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Volume comparison at 10 µL, 100 µL and 1000 µL – Calibration of micropipettes
Volume comparison at 10 mL – Calibration of Burette

RMO Supplementary Comparison

COOMET.M.FF-S8
(COOMET 767/GE/18)

Final Report

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Abstract:

This report describes the results of international comparison within COOMET.M.FF-S8 (also known as supplementary comparison COOMET.M no. 767/GE/18). The comparison measurements between the two participants GEOSTM (pilot laboratory) and IPQ started in August 2018 and finished in December 2018.

The determined |E_n|-values of the comparison measurement are ≤ 1

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1. Introduction

The purpose of this comparison is to perform a bilateral comparison between IPQ - Portugal and GEOSTM - Georgia in order to test the agreement of their results and uncertainties in the calibration of micropipettes and a digital burette despite the different equipment used and calibration processes.

The comparison was registered at COOMET with the number 767/GE/18.

In order to assess the stability of the micropipettes IPQ preformed two measurements, one at the beginning and another at the end of the comparison.

Table 1 - Participants in 767/GE/18

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	May and September 2018	Elsa Batista	Email: ebatista@mail.ipq.pt Tel: +351212948167
Georgia	GEOSTM	August 2018	Irma Rurua	Email: irmarurua@yahoo.com Tel: +995 599 424 888

2. The instruments

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 easier 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 figures there are all 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].

Moreover, a digital burette of 50 mL was also used in this comparison, see Figure 1.

Table 2 – Instruments used in this comparison

Manufacturer	Model	Nominal Volume	Type	Serial number
Eppendorf	Research plus	1000µL	Variable micropipette	P34557G
Sartorius	Tacta	100µL	Variable micropipette	17028127
Eppendorf	Research	10µL	Variable micropipette	1839034
Brand	Burette digital III	50 mL	Burette	02C9621

Figure 1 – 1000 µL Micropipette

Figure 2 – 100µL Micropipette

Figure 3 – 10 µL Micropipette

Figure 4 – 50 mL Burette

Figures 1-4. Micropipettes and burette

3. Calibration procedure and mathematical model

The participating NMIs applied a gravimetric method, with direct weighing, to determine the amount of water that the instrument delivers at a reference temperature of 20 ºC, based on ISO 8655 [1] and ISO 4787 [3], see equation (1):

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

Where:

V_{20}, µL	volume at reference temperature, 20 ºC;
I_I, mg	weighing result of the recipient full of liquid;
I_E, mg	weighing result of the empty recipient;
ρ_W, mg/µL	water density at the calibration temperature, using Tanaka density formula;
ρ_A, mg/µL	air density;
ρ_B, mg/µL	density of masses used during measurement (substitution) or during calibration of the balance;
γ, °C^{-1}	cubic thermal expansion coefficient of the material of the instrument;
t, °C	water temperature during the calibration process.

During the comparison, the participants were not allowed to adjust, clean or re-grease the equipment.

3.1. Equipment

Each laboratory described the equipment used in the calibrations.

Table 3 – Equipment characteristics

Balance	**Type**	**Range**	**Resolution**
IPQ	Electronic	(0 to 22) g	0,001 mg
	Electronic	(0 to 2) kg	0,001 g
GEOSTM	Electronic	(0 to 22) g	0,001 mg
		(0 to 220) g	0,1 mg
Water thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(-30 to 150) ºC	0,001 ºC
GEOSTM	Digital	(-200 to 850) ºC	0,001 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 100) ºC	0,1 ºC
GEOSTM	Digital	(-20 to 80) ºC	0,01 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(800 to 1150) hPa	0,01 hPa
GEOSTM	Digital	(600 to 1100) hPa	0,01 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 100) %	0,1%
GEOSTM	Digital	(0 to 100) %	0,1%

3.2. Type of water

It was required that the water had a quality suitable for the purpose of the calibrations. The participants reported some of the water characteristics to allow its quality to be evaluated.

Table 4 – Water characteristics

Laboratory	Type	Density reference	Conductivity (µS/cm)
IPQ	**Ultra-pure**	**Tanaka**	**0,046**
GEOSTM	**Bi-distilled**	**Tanaka**	**< 5**

Both laboratories used water of adequate purity.

3.3. Mass standards

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

Table 5 – Mass Standards

Laboratory	OIML Accuracy Class	Density (kg/m^3)
IPQ	E2	7960 - 8600
GEOSTM	E2	8000

4. Ambient conditions

Both laboratories described the ambient conditions where the calibrations were performed.

Table 6 - Ambient conditions

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ - 1	19,4	1005,66	70,7	0,0012
GEOSTM	19,72	956,08	51,9	0,0012
IPQ - 2	22,6	1008,65	55,7	0,0012

5. Measurement results

5.1. Stability of the micropipettes and burette

Two different measurements of the 3 micropipettes and a burette were performed by the pilot laboratory during the comparisons in order to verify the stability of the standards (nominal values). The first measurement was chosen to be the IPQ official result. The results are presented in the following table:

Table 7 - Stability of the instruments

Instrument	Measurement	Date	Volume	Uncertainty	Variation
O34557G	**1**	May	1001,1 µL	1,0 µL	1,0 µL
	2	September	1000,1 µL	1,8 µL	
17028127	**1**	May	99,61 µL	0,10 µL	0,01 µL
	2	September	99,62 µL	0,26 µL	
1839034	**1**	May	10,345 µL	0,038 µL	0,015 µL
	2	September	10,330 µL	0,020 µL	
02C9621	**1**	May	50,010 mL	0,009 mL	0,005 mL
	2	September	50,015 mL	0,008 mL	

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The results obtained by IPQ, for the three micropipettes, are consistent with each other; all the results are within the presented uncertainty. This demonstrates that the micropipettes and the burette had a stable volume during the entire comparison.

