Calibration of small volume instruments

Final Report

EURAMET Project no. 1602

Coordination Elsa Batista
IPQ-DMET - Volume and Flow Laboratory

March 2024

Contents

1. Introduction

The purpose of this comparison between IPQ - Portugal and KMA - Kosovo is to verify the agreement of results and uncertainties in the calibration of 3 different volume instruments: micropipette, pycnometer and digital burette despite the different equipment used and calibration process by each laboratory. Kosovo is not yet a member of BIPM and does not have CMC in this field of activity.
This document presents the guidelines and results of this bilateral comparison. The measurements were performed in December 2023 and February 2024.

Table 1 – Participants

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	December 2023/January 2024	Elsa Batista	ebatista@ipq.pt
Kosovo	KMA	January 2024	Agim Xhuraj	agim.xhuraj@rks-gov.net

2. The instrument

Tree different volume standards were provided by IPQ: one single channel micropipette of fixed capacity (figure1), one glass Gay Lussac pycnometer of 50 mL (figure 2) and a 50 mL digital burette (figure 3). All instruments' characteristics are described in table 2.

Table 2 – Instruments used in the bilateral comparison

Manufacturer	Model	Nominal Volume	Type	Serial number
Eppendorf	Reference	10 μL	Fixed	1863594
Fortuna	Gay Lussac	50 mL	Glass	58
Brand	Titrette	50 mL	Digital	-

Figure 1 – 10 μL Micropipette

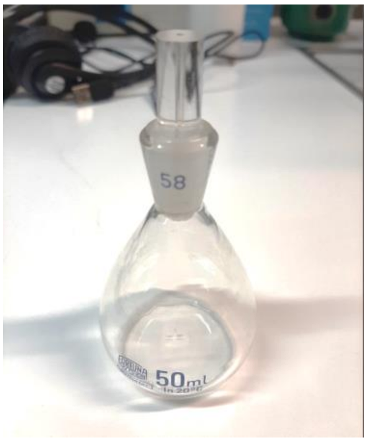

Figure 2 – 50 mL Pycnometer


Figure 3 – 50 mL digital burette

3. Experimental tests

The three chosen instruments were calibrated at the following nominal volumes:

- Calibration of a fixed micropipette of 10 μL.
- Calibration of a glass pycnometer at its nominal volume of 50 mL.
- Calibration of digital burette at 50 mL, 25 mL and 5 mL.

Each test was performed with 10 replicates.

The ambient conditions of the laboratory room during the measurements should be the following:

- humidity higher than 50 %,
- ambient temperature between 17 ºC up to 23 ºC,

the water temperature must be near the air temperature and shall not vary more than 0,5 ºC during the measurements.

4. Calibration method

The suggest method to perform the calibration of volume instruments is the gravimetry. The following formula described in ISO 4787 [1] can be used for the calculation of the delivered or contained volume:

$$V_{20} = (I_L - I_E) \times \frac{1}{\rho_W - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(t - 20)] \qquad (1)$$

The results must be given for a reference temperature of 20 ºC, and the calibration liquid should be distilled water. The volume for each artefact should be determined using 10 repeated measurements.
The calibration procedure, in detail, is described in ISO 8655 [2] for the micropipette and the burette and in ISO 4787 for the pycnometer [1].

5. Evaluation of the measurement results

5.1 Reference value

To determine the reference value the formula of the weighted mean is used, by means of the inverses of the squares of the associated standard uncertainty are the weighting factors [3]:

$$y = \frac{x_1/u^2(x_1) + ... + x_n/u^2(x_n)}{1/u^2(x_1) + ... + 1/u^2(x_n)} \qquad (2)$$

To determine the standard uncertainty u(y) associated with y is used the following expression:

$$u(y) = \sqrt{\frac{1}{1/u^2(x_1) + ... + 1/u^2(x_n)}} \qquad (3)$$

5.2 Consistency determination

To identify an overall consistency of the results a chi-square test can be applied to all *n* calibration results.

