Final Report

Bilateral comparison of a 1000 μl micropipette and a 50 μl micropipette

BILATERAL COMPARISON SIM.M.FF-S11

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1. Introduction

The purpose of this comparison is to perform a bilateral comparison between IPQ - Portugal and INTI - Argentina in order to test the agreement of their results and uncertainties in the calibration of micropipettes despite the different equipment used and calibration process.
In order to access the stability of the micropipettes INTI preformed two measurements, one at the beginning and another at the end of the comparison.

Table 1 - Participants in SIM.M.FF-S11

Country	Laboratory	Periods	Responsible	Contact
Argentina	INTI	December 2016/November 2017	Mauricio Alberini	Email: alberini@inti.gob.ar Tel: +543492440471
Portugal	IPQ	September 2017	Elsa Batista	Email: ebatista@mail.ipq.pt Tel: +351212948167

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. INTI supplied 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 is a fixed and a 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 a fixed volume micropipette of 50 µl and a variable volume micropipette of 1000 µl in 1000 µl , 500 µl , 100 µl, figure 1.

Figure 1- Micropipettes

3. Calibration procedure and mathematical model

The participating NMIs applied a gravimetric method, with direct weighing, to determine the amount of water that the micropipettes deliver at 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 [4]

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 piston pipette

t/°C: water temperature during the calibration process

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

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
INTI	Electronic	(0-22) g	0.001 mg
Water thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(-30 to 150) ºC	0.01 ºC
INTI	Liquid in glass	(19 to 35) ºC	0.02 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 50) ºC	0.1 ºC
INTI	Digital	(-10 to 60) ºC	0.1 ºC

Barometer	Type	Range	Resolution
IPQ	Digital	(800 - 1150) hPa	0.01 hPa
INTI	Analogic	(920 – 1050) hPa	0.5 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0-100) %	0.1%
INTI	Digital	(10-99) %	1%

3.2. Type of water

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

Table 3 – Water characteristics

Laboratory	Type	Density reference	Conductivity (μS/cm)
IPQ	Ultra-pure	Tanaka	0.055
INTI	Bi-distilled	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/m^3)
IPQ	E2	7960-8600
EIM	F2/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)
INTI - 1	21.1	998.4	58	0.0012
IPQ	20.7	1012.02	69.1	0.0012
INTI- 2	20.6	998.2	49	0.0012

Since the ambient pressure is very close to the reference pressure 1013.25 hPa, no corrections will be applied in previous volume data.

5. Measurement results

5.1 Volume measurements fixed micropipette

A 50 μl micropipette was calibrated at its nominal volume. The volume measurements obtained by INTI in the beginning of the comparison (INTI-1) and in the end of the comparison (INTI-2) and by IPQ are presented in the following table and figure:

Table 6 – Volume measurement results

Laboratory	Volume (μl)	U_{exp} (μl)
INTI-1	54.36	0.22
IPQ	56.90	0.09
INTI-2	67.05	0.20

