Calibration of volumetric and piston operating instruments

Final Report

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Coordination Elsa Batista
IPQ-DMET - Volume and Flow Laboratory

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Contents

1. Introduction

The purpose of this comparison between IPQ – Portugal, INM – Moldavia and GEOSTM - Georgia is to verify the agreement of results and uncertainties in the calibration of 4 different volume instruments: micropipette, pycnometer, flask and dispenser despite the different equipment used and calibration process by each laboratory.
This document presents the guidelines and results of this comparison. The measurements were performed from March 2024 to June 2024.

Table 1 – Participants

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	March 2024/June 2024	Elsa Batista	ebatista@ipq.pt
Moldova	INM	April 2024	Ana Rusu	ana.rusu@inm.gov.md
Georgia	GEOSTM	June 2024	Irma Rurua	irmarurua@yahoo.com

2. The instrument

Four different volume standards were provided by IPQ:one single channel micropipette of fixed capacity (figure 1), one glass Gay Lussac pycnometer of 50 mL (figure 2), a 100 mL flask (figure 3) and a 10 mL bottle dispenser (figure 4). All instruments' characteristics are described in table 2.

Table 2 – Instruments used in the comparison

Instrument	Manufacturer	Model	Nominal Volume	Serial number
Micropipette	Eppendorf	Reference	100 μL	J25622E
Pycnometer	Fortuna	Gay Lussac	50 mL	58
Flask	Normax		100 mL	9573
Dispenser	Brand	Dispensette	10 mL	08E08071

Figure 1 – 100 μL Micropipette

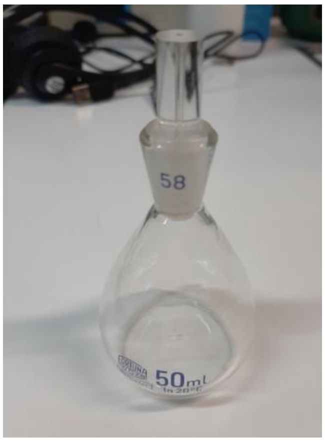

Figure 2 – 50 mL Pycnometer

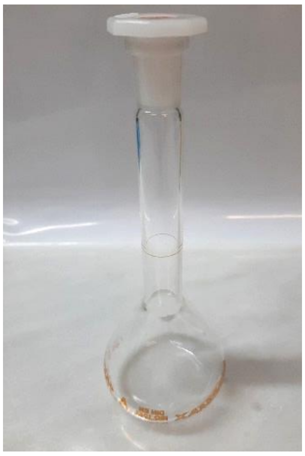

Figure 3 – 100 mL flask

Figure 4 – 10 mL dispenser

3. Experimental tests

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

- Calibration of a fixed micropipette of 100 μL.
- Calibration of a glass pycnometer at its nominal volume of 50 mL.
- Calibration of a flask at its nominal volume of 100 mL.
- Calibration of bottle dispenser at nominal volume of 10 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:

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

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 dispenser and in ISO 4787 for the pycnometer and flask [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 regarded as failed 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 did not fail then y was accepted as the KCRV x_{ref} and $U(x_{ref})$ was accepted as the expanded uncertainty of the KCRV.

If the consistency check failed 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
INM	XA.200.4Y.A.KB; RADWAG	210 g	0,01 mg
	XPR26/A, Mettler Toledo	22 g	0,001 mg
GEOSTM	XPE 2024/Mettler Toledo	200 g	0,0001 g
	XPE 26/Mettler Toledo	22 g	0,001 mg
Liquid thermometer	**Type**	**Range**	**Resolution**
IPQ	Luft, PT100	(-30 to 150) ºC	0,001 ºC
INM	1523 Fluke	-10 °C to 60 °C	0,001 °C
GEOSTM	XP 100 Luft	(-30 to 150) ºC	0,001 ºC
Air Thermometer	**Type**	**Range**	**Resolution**

IPQ	Rotronic HP32	(0 to 70) ºC	0,01 ºC
INM	THB 1B, UNITESS	0 °C to 50 °C	0,01 ºC
GEOSTM	OPUS 20 THIP		0,1 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Druck, DPI 142	(900 - 1200) hPa	0,01 hPa
INM	THB 1B, UNITESS	86 kPa to 106 kPa	0,1 kPa
GEOSTM	OPUS 20 THIP		0,1 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Rotronic HP32	(0-100) %	0,01%
INM	THB 1B, UNITESS	10 % to 90 %	0,1 %
GEOSTM	OPUS 20 THIP		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	19,89 – 20,42	995,10 - 1007,09	55,41 – 74,0	0,0012
INM	20,43 - 20,78	1012-1022	45,4-49,5	0,0012
GEOSTM	21,1 - 21,3	955,0 - 955,3	57,3 – 66,0	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 4 instruments.

