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BIPM/CIPM key comparison CCM.FF-K4.2.2011
Volume comparison at 100 μL – Calibration of micropipettes (piston pipettes)

Final Report

Pilot
Elsa Batista, IPQ

Co-pilots
Roberto Arias, CENAM and Wang Jintao, NIM

January 2013

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1. Introduction

During the 10th WGFF meeting, held in Taiwan in October 2010, it was agreed to start a Key Comparison (KC) on volume measurements in the range of the microliter. The main purpose of this KC was to compare the results and calibration methods of 100 µL micropipettes in order to permit the participating laboratories to test the agreement of their results and uncertainties despite the different equipment and calibration methods.

The Volume Laboratory of the Portuguese Institute for Quality (IPQ) - Central Laboratory of Metrology (LCM), volunteered to be the pilot laboratory. NIM from China and CENAM from Mexico were also involved as co-pilots.

A questionnaire circulated among all the WGFF members and 7 positive answers were received. A protocol was then developed by the pilot and co-pilot laboratories and later approved by all the participants.

Five 100 µL micropipettes (transfer package) were tested in order to have at least one back-up for each RMO. After the completion of this CCM.FF-K4.2.2011 comparison, one micropipette will be sent to each RMO in order to start a regional comparison.

Measurements were performed by the pilot laboratory on three different occasions during the comparison in order to verify the stability of the travelling standards (TS). Behaviour tests were also performed in order to test the experimental procedure in detail.

One participant withdrew from the comparison and another joined it at the end of the circulation scheme.

The comparison started in July 2011 and ended in July 2012.

2. Participants

Each participant had 4 weeks to receive the micropipettes, perform the measurements and send the instruments to the next participant according to the following schedule:

Table 1 – Time schedule

NMI	Country	Contact	Arrival Date
IPQ	Portugal	Elsa Batista	July 2011
NMISA	South Africa	Ronél Steyn/Thomas Mautjana	11 August 2011
NIM	China	Liu Ziyong/ Wang Jintao	1 October 2011
CENAM	Mexico	Roberto Arias /Sonia Trujillo	4 November 2011
INMETRO	Brazil	Dalni Malta	25 November 2011
NMO	United Kingdom	John Pain	Withdrew
UME	Turkey	Umit Akcadag	23 January 2012
LNE	France	Tanguy Madec / Paul-Andre Meury	29 February 2012
IPQ	Portugal	Elsa Batista	27 March 2012

KEBS	Kenya	Dominic Ondoro	23 May 2012
IPQ	Portugal	Elsa Batista	15 June 2012

Due to a delay in transport NMO from United Kingdom did not make any measurements. They withdrew before the instruments arrive at UK.

3. The transfer standard

The chosen instruments were air displacement, single channel, fixed micropipettes (piston pipettes) of low nominal value, 100 μL (see figure 1). The micropipettes need to have attached a removable plastic tip in order to aspirate the liquid. IPQ-LCM acting as the pilot laboratory supplied these tips that were directly obtained from the manufacturer and from the same lot.

The fixed micropipettes used for this comparison are essentially of plastic material with a coefficient of thermal expansion of 2,4 × 10^{-4} / ºC [1]. The serial numbers of the 5 micropipettes used in this comparison are the following; 354828Z, 354853Z, 354864Z, 354868Z, 354872Z.

Figure 1- Fixed micropipette of 100 μL

4. The measurement procedure

4.1 Experimental method

All 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 [2] and ISO 4787 [3], see equation (1):

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

Where:

V_0/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]

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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.

An equation for correction of volume at the standard atmospheric pressure, 1013,25 hPa, was applied to the results of all participants, see point 5.3.

4.2 Water characteristics

The participants differed in the used water characteristics. A summary is found in table 2.

Table 2 – Water characteristics

NMI	Type of water	Density Formula	Conductivity (µS/cm)
IPQ	Ultra-pure	Tanaka	0,054
CENAM	De-aired and De-Ionized	Tanaka	1,1 to 2
LNE	Bi - distilled water	Tanaka	< 15
UME	Reverse osmosis and degassing process	Tanaka	0,67
NMISA	Triple distilled	Tanaka	-
NIM	De-ionized	Tanaka	5
INMETRO	Bi - distilled water	Tanaka	-
KEBS	Distilled	Tanaka	6,02

The method of water purification or type of water is listed in Table 2. The Tanaka formula was used as the reference for water density and the conductivity was only determined by some laboratories, the maximum value allowed is 5 µS/cm [3].

4.3 Equipment

Each laboratory described the equipment used in the calibration and the respective traceability following a prepared form sent along with the protocol. The majority of the participants used equipment with the following characteristics:

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Table 3 – Equipment characteristics

Equipment	Type	Resolution
Balance	Comparator	(0,001 - 0,01) mg
Weights	E1, E2	-
Water thermometer in sampling vessel	Digital	(0,001 - 0,1) ºC
Air thermometer	Digital	(0,02 - 0,1) ºC
Barometer	Digital	(0,01 - 1) hPa
Hydrometer	Digital	(0,1 - 1) %

The last three instruments were used to calculate the air buoyancy effect. The details of the equipment used by each NMI are described in Annex 1.

4.4 Ambient conditions of the measurements

The ambient conditions were described by all participants for the 5 micropipettes. The values given in table 4 refer to mean values for the micropipette 354828Z. The ambient conditions for the other 4 micropipettes are presented in Annex 2.

Table 4 - Ambient conditions

NMI	Air Temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water Temperature (ºC)
IPQ	22,3	1006,67	53,2	22,05
CENAM	17,8	814,13	48	17,56
LNE	20,729	1029,11	56,1	20,360
UME	20,55	1002,14	50,7	19,497
NMISA	20,46	872,89	60,43	20,04
NIM	20,9	1044	51	19,17
INMETRO	20,0	1011	59	20,00
KEBS	22,56	839,50	63,52	22,70

The majority of the laboratories presented values that are in agreement with the conditions proposed in the protocol: humidity higher than 50 % and ambient temperature between 17 ºC and 23 ºC. Also and in all cases, the water temperature did not vary more than 0,5 ºC during the 10 measurements.
It can also be seen, from this table, the differences in atmospheric pressure due to altitude, leading to different values of the air density.

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5. Measurement results

5.1 Stability of the micropipettes

Three different measurements of the five micropipettes were performed by the pilot laboratory during the comparisons in order to verify the stability of the standards. The measurement with the largest uncertainty was chosen to be the IPQ result. The results are presented in the following table:

Table 5 - Stability of the instruments

Micropipette	Measurement	Date	Volume (□L)	Uncertainty (□L)	Standard Deviation (□L)
354828Z	**1**	July 2011	99,98	0,10	0,06
	2	February 2012	100,04	0,06	
	3	June 2012	99,92	0,08	
354853Z	**1**	July 2011	99,92	0,04	0,06
	2	February 2012	100,03	0,10	
	3	June 2012	99,98	0,08	
354864Z	**1**	July 2011	99,93	0,07	0,06
	2	February 2012	99,96	0,09	
	3	June 2012	100,05	0,07	
354868Z	**1**	July 2011	100,05	0,06	0,05
	2	February 2012	100,07	0,13	
	3	June 2012	100,15	0,07	
354872Z	**1**	July 2011	99,74	0,06	0,06
	2	February 2012	99,76	0,09	
	3	June 2012	99,85	0,08	

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

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5.2 Laboratory results

The results for the five micropipettes reported by the participating laboratories are included in table 6.

Table 6 – Volume measurement results

NMI	354828Z		354853Z		354864Z		354868Z		354872Z	
	V/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL
IPQ	99,98	0,10	100,03	0,10	99,96	0,09	100,07	0,13	99,76	0,09
CENAM	99,61	0,21	99,37	0,26	99,41	0,18	99,66	0,14	99,12	0,28
LNE	100,28	0,22	100,16	0,21	100,15	0,21	100,31	0,21	99,98	0,21
UME	100,01	0,17	100,07	0,18	100,04	0,17	100,16	0,17	99,86	0,17
NMISA	99,92	0,13	99,87	0,14	99,86	0,19	99,91	0,13	99,72	0,13
NIM	99,89	0,04	99,91	0,06	99,99	0,06	99,91	0,08	99,65	0,04
INMETRO	100,32	0,20	100,28	0,34	100,35	0,17	100,59	0,29	100,22	0,37
KEBS	99,62	0,09	99,65	0,11	99,97	0,13	99,59	0,10	99,08	0,51

A very large dispersion was found among these values. The source of this dispersion in explained in the next section.

5.3 Pressure correction

Piston stroke pipettes (air displacement) have an air-cushion which moves between the piston and the sample liquid, and which aspirates and dispenses the sample. With the decreasing atmospheric pressure the density of the air cushion decreases leading to a reduction in the dispensed volume of the micropipette.
If the dead volume and the capillary rise of the liquid column in the micropipette are known, the change in volume that results from calibration at a location X2 (with $p_{L,X2}$ atmospheric pressure) compared to a location X1 (with $p_{L,X1}$ atmospheric pressure) can be calculated by using the following formula [5]:

$$\Delta V = V_t \times \rho_W \times g \times h_W \times \left(\frac{1}{p_{L,X2} - \rho_W \times g \times h_W} \quad \frac{1}{p_{L,X1} - \rho_W \times g \times h_W} \right) \qquad (2)$$

Where,

ΔV/µL:	Volume change that results in the calibration at location X1 over a location X2
V_t/ µL:	Volume of the air cushion
g/(m/s^2):	Acceleration of gravity
h_w/m:	Rising height of the liquid column in the pipette tip
$p_{L,X1}$/Pa:	Atmospheric pressure at location X1
$p_{L,X2}$/Pa:	Atmospheric pressure at location X2
ρ_w/(kg/m^3):	Water density at X2

One of the possible reasons for the dispersion of the results is that the altitude difference between the geographical locations of the participating laboratories leads to a difference in atmospheric pressure. If the

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results in table 6 are corrected for a standard atmospheric pressure 1013,25 hPa using equation 2, the dispersion is much smaller, see table 7.
The values for h_w/m = 0,030 and V_t/ µL = 437 for the air pressure correction were obtained from the micropipette manufacturer (Eppendorf).

Table 7 – Volume measurement results corrected for atmospheric pressure

NMI	354828Z		354853Z		354864Z		354868Z		354872Z	
	V/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL
IPQ	99,99	0,10	99,99	0,10	99,97	0,09	100,08	0,13	99,77	0,09
CENAM	99,92	0,21	99,68	0,26	99,72	0,18	99,97	0,14	99,43	0,28
LNE	100,26	0,22	100,14	0,21	100,13	0,21	100,29	0,21	99,96	0,21
UME	100,02	0,17	100,08	0,18	100,05	0,17	100,17	0,17	99,87	0,17
NMISA	100,12	0,13	100,08	0,14	100,08	0,19	100,12	0,13	99,93	0,13
NIM	99,86	0,04	99,88	0,06	99,96	0,06	99,88	0,08	99,62	0,04
INMETRO	100,32	0,20	100,28	0,34	100,36	0,17	100,60	0,29	100,23	0,37
KEBS	99,88	0,09	99,91	0,11	100,23	0,13	99,85	0,10	99,34	0,51

An example of the volume change determination for the micropipette 354828Z is presented in table 8. All the results for the other 4 micropipettes are presented in Annex 3.