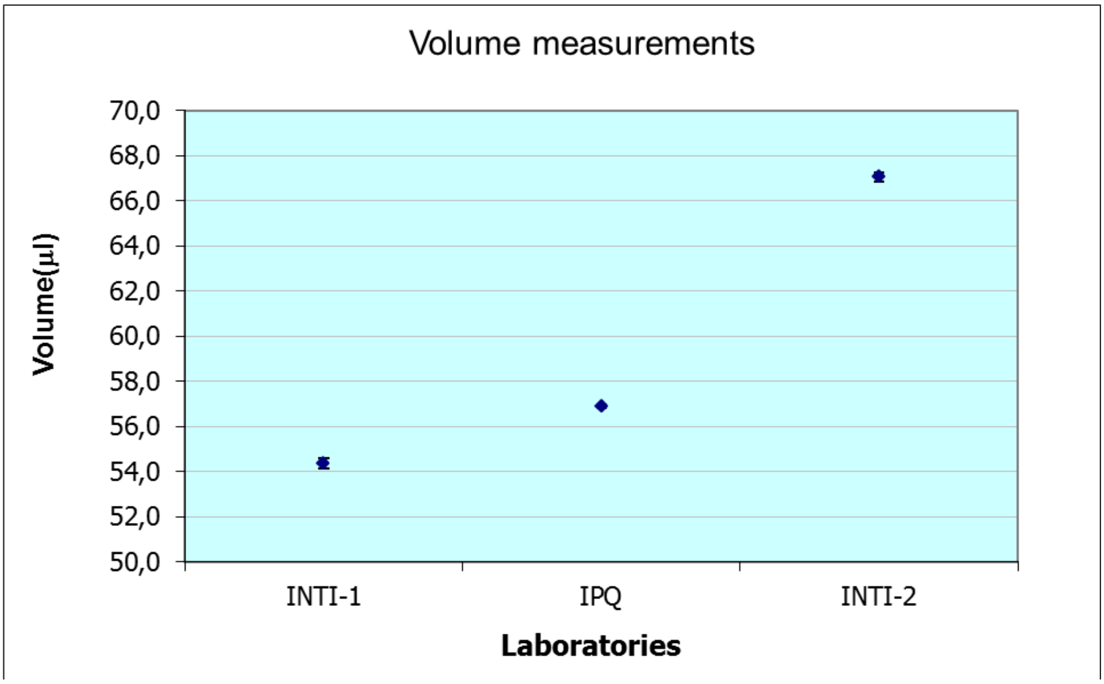

Figure 2 – Volume measurements fixed micropipette

This micropipette was damaged during the transportation between laboratories.

From figure 2 it can verify that the micropipette had a drift during the comparison so the results cannot be used.

5.2 Volume measurements with the variable micropipette

A 1000 μl variable micropipette was calibrated at its nominal volume, 50 % of the nominal volume and 10 % of the nominal volume.

Two different measurements of the variable micropipette were performed by INTI during the comparisons in order to verify the stability of the standards. The results are presented in the following table:

Table 7 – Stability of the variable micropipette

Laboratory	Volume (μl)	U_{exp} (μl)	Volume (μl)	U_{exp} (μl)	Volume (μl)	U_{exp} (μl)
INTI-1	997.8	2.3	496.2	1.5	98.8	1.2
INTI-2	997.9	2.2	496.5	1.6	99.7	1.2
ΔV	0.1		0.3		0.9	

The result variation is smaller than the declared uncertainty therefore it is assumed that the variable micropipette was stable during the comparison.

The volume results of IPQ and INTI are presented in the following table and figures:

Table 8 – Volume measurement results

Laboratory	Volume (μl)	U_{exp} (μl)	Volume (μl)	U_{exp} (μl)	Volume (μl)	U_{exp} (μl)
INTI-1	997.8	2.3	496.2	1.5	98.8	1.2
IPQ	999.6	1.5	496.35	0.85	99.01	0.60
INTI-2	997.9	2.2	496.5	1.6	99.7	1.2

