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Gravimetric calibration of piston pipette

SUPPLEMENTARY COMPARISON

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Final report

Gravimetric Calibration of 1000 µl Piston pipette

GULFMET.M. FF-S1

Elsa Batista - IPQ

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1. Introduction

The purpose comparison between IPQ - Portugal and SASO – Saudi Arabia is to verify the agreement of results and uncertainties in the calibration a micropipette despite the different equipment used and calibration process by each laboratory.
In order to access the stability of the micropipettes IPQ preformed two measurements, one at the beginning and another at the end of the comparison.

Table 1 - Participants

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	March 2025/January 2026	Elsa Batista	Email: ebatista@mail.ipq.pt Tel: +351212948167
Saudi Arabia	SASO	October 2025	Abdulkarim AlShahrany	Email:a.shahrany@saso.gov.sa Tel: +966 55 824 3530

2. The instrument

There are several types of micropipettes, single channel or multichannel. The type suggested for this comparison is the single-channel piston pipette, which is the most common, used in laboratories and easy to handle. The micropipette needs to have a removable plastic tip attached in order to aspirate the liquid. IPQ will supply these tips.

Micropipettes may be factory-pre-set to deliver volume or have selectable volumes within a useful volume range [1]. In the following figure there are two variable micropipette that will be used for this comparison. They are made essentially of plastic with a coefficient of thermal expansion of 2.4 × 10-4 °C $^{-1}$ [2].

Each laboratory had to calibrate the variable volume micropipette of 1000 μl in 1000 μl, 500 μl, 200 μL, and 100 μl, figure 1.

Figure 1 – 1000 μL Micropipette

3. Calibration procedure and mathematical model

The suggest method to perform the calibration of volume instruments is the gravimetric. The following formula described in ISO 4787 [1] and ISO 8655-6 [2] can be used for the calculation of the delivered or contained volume:

$$V_{i,\mathrm{ref}} = \left(m_\mathrm{L} - m_\mathrm{E} + m_\mathrm{evap}\right) \times \frac{1}{\rho_\mathrm{W} - \rho_\mathrm{A}} \times \left(1 - \frac{\rho_\mathrm{A}}{\rho_\mathrm{B}}\right) \times \left[1 - \gamma\left(t_\mathrm{W} - t_\mathrm{ref}\right)\right] \qquad (1)$$

The results must be given for a reference temperature of 20 °C, 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 8655-6 [2].
During the comparison, the participants were not allowed to adjust, clean or re-grease the micropipettes.
The uncertainty budget should be performed according to the Guide to the Expression of uncertainty in measurement [3].

3.1. Equipment

Each laboratory described the equipment used in the calibration.

Table 2 – Equipment characteristics

Balance	**Type**	**Range**	**Resolution**
IPQ	Electronic	(0-22) g	0,001 mg
SASO	Electronic	(0-220) g	0,01 mg
Water thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(-30 to 150) ºC	0,001 ºC
SASO	Digital	(-100 to 300) ºC	0,001 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 50) ºC	0,01 ºC
SASO	Digital	(-40 to 60) ºC	0,01 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(800 - 1150) hPa	0,01 hPa
SASO	Digital	(50 – 1100) hPa	0,01 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0-100) %	0,01%
SASO	Digital	(0-100) %	0,01%

3.2. Type of water

It was required that the water had a quality suitable for the purpose of calibration. The participants reported some of the water characteristics in order to be evaluated as its quality.

Table 3 – Water characteristics

Laboratory	Type	Density reference	Conductivity (μS/cm)
IPQ	Ultra-pure	Tanaka	0,046
SASO	Ultra-pure	Tanaka	1

Both laboratories used water of adequate purity.

3.3. Mass standards

Some information about the type of mass standard used was also requested:

Table 4 – Mass Standards

Laboratory	OIML Accuracy Class	Density (kg/m³)
IPQ	E2	8000
SASO	E2	8000

4. Ambient conditions

Both laboratories described the ambient conditions which the calibration was performed.