$$\chi^2_{obs} = \frac{(x_1 - y)^2}{u^2(x_1)} + ... + \frac{(x_n - y)^2}{u^2(x_n)} \qquad (4)$$

where the degrees of freedom are: $\nu = n - 1$

The consistency check is failing if: $\Pr\{\chi^2(\nu) > \chi^2_{obs}\} < 0{,}05$. The function *CHIINV(0,05; n-1)* in MS Excel was used. The consistency check was failing if *CHIINV(0,05; n-1) < $\chi^2_{obs.}$*

If the consistency check passes, then *y* was accepted as the KCRV x_{ref} and $U(x_{ref})$ is accepted as the expanded uncertainty of the KCRV.

If the consistency check fails then the laboratory with the highest value of $\frac{(x_i - y)^2}{u^2(x_i)}$ is excluded from the next round of evaluation and the new reference value, reference standard uncertainty and chi-squared value is calculated again without the excluded laboratory.

The En value was also calculated. This value is defined as [4]:

$$E_{n_{lab-i}} = \frac{\varepsilon_{lab-i} - \varepsilon_{RV}}{\sqrt{U^2(\varepsilon_{lab-i}) + U^2(\varepsilon_{RV})}} \qquad (5)$$

where ε_{lab-i} is the error of lab-i for a certain point, ε_{RV} is the comparison reference value (RV) for the error and $U(\varepsilon_{lab-i})$ and $U(\varepsilon_{RV})$ and the expanded uncertainties (*k*=2) of those values.

With the value of E_n one can conclude that:

- The results of the laboratory for a certain point are consistent (passed) if $E_n \leq 1$

- The results of the laboratory for a certain point are inconsistent (failed) if $E_n > 1$

IPQ performed two calibrations, one at the beginning and another at the end of the to access the stability of the artefacts.
The first result of IPQ was considered for the determination of reference value, along with its value of uncertainty.

6. Equipment used

Table 3 – Equipment characteristics

Balance	Type	Range	Resolution
IPQ	Electronic, Mettler AX26 with evaporation trap	(0-22) g	0,001 mg
	Electronic, Sartorius CE2004	(0-2200) g	0,00001 g
KMA	Electronic, Mettler XPR 56	(0-52) g	0,001 mg
	Eletronic, Radwag XA.210.4Y.A Plus	(1-210) g	0,00001 g
Liquid thermometer	**Type**	**Range**	**Resolution**
IPQ	Luft, PT100	(-30 to 150) ºC	0,001 ºC
KMA	Almemo, FNA32L025	(0 to 23) ºC	0,001 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Rotronic HP32	(0 to 70) ºC	0,01 ºC
KMA	Almemo, ZAD6492	(15 to 25) ºC	0,01 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Druck, DPI 142	(900 - 1200) hPa	0,01 hPa
KMA	Almemo	(700 - 1050) hPa	0,01 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Rotronic HP32	(0-100) %	0,01%
KMA	Almemo, 11120207	(30-70) %	0,1%

7. Ambient conditions

The ambient conditions of both laboratories were the following:

Table 4 - Ambient conditions

Laboratory	Air Temperature (°C)	Pressure (hPa)	Relative Humidity (%)	Air Density (g/ml)
IPQ	20,61 – 20,80	1004,36 – 1012,37	64,9 – 78,7	0,0012
KMA	20,33 – 20,64	943,9 – 944,1	53,7 – 55,1	0,0012

8. Measurement results

8.1. Determination of the stability of the artefacts

In order to determine the reference value and access the stability of the instrument two measurements were performed by IPQ - one at the beginning and other at the end of the comparison for the 3 instruments.