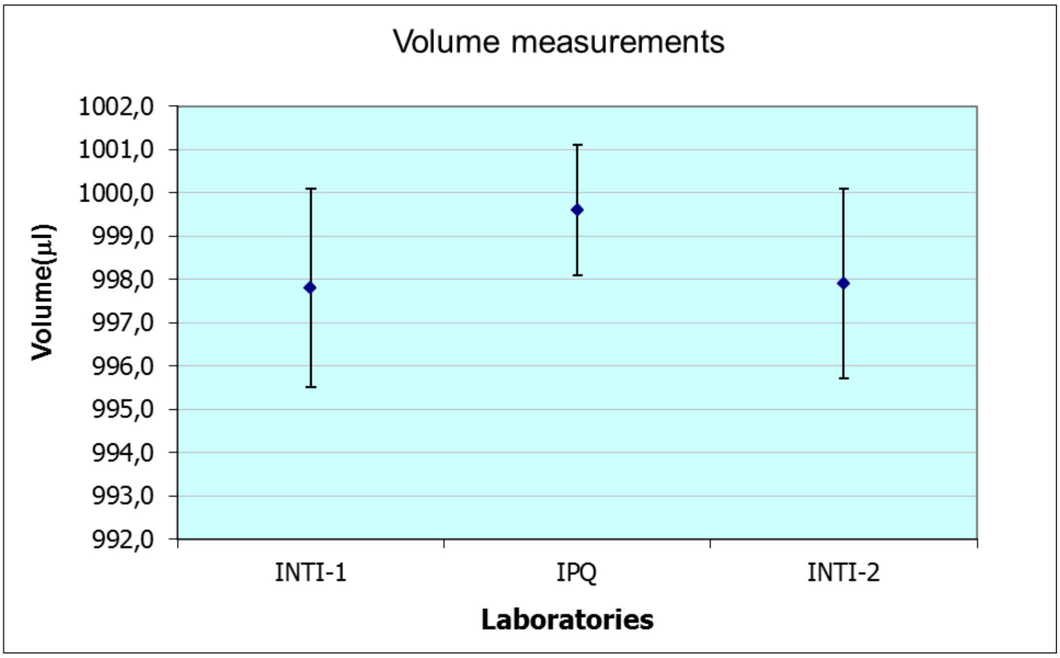

Figure 3 – Volume measurements 1000 µL

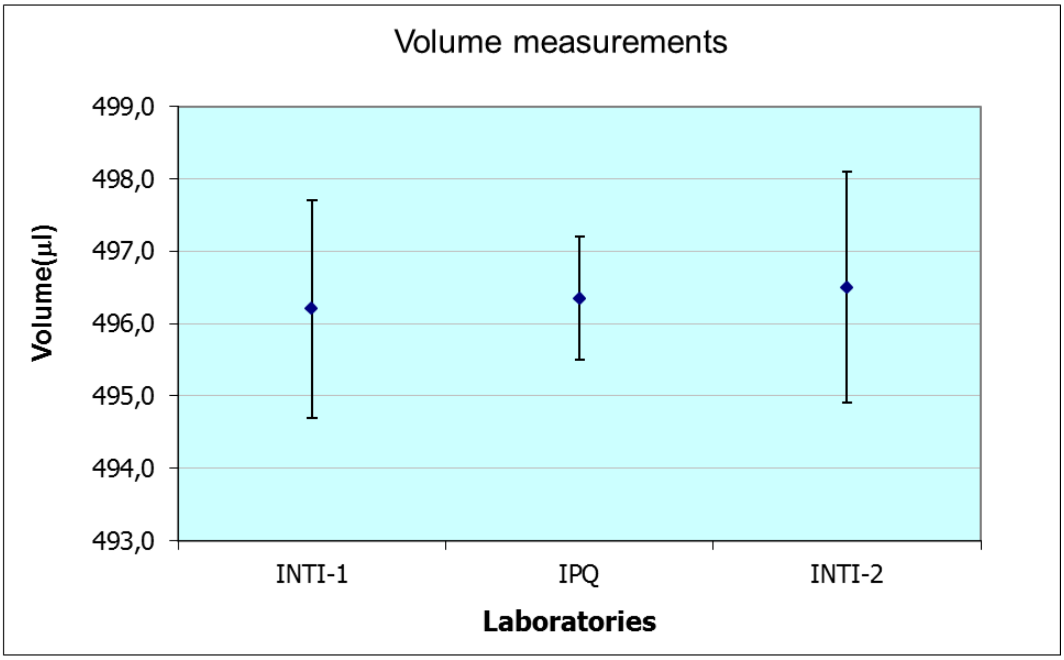

Figure 4 – Volume measurements 500 µL

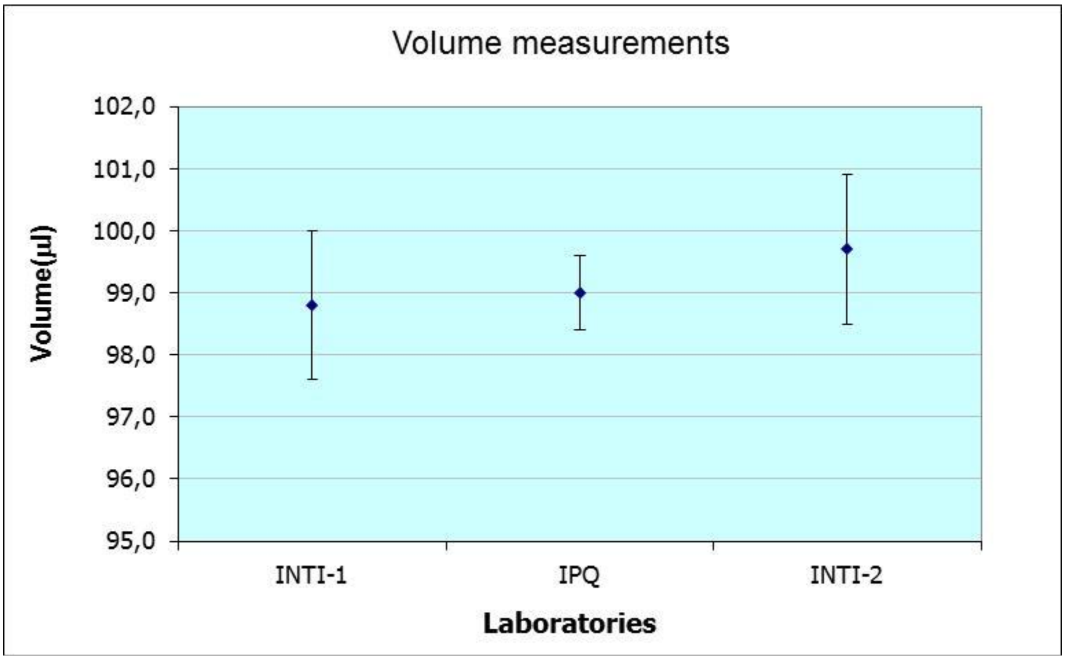

Figure 5 – Volume measurements 100 µL

5.3. Determination of the reference value for the variable micropipette

To determine the reference value of this bilateral comparison (RV) the weighted mean, equation (2) was selected, using the inverses of the squares of the associated standard uncertainties as the weights [5], according to the instructions given by the BIPM, only the one result from INTI 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 $U(y) = 2 \times u(y)$.

The determined values are y = 999.06 µl and $U(y)$ = 1.3 µl, y = 496.31 µl and $U(y)$ = 0.74 µl and y = 98.98 µl and $U(y)$ = 0.48 µl

The next figures show the measurement results with reference values and associated uncertainties, for each volume.