Table 8 – Volume change determination for micropipette 35428Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/µL	*V*/µL	V_{corr}/µL
IPQ	997,76	100 667	-0,008	99,98	99,99
CENAM	998,664	81 413	-0,312	99,61	99,92
LNE	998,13	102 911	0,020	100,28	100,26
UME	998,31	100 214	-0,014	100,01	100,02
NMISA	998,1955	87 289	-0,205	99,92	100,12
NIM	998,038	104 400	0,037	99,89	99,86
INMETRO	998,204	101 100	-0,003	100,32	100,32
KEBS	997,607	83 950	-0,263	99,62	99,88

5.4 Uncertainty of the corrections and stability of the micropipettes

Several experimental tests were performed by the pilot laboratory in the calibration of micropipettes in order to identify possible handling problems (immersion depth, aspiration angle, aspiration velocity, delivery angle, etc). A maximum error of 0,4 % was found during the experiments (E_{exp}). This value along with the standard

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deviation (s) of the three pilot measurements (n) for each micropipette will be added to the uncertainty budget of the laboratories in order to have a more realistic uncertainty result.

$$u_{st} = \sqrt{\left(\frac{s}{\sqrt{n}}\right)^2 + \left(\frac{E_{exp}}{2\sqrt{3}}\right)^2} \qquad (3)$$

This value, u_{st}, corresponds to 0,13 µL for all micropipettes.

The standard uncertainties of each laboratory (u_{xi}) can then be the calculated according to the following equation:

$$u_{xi} = \sqrt{\left(\frac{U(x_i)}{2}\right)^2 + u^2_{st}} \qquad (4)$$

The final results with all corrections applied for volume and uncertainty are found in table 9.

Table 9 - Volume measurement results and uncertainty corrected

NMI	354828Z		354853Z		354864Z		354868Z		354872Z	
	V/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL	*V*/µL	*U*/µL
IPQ	99,99	0,27	99,99	0,27	99,97	0,27	100,08	0,28	99,77	0,27
CENAM	99,92	0,32	99,68	0,34	99,72	0,31	99,97	0,29	99,43	0,35
LNE	100,26	0,34	100,14	0,33	100,13	0,33	100,29	0,33	99,96	0,33
UME	100,02	0,31	100,08	0,32	100,05	0,31	100,17	0,31	99,87	0,31
NMISA	100,12	0,29	100,08	0,30	100,08	0,32	100,12	0,29	99,93	0,29
NIM	99,86	0,26	99,88	0,27	99,96	0,27	99,88	0,27	99,62	0,26
INMETRO	100,32	0,33	100,28	0,43	100,36	0,31	100,60	0,39	100,23	0,45
KEBS	99,88	0,27	99,91	0,28	100,23	0,28	99,85	0,27	99,34	0,51

6. Determination of the key comparison reference value, uncertainty, consistency and degree of equivalence

To determine the reference value of this key comparison (KCRV) the weighted mean, equation (5) was selected, using the inverses of the squares of the associated standard uncertainties as the weights [6], according to the instructions given by the BIPM:

$$y = \frac{x_1/u^2(x_1) + ... + x_n/u^2(x_n)}{1/u^2(x_1) + ... + 1/u^2(x_n)} \qquad (5)$$

To calculate the standard deviation $u(y)$ associated with the volume y [6] equation (6) was used:

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$$u(y) = \sqrt{\frac{1}{1/u^2(x_1) + \ldots + 1/u^2(x_n)}} \quad (6)$$

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

To identify an overall consistency of the results a chi-square test can be applied to all n calibration results [6].

$$\chi^2_{obs} = \frac{(x_1 - y)^2}{u^2(x_1)} + \ldots + \frac{(x_n - y)^2}{u^2(x_n)} \quad (7)$$

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) < χ^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 $\frac{(x_i - y)^2}{u^2(x_i)}$ was excluded from the next round of evaluation and the new reference value, reference standard uncertainty and chi-squared value was calculated again without the excluded laboratory. When the consistency check passed, for each laboratory result, x_i the degree of equivalence d_i between each laboratory and the KCRV (x_{ref}) was calculated using the following formulas [6]:

$d_i = x_I - x_{ref}$ (8)

$U(d_i) = 2 \times u(d_i)$ (9)

where $u(d_i)$ was calculated from

$u^2(d_i) = u^2(x_i) - u^2(x_{ref})$ (10)

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

To calculate the degrees of equivalence d_{ij} between the laboratories the following formulas were used [6]:

$d_{i,j} = x_i - x_j$ (11)

$U(d_{i,j}) = 2 \times u(d_{i,j})$ (12)

Where $u(d_{i,j})$ was calculated from

$u^2(d_{i,j}) = u^2(x_i) + u^2(x_j)$ (13)

The factor 2 in equation (9 and 12) corresponds to a 95 % coverage under the assumption of normality.

6.1 Micropipette 354828Z

The obtained KCRV for micropipette 354828Z is 100,03 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0,10 µL.
The calculated value $\chi^2(\nu)$ = 14,07 is larger than χ^2_{obs} = 8,99, the observed value, therefore the results are consistent with each other and with the reference value from a statistical point of view.
All the measurement results, the reference value and its uncertainty are presented in the following figure 2:

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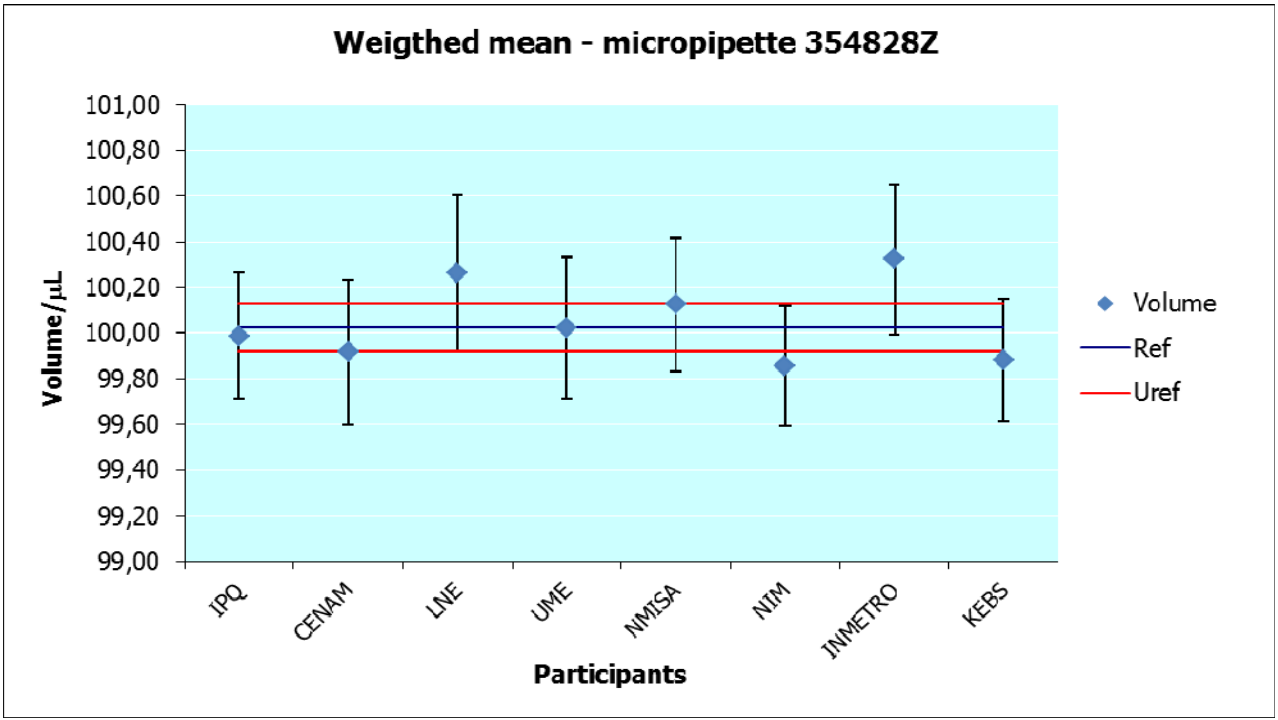

Figure 2- Measurement results of micropipette 354828Z with reference value and uncertainty

The degree of equivalence with the KCRV is presented in figure 3:

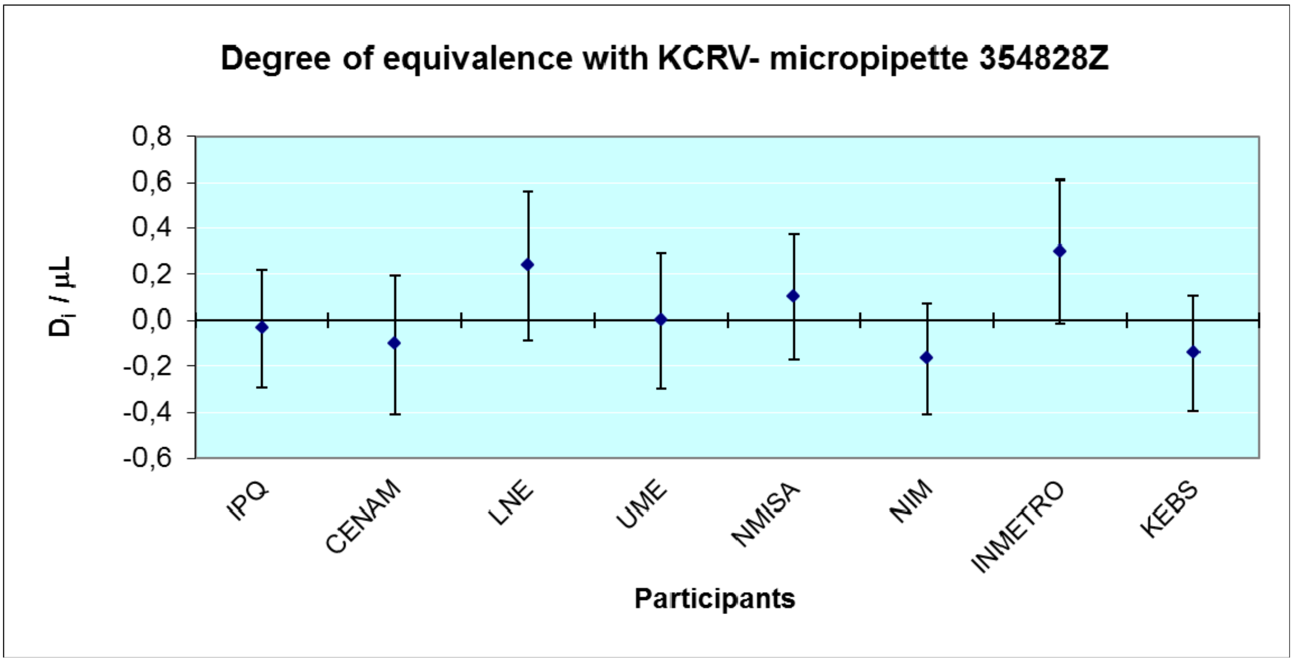

Figure 3- Degree of equivalence with reference value of micropipette 354828Z with reference value and uncertainty

Table 10 – Degree of equivalence with KCRV for micropipette 354828Z

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NMI	d_i/µL	$U(d_i)$/µL
IPQ	-0,04	0,25
CENAM	-0,11	0,30
LNE	0,24	0,32
UME	0,00	0,29
NMISA	0,10	0,27
NIM	-0,17	0,24
INMETRO	0,30	0,31
KEBS	-0,14	0,25

The degrees of equivalence (DoE) between the laboratories are presented in Annex 4.