5.2. Volume measurements burette

A 50 mL burette was calibrated at its nominal volume. The volume measurements obtained by IPQ at the beginning of the comparison (IPQ - 1) and at the end of the comparison (IPQ - 2) and by GEOSTM are presented in the following tables and figures.

Table 8 – Volume measurement results burette

Laboratory	Volume (mL)	U_{exp} (mL)	*En*
IPQ - 1	50,010	0,009	-0,42
GEOSTM	50,014	0,014	-0,08
IPQ - 2	50,015	0,008	0,33
V_{ref}	50,013	0,0054	

5.3. Volume measurements variable micropipette 1000 µL

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

Table 9 – Volume measurement results 1000 µL micropipette

Laboratory	Volume (µL)	U_{exp} (µL)	*En*	Volume (µL)	U_{exp} (µL)	*En*	Volume (µL)	U_{exp} (µL)	*En*
IPQ - 1	1001,1	1,2	-0,49	498,75	0,83	0,03	99,21	0,36	-0,19
GEOSTM	1002,0	1,2	0,49	498,72	0,63	0,03	99,29	0,16	-0,10
IPQ - 2	1000,1	1,8	-0,91	498,54	0,84	-0,28	99,23	0,16	-0,48
V_{ref}	1001,56	0,83		498,73	0,50		99,28	0,13	

5.4. Volume measurements variable micropipette 100 µL

A 100 µl variable micropipette was calibrated at its nominal volume, 50 % of the nominal volume and 10 % of the nominal volume. The volume measurements obtained by IPQ at the beginning of the comparison (IPQ - 1) and at the end of the comparison (IPQ - 2) and by GEOSTM are presented in the following tables and figures.

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Table 10 – Volume measurement results 100 µL micropipette

Laboratory	Volume (µL)	U_{exp} (µL)	*En*	Volume (µL)	U_{exp} (µL)	*En*	Volume (µL)	U_{exp} (µL)	*En*
IPQ -1	99,61	0,12	0,00	49,630	0,060	0,15	9,780	0,020	0,74
GEOSTM	99,61	0,13	0,00	49,617	0,073	0,12	9,745	0,049	0,63
IPQ - 2	99,62	0,26	0,04	49,66	0,13	0,30	9,779	0,018	0,21
V_{ref}	99,610	0,082		49,624	0,044		9,774	0,018	

5.5. Volume measurements variable micropipette 10 µL

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

Table 11 – Volume measurement results 10 µL micropipette

Laboratory	Volume (µL)	U_{exp} (µL)	E_n	Volume (µL)	U_{exp} (µL)	E_n	Volume (µL)	U_{exp} (µL)	E_n
IPQ -1	10,345	0,038	-0,19	5,320	0,013	0,74	1,313	0,021	0,73
GEOSTM	10,354	0,031	-0,14	5,306	0,016	0,58	1,297	0,006	0,28
IPQ-2	10,330	0,020	-0,71	5,324	0,022	0,53	1,309	0,020	0,56
V_{ref}	10,351	0,022		5,313	0,009		1,298	0,005	

5.6. Determination of the reference value

To determine the reference values of this bilateral comparison (RV) the weighted mean, equation (2) was selected, using the inverses of the squares of the associated standard uncertainty as the weights [5], according to the instructions given by the BIPM, only the first result from IPQ was considered for the reference value determination:

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

To calculate the standard deviation *u(y)* associated with the volume *y* [5] equation (3) was used:

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

The expanded uncertainty of the reference value is $U(y) = 2 \times u(y)$.

In the next figuresthe measurement results are presented with reference values and associated uncertainties, for each micropipette and burette at each volume.