Table 5 – Stability of the transfer standards

	IPQ1		IPQ2		
Micropipette	**Volume (μL)**	**Uncertainty (μL)**	**Volume (μL)**	**Uncertainty (μL)**	**ΔV(μL)**
10	10,041	0,033	10,036	0,032	0,005
Pycnometer	**Volume (mL)**	**Uncertainty (mL)**	**Volume (mL)**	**Uncertainty (mL)**	**ΔV(mL)**
50	49,8857	0,0031	49,8853	0,0038	0,0004
Burette	**Volume (mL)**	**Uncertainty (mL)**	**Volume (mL)**	**Uncertainty (mL)**	**ΔV(mL)**
50	50,0101	0,0071	50,0113	0,0070	0,0012
25	25,0080	0,0073	25,0060	0,0071	0,0020
5	5,0028	0,0062	5,0010	0,0060	0,0018

The result variation of IPQ is smaller than the declared uncertainty and therefore it is assumed that all instruments were stable during the comparison.

8.2. Volume results with reference value

8.2.1 Micropipette

Table 6 – Volume measurement results – Micropipette

Laboratory	Volume (μL)	Uncertainty (μL)	En value
IPQ - 1	10,041	0,033	0,77
KMA	10,011	0,021	-0,77
IPQ - 2	10,036	0,032	
Vref	10,020	0,018	

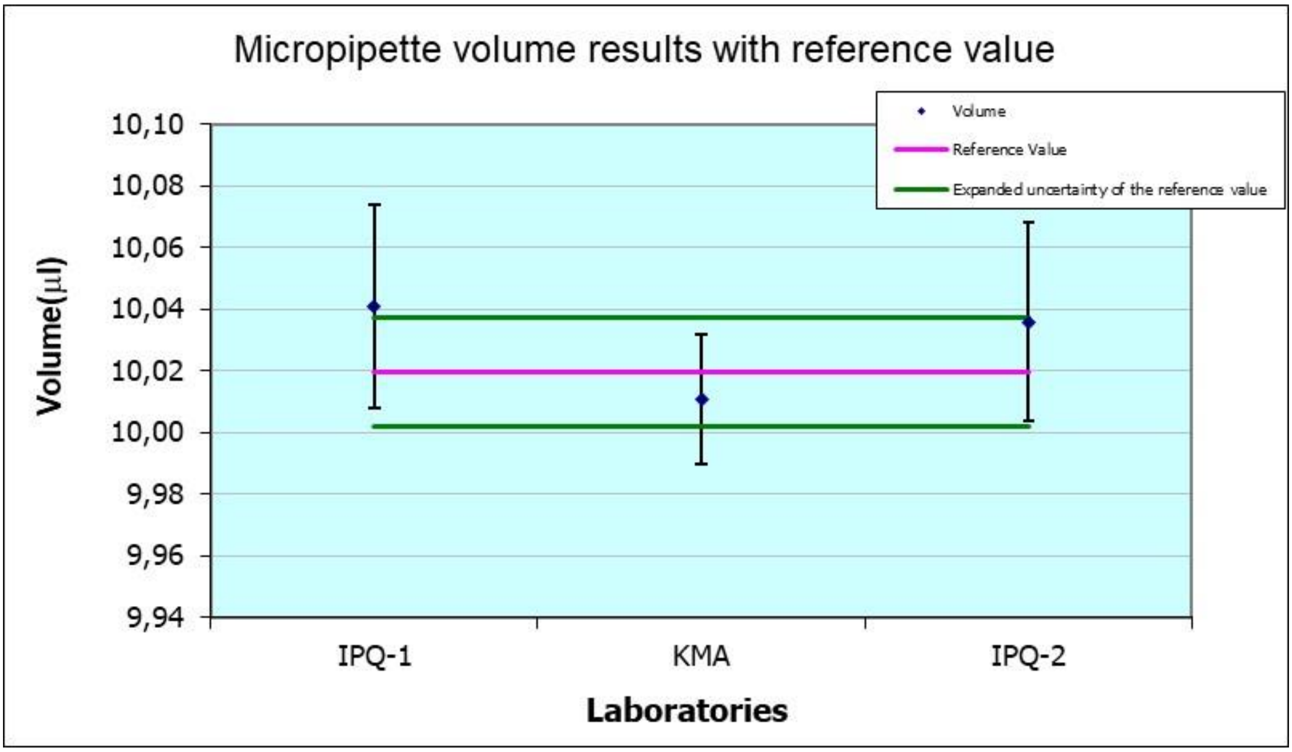

Figure 4 – Volume results with reference value – micropipette

8.2.2. Pycnometer

Table 7 – Volume measurement results – pycnometer

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ - 1	49,8857	0,0031	0,81
KMA	49,8810	0,0049	-0,81
IPQ - 2	49,8853	0,0038	
Vref	49,8844	0,0026	

