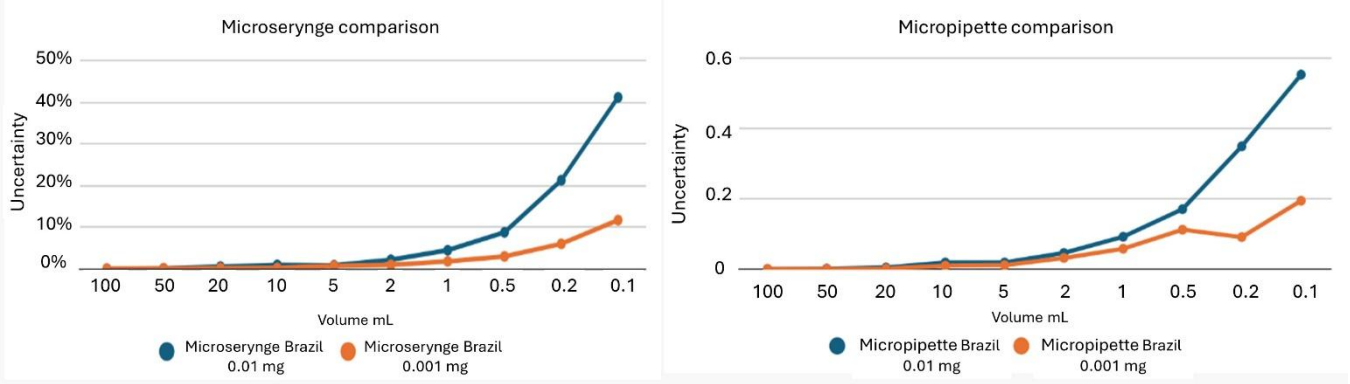


Figure 5. Comparison between microsyringes and micropipettes volume measurements performed in both 0.01 mg and 0.001 mg resolution scales. The x-axis refers to Nominal volume (µL) and the y-axis is Expanded uncertainty U (%).

should be further investigated through structured inter-operator studies. Therefore, conclusions regarding operator influence are limited to the observed repeatability patterns.

In summary, the methodology presented here contributes to improving the metrological reliability of microvolume measurements, particularly in the sub-microliter range. However, future work should refine the evaluation of operator effects, explore additional fluids, and extend the method's validation to other temperature and humidity conditions.

## AUTHORS' CONTRIBUTION

All authors contributed significantly to the completion of this work.

Leandro S. Sampaio was responsible for the design and execution of the study, in addition to performing the statistical analysis and writing the manuscript.

Mila R. Avelino and Luis V. Tarelho acted as doctoral advisors, contributing with theoretical and methodological guidance throughout the entire research process, and participated in the literature review and data collection.

All authors participated in the preparation of the manuscript and in the final formatting of the document, reviewed and approved the final version of the article, ensuring the integrity and accuracy of the information presented.

## REFERENCES


[1] M. Rosendahl, R. S. Nazareth, M. R. Magalhaes, W. S. Silva, P. L. S. Ferreira, (+ 4 more author), New calibration procedure for differential pressure using twin pressure scales for flowrate measurement, Journal of Physics: Conf. Series vol. 1044 (2018) no. 1, p. 012053.
DOI: 10.1088/1742-6596/1044/1/012053

[2] J. J. P. Santos Junior, R. G. Pereira, M. Rosendahl, D. M. Santo Filho, J. M. G. Gouveia, A. P. F. Barbosa, C. S. C. Castro, M. H. Farias, Inmetro hydrostatic weighing system–determination of solids volume, Journal of Physics: Conference Series vol. 1044 (2018) no. 1, p. 012036.
DOI: 10.1088/1742-6596/1044/1/012036

[3] E. Batista, L. Pinto, E. Filipe, A. van der Veen, Calibration of micropipettes – Test methods and uncertainty analysis, Measurement vol. 40 (2007) no. 3, pp. 338–342.
DOI: 10.1016/j.measurement.2006.05.012

[4] E. Batista, I. Godinho, G. Rodrigues, S. Schork, Pilot study on calibration of micropipettes using the photometric method, J. Phys.: Conf. Ser. vol. 1065, issue 7, 072006.
DOI: 10.1088/1742-6596/1065/7/072006

[5] ISO 8655-2: 2022, Piston-operated volumetric apparatus – Part 2: Piston pipettes, International Organization for Standardization, Geneva, Switzerland, 2022. Online [Accessed 19 May 2026] https://www.iso.org/standard/68797.html