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5.6.1. Burette

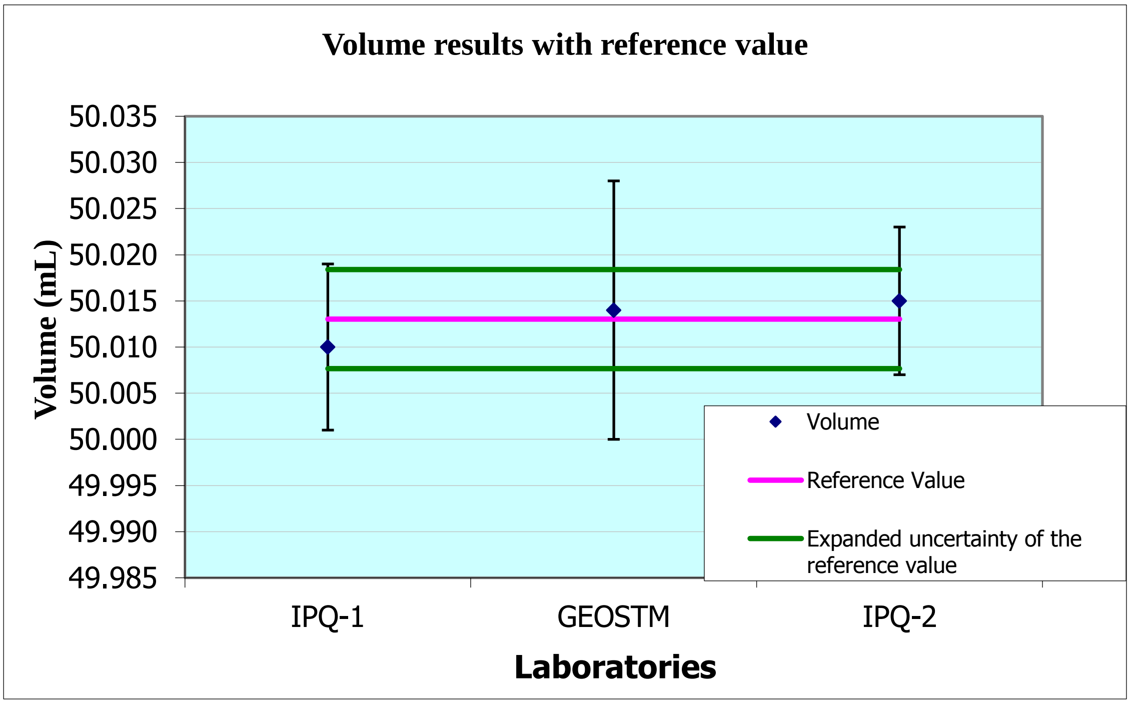

Figure 5 – Volume results with reference value for the burette

5.6.2. Variable micropipette 1000 µL

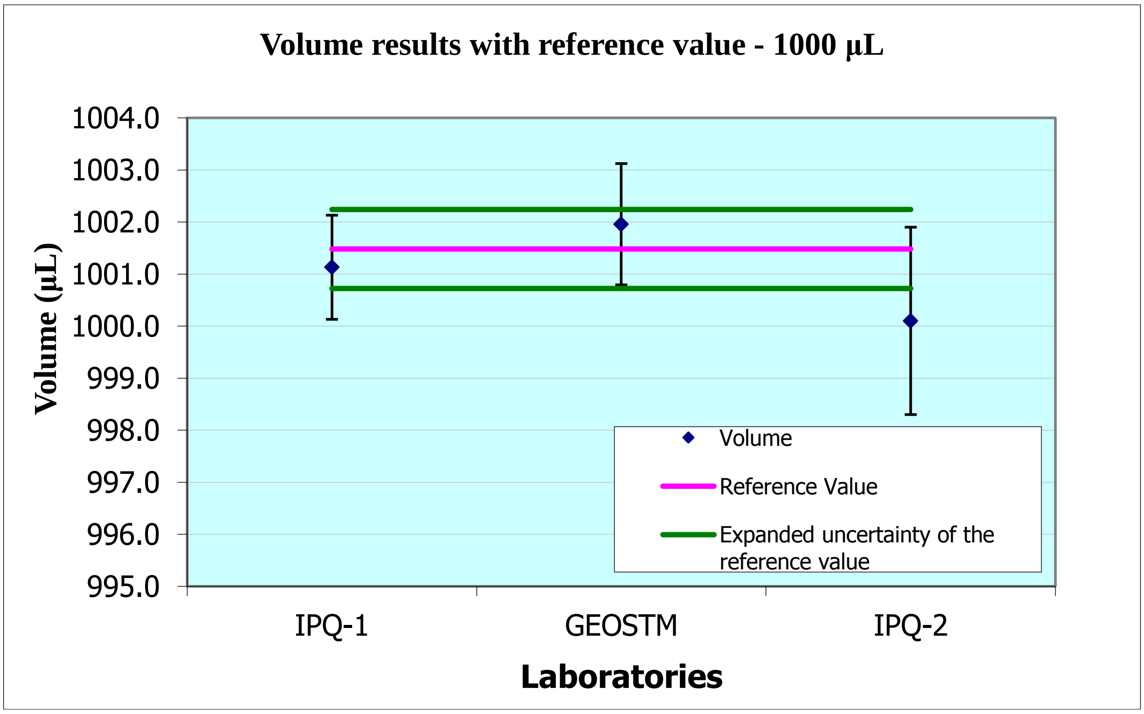

Figure 6 – Volume results with reference value for 1000 µl