Table 5 – Stability of the transfer standards

	IPQ1		IPQ2		
Micropipette	**Volume (μL)**	**Uncertainty (μL)**	**Volume (μL)**	**Uncertainty (μL)**	**ΔV(μL)**
100	99,98	0,12	100,00	0,12	0,02
Pycnometer	**Volume (mL)**	**Uncertainty (mL)**	**Volume (mL)**	**Uncertainty (mL)**	**ΔV(mL)**
50	49,8933	0,0040	49,8935	0,0038	0,0002
Flask	**Volume (mL)**	**Uncertainty (mL)**	**Volume (mL)**	**Uncertainty (mL)**	**ΔV(mL)**
100	99,905	0,010	99,900	0,010	0,005
Dispenser	**Volume (mL)**	**Uncertainty (mL)**	**Volume (mL)**	**Uncertainty (mL)**	**ΔV(mL)**
10	9,9739	0,0044	9,9720	0,040	0,0019

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	99,98	0,12	-0,06
INM	99,79	0,23	-0,89
GEOSTM	100,03	0,12	0,44
IPQ - 2	100,00	0,12	
Vref	99,986	0,066	

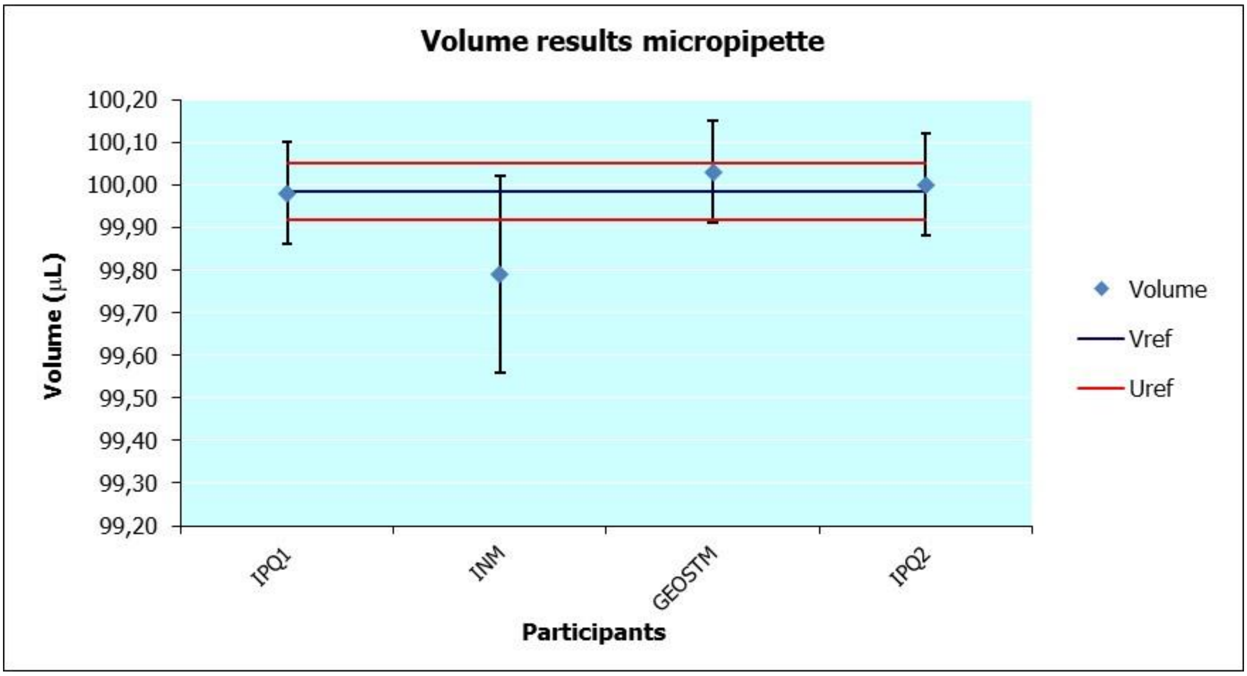

Figure 4 – Volume results with reference value – micropipette

As can be seen from the table and figure above, all the results of IPQ, INM and GEOSTM are satisfactory for the micropipette.