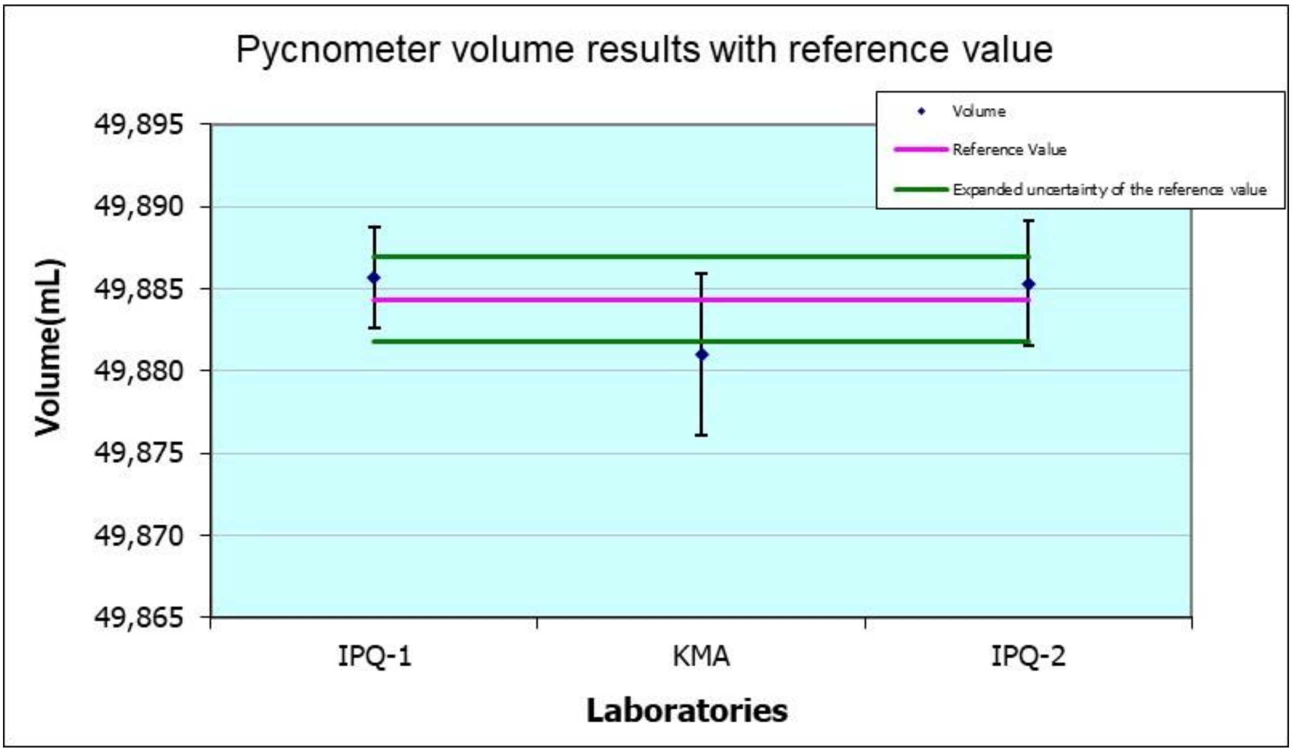

Figure 5 – Volume results with reference value – pycnometer

8.2.3. Burette at 50 mL

Table 8 – Volume measurement results - burette at 50 mL

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ – 1	50,0101	0,0071	0,05
KMA	50,0096	0,0064	-0,05
IPQ – 2	50,0113	0,0070	
Vref	50,0098	0,0048	