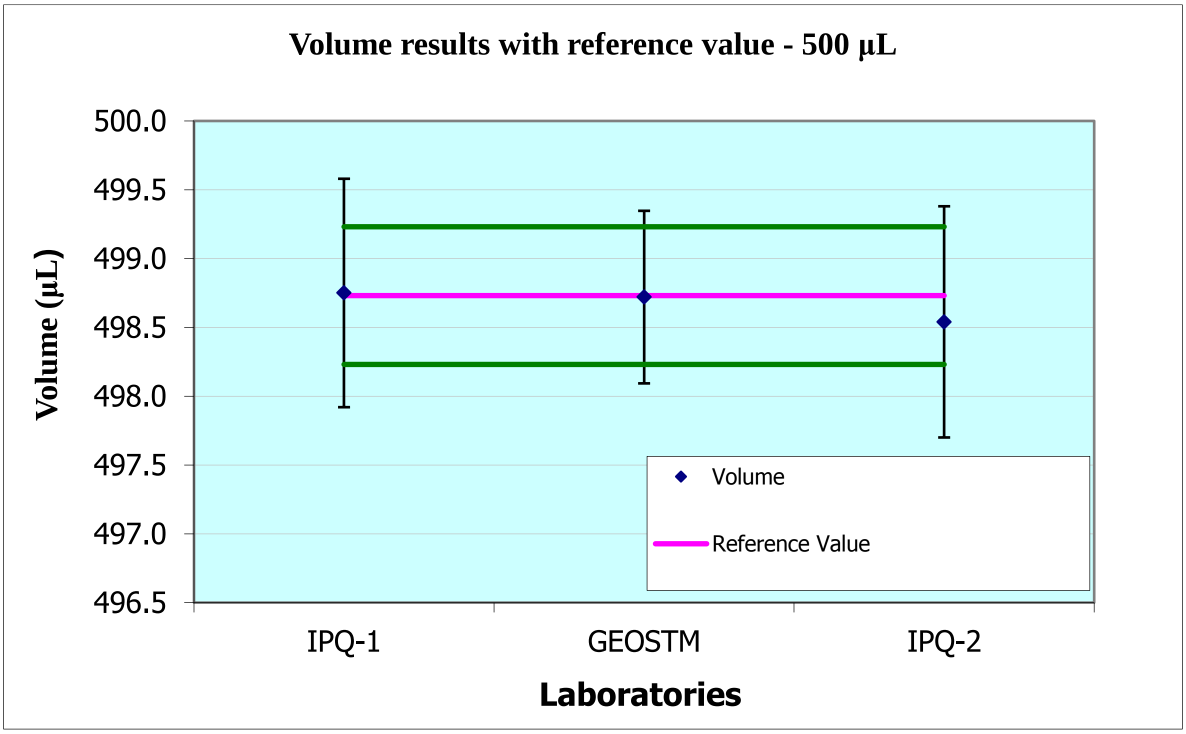

Figure 7 – Volume results with reference value for 500 µL

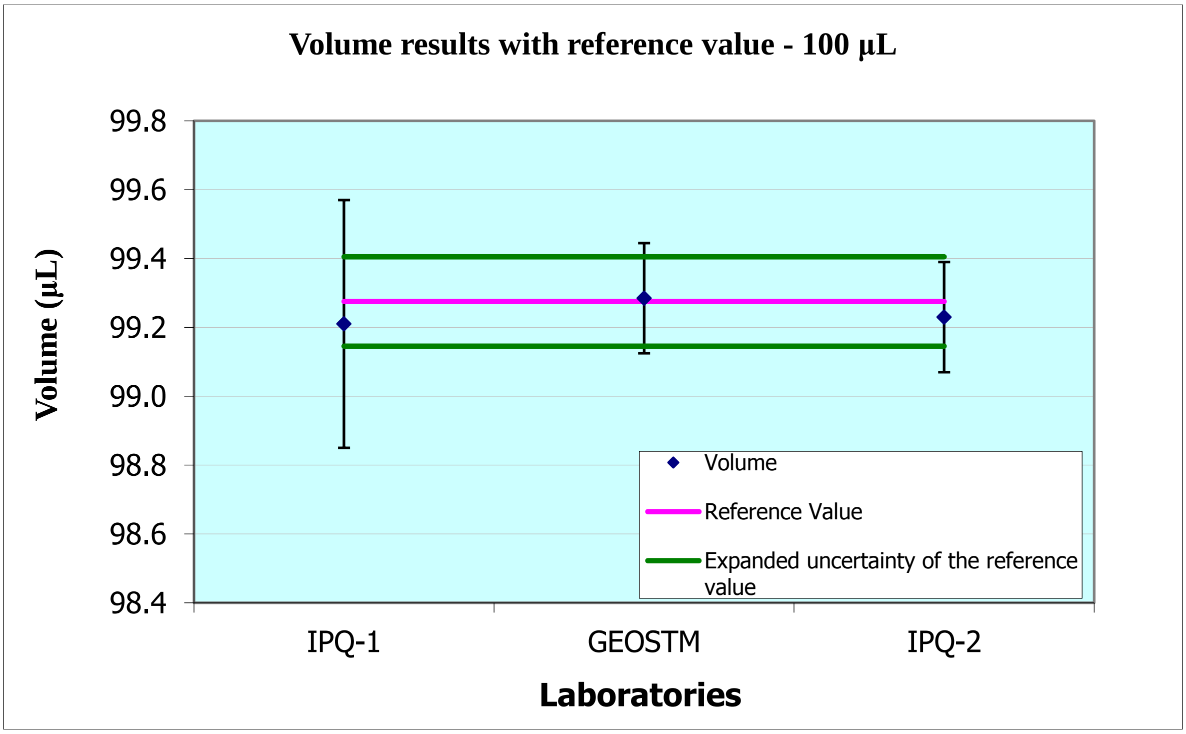

Figure 8 – Volume results with reference value for 100 µL

From this figure it can be observed that the volume results are all consistent with the reference value.