8.2.2. Pycnometer

Table 7 – Volume measurement results – pycnometer

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ - 1	49,8933	0,0040	-0,83
INM	49,8994	0,0056	0,89
GEOSTM	49,8670	0,0020	-7,5
IPQ - 2	49,8935	0,0038	
Vref	49,8952	0,0032	

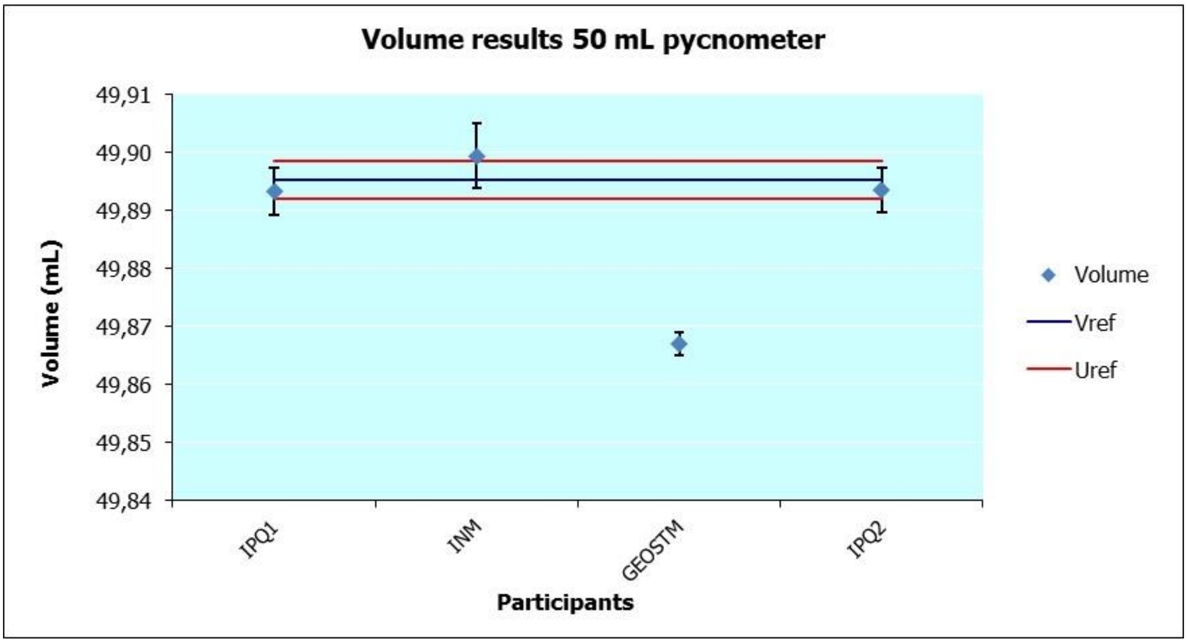

Figure 5 – Volume results with reference value – pycnometer

As can be seen from the table and figure above, all the results of IPQ and INM are satisfactory for the pycnometer. GEOSTM has inconsistent results.

8.2.3. Flask

Table 8 – Volume measurement results - flask

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ – 1	99,905	0,010	0,03
INM	99,904	0,027	-0,03
GEOSTM	99,865	0,004	-3,69
IPQ-2	99,900	0,010	
Vref	99,905	0,009	