6.2 Micropipette 354853Z

The obtained KCRV for micropipette 354853Z is 99,99 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0,11 µL.
The calculated value $\chi^2(\nu)$ = 14,07 is larger than χ^2_{obs} = 7,57, the observed value, therefore the results are consistent with each other and with the reference value from a statistical point of view.
All the measurement results, the reference value and its uncertainty are presented in the following figure 4:

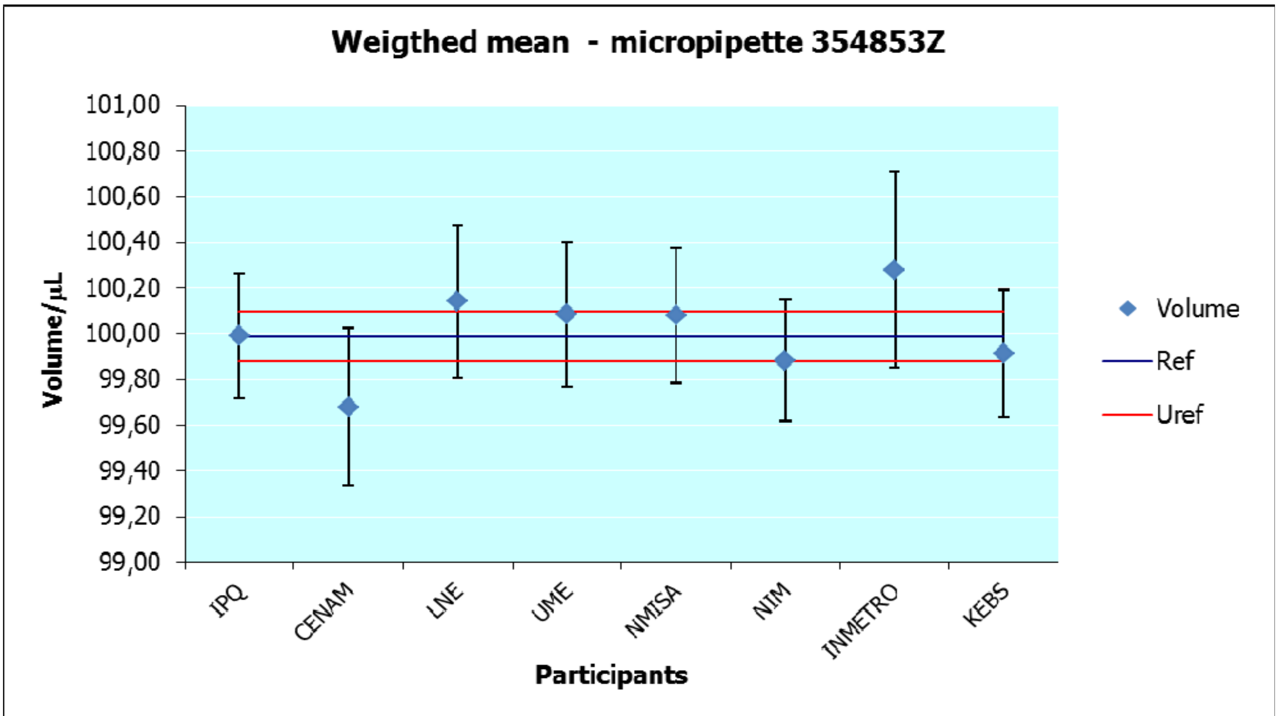

Figure 4- Measurement results of micropipette 354853Z with reference value and uncertainty

The degree of equivalence with the KCRV is presented in figure 5:

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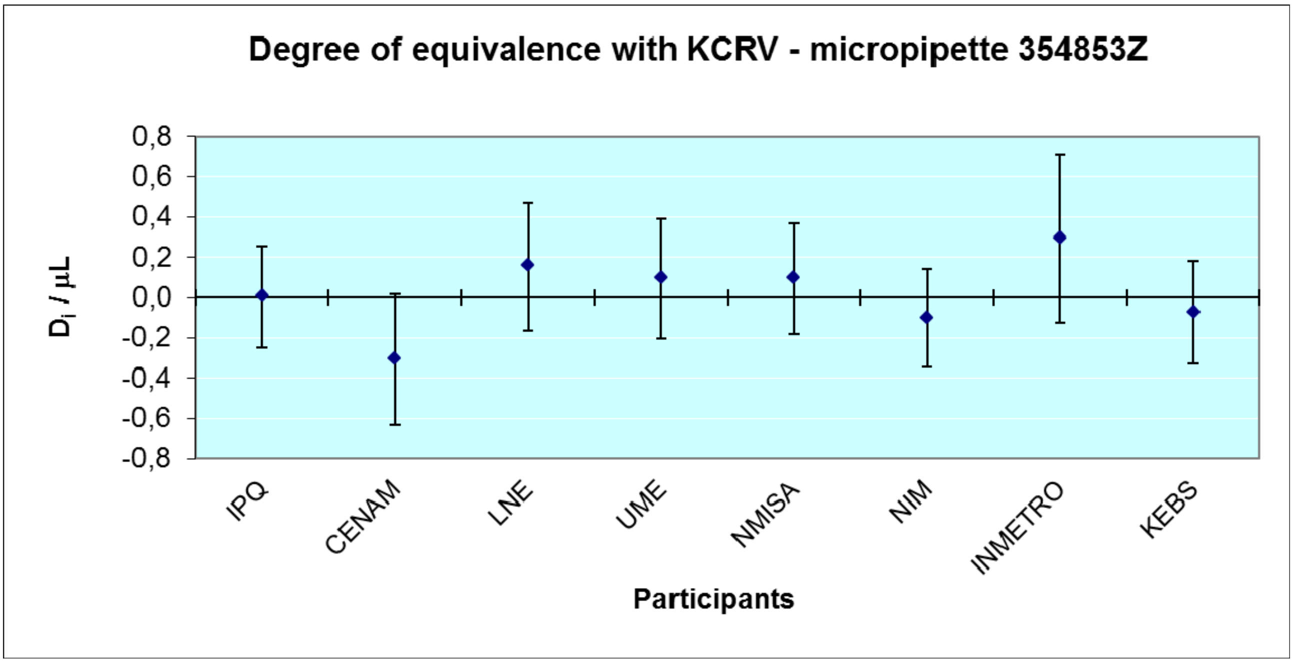

Figure 5- Degree of equivalence with reference value of micropipette 354853Z with reference value and uncertainty

Table 11 – Degree of equivalence with KCRV for micropipette 354853Z

NMI	d_i/µL	$U(d_i)$/µL
IPQ	0,00	0,25
CENAM	-0,31	0,33
LNE	0,15	0,32
UME	0,10	0,30
NMISA	0,09	0,27
NIM	-0,10	0,24
INMETRO	0,29	0,41
KEBS	-0,07	0,26

The degrees of equivalence (DoE) between the laboratories are presented Annex 4.

6.3 Micropipette 354864Z

The obtained KCRV for micropipette 354864Z is 100,06 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0,11 µL.
The calculated value, $\chi^2(\nu)$ = 14,07 is larger than χ^2_{obs} = 11,33, the observed value, therefore the results are consistent with each other and with the reference value from a statistical point of view.
All the measurement results, the reference value and its uncertainty are presented in the following figure 6:

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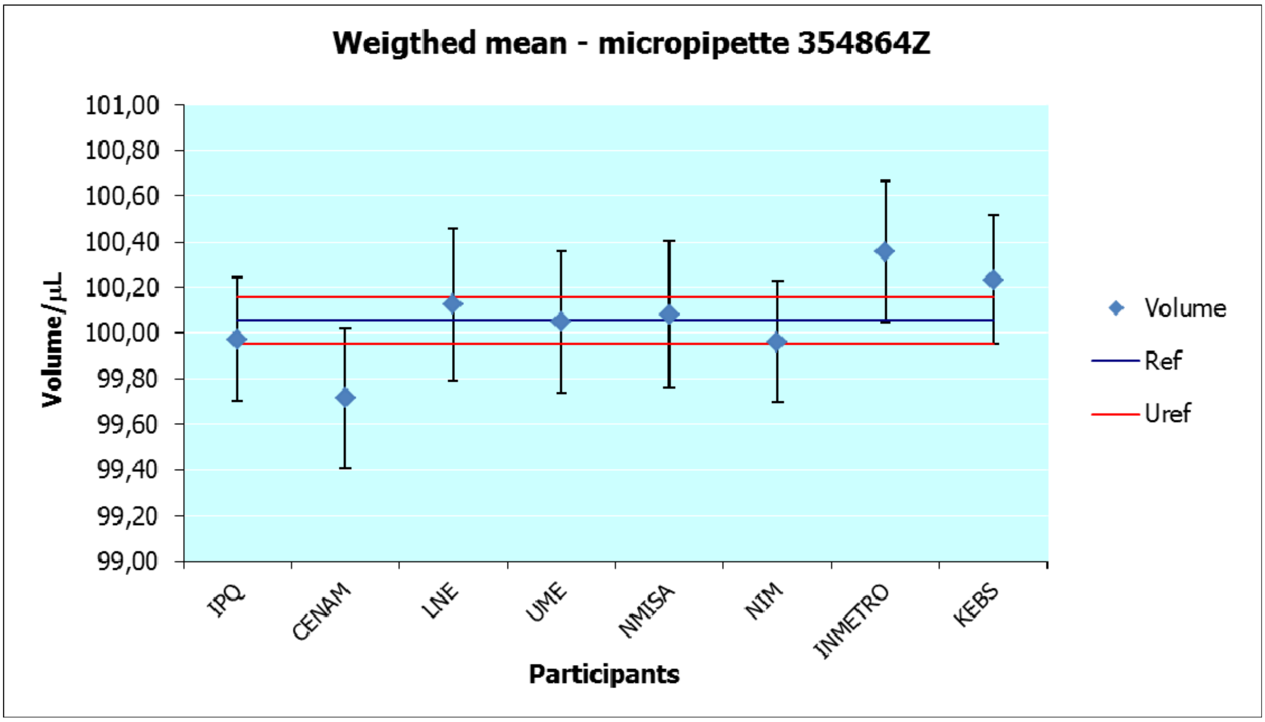