Table 5 - Ambient conditions

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ - 1	18,5	1007,72	68,2	0,0012
SASO	20,09-21,00	943,19 - 948,45	46,91 – 49,69	0,0012
IPQ - 2	20,7	1002,96	51,4	0,0012

The values of humidity are all within the humidity recommended.

5. Determination of the reference value and results analysis

The reference value corresponds to the weighted mean of all participants. The methodical approach from Cox (see metrologia 2002) [4] according to chapter A will be applied. As performance criteria the normalized error will be applied.

The results analysis will be performed according to the E_n number [5]:

$$|E_n| = \frac{x_{lab} - x_{ref}}{\sqrt{(U_{lab}^2 - U_{ref}^2)}} \qquad (2)$$

Where x_{lab} and U_{ab} are the volume and the uncertainty obtained by the laboratory and, x_{ref} and U_{ref} are the volume and the uncertainty obtained by the reference.
IPQ will perform two calibrations, one at the beginning and another at the end of the to access the stability of the artefacts. One the first result was used for the determination of the reference values.

Absolute values of $E_n \leq 1$ represent a satisfactory performance by the laboratory.

6. Measurement results

6.1 Volume measurements 1000 μl variable micropipette

A 1000 μl variable micropipette was calibrated at its nominal volume, 50 % of the nominal volume, 20 % of the nominal volume and 10 % of the nominal volume. The volume measurements obtained by IPQ in the beginning of the comparison (IPQ-1) and in the end of the comparison (IPQ-2) and by SASO are presented in the following table and figures:

Table 6 – 1000 μl micropipette volume measurement results

Laboratory	**Volume (μl)**	U_{exp} **(μl)**	**Volume (μl)**	U_{exp} **(μl)**	**Volume (μl)**	U_{exp} **(μl)**	**Volume (μl)**	U_{exp} **(μl)**
IPQ - 1	1001,1	1,2	499,64	0,60	198,50	0,29	98,89	0,19
SASO	1000,0	1,7	498,87	0,85	198,27	0,36	98,67	0,31
IPQ - 2	1002,0	1,3	499,35	0,58	198,80	0,20	98,80	0,20