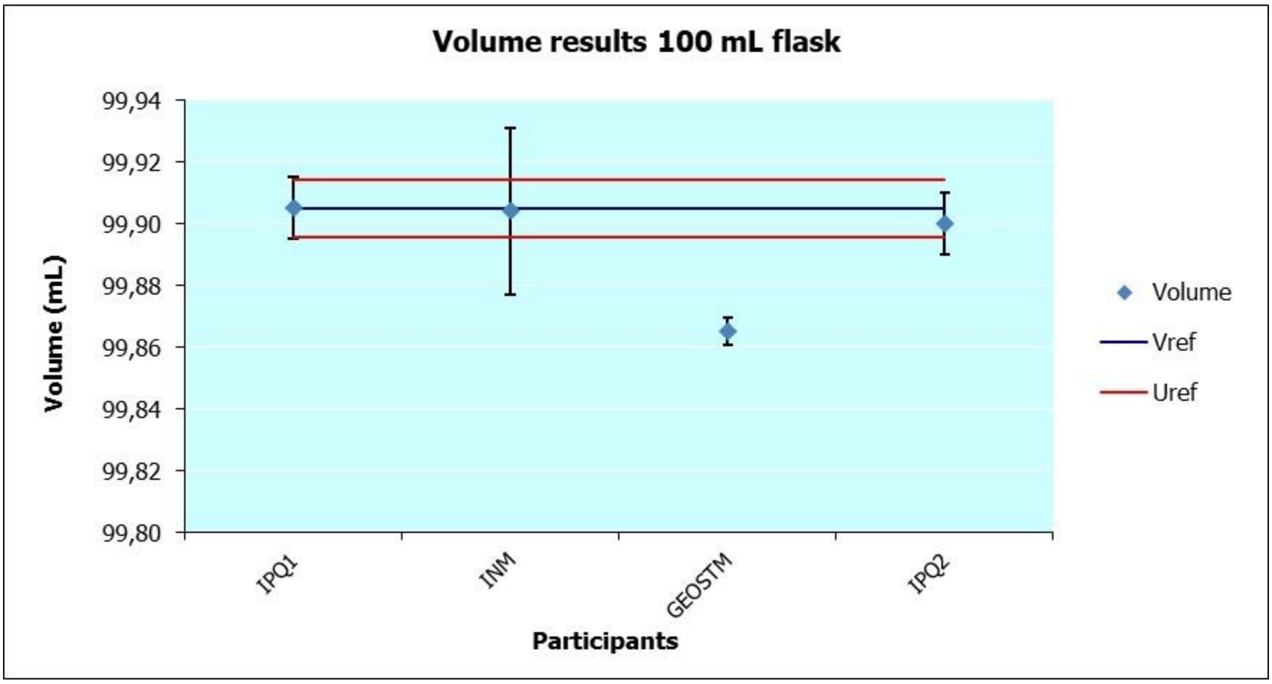

Figure 6– Volume results with reference value – flask

As can be seen from the table and figure above, all the results of IPQ and INM are satisfactory for the flask. GEOSTM has inconsistent results.

8.2.4. Dispenser at 10 mL

Table 9 – Volume measurement results - dispenser at 10 mL

Laboratory	Volume (mL)	Uncertainty (mL)	En value
IPQ – 1	9,9739	0,0044	0,28
INM	9,9728	0,0018	0,04
GEOSTM	9,9720	0,0056	-0,14
IPQ – 2	9,9720	0,0040	
Vref	9,9728	0,0015	

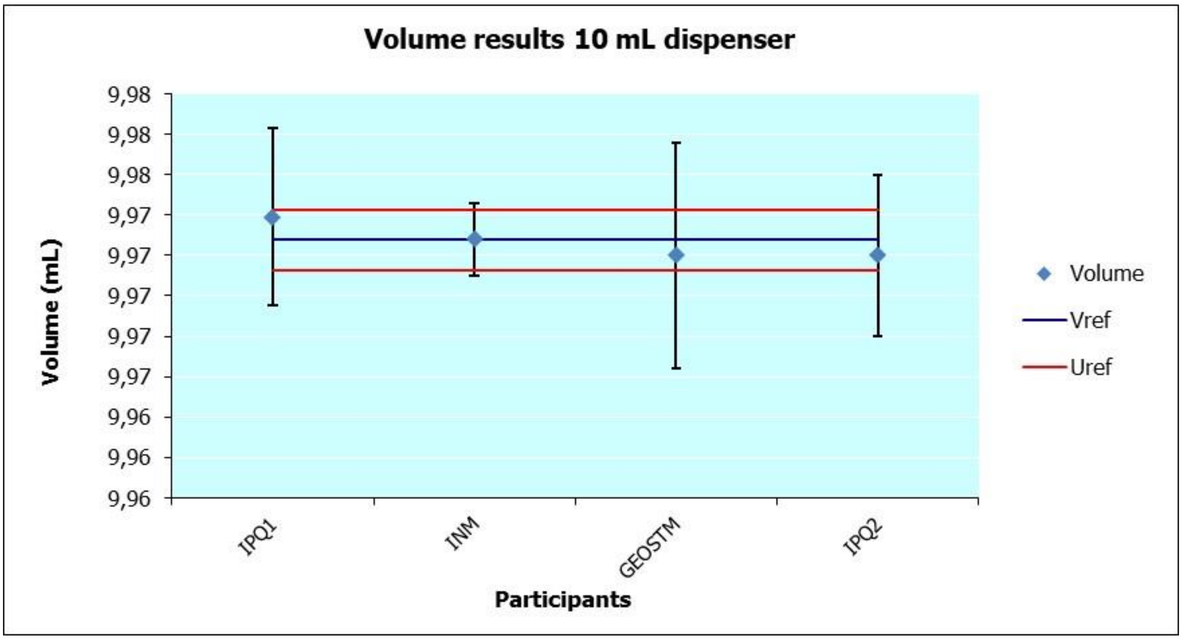

Figure 7– Volume results with reference value – dispenser 10 mL

As can be seen from the table and figure above, all the results are consistent with the reference value.

9. Uncertainty calculation

The laboratories calculated the uncertainty according to GUM [5]. In general, GEOSTM has smaller uncertainty than INM and IPQ and this could be one of the reasons for inconsistent results

IPQ and INM declared the same uncertainty components: mass, air density, water density, mass standards density, expansion coefficient of the instruments, water temperature, repeatability, evaporation and meniscus adjustment.

GEOSTM did not declared the uncertainty components.

10. Conclusions

In this comparison between IPQ, INM and GEOSTM, 4 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 between IPQ and INM for all artifacts. GEOSTM had consistent results for the micropipette and dispenser and inconsistent results for the pycnometer and flask. GEOSTM does not have CMC for glassware.

The uncertainty values and components of the determined volumes are very similar for IPQ and INM 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)