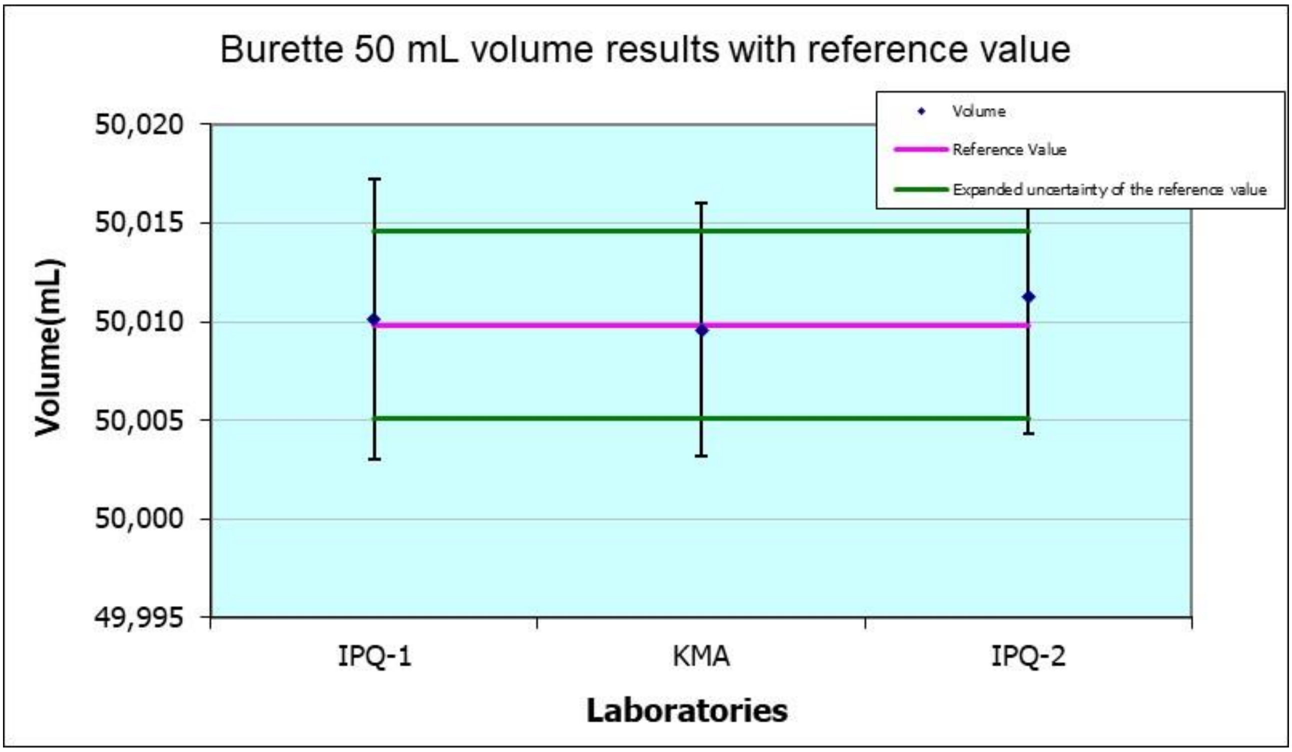

Figure 6– Volume results with reference value – burette 50 mL

8.2.4. Burette at 25 mL

Table 9 – Volume measurement results - burette at 25 mL

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ – 1	25,0080	0,0073	0,35
KMA	25,0045	0,0069	-0,35
IPQ – 2	25,0060	0,0071	
Vref	25,0061	0,0050	