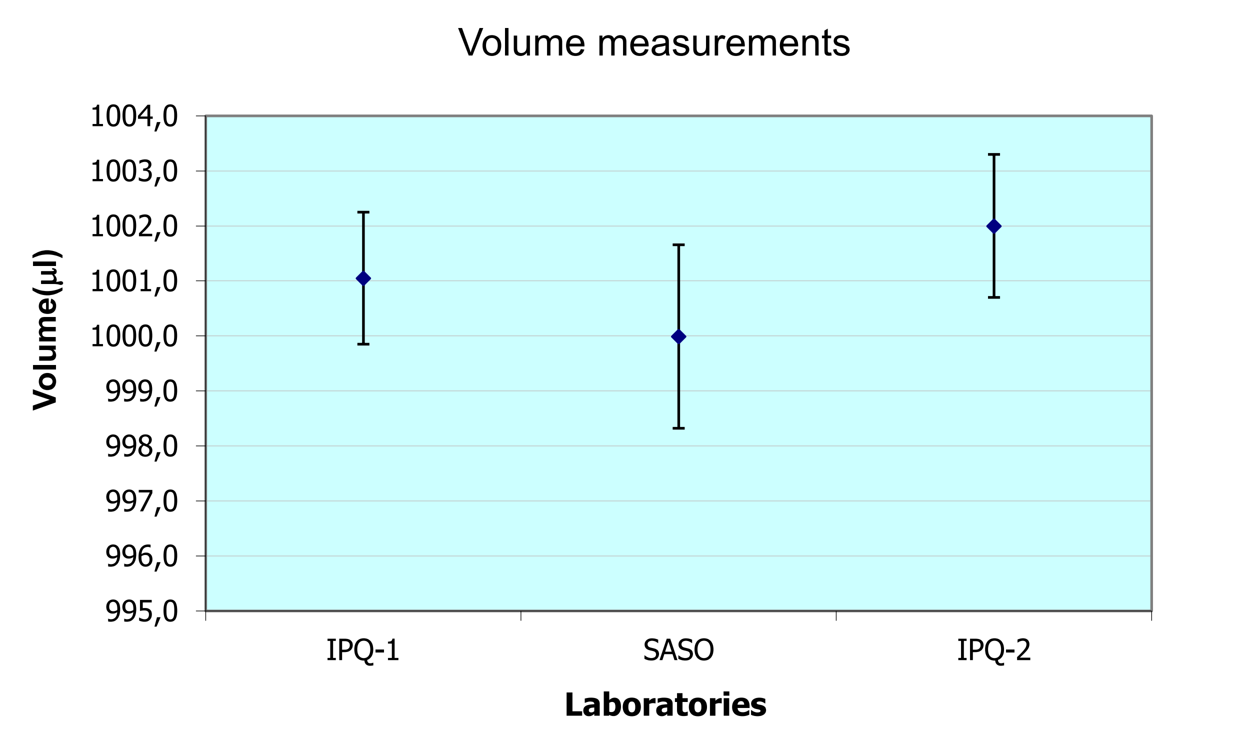

Figure 2 – Volume measurements 1000 µl

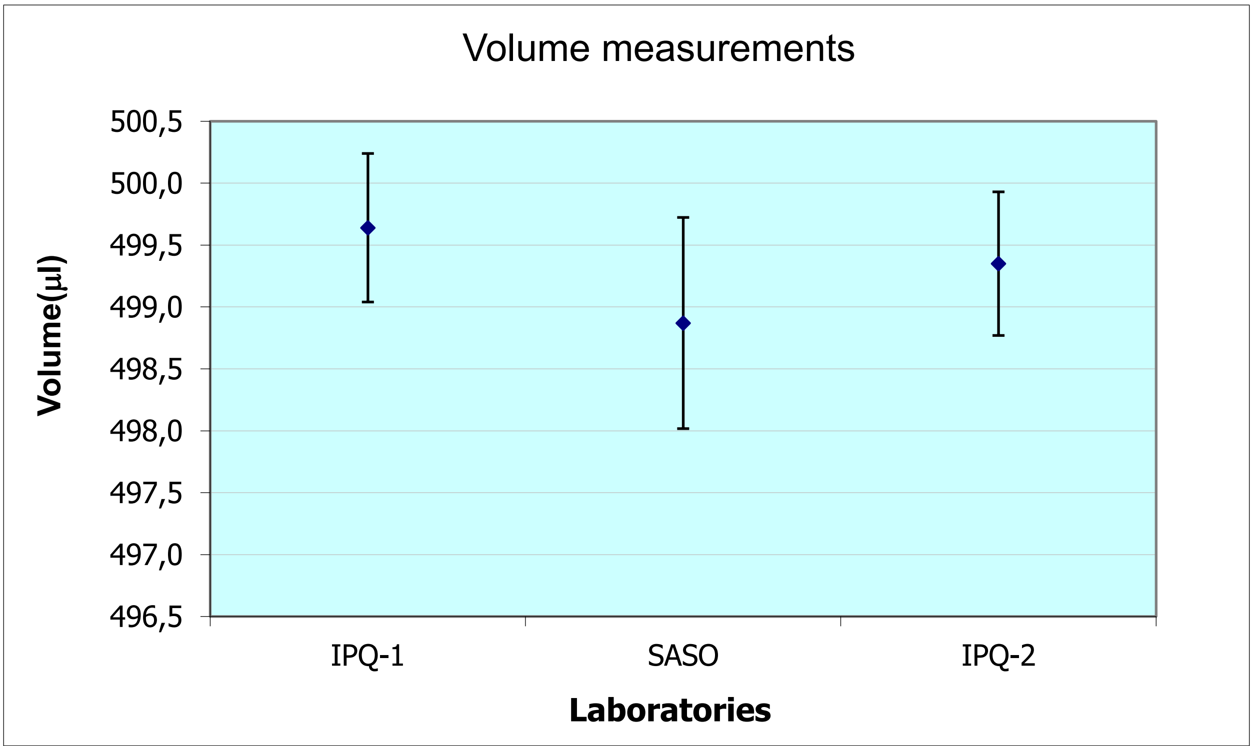

Figure 3 – Volume measurements 500 µl