Figure 6- Measurement results of micropipette 354864Z with reference value and uncertainty

The degree of equivalence with the KCRV is presented in figure 7:

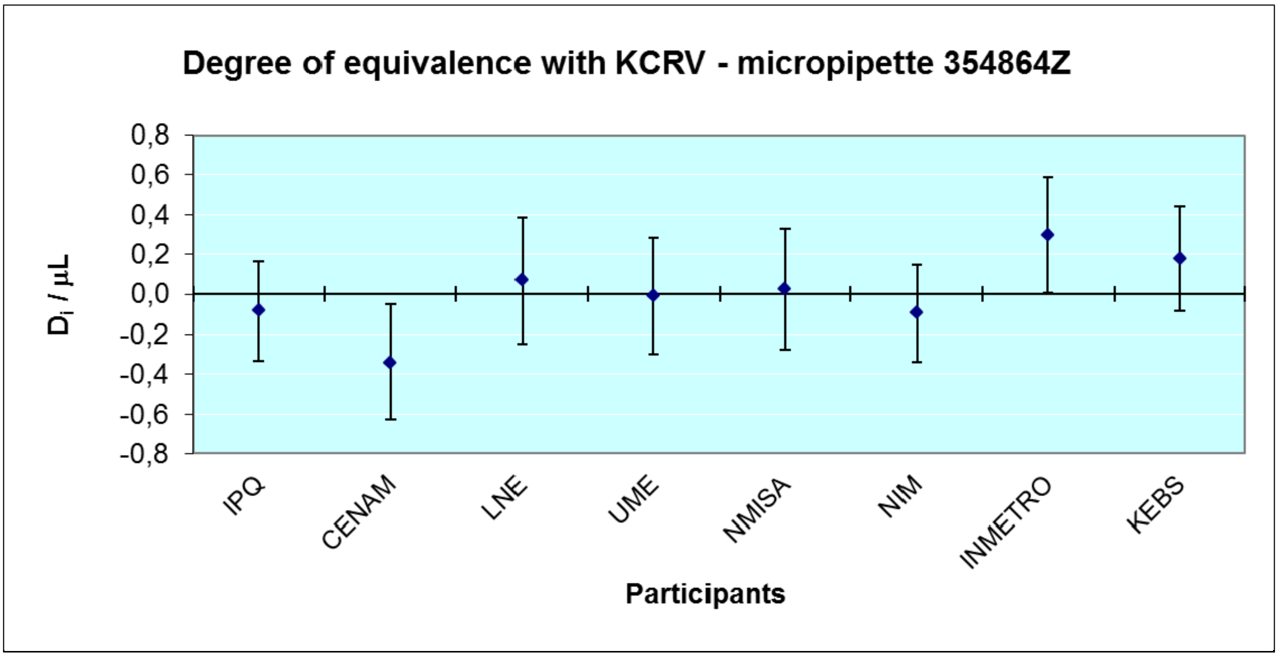

Figure 7- Degree of equivalence with reference value of micropipette 354864Z with reference value and uncertainty

Table 12 – Degree of equivalence with KCRV for micropipette 354864Z

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NMI	d_i/µL	$U(d_i)$/µL
IPQ	-0,08	0,25
CENAM	-0,34	0,29
LNE	0,07	0,32
UME	-0,01	0,29
NMISA	0,03	0,30
NIM	-0,09	0,24
INMETRO	0,30	0,29
KEBS	0,18	0,26

The degrees of equivalence (DoE) between the laboratories are presented Annex 4.

6.4 Micropipette 354868Z

The obtained KCRV for micropipette 354868Z is 100,08 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0,11 µL.
The calculated value $\chi^2(\nu)$ = 14,07 is smaller than χ^2_{obs}= 14,54, the observed value, therefore the results are not consistent with each other and with the reference value from a statistical point of view. If the result of the INMETRO is removed from the subset, because is the most discrepant value; now, with ν= 6, the calculated value $\chi^2(\nu)$ = 12,59 is larger than χ^2_{obs}= 6,92, then the remaining values are consistent with each other and with the new reference value 100,04 µL, and expanded uncertainty, 0,11 µL .
All the measurement results, the reference value and its uncertainty are presented in the following figure 8:

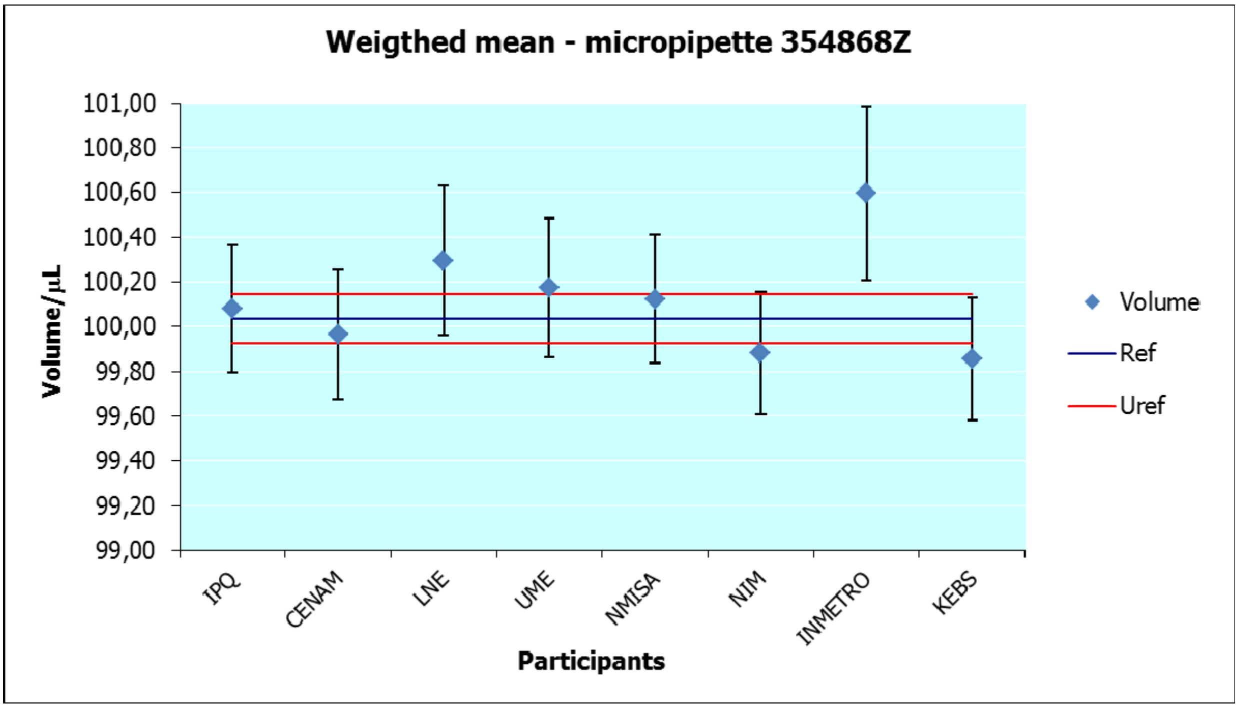

Figure 8- Measurement results of micropipette 354868Z with reference value and uncertainty

The degree of equivalence with the KCRV is presented in figure 9:

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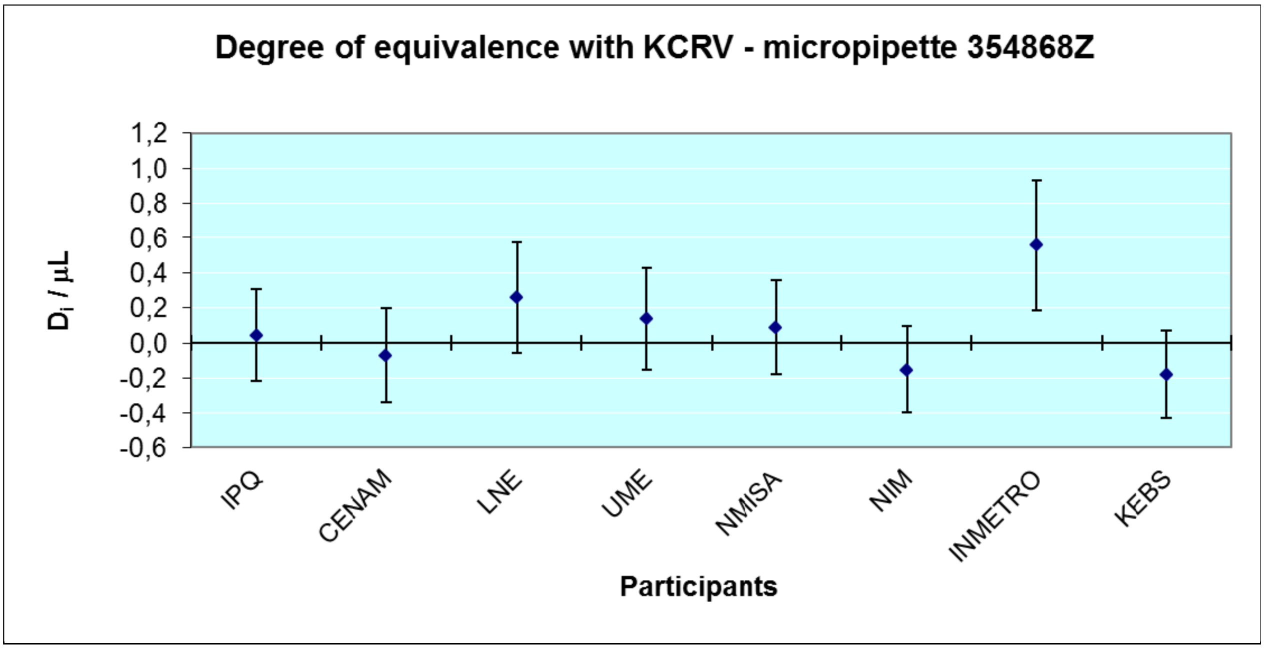

Figure 9- Degree of equivalence with reference value of micropipette 354868Z with reference value and uncertainty

Table 13 – Degree of equivalence with KCRV for micropipette 354868Z

NMI	d_i/µL	$U(d_i)$/µL
IPQ	0,05	0,26
CENAM	-0,07	0,27
LNE	0,26	0,32
UME	0,14	0,29
NMISA	0,09	0,27
NIM	-0,15	0,25
INMETRO	0,56	0,37
KEBS	-0,18	0,25

The degrees of equivalence (DoE) between the laboratories are presented Annex 4.