5.6.3. Variable micropipette 100 µL

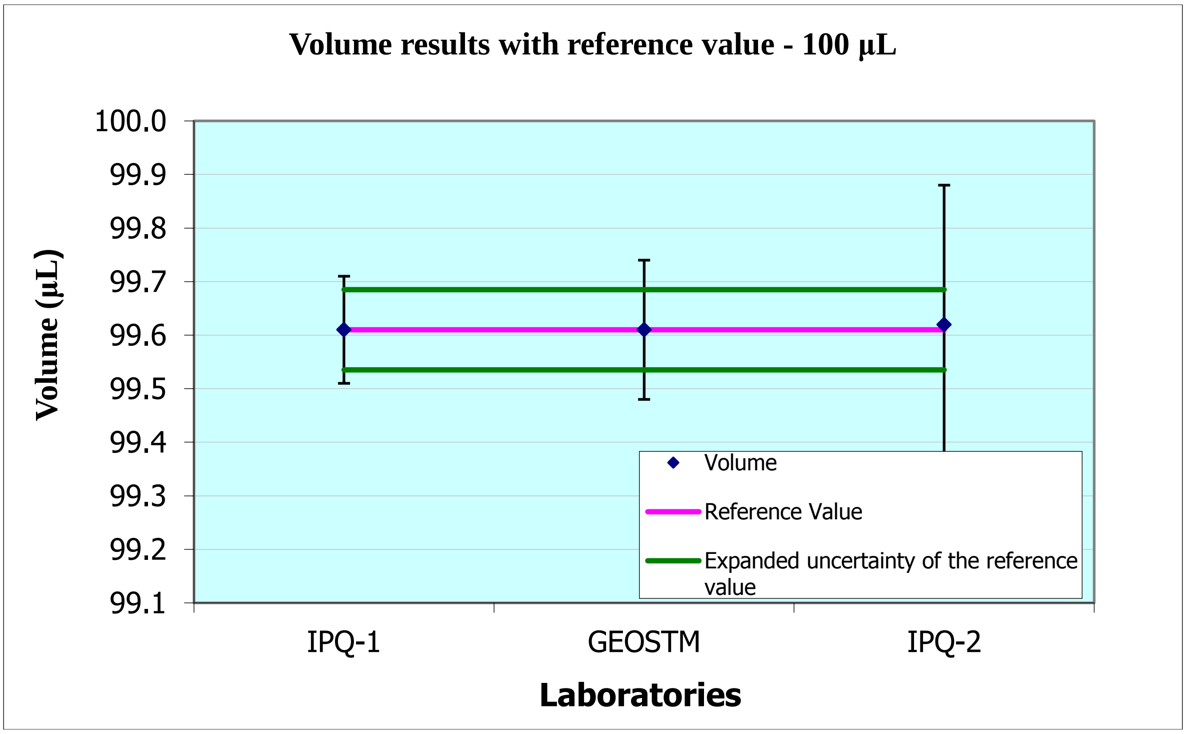

Figure 9 – Volume results with reference value for 100 µL

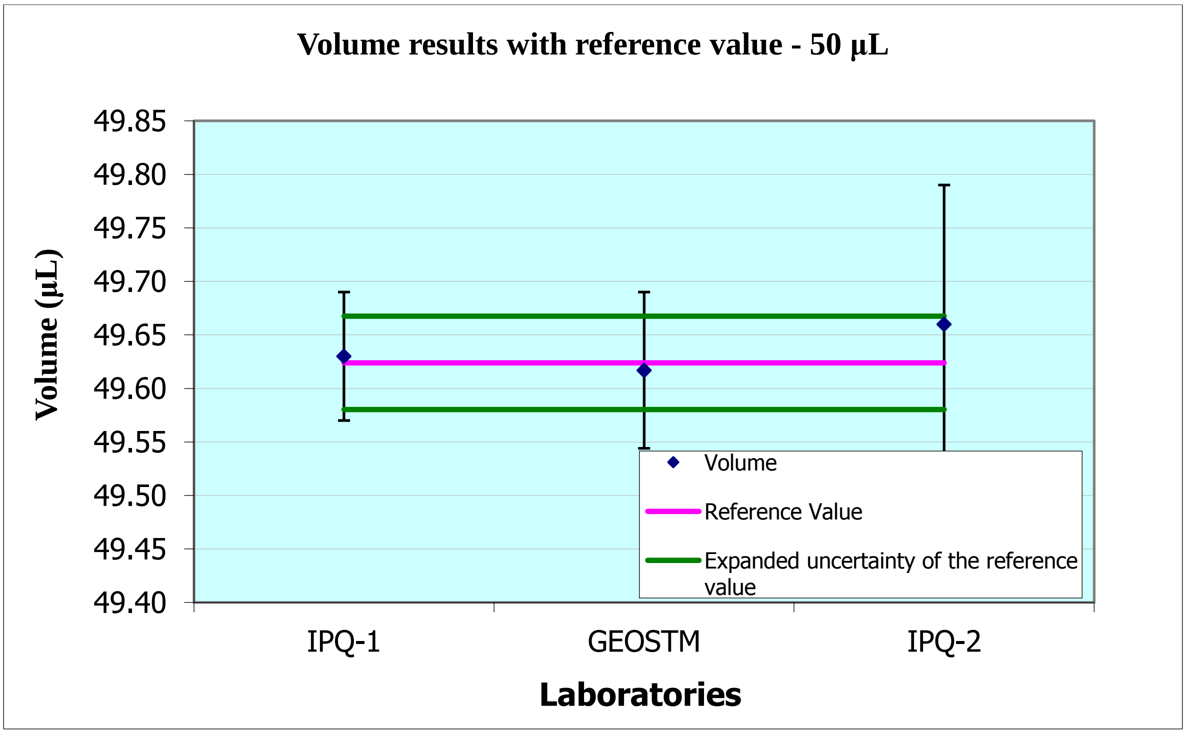

Figure 10 – Volume results with reference value for 50 µL