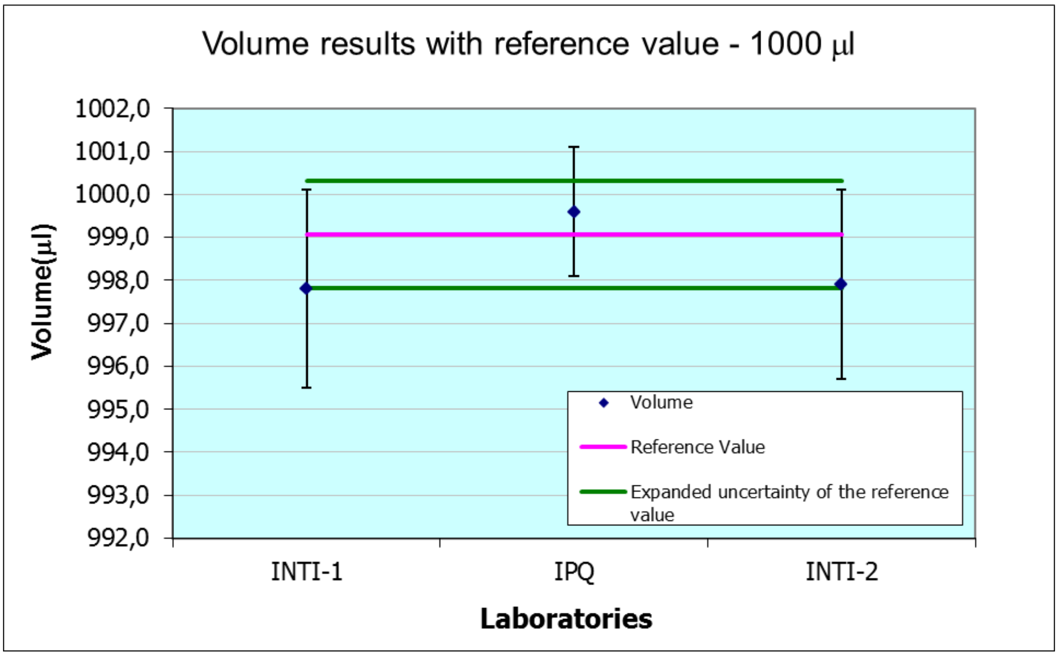

Figure 6 – Volume results with reference value for 1000 µl

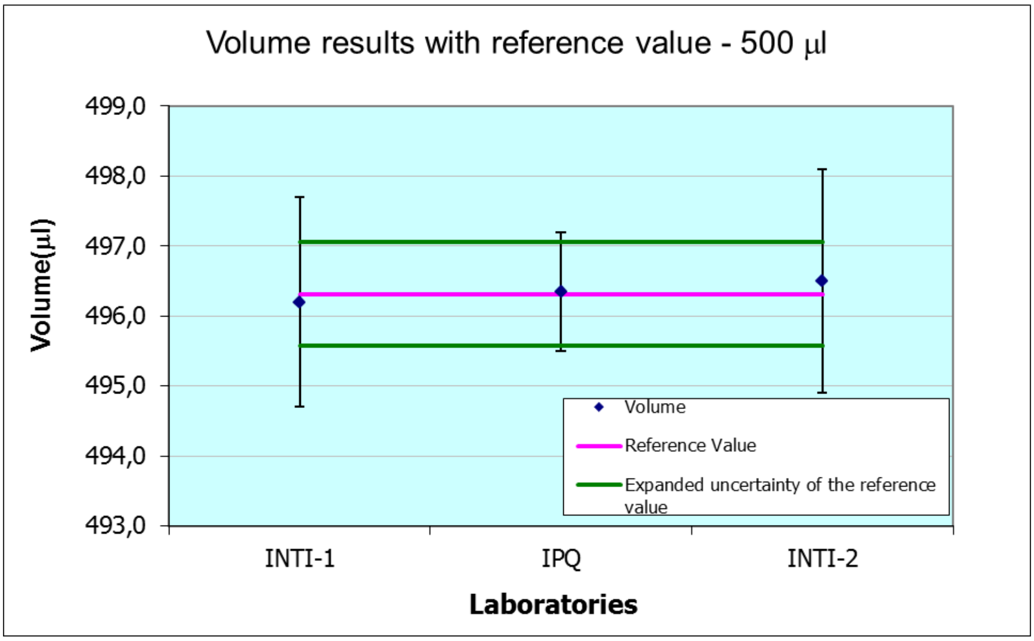

Figure 7 – Volume results with reference value for 500 µl

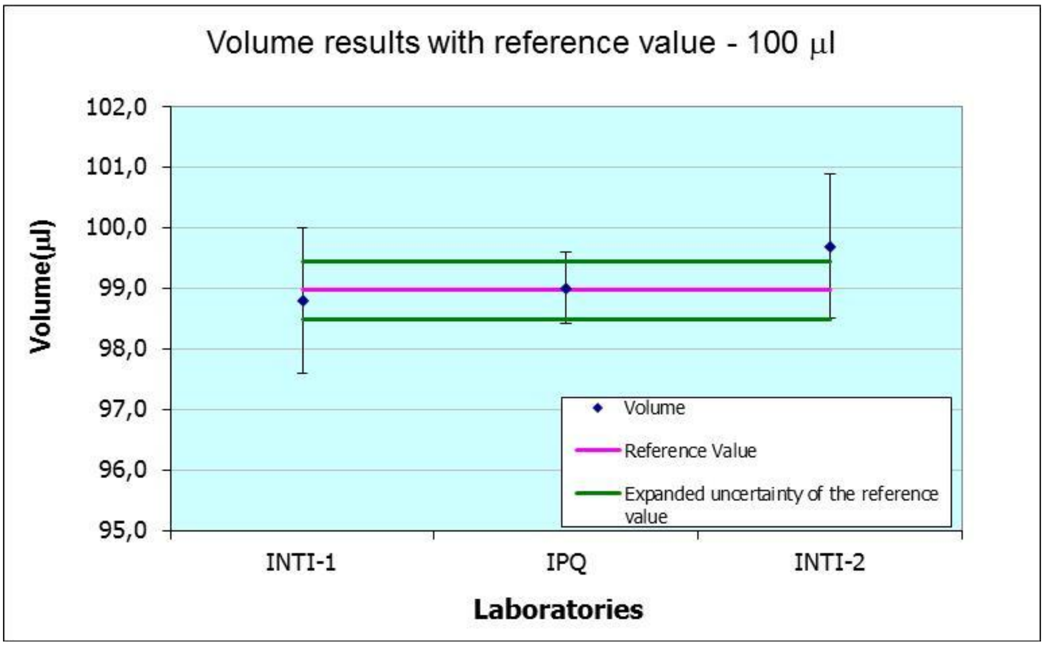

Figure 8 – Volume results with reference value for 100 µl

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

5.4. Determination of the degree of equivalence for the variable micropipette

For each laboratory result, x_i the degree of equivalence d_i between each laboratory and the RV (x_{ref}) was calculated using the following formulas [5]:

$$d_i = x_i - x_{ref} \quad (4)$$

$$U(d_i) = 2 \times u(d_i) \quad (5)$$

where $u(d_i)$ was calculated from

$$u^2(d_i) = u^2(x_i) - u^2(x_{ref}) \quad (6)$$

Discrepant values can be identified if $|d_i| > 2u(d_i)$.