6.5 Micropipette 354872Z

The obtained KCRV for micropipette 354872Z is 99,78 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0,11 µL.
The calculated value $\chi^2(\nu)$ = 14,07 is smaller than χ^2_{obs} = 14,82 the observed value, therefore the results are not consistent with each other and with the reference value from a statistical point of view. If the result of INMETRO is removed from the subset, because is the most discrepant value; now with $\nu = 6$, $\chi^2(\nu)$ = 12,59 is larger than χ^2_{obs} = 10,56, then the remaining values are consistent with each other and with the new reference value 99,74 µL, and expanded uncertainty, 0,12 µL.

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All the measurement results, the reference value and its uncertainty are presented in the following figure 10:

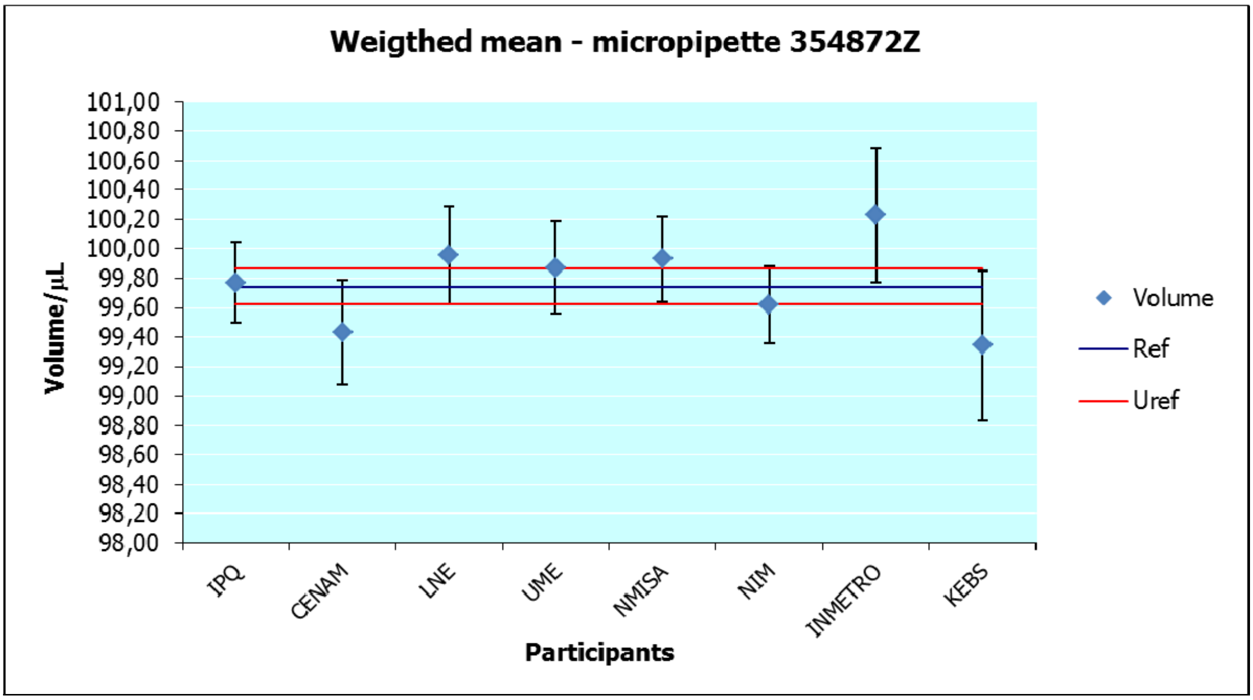

Figure 10- Measurement results of micropipette 354872Z with reference value and uncertainty

The degree of equivalence with the KCRV is presented in figure 11:

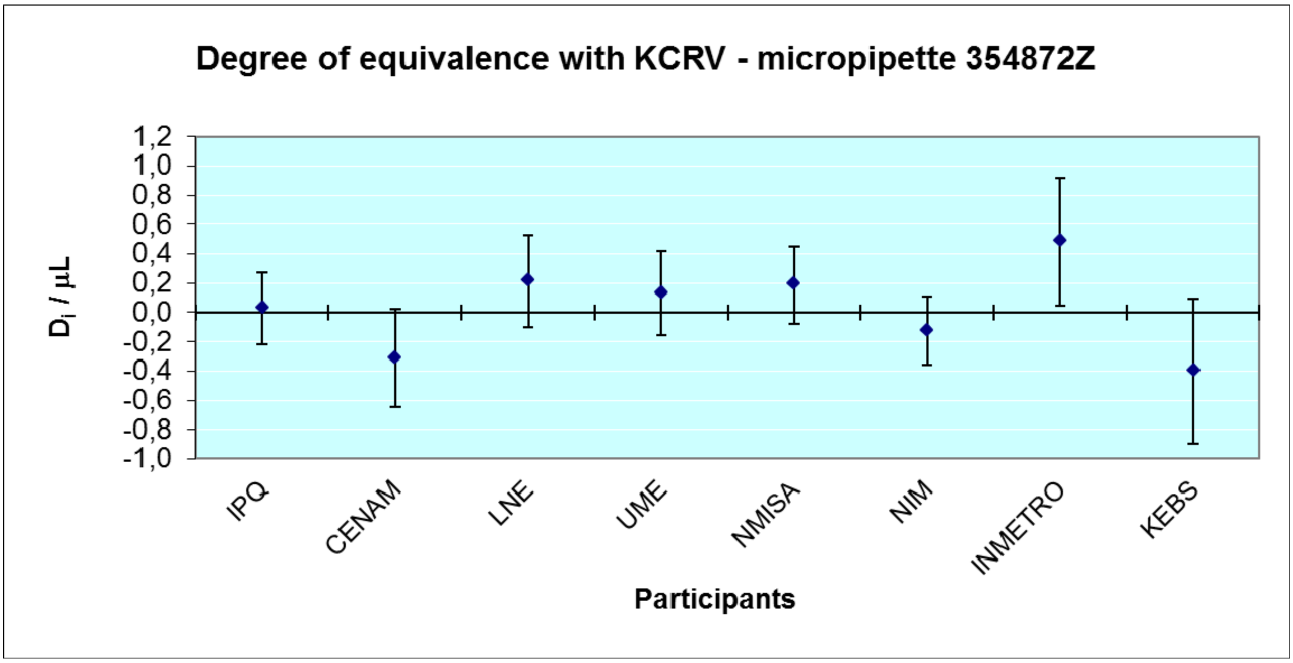

Figure 11- Degree of equivalence with reference value of micropipette 354872Z with reference value and uncertainty

Table 14 – Degree of equivalence with KCRV for micropipette 354872Z

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NMI	d_i/µL	$U(d_i)$/µL
IPQ	0,03	0,24
CENAM	-0,31	0,33
LNE	0,21	0,31
UME	0,13	0,29
NMISA	0,19	0,26
NIM	-0,13	0,23
INMETRO	0,48	0,44
KEBS	-0,40	0,50

The degrees of equivalence (DoE) between the laboratories are presented Annex 4.

7. Uncertainty calculation

It was requested that all participants present their uncertainty budget according to the spreadsheet supplied by the pilot laboratory in the comparison protocol. As an example, the results for the micropipette 354828Z are presented in table 15, the values for the other 4 micropipettes are presented in Annex 5.

Table 15 – Uncertainty contributions

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Uncertainty contributions (µl)	IPQ	CENAM	LNE	UME	NMISA	NIM	INMETRO	KEBS
Repeatability	0,044	0,087	0,04	0,008	0,038	0,012	0,0316	0,036
Balance	$5{,}62\times10^{-3}$	0,017	0,011	0,083		$5{,}77\times10^{-3}$	0,01	0,058
Air density	$2{,}53\times10^{-5}$	$1{,}30\times10^{-4}$	3×10^{-6}	$3{,}6\times10^{-4}$	$5{,}7\times10^{-6}$	$5{,}94\times10^{-5}$	$8{,}22\times10^{-8}$	$3{,}9\times10^{-5}$
Water density	$1{,}32\times10^{-4}$	$1{,}04\times10^{-3}$	2×10^{-3}	$1{,}2\times10^{-3}$	0,048	$5{,}80\times10^{-3}$	$3{,}52\times10^{-7}$	-
Density of the mass pieces	$6{,}46\times10^{-5}$	$1{,}07\times10^{-4}$	-	-	$6{,}49\times10^{-5}$	$1{,}31\times10^{-4}$	$1{,}086\times10^{-7}$	-
Expansion coefficient	$1{,}42\times10^{-3}$	$3{,}30\times10^{-3}$	8×10^{-3}	$6{,}95\times10^{-3}$	$4{,}61\times10^{-5}$	$8{,}024\times10^{-4}$	$1{,}15\times10^{-9}$	$7{,}0\times10^{-3}$
Water temperature	$1{,}20\times10^{-4}$	$1{,}35\times10^{-3}$	3×10^{-3}	-	$3{,}9\times10^{-4}$	$1{,}34\times10^{-3}$	$1{,}40\times10^{-8}$	$2{,}2\times10^{-3}$
Evaporation	$1{,}90\times10^{-3}$	$1{,}48\times10^{-3}$	0,055	-	0,014	0,0115	-	-
Others	-	-	0,087	$5{,}8\times10^{-3}$	1×10^{-3}	-	0,0926	$2{,}3\times10^{-3}$
Combined Uncertainty (µl)	0,044	0,09	0,111	0,0084	0,063	0,0186	0,098	0,036
Expanded uncertainty (µl)	**0,1**	**0,21**	**0,22**	**0,17**	**0,12**	**0,037**	**0,2**	**0,085**
Stability and method standard uncertainty (µl)	0,13	0,13	0,13	0,13	0,13	0,13	0,13	0,13
Corrected expanded uncertainty (µl)	**0,27**	**0,28**	**0,32**	**0,29**	**0,27**	**0,25**	**0,38**	**0,26**

The presented values of the uncertainty components and expanded uncertainty are quite similar, except for one or two participants. The major source of uncertainty (in red letters) varies from laboratory to laboratory but the repeatability uncertainty component is one of the largest for the majority of the laboratories, figure 12.