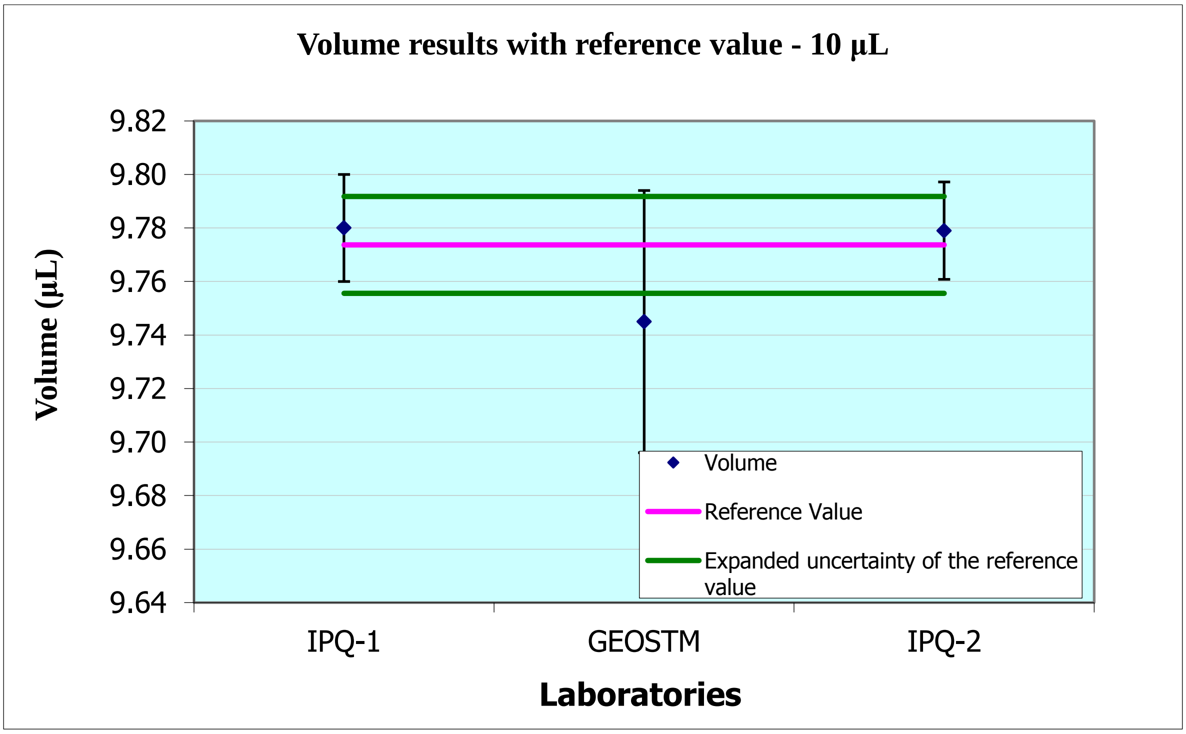

Figure 11 – Volume results with reference value for 10 µL

From this figure it can be observed that the volume results are all consistent with the reference value.