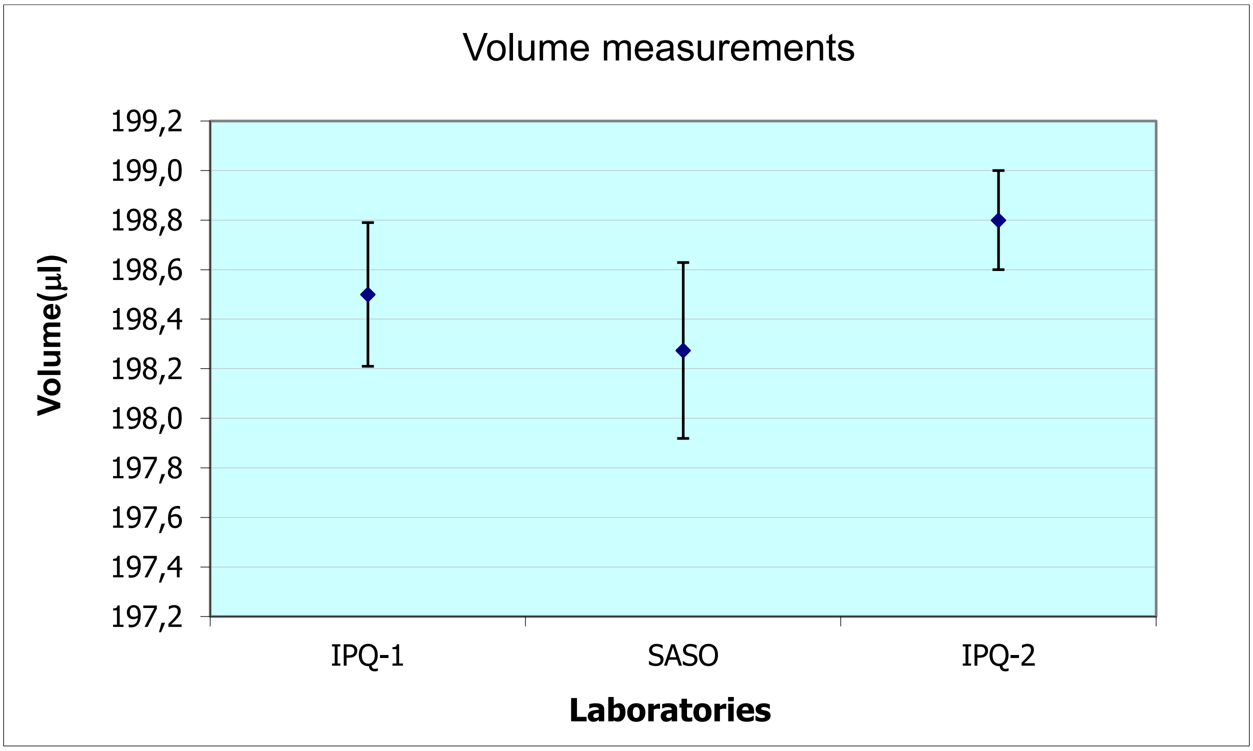

Figure 4 – Volume measurements 200 µl

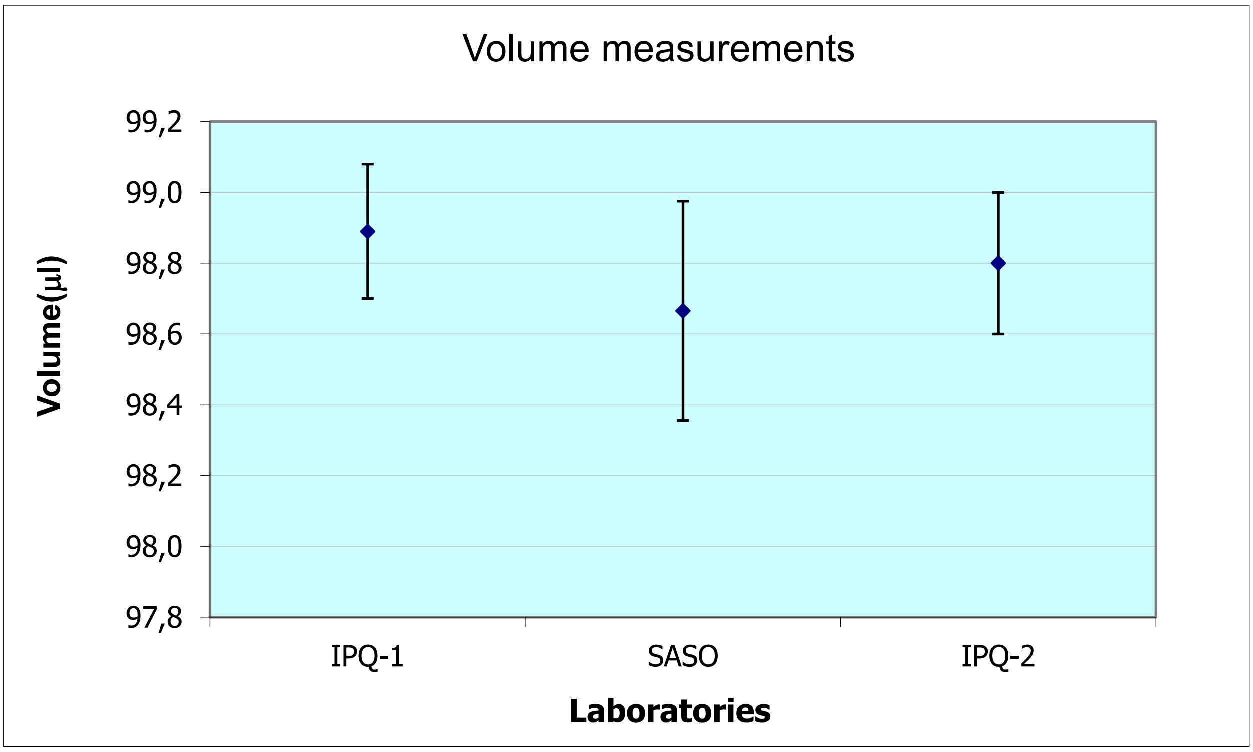

Figure 5 – Volume measurements 100 µl

6.2. Results analysis for the 1000 μl variable micropipette

In the next figures shown the measurement results with reference value and associated uncertainty, for each volume of the 1000 μl micropipette.