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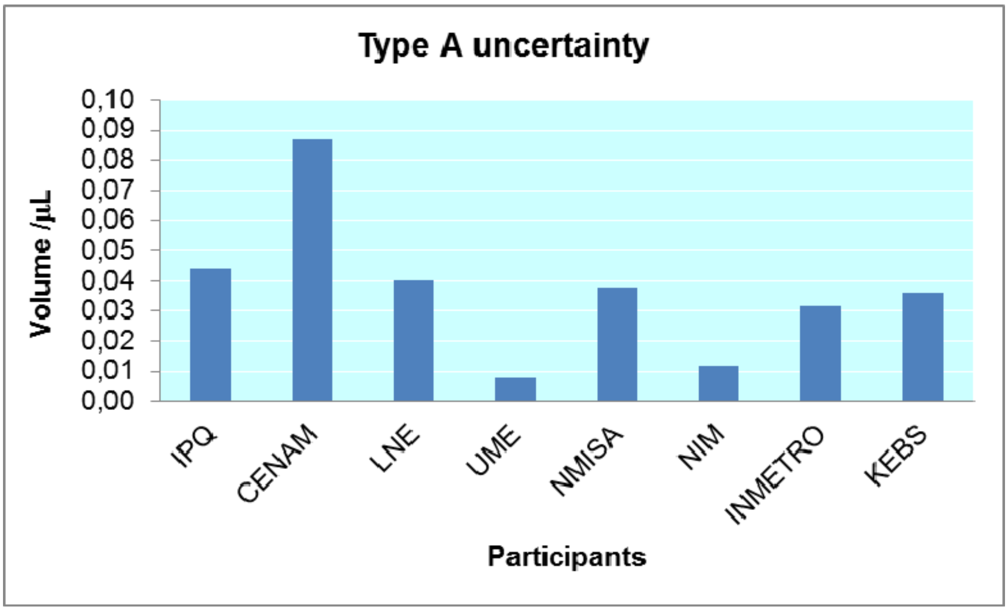

Figure 12 – Type A uncertainty (repeatability)

When the stability and method uncertainties (0,13 µL) are applied to all uncertainty budgets this source becomes the largest uncertainty component for all laboratories and it represents a 50 % average increase in the laboratories declared expanded uncertainty, see figure 13.

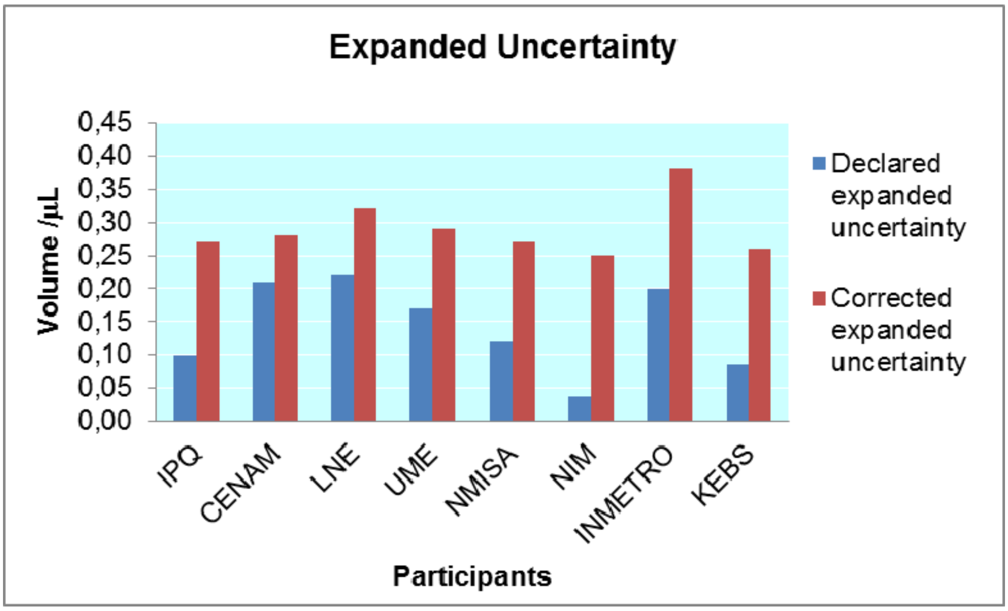

Figure 13 – Expanded uncertainty declared and corrected

It is recommended, that in the future, all laboratories should take into consideration in there uncertainty budget the procedure variability or operator errors as a source of uncertainty.

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8. Conclusions

This key comparison, in the range of the microliter, started in July 2011 and ended in June 2012.

Five fixed micropipettes of 100 µl were tested by 8 different NMIs from different RMOs.

The micropipettes had a stable volume, during the whole comparison, with a maximum standard deviation of 0,06 µl.

After a careful analysis of the original results it was decided to make corrections to the standard atmospheric pressure in order to compare results under the same calibration conditions. These corrections lead to a decrease of variability within the laboratories. It was also decided to include the stability of the standard and the method variability, determined by the pilot laboratory, into the uncertainty budget of each laboratory.

The corrected results (volume and uncertainty) are consistent and overlap with the KCRV for the micropipettes 354828Z, 354853Z and 354864Z. For the other two micropipettes only one result is not consistent with the reference value. Most results also overlap with those of the other laboratories $d_{i,j} < U(d_{i,j})$.

An important outcome of this comparison is that in order to have comparable results, corrections for the working conditions must be applied to the laboratory results. Laboratories must always correct their reported volume results to a reference pressure condition and temperature (for example 1013,25 hPa and 20 ºC) and this information should be stated in the calibration certificate of the micropipette. Also the standard uncertainty of the method variability and reproducibility values should always be included in the uncertainty budget (around 0,1 %) to lead to more realistic results.

9. References

1. ASTM E 542:2000 - Standard practice for calibration of laboratory volumetric apparatus;
2. ISO 8655-1/2/6:2002, Piston-operated volumetric apparatus;
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. Calibration guide DKD-R 8-1; calibration of piston pipettes, 2011;
6. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595.
7. OIML R111:2004 - Weights of classes E1, E2, F1, F2, M1, M1–2, M2, M2–3 and M3. Part 1: Metrological and technical requirements

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Annex 1 - Equipment

Balance

NMI	Manufacturer	Model	Range (g)	Resolution (mg)	Standard uncertainty (mg)	Calibration date
IPQ	Mettler	AX26	0-20	0,001	0,005	January 2012
CENAM	Sartorius	MC5	5,1	0,001	0,034	25-03-2011
LNE	Mettler	AT201	205	0,01	0,017	Sep-11
UME	Sartorius AG	ME 235 S	0 - 230	0,01	0,015	18-08-2011
NMISA	Mettler Toledo	AX 205	0 - 200	0,01	0,03	2011/08/23
NIM	Mettler Toledo	XP26PC	0 - 22	0,001	0,01	01-Sep-12
INMETRO	Sartorius	ME 215S	210	0,01	0,01	17-12-2010
KEBS	Sartorius	CC1200	0 - 1200	0,1	0,058	Before use

Weights

NMI	Manufacturer	OIML R111 Class	Mass	Standard uncertainty from the calibration certificates	Calibration date
IPQ	Sartorious	E2	100 mg	0,001 mg	09-12-2009
CENAM	-	-	-	-	-
LNE	Mettler	E2	1 mg to 500 g	Class E2/6	Jan-12
UME	Hafner	E1	1 mg-10 kg	1 mg (for 200 g)	03-05-2010
NMISA	Mettler Toledo	E1	0,1 g	0,002 mg	07-04-2011
NIM	Mettler Toledo	E2	(1-500) mg	0,008 mg	07-Jun-12
INMETRO	Häfner	E2	weight collection: 1 mg a 10 Kg	0,0021 mg	25-06-2010
KEBS	Hafner	E1	1 mg – 1 kg	0,0006 mg – 0,16 mg	Sep-11

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Water thermometer

NMI	Manufacturer	Model	Range (ºC)	Resolution (ºC)	Standard uncertainty (ºC)	Calibration date
IPQ	Luft	C100	0 to 200	0,01	0,01	24-03-2011
CENAM	Burns /ASL	LY118/F250	0 to 60	0,001	0,01	08-05-2007
LNE	Hewlett Packard	HP34420	0 to 80	0,001	0,007	Dec-10
UME	Thermometrics	TS8504	-40 to +180	0,001	0,015	09-09-2011
NMISA	AMA	-	15 to 25	0,1	0,04	16-07-2010
NIM	P.R.China	JW-1	0 to 90	0,01	0,1	01-Sep-12
INMETRO	Incoterm	TLV	18,9 to 21,10	0,01	0,004	19-05-2009
KEBS	Technetics of Germany	MLm624n	0 to 30	0,01	0,05	Sep-11

Air thermometer

NMI	Manufacturer	Model	Range (ºC)	Resolution (ºC)	Standard uncertainty (ºC)	Calibration date
IPQ	Hygroclip	Electronic	0 - 100	0,1	0,05	06-07-2012
CENAM	Vaisala	PT-100 HM34C	N.A.	0,1	0,1	12-07-2011
LNE	AOIP	ITI20	0 to 20	0,02	0,05	Mar-10
UME	Vaisala	HMI 36	-40 to 80	0,1	0,2	30-09-2011
NMISA	Rotronic	Hygrolog	-10 to 15	0,1	0,4	09-11-2010
NIM	SHINYEI	M288-CTH	-10 to 50	0,1	1	01-Sep-12
INMETRO	Thermoschneider	TLV	-3,5 to 38,5	0,1	0,02	08-09-2008
KEBS	Technetics of Germany	MLm624n	0 to 30	0,01	0,05	Sep-11

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Barometer

NMI	Manufacturer	Model	Range	Resolution	Standard uncertainty	Calibration date
IPQ	Druck	DPI142	(0-115) kpa	1 pa	15 pa	28-12-2011
CENAM	Druck	DPI740	(60 – 100) kPa	0,1 kPa	3,0 Pa	25-04-2011
LNE	Vaisala	PTB220	(950 – 1050) hPa	0,01 hPa	11 Pa	Mar-10
UME	Setra	Digital	(0 – 1,6) bar	0,01 mbar	0,05 mbar	14-09-2011
NMISA	Druck	DPI 141	(800 – 1150) mbar	0,01 mbar	0,06 mbar	19-11-2010
NIM	Barigo	-	(960–1070) hPa	10 Pa	50 Pa	01-Sep-12
INMETRO	Oregon Scientific	BAR 988 HGT/Digital	(500 – 1050) mbar	1 mbar	1,2 mbar	10-02-2011
KEBS	Technetics of Germany	MLm624n	(900 – 1100) mbar	0,1 mbar	0,19 mbar	Sep-11