5.6.4. Variable micropipette 10 µL

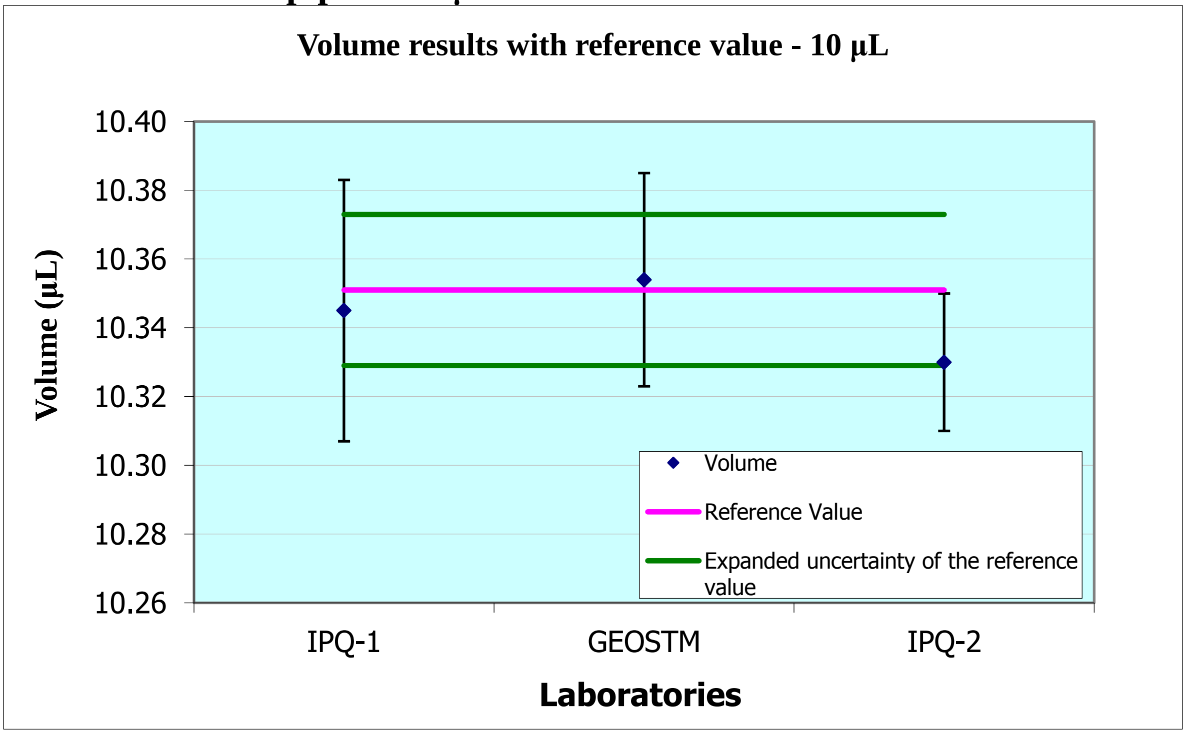

Figure 12 – Volume results with reference value for 10 µL

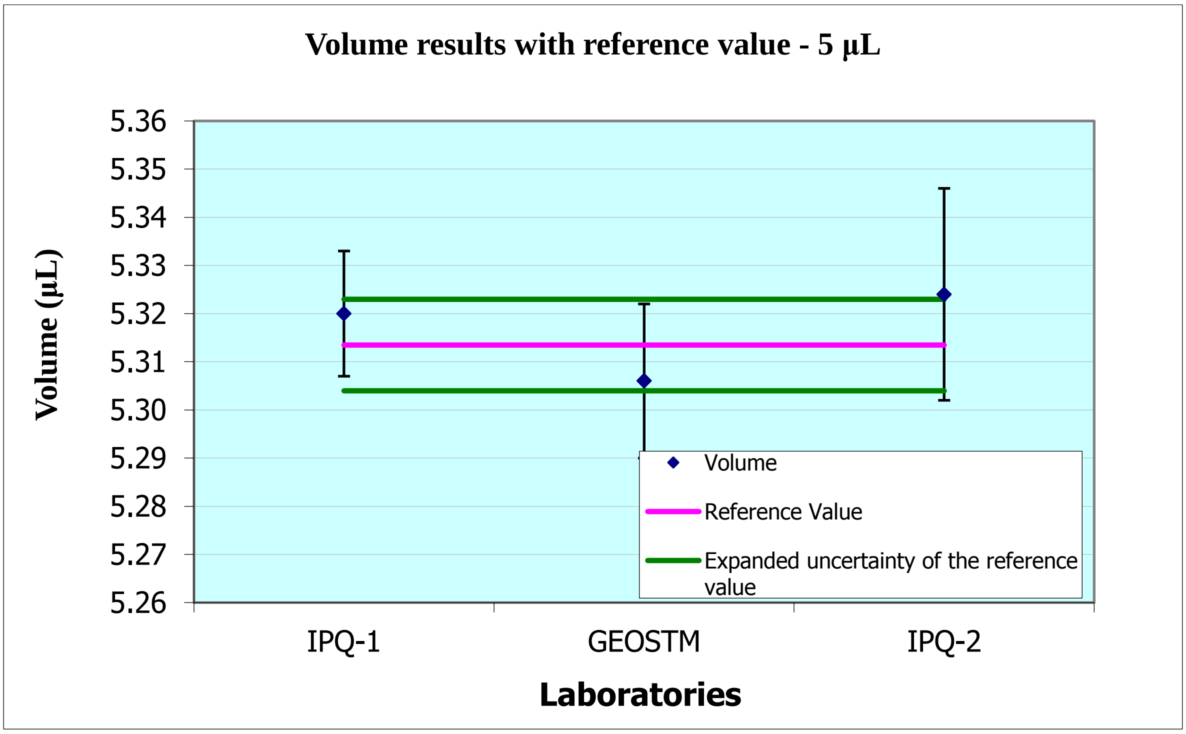

Figure 13 – Volume results with reference value for 5 µL

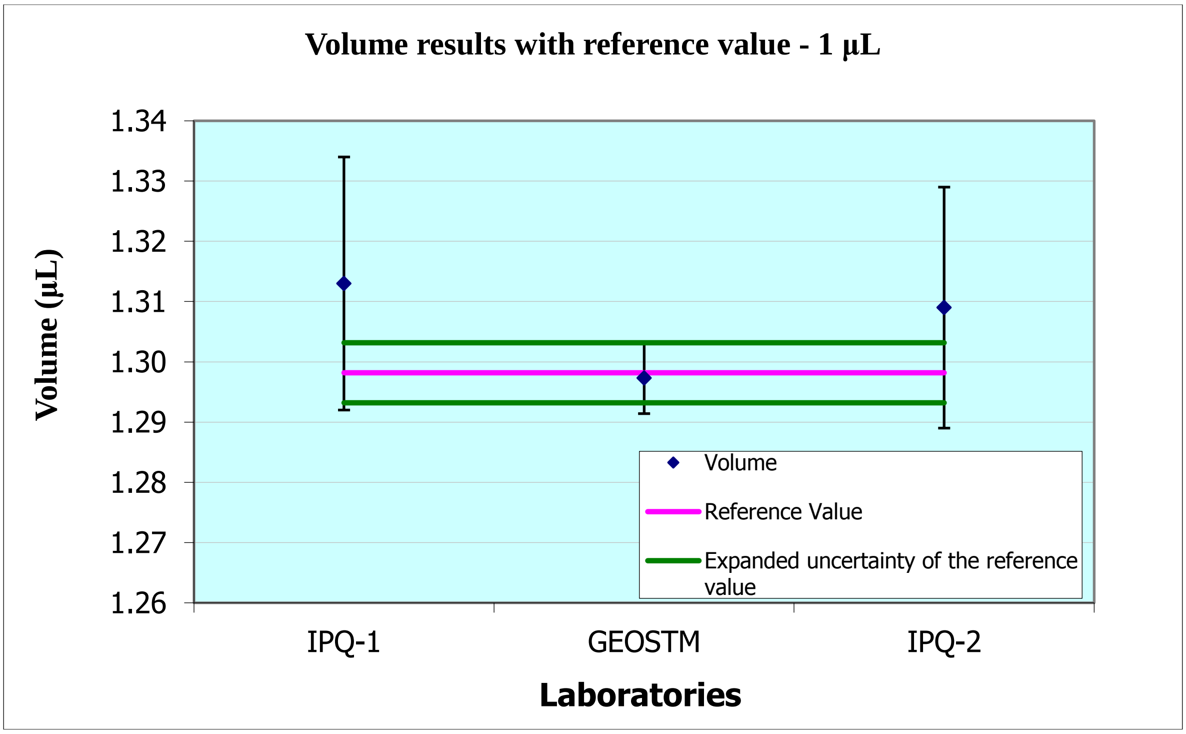

Figure 14 – Volume results with reference value for 1 µL

From this figure it can be observed that the volume results are all consistent with the reference value.

6. Uncertainty calculation

The participant laboratories presented there uncertainty determination according to GUM [6]. The main uncertainty components were repeatability, reproducibility, mass and density of water. Other components were also considered, such as the density of weights, density of air, evaporation, expansion coefficient of material and water temperature. For the micropipettes only the values of the nominal volumes are presented as examples.

6.1. Burette

Uncertainty contributions (µl)	IPQ	GEOSTM
Repeatability	0,0028	0,006
Balance	$1,69\times10^{-3}$	0,0002
Air density	$4,89\times10^{-5}$	$4,9\times10^{-5}$
Water density	$2,51\times10^{-3}$	$2,5\times10^{-6}$
Density of the mass pieces	$3,27\times10^{-5}$	$6,2\times10^{-8}$
Expansion coefficient	$5,03\times10^{-3}$	$8,3\times10^{-8}$
Water temperature	$1,91\times10^{-3}$	$4,9\times10^{-6}$
Evaporation	0	0
Others	-	-
Combined Uncertainty (µl)	0,0046	0,006
Expanded uncertainty (µl)	**0,009**	**0,014**

6.2. Variable micropipette 1000 µL

Uncertainty contributions (µl)	IPQ	GEOSTM
Repeatability	0,16	0,078
Balance	$6{,}44\times10^{-3}$	$5{,}67\times10^{-5}$
Air density	$9{,}73\times10^{-4}$	$9{,}9\times10^{-4}$
Water density	$5{,}02\times10^{-2}$	$5{,}0\times10^{-5}$
Density of the mass pieces	$6{,}50\times10^{-4}$	$1{,}25\times10^{-6}$
Expansion coefficient	$8{,}60\times10^{-3}$	$2{,}1\times10^{-6}$