[6] ISO 8655-6: 2022, Piston-operated volumetric apparatus – Part 6: Gravimetric methods for testing piston-operated volumetric apparatus, International Organization for Standardization, Geneva, Switzerland, 2022. Online [Accessed 19 May 2026] https://www.iso.org/standard/75211.html

[7] L. S. Sampaio, M. R. Avelino, L. V. G. Tarelho, Uncertainty evaluation in microvolume measurements using gravimetric method, Proc. VIII CIMMEC 2023 – Congresso Internacional de Metrologia Mecânica, Rio de Janeiro, Brazil (2023) 1–6. Online [Accessed 19 May 2026] https://metrologia2023.org.br/wp-content/uploads/2023/11/303738_on-the-microvolume-measurement-from-01-ul-to-100-ul-using-a-micropipette.pdf

[8] E. Batista, L. Pinto, E. Filipe, A. van der Veen, Micropipette calibration and uncertainty evaluation at very small volumes, Proc. FLOMEKO 2016 – 18th IMEKO Symposium on Flow Measurement, Lima, Peru (2016), pp. 1–6.

[9] E. Batista, N. Almeida, E. Filipe, A Study of Factors that Influence Micropipette Calibrations, NCSLI Measure 11 (2016), pp. 44–49.
DOI: 10.1080/19315775.2015.11721717

[10] L. S. Sampaio, M. R. Avelino, L. V. G. Tarelho, J. N. S. Lima, Statistical analysis of measurement uncertainty in microvolumes using the gravimetric method, Proc. 8th Int. Congress of Mechanical Metrology (CIMMEC 2025), Fortaleza, Brazil (2025).

[11] F. Kong, L. Yuan, Y. F. Zheng, W. Chen, Automatic liquid handling for life science: a critical review of the current state of the art, Journal of laboratory automation, 17(3), pp. 169-185.
DOI: 10.1177/2211068211435302

[12] F. Boineau, M. D. Plimmer, E. Mahé, Volume calibration using a comparison method with a transfer leak flow rate, Acta IMEKO vol. 9 (2020) no. 5, pp. 343–348.
DOI: 10.21014/acta_imeko.v9i5.997

[13] L. S. Sampaio, M. R. Avelino, L. V. G. Tarelho, Application of uncertainty evaluation for microvolume calibration in laboratory practice, Proc. Enqualab 2019 – Encontro da Qualidade em Metrologia, São Paulo, Brazil (2019), pp. 1–6.

[14] A. Cruz Serra, L. Van Biesen, Measurement uncertainty in complex metrological systems, Proc. 12th IMEKO TC1 & TC7 Joint Symposium on Man, Science and Measurement, Annecy, France (2008) 1–5. Online [Accessed 19 May 2026] https://www.imeko.org/publications/tc7-2008/IMEKO-TC1-TC7-2008-IKL-001.pdf

[15] OIML R 111-1: 2004, Weights of classes E1, E2, F1, F2, M1, M1-2, M2, M2-3 and M3 – Part 1: Metrological and technical requirements, International Organization of Legal Metrology, Paris, France, 2004. Online [Accessed 19 May 2026] https://www.oiml.org/en/files/pdf_r/r111-1-e04.pdf

[16] J. Barbosa, E. Batista, L. Pinto, E. Filipe, A. van der Veen, Comparative study of gravimetric methods for small-volume calibration, Proc. II CIMMEC 2011 – Congresso Internacional de Metrologia Mecânica, Belo Horizonte, Brazil (2011), pp. 1–5.

[17] EURAMET CG-19: 2018, Guidelines on the determination of uncertainty in gravimetric volume calibration, EURAMET, Braunschweig, Germany, 2018.

[18] INMETRO GUM: 2012, Avaliação de dados de medição – Guia para a expressão da incerteza de medição (GUM 2008), INMETRO, Rio de Janeiro, Brazil, 2012. Online [Accessed 19 May 2026] [in Portuguese] https://www.gov.br/inmetro/pt-br/assuntos/metrologia-cientifica/documentos-tecnicos-em-metrologia/gum_final.pdf/@@download/file

[19] L. S. Sampaio, M. R. Avelino, L. V. G. Tarelho, A. J. M. Mendonça, D. Soares, Calibração de volume em escala nanométrica – Escolha da correta balança, Enqualab, 1-6. 2019. [in Portuguese]

[20] B. G. Soares, Development of a procedure for calibration of a microvolume system, MSc Dissertation, Inmetro, Rio de Janeiro, Brazil, 2021