Hygrometer

NMI	Manufacturer	Model	Range (%)	Resolution (%)	Standard uncertainty (%)	Calibration date
IPQ	Hygroclip	Electronic	0-100	0,1	0,05	06-07-2012
CENAM	Vaisala	HM34C	N.A.	0,1	0,5	12-07-2011
LNE	Vaisala	HMP233	20-80	0,1	1,2	Apr-10
UME	Vaisala	HMI 36	0-100	0,1	1,0	30-09-2011
NMISA	Rotronic	Hygrolog	0-100	0,1	1,6	09-11-2010
NIM	SHINYEI	M288-CTH	20-99	1	4	01-Sep-12
INMETRO	Oregon Scientific	BAR 988 HGT/Digital	25- 95	1	0,7	09-12-2010
KEBS	Technetics of Germany	MLm624n	0-80	0,1	1	Sep-11

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Annex 2 - Ambient conditions of the measurements

Micropipette 354853Z

NMI	Air temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water temperature (ºC)
IPQ	21,3	1003,73	51,7	21,72
CENAM	17,7	814,13	47,9	17,54
LNE	20,715	1028,74	56,3	20,360
UME	20,45	1002,07	54,2	19,628
NMISA	20,53	870,12	63,43	20,33
NIM	20,9	1037	54	19,88
INMETRO	19,78	1013	56	19,97
KEBS	22,56	839,50	63,52	22,70

Micropipette 354864Z

NMI	Air temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water temperature (ºC)
IPQ	21,3	1003,54	51,8	21,71
CENAM	17,9	814,23	46,9	17,60
LNE	20,665	1029,02	56,1	20,360
UME	20,50	1005,38	50,8	19,550
NMISA	21,86	862,59	58,11	20,47
NIM	21,4	1037	51	20,12
INMETRO	20,0	1009	60	19,9
KEBS	22,56	839,50	63,52	22,70

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Micropipette 354868Z

NMI	Air temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water temperature (ºC)
IPQ	22,7	1004,11	53,8	22,66
CENAM	18,1	814,47	46,9	17,73
LNE	20,667	1029,10	56,7	20,360
UME	20,60	1001,93	53,5	19,593
NMISA	21,8	869,14	55,31	20,3
NIM	21,2	1037	54	19,88
INMETRO	20,08	1009	60	19,99
KEBS	22,56	839,50	63,52	22,70

Micropipette 354872Z

NMI	Air temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water temperature (ºC)
IPQ	21,3	1003,64	51,3	21,70
CENAM	18,2	814,30	46,8	17,66
LNE	20,535	1028,95	56,2	20,360
UME	20,40	1002,13	51,2	19,561
NMISA	20,46	872,35	63,41	20,20
NIM	20,9	1037	47	20,30
INMETRO	20,0	1008	60	20,00
KEBS	22,56	839,50	63,52	22,70

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Annex 3 - Volume change determination for micropipette

Micropipette 354853Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/µL	V/µL	V_{corr}/µL
IPQ	997,84	100373	-0,012	99,98	99,99
CENAM	998,667	81413	-0,312	99,37	99,68
LNE	998,13	102874	0,019	100,16	100,14
UME	998,28	100207	-0,014	100,07	100,08
NMISA	998,1351	87012	-0,209	99,87	100,08
NIM	998,227	103700	0,029	99,91	99,88
INMETRO	998,209	101300	0,000	100,28	100,28
KEBS	997,63	83950	-0,263	99,65	99,91

Micropipette 354864Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/µL	V/µL	V_{corr}/µL
IPQ	997,84	100354	-0,012	99,96	99,97
CENAM	998,657	81423	-0,312	99,41	99,72
LNE	998,13	102902	0,020	100,15	100,13
UME	998,3	100538	-0,010	100,04	100,05
NMISA	998,1058	86259	-0,222	99,86	100,08
NIM	998,182	103700	0,029	99,99	99,96
INMETRO	998,205	100900	-0,005	100,35	100,36
KEBS	997,84	100354	-0,264	99,97	100,23

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Micropipette 354868Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/µL	V/µL	V_{corr}/µL
IPQ	997,62	100411	-0,012	100,07	100,08
CENAM	998,633	81447	-0,311	99,66	99,97
LNE	998,13	102910	0,020	100,31	100,29
UME	998,29	100193	-0,014	100,16	100,17
NMISA	998,1414	86912	-0,211	99,91	100,12
NIM	998,231	103700	0,029	99,91	99,88
INMETRO	998,206	100900	-0,005	100,59	100,60
KEBS	997,584	83940	-0,264	99,59	99,85

Micropipette 354872Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/µL	V/µL	V_{corr}/µL
IPQ	997,84	100364	-0,012	99,76	99,77
CENAM	998,646	81430	-0,311	99,12	99,43
LNE	998,12	102895	0,019	99,98	99,96
UME	998,30000	100213	-0,014	99,86	99,87
NMISA	998,1414	86914,2	-0,211	99,72	99,93
NIM	998,144	103700	0,029	99,65	99,62
INMETRO	998,205	100800	-0,007	100,22	100,23
KEBS	997,654	83960	-0,263	99,08	99,34

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Annex 4 - Degree of equivalence between the laboratories

(all results are in µL)

Micropipette 354828Z

	IPQ		CENAM		LNE		UME		NMISA		NIM		INMETRO		KEBS	
	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}
IPQ			-0,07	0,42	0,27	0,44	0,04	0,41	0,14	0,40	-0,13	0,38	0,33	0,43	-0,11	0,39
CENAM	0,07	0,42			0,34	0,47	0,11	0,45	0,21	0,43	-0,06	0,41	0,41	0,46	-0,04	0,42
LNE	-0,27	0,44	-0,34	0,47			-0,24	0,46	-0,14	0,45	-0,40	0,43	0,06	0,47	-0,38	0,43
UME	-0,04	0,41	-0,11	0,45	0,24	0,46			0,10	0,43	-0,17	0,41	0,30	0,45	-0,14	0,41
NMISA	-0,14	0,40	-0,21	0,43	0,14	0,45	-0,10	0,43			-0,27	0,39	0,20	0,44	-0,24	0,40
NIM	0,13	0,38	0,06	0,41	0,40	0,43	0,17	0,41	0,27	0,39			0,47	0,42	0,03	0,38
INMETRO	-0,33	0,43	-0,41	0,46	-0,06	0,47	-0,30	0,45	-0,20	0,44	-0,47	0,42			-0,44	0,42
KEBS	0,11	0,39	0,04	0,42	0,38	0,43	0,14	0,41	0,24	0,40	-0,03	0,38	0,44	0,42		

Micropipette 354853Z

	IPQ		CENAM		LNE		UME		NMISA		NIM		INMETRO		KEBS	
	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}
IPQ			-0,31	0,44	0,15	0,43	0,09	0,42	0,09	0,40	-0,11	0,38	0,29	0,51	-0,08	0,39
CENAM	0,31	0,44			0,46	0,48	0,40	0,47	0,40	0,45	0,20	0,43	0,60	0,55	0,23	0,44
LNE	-0,15	0,43	-0,46	0,48			-0,06	0,46	-0,06	0,45	-0,26	0,43	0,14	0,54	-0,23	0,43
UME	-0,09	0,42	-0,40	0,47	0,06	0,46			0,00	0,43	-0,20	0,41	0,20	0,53	-0,17	0,42
NMISA	-0,09	0,40	-0,40	0,45	0,06	0,45	0,00	0,43			-0,20	0,40	0,20	0,52	-0,17	0,41
NIM	0,11	0,38	-0,20	0,43	0,26	0,43	0,20	0,41	0,20	0,40			0,40	0,50	0,03	0,38
INMETRO	-0,29	0,51	-0,60	0,55	-0,14	0,54	-0,20	0,53	-0,20	0,52	-0,40	0,50			-0,37	0,51
KEBS	0,08	0,39	-0,23	0,44	0,23	0,43	0,17	0,42	0,17	0,41	-0,03	0,38	0,37	0,51		

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Micropipette 354864Z

	IPQ		CENAM		LNE		UME		NMISA		NIM		INMETRO		KEBS	
	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}
IPQ			-0,26	0,41	0,15	0,43	0,08	0,41	0,11	0,42	-0,01	0,38	0,38	0,41	0,26	0,39
CENAM	0,26	0,41			0,41	0,45	0,33	0,44	0,37	0,44	0,25	0,41	0,64	0,44	0,52	0,42
LNE	-0,15	0,43	-0,41	0,45			-0,08	0,46	-0,04	0,46	-0,16	0,43	0,23	0,46	0,11	0,44
UME	-0,08	0,41	-0,33	0,44	0,08	0,46			0,03	0,45	-0,09	0,41	0,31	0,44	0,18	0,42
NMISA	-0,11	0,42	-0,37	0,44	0,04	0,46	-0,03	0,45			-0,12	0,42	0,27	0,45	0,15	0,43
NIM	0,01	0,38	-0,25	0,41	0,16	0,43	0,09	0,41	0,12	0,42			0,39	0,41	0,27	0,39
INMETRO	-0,38	0,41	-0,64	0,44	-0,23	0,46	-0,31	0,44	-0,27	0,45	-0,39	0,41			-0,12	0,42
KEBS	-0,26	0,39	-0,52	0,42	-0,11	0,44	-0,18	0,42	-0,15	0,43	-0,27	0,39	0,12	0,42		

Micropipette 354868Z

	IPQ		CENAM		LNE		UME		NMISA		NIM		INMETRO		KEBS	
	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}
IPQ			-0,12	0,41	0,21	0,44	0,09	0,42	0,04	0,41	-0,20	0,39	0,51	0,48	-0,23	0,40
CENAM	0,12	0,41			0,33	0,44	0,21	0,42	0,16	0,41	-0,08	0,40	0,63	0,49	-0,11	0,40
LNE	-0,21	0,44	-0,33	0,44			-0,12	0,46	-0,17	0,44	-0,41	0,43	0,30	0,51	-0,44	0,43
UME	-0,09	0,42	-0,21	0,42	0,12	0,46			-0,05	0,42	-0,29	0,41	0,42	0,50	-0,32	0,41
NMISA	-0,04	0,41	-0,16	0,41	0,17	0,44	0,05	0,42			-0,24	0,40	0,47	0,48	-0,27	0,40
NIM	0,20	0,39	0,08	0,40	0,41	0,43	0,29	0,41	0,24	0,40			0,71	0,48	-0,03	0,39
INMETRO	-0,51	0,48	-0,63	0,49	-0,30	0,51	-0,42	0,50	-0,47	0,48	-0,71	0,48			-0,74	0,48
KEBS	0,23	0,40	0,11	0,40	0,44	0,43	0,32	0,41	0,27	0,40	0,03	0,39	0,74	0,48		