The results are presented in table 9.

Table 9 – Degree of equivalence with RV for the variable micropipette

	1000 µL		500 µL		100 µL	
Laboratory	***d_i*/µL**	***U*(*d_i*)/µL**	***d_i*/µL**	***U*(*d_i*)/µL**	***d_i*/µL**	***U*(*d_i*)/µL**
INTI-1	-1.26	1.93	-0.11	1.31	-0.18	1.10
IPQ	0.54	0.82	0.04	0.42	0.03	0.21
INTI-2	-1.16	1.81	0.19	1.42	0.72	1.10

No discrepant values were found.

The degree of equivalence between the laboratories can also be calculated using:

$d_{i,j} = x_i - x_j$

$U(d_{i,j}) = 2 \times u(d_{i,j})$ (7)

where $u(d_i)$ was calculated from

$u^2(d_{i,j}) = u^2(x_i) + u^2(x_j)$ (8)

Discrepant values can be identified if $|d_{i,j}| > 2u(d_{i,j})$.

The results are presented in table 10.

Table 10 – Degree of equivalence between the labotaories for the variable micropipette

Volume		1000 μL	500 μL	100 μL
i \ j		IPQ	IPQ	IPQ
INTI -1	$d_{i,j}$	-1.8	-0.2	-0.2
	$U(d_{ij})$	2.7	1.7	1.3
INTI - 2	$d_{i,j}$	-1.7	0.1	-0.7
	$U(d_{ij})$	2.2	1.6	1.2

No discrepant values were found.

6. Uncertainty calculation

The participant laboratories presented there uncertainty determination according to GUM [6]. The results are presented in table 11, for the 1000 μL micropipette at its nominal volume.

Table 11 – Uncertainty contributions

Uncertainty contributions (μl)	IPQ	INTI
Repeatability	0.388	0.529
Balance	6.02×10^{-3}	0.474
Air density	2.52×10^{-3}	2.15×10^{-3}
Water density	-1.22×10^{-2}	-7.53×10^{-3}
Density of the mass pieces	6.50×10^{-4}	1.28×10^{-3}
Expansion coefficient	-3.38×10^{-3}	-1.28×10^{-3}

Water temperature	1.20×10^{-2}	0.008
Evaporation	0.0036	0.087
Handling	0.574	0.576
Resolution	0.289	0.289
Temp. Difference water-pipette-air	-	0.634
Humidity	-	0.020
Atmospheric pressure	-	0.067
Combined Uncertainty (μl)	0.75	1.2
Expanded uncertainty (μl)	**1.5**	**2.3**

7. Conclusions

This bilateral comparison of a variable 1000 μl micropipette and a fixed 50 μl micropipette involved IPQ and INTI.

The fixed micropipette was damaged during the transportation between laboratories and therefore the results cannot be used.

For the variable micropipette all results for both laboratories are consistent with the reference value obtained by the weighted mean. Also the results between laboratories are also consistent.

The value of the expanded uncertainty is quite similar for the higher volumes but there are some differences in the values of the uncertainty components. The uncertainty component that has a major contribution to the final uncertainty was the handling for IPQ and the temperature difference between water and pipette for INTI.

8. References

1. ISO 8655-1/2/6, Piston-operated volumetric apparatus, 1st ed., Genève, International Organization for Standardization, 2002.
2. ASTM E542: Standard Practice for Calibration of laboratory Volumetric Apparatus, 1st ed., American Standard, 1st ed., 2012
3. ISO 4787:2010; Laboratory glassware – Volumetric glassware – Methods for use and testing of capacity;
4. Tanaka, M., et. al; Recommended table for the density of water between 0 °C and 40 °C based on recent experimental reports, Metrologia, 2001, Vol.38, 301-309;
5. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595.
6. BIPM et al, Guide to the Expression of Uncertainty in Measurement (GUM), 2nd ed., International Organization for Standardization, Genève, 1995.