Water temperature	$2{,}36\times10^{-2}$	$2{,}9\times10^{-6}$
Evaporation	0,0036	0,007
Reprodutibility	0,57	0,57
Combined Uncertainty (µl)	0,60	0,58
Expanded uncertainty (µl)	**1,2**	**1,2**

6.3. Variable micropipette 100 µL

Uncertainty contributions (µl)	IPQ	GEOSTM
Repeatability	0,16	0,023
Balance	$4{,}17\times10^{-3}$	$5{,}67\times10^{-5}$
Air density	$9{,}68\times10^{-5}$	$9{,}79\times10^{-4}$
Water density	$4{,}99\times10^{-3}$	$5{,}0\times10^{-6}$
Density of the mass pieces	$6{,}47\times10^{-5}$	$1{,}23\times10^{-7}$
Expansion coefficient	$8{,}90\times10^{-4}$	$3{,}49\times10^{-7}$
Water temperature	$3{,}3\times10^{-3}$	$3{,}27\times10^{-6}$
Evaporation	0,0019	0,004
Reproducibility	0,057	0,057
Combined Uncertainty (µl)	0,06	0,062
Expanded uncertainty (µl)	**0,12**	**0,13**

6.4. Variable micropipette 10 µL

Uncertainty contributions (µl)	IPQ	GEOSTM
Repeatability	0,015	0,012
Balance	$4{,}11\times10^{-3}$	$5{,}67\times10^{-5}$
Air density	1×10^{-5}	$1{,}03\times10^{-5}$
Water density	$5{,}02\times10^{-4}$	$5{,}0\times10^{-7}$
Density of the mass pieces	$6{,}72\times10^{-6}$	$1{,}23\times10^{-7}$
Expansion coefficient	$8{,}81\times10^{-5}$	$1{,}3\times10^{-8}$
Water temperature	$3{,}08\times10^{-4}$	$2{,}8\times10^{-7}$
Evaporation	0,0012	0,003
Reproducibility	0,006	0,006
Combined Uncertainty (µl)	0,017	0,014
Expanded uncertainty (µl)	**0,037**	**0,031**

7. Conclusions

This bilateral comparison for small volume measurements involved IPQ and GEOSTM.
For the digital burette and the variable micropipettes all volume results for both laboratories are consistent with the reference value obtained by the weighted mean.
The value of the expanded uncertainty is quite similar in both laboratories as well as the largest uncertainty source, which is the repeatability and reproducibility of the measurements.

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