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Micropipette 354872Z

	IPQ		CENAM		LNE		UME		NMISA		NIM		INMETRO		KEBS	
	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}	D_{ij}	Ud_{ij}
IPQ			-0,34	0,45	0,19	0,43	0,10	0,41	0,16	0,40	-0,15	0,38	0,45	0,53	-0,43	0,58
CENAM	0,34	0,45			0,53	0,49	0,44	0,47	0,50	0,46	0,19	0,44	0,80	0,57	-0,09	0,62
LNE	-0,19	0,43	-0,53	0,49			-0,08	0,46	-0,03	0,44	-0,34	0,42	0,27	0,56	-0,61	0,61
UME	-0,10	0,41	-0,44	0,47	0,08	0,46			0,06	0,42	-0,26	0,41	0,35	0,55	-0,53	0,60
NMISA	-0,16	0,40	-0,50	0,46	0,03	0,44	-0,06	0,42			-0,31	0,39	0,30	0,54	-0,59	0,59
NIM	0,15	0,38	-0,19	0,44	0,34	0,42	0,26	0,41	0,31	0,39			0,61	0,52	-0,27	0,57
INMETRO	-0,45	0,53	-0,80	0,57	-0,27	0,56	-0,35	0,55	-0,30	0,54	-0,61	0,52			-0,88	0,68
KEBS	0,43	0,58	0,09	0,62	0,61	0,61	0,53	0,60	0,59	0,59	0,27	0,57	0,88	0,68		

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Annex 5 - Uncertainty contributions

Micropipette 354853Z

Uncertainty contributions (µL)	IPQ	CENAM	LNE	UME	NMISA	NIM	INMETRO	KEBS
Repeatability	0,045	0,1067	0,024	0,027	0,051	0,025	0,059	0,048
Balance	$3,7\times10^{-3}$	0,017	0,011	0,083		$5,77\times10^{-3}$	0,01	0,058
Air density	$2,89\times10^{-7}$	$1,31\times10^{-4}$	3×10^{-6}	$3,6\times10^{-4}$	$6,18\times10^{-6}$	$5,94\times10^{-5}$	$8,23\times10^{-8}$	$3,9\times10^{-5}$
Water density	$1,29\times10^{-6}$	$1,04\times10^{-3}$	2×10^{-3}	$1,2\times10^{-3}$	0,048	$5,80\times10^{-3}$	$3,52\times10^{-7}$	-
Density of the mass pieces	0,0346	$1,07\times10^{-4}$	-	-	$6,49\times10^{-5}$	$1,31\times10^{-4}$	$1,086\times10^{-7}$	-
Expansion coefficient	$6,93\times10^{-6}$	$3,36\times10^{-3}$	8×10^{-3}	5×10^{-3}	$3,8\times10^{-5}$	$8,024\times10^{-4}$	$1,62\times10^{-9}$	$7,0\times10^{-3}$
Water temperature	5×10^{-3}	$8,99\times10^{-4}$	3×10^{-3}	-	$3,9\times10^{-4}$	$1,34\times10^{-3}$	$2,59\times10^{-8}$	$2,2\times10^{-3}$
Evaporation	$1,90\times10^{-3}$	$1,48\times10^{-3}$	0,055	-	0,014	0,0115	-	-
Others			0,087	$5,8\times10^{-3}$	1×10^{-3}		0,159	$2,3\times10^{-3}$
Combined uncertainty (µL)	0,046	0,11	0,106	0,0084	0,071	0,0285	0,17	0,048
Expanded uncertainty (µl)	**0,10**	**0,26**	**0,21**	**0,18**	**0,14**	**0,06**	**0,34**	**0,11**
Stability and method standard uncertainty (µL)	0,13	0,13	0,13	0,13	0,13	0,13	0,13	0,13
Corrected expanded uncertainty (µL)	**0,27**	**0,34**	**0,33**	**0,32**	**0,30**	**0,27**	**0,43**	**0,28**

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Micropipette 354864Z

Uncertainty contributions (µL)	IPQ	CENAM	LNE	UME	NMISA	NIM	INMETRO	KEBS
Repeatability	0,038	0,1067	0,027	0,011	0,08	0,0247	0,027	0,054
Balance	$3{,}7\times10^{-3}$	0,017	0,011	0,083		$5{,}77\times10^{-3}$	0,01	0,058
Air density	$2{,}89\times10^{-7}$	$1{,}31\times10^{-4}$	3×10^{-6}	$3{,}5\times10^{-4}$	7×10^{-5}	$5{,}94\times10^{-5}$	$8{,}23\times10^{-8}$	$3{,}9\times10^{-5}$
Water density	$1{,}29\times10^{-6}$	$1{,}04\times10^{-3}$	2×10^{-3}	$1{,}2\times10^{-3}$	0,048	$5{,}80\times10^{-3}$	$3{,}52\times10^{-7}$	-
Density of the mass pieces	0,0346	$1{,}07\times10^{-4}$	-	-	$6{,}38\times10^{-5}$	$1{,}31\times10^{-4}$	$1{,}087\times10^{-7}$	-
Expansion coefficient	$6{,}93\times10^{-6}$	$3{,}26\times10^{-3}$	8×10^{-3}	$6{,}22\times10^{-3}$	$5{,}4\times10^{-4}$	$8{,}024\times10^{-4}$	$3{,}47\times10^{-9}$	$7{,}0\times10^{-3}$
Water temperature	5×10^{-3}	$1{,}69\times10^{-3}$	3×10^{-3}	-	$3{,}9\times10^{-4}$	$1{,}34\times10^{-3}$	$2{,}24\times10^{-8}$	$2{,}2\times10^{-3}$
Evaporation	$1{,}90\times10^{-3}$	$1{,}48\times10^{-3}$	0,055	-	0,014	0,0115	-	-
Others			0,087	$5{,}8\times10^{-3}$	1×10^{-3}		0,078	$2{,}3\times10^{-3}$
Combined uncertainty (µL)	0,038	0,078	0,107	0,0084	0,095	0,0285	0,083	0,054
Expanded uncertainty (µL)	**0,09**	**0,18**	**0,21**	**0,17**	**0,19**	**0,06**	**0,17**	**0,13**
Stability and method standard uncertainty (µL)	0,13	0,13	0,13	0,13	0,13	0,13	0,13	0,13
Corrected expanded uncertainty (µL)	**0,27**	**0,31**	**0,33**	**0,31**	**0,32**	**0,27**	**0,31**	**0,28**

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Micropipette 354868Z

Uncertainty contributions (µL)	IPQ	CENAM	LNE	UME	NMISA	NIM	INMETRO	KEBS
Repeatability	0,055	0,0539	0,015	0,018	0,037	0,038	0,0487	0,043
Balance	$3,70\times10^{-3}$	0,017	0,011	0,083		$5,77\times10^{-3}$	0,01	0,058
Air density	$2,89\times10^{-7}$	$1,31\times10^{-4}$	3×10^{-6}	$3,6\times10^{-4}$	$1,8\times10^{-6}$	$5,94\times10^{-5}$	$8,24\times10^{-8}$	$3,9\times10^{-5}$
Water density	$1,35\times10^{-6}$	$1,04\times10^{-3}$	2×10^{-3}	$1,2\times10^{-3}$	0,048	$5,80\times10^{-3}$	$3,52\times10^{-7}$	-
Density of the mass pieces	0,0346	$1,07\times10^{-4}$	-	-	$6,4\times10^{-5}$	$1,31\times10^{-4}$	$1,088\times10^{-7}$	-
Expansion coefficient	$6,93\times10^{-6}$	$3,08\times10^{-3}$	8×10^{-3}	$5,63\times10^{-3}$	$3,4\times10^{-4}$	$8,024\times10^{-4}$	$6,38\times10^{-9}$	$7,0\times10^{-3}$
Water temperature	5×10^{-3}	$2,08\times10^{-3}$	3×10^{-3}	-	$3,9\times10^{-4}$	$1,34\times10^{-3}$	$1,69\times10^{-7}$	$2,2\times10^{-3}$
Evaporation	$1,90\times10^{-3}$	$1,48\times10^{-3}$	0,055	-	0,014	0,0115	-	-
Others			0,087	$5,8\times10^{-3}$	1×10^{-3}		0,133	$2,3\times10^{-3}$
Combined uncertainty (µL)	0,055	0,060	0,105	0,0084	0,063	0,0408	0,14	0,044
Expanded uncertainty (µL)	**0,13**	**0,14**	**0,21**	**0,17**	**0,13**	**0,08**	**0,29**	**0,10**
Stability and method standard uncertainty (µL)	0,13	0,13	0,13	0,13	0,13	0,13	0,13	0,13
Corrected expanded uncertainty (µL)	**0,28**	**0,29**	**0,33**	**0,31**	**0,29**	**0,27**	**0,39**	**0,27**

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Micropipette 354872Z

Uncertainty contributions (μL)	IPQ	CENAM	LNE	UME	NMISA	NIM	INMETRO	KEBS
Repeatability	0,039	0,1147	0,024	0,017	0,037	0,0104	0,061	0,21
Balance	$3,70\times10^{-3}$	0,017	0,011	0,083		$5,77\times10^{-3}$	0,01	0,058
Air density	$2,89\times10^{-7}$	$1,31\times10^{-4}$	3×10^{-6}	$3,6\times10^{-4}$	$2,75\times10^{-6}$	$5,94\times10^{-5}$	$8,22\times10^{-8}$	$3,9\times10^{-5}$
Water density	$1,29\times10^{-6}$	$1,04\times10^{-3}$	2×10^{-3}	$1,2\times10^{-3}$	0,048	$5,80\times10^{-3}$	$3,52\times10^{-7}$	-
Density of the mass pieces	0,0346	$1,07\times10^{-4}$	-	-	$6,47\times10^{-5}$	$1,31\times10^{-4}$	$1,085\times10^{-7}$	-
Expansion coefficient	$6,93\times10^{-6}$	$3,13\times10^{-3}$	8×10^{-3}	$6,05\times10^{-3}$	$2,3\times10^{-4}$	$8,024\times10^{-4}$	$2,31\times10^{-9}$	$7,0\times10^{-3}$
Water temperature	5×10^{-3}	$3,03\times10^{-3}$	3×10^{-3}	-	$3,9\times10^{-4}$	$1,34\times10^{-3}$	$1,76\times10^{-8}$	$2,2\times10^{-3}$
Evaporation	$1,90\times10^{-3}$	$1,48\times10^{-3}$	0,055	-	0,014	0,0115	-	-
Others			0,087	$5,7\times10^{-3}$	1×10^{-3}		0,173	$2,3\times10^{-3}$
Combined uncertainty (μL)	0,039	0,12	0,106	0,0084	0,063	0,0176	0,184	0,21
Expanded uncertainty (μL)	**0,09**	**0,28**	**0,21**	**0,17**	**0,13**	**0,04**	**0,37**	**0,51**
Stability and method standard uncertainty (μL)	0,13	0,13	0,13	0,13	0,13	0,13	0,13	0,13
Corrected expanded uncertainty (μL)	**0,27**	**0,35**	**0,33**	**0,31**	**0,29**	**0,26**	**0,45**	**0,51**