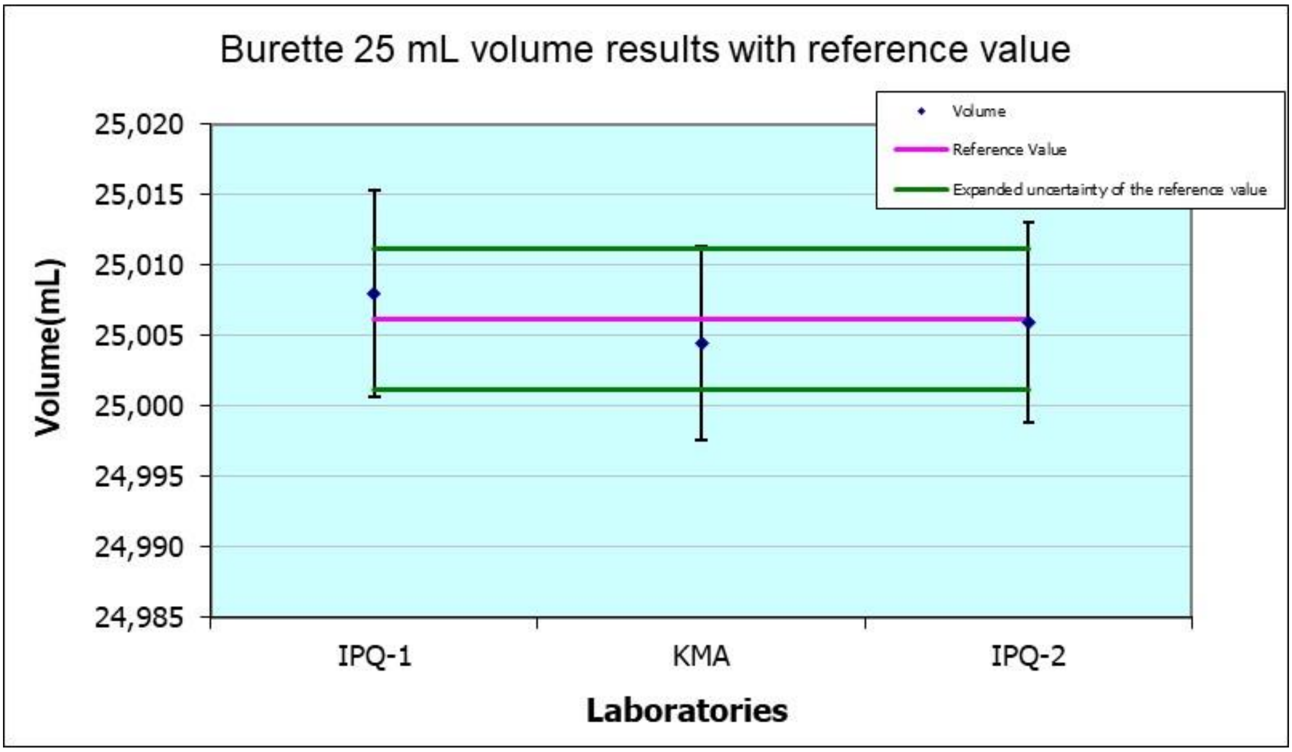

Figure 7– Volume results with reference value – burette 25 mL

8.2.4. Burette at 5 mL

Table 10 – Volume measurement results - burette at 5 mL

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ - 1	5,0028	0,0062	0,63
KMA	4,9973	0,0061	-0,63
IPQ - 2	5,0010	0,0060	
Vref	5,0000	0,0044	