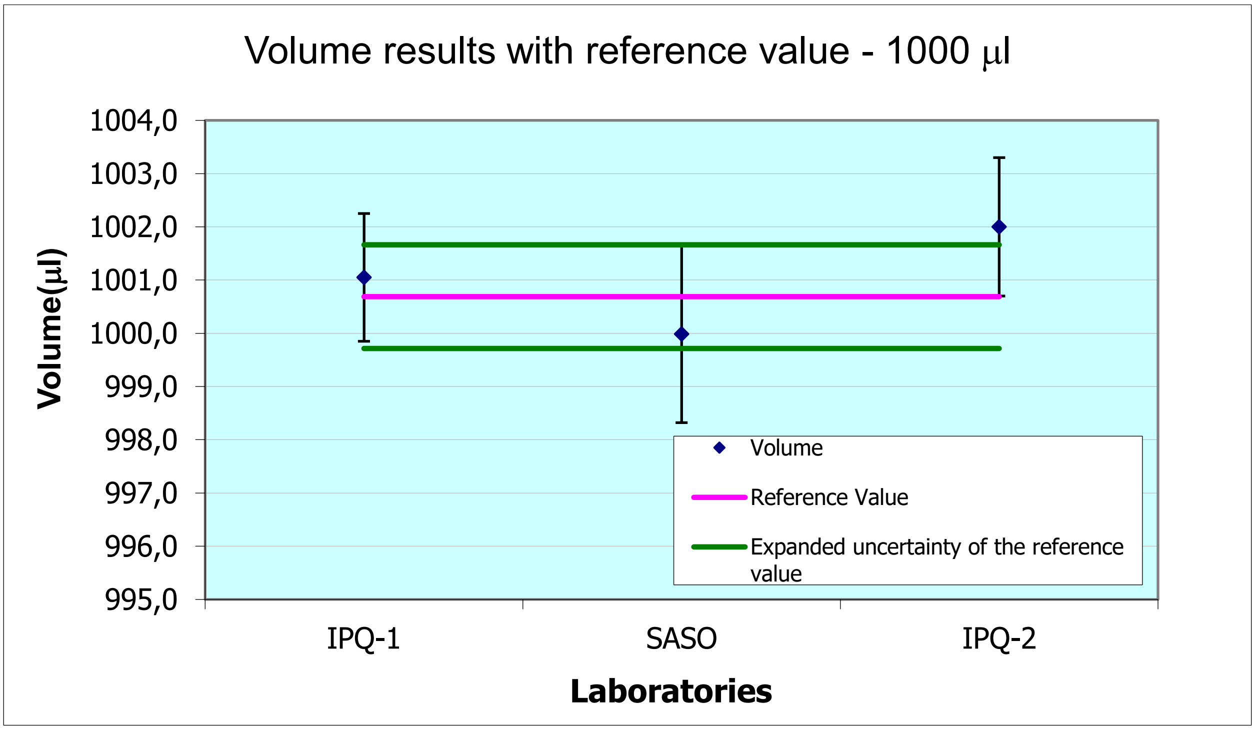

Figure 6 – Volume results with reference value for 1000 μl

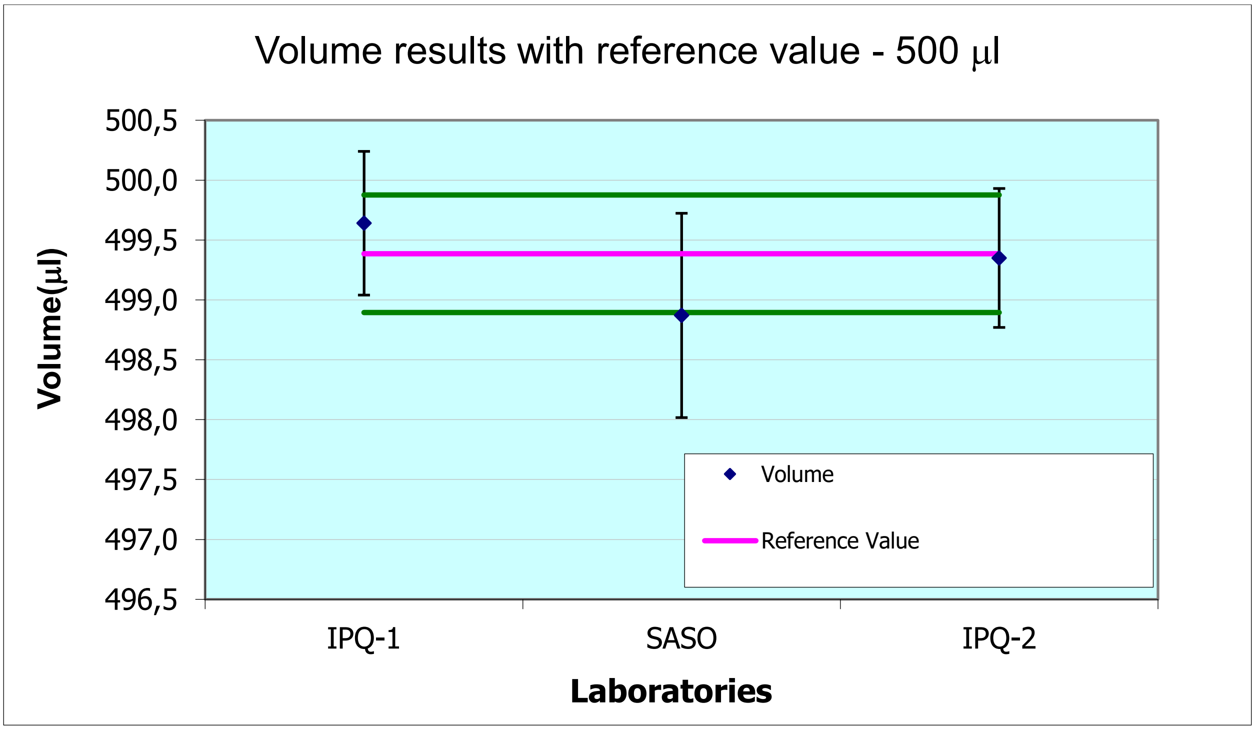

Figure 7 – Volume results with reference value for 500 μl

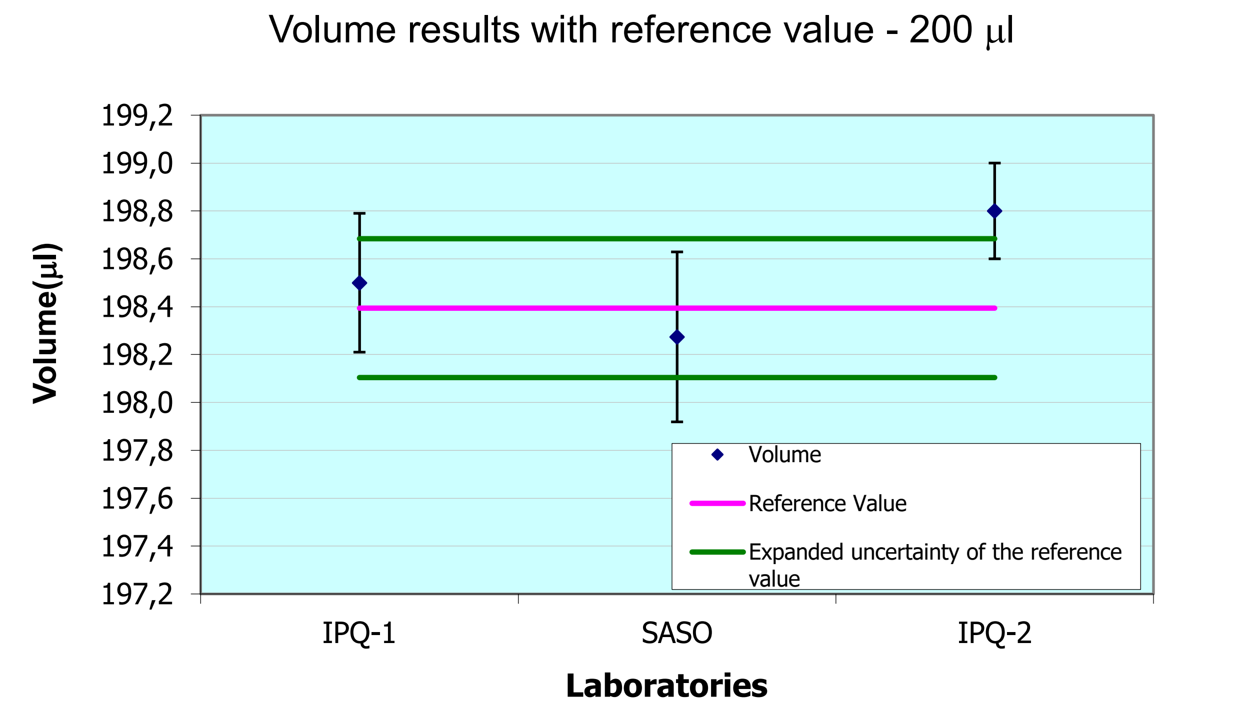

Figure 8 – Volume results with reference value for 200 µl

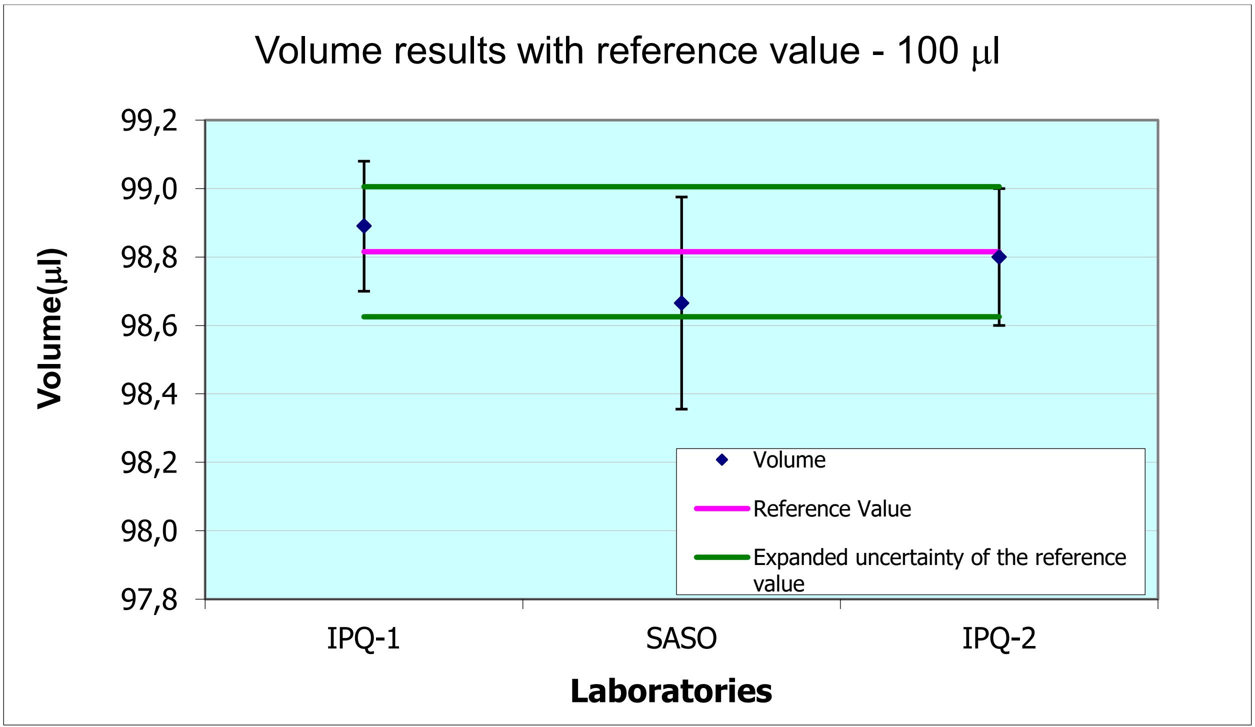

Figure 9 – Volume results with reference value for 100 µl

Table 7 – 1000 μl micropipette En values

	1000 μl	500 μl	200 μl	100 μl
IPQ En	0,52	0,74	0,26	0,61
SASO En	-0,52	-0,74	-0,26	-0,61

From the figures and table above, it can be observed that the volume results are all consistent. In general, the values of SASO are lower than IPQ possibly due to the pressure values and altitude.

7. Uncertainty calculation

The participant laboratories presented their uncertainty determination according to GUM [4].

The uncertainty value declared by SASO is similar to IPQ. Also, the uncertainty declared by both laboratories are similar. Both included the reproducibility of the measurements.

8. Conclusions

This bilateral comparison of one variable micropipette involved IPQ and SASO.

All presented results are consistent with the reference value for both laboratories in all tested points.

The value of the expanded uncertainty and the uncertainty components declared are quite similar for in both laboratories.

9. 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. **JCGM 100:2008** - *Guide to the expression of uncertainty in measurement* (GUM). (1993, amended 1995) (published by ISO in the name of BIPM, IEC, IFCC, IUPAC, IUPAP and OIML)
4. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595.
5. **ISO 13528:2005** - Statistical methods used in proficiency testing by interlaboratory comparison