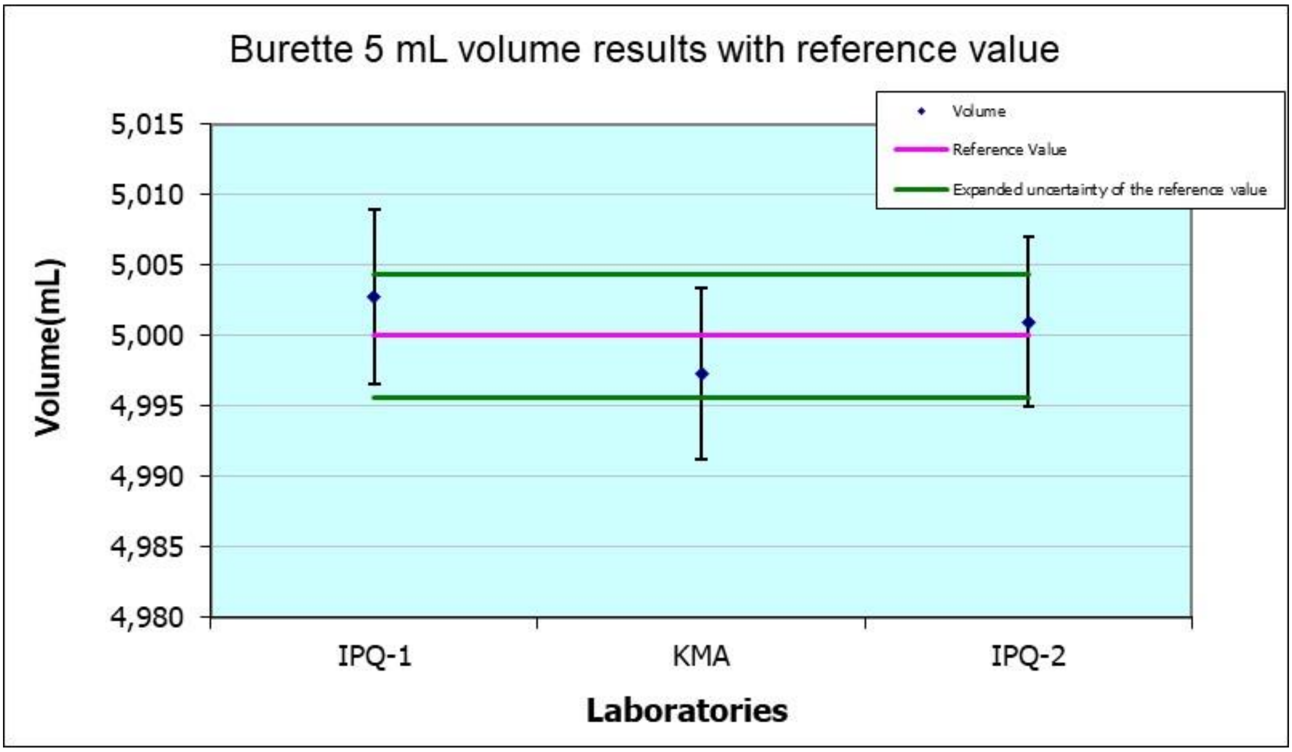

Figure 8– Volume results with reference value – burette 5 mL

As can be seen from the tables and figures above, all of the results for all artifacts in al tested nominal volumes are satisfactory.

9. Uncertainty calculation

Both laboratories calculated the uncertainty according to GUM [5] and presented similar uncertainty components and values for the 3 artifacts.

Both laboratories declared the same uncertainty components: mass, air density, water density, mass standards density, expansion coefficient of the instruments, water temperature, repeatability, evaporation and resolution of the device. KMA did not calculate the operator effect in the micropipette, and this is why the values are smaller than for IPQ.

The largest uncertainty component for both laboratories was the repeatability for the pycnometer and micropipette, the resolution for the burette.

10. Conclusions

In this bilateral comparison between IPQ and KMA, 3 volume artifacts were calibrated. The stability of the instruments was confirmed by the initial and final calibration of IPQ.

The volume results are quite similar and consistent with each other and with the determined reference value.

The uncertainty values and components of the determined volumes are very similar for both laboratories.

11. References

1. ISO 4787: 2021 Laboratory glass and plastic ware - Volumetric instruments - Methods for testing of capacity and for use
2. ISO 8655-6:2022 – Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume
3. ISO 13528:2005 - Statistical methods for used in proficiency testing by interlaboratory comparisons
4. ISO/IEC 17043:2010 - Conformity assessment — General requirements for proficiency testing
5. JCGM 100:2008 - Guide to the expression of uncertainty in measurement (GUM)