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EURAMET.M.FF-K4.2.2014

Volume comparison at 100 µL – Calibration of micropipettes

Final Report

Pilot
Elsa Batista, IPQ

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1. Introduction

During the EURAMET TC-F meeting of 2014 and following the finalization of CCM.FF-K4.2.2011 comparison, it was agreed to start a Regional Key Comparison (KC) on volume measurements in the range of the microliter. This Regional Key Comparison registered at the JCRB website as EURAMET.M.FF-K4.2.2014 had the main purpose of comparing the results and methods of calibration of two 100 µL micropipettes (piston pipettes) allowing the participating laboratories to assess the agreement of their results and uncertainties despite their use of different equipment and calibration methods.

The Volume and Flow Laboratory of the Portuguese Institute for Quality (IPQ) - National Metrology Institute (NMI), as the pilot laboratory performed the initial and final measurements of the micropipettes.

Two 100 µL micropipettes (transfer package) were tested.

15 participants agreed to participate in this EURAMET key comparison, one was not a member or associated of BIPM and was be removed from this report.

The comparison started in July 2015 and ended in March 2016. The last result was received in May 2016.

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	Responsible	Date	Results sent
IPQ	Portugal	Elsa Batista	January 2015	
BEV	Austria	Michael Matus	February	31-03-2015
EIM	Greece	Zoe Metaxiotou	March	19-03-2015
INM	Romania	Maria Tudor	April	16-05-2015
GUM	Poland	Elzbieta Lenard	May	30-06-2015
SP	Sweden	Oliver Buker/ Per Wennergren	June	12-06-2016
DPM	Albania	Erinda Piluri	July	11-04-2016
BIM	Bulgaria	Mariana Miteva	August	24-09-2016
CMI	Czech Republic	Martina Vicarova/ Alena Vospělová	September	24-09-2015
MIRS	Slovenia	Urska Turnsek	October	13-11-2015
DMDM	Serbia	Ljiljana Micic	November	18-02-2016
FORCE	Denmark	Lise-Lote Grue	December	17-12-2015
MBM	Montenegro	Mirjana Mihailovic	January 2016	23-03-2016
BOM	Macedonia	Anastazija Sarevska	March 2016	06-05-2016
IPQ final	Portugal	Elsa Batista	April 2016	

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3. The transfer standard

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

The fixed micropipettes used for this comparison are made essentially of plastic material with a thermal expansion coefficient of 2.4×10^{-4} / ºC [1]. The serial numbers of the 2 micropipettes used are: 354828Z and 354853Z.

Figure 1- Fixed micropipette of 100 µL

4. The measurement procedure

4.1 Experimental method

All the participating NMIs used the gravimetric method to determine the amount of water that the micropipettes deliver at a reference temperature of 20 ºC, based on ISO 8655-6 [2] and ISO 4787 [3], with 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 balance calibration

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.

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4.2 Water characteristics

The water used by the participants NMIs had different characteristics summarised in Table 2.

Table 2 – Water characteristics

NMI	Type of water	Density Formula	Conductivity/µS/cm
IPQ	Ultra-pure	Tanaka	0.054
BEV	Type 3	ISO 8655-6	-
DMDM	Grade 3	Tanaka	1.9
INM	Ultra-pure	Tanaka	0.06
GUM	Distilled	Tanaka	1.32
SP	De-ionized	Tanaka	-
DPM	Distilled	Spieweck	0.1
BIM	Grade 3	Tanaka	1.4
CMI	De-ionized	Tanaka	1-2
MIRS	De-ionized	Kell	1.9
EIM	Grade 3	Tanaka	-
FORCE	Distilled	Spieweck	1.25
MBM	Distilled	Tanaka	<5
BOM	Distilled	Tanaka	2.2

All participants used at least distilled water. The majority used the Tanaka formula as the reference for water density. The conductivity was determined by the majority of the NMIs, and all of them presented lower values than the maximum allowed value of 5 µS/cm [3].

4.3 Equipment

Each NMI described the equipment used in the calibration and respective traceability by filling a form that was sent with the protocol. The summary of these characteristics is presented in the following table:

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Table 3 – Equipment characteristics

Equipment	Type	Resolution
Balance	Comparator	(0.001 – 0.01) mg
Weights	E1, E2	-
Water thermometer	Digital	(0.0001 – 0.1) ºC
Air thermometer	Digital	(0.001 – 0.1) ºC
Barometer	Digital	(0.01 – 0.5) hPa
Hydrometer	Digital	0.1 %

The last three instruments in the table were used to calculate the air buoyancy effect.

4.4 Ambient conditions of the measurements

The ambient conditions were described by all participants NMIs for the use of the 2 micropipettes. The first values given in Table 4 refer to mean values obtained for the micropipette 354828Z and the second to the micropipette 354853Z.

Table 4 - Ambient conditions

NMI	Air Temperature (ºC)	Pressure (hPa)	Relative humidity (%)	Water Temperature (ºC)
IPQ	17.9 – 21.0	1019.49 – 1005.11	50.9 – 63.4	17.46 – 20.60
BEV	20	972	32	20
DMDM	21.11 – 21.00	1005.66 – 1004.95	50.12-50.40	20.9 – 20.84
INM	21.0 – 20.9	1014.0 -1007.7	69	20.6 – 20.4
GUM	21.5 -20.95	1006.07 – 992.33	60.97 – 58.97	21.15 – 20.75
SP	20.672 – 20.739	995.38 – 995.40	49.4-49.3	19.885 – 19.907
DPM	20.17 – 20.56	1009.3 – 1008.3	56.7 – 56.51	19.93 – 20.30
BIM	25.65 – 25.67	954.45 – 954.42	45.39 – 42.46	25.45 -25.37
CMI	20.3 - 21	974.41 – 974.57	47.1 – 47.5	20.53 – 20.75
MIRS	20.44 – 20.38	991.02 – 990.68	55.07 – 55.02	19.68 – 17.72
EIM	20.4	1024.92 – 1024.17	41.7 – 42.3	19.84 – 20.05
FORCE	22.62	1014.15	42.6 – 42.4	22.47
MBM	19.55-20.3	1002.15 – 1005.6	57.7 – 58.55	19.30 – 19.75
BOM	21.5 – 22.1	978.7 – 984.2	50 – 50.3	20.5 – 22.1

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The majority of the NMIs presented values that are in agreement with the protocol proposal: humidity higher than 50 % and ambient temperature between 17 ºC and 23 ºC, although some declared values outside this specification. In all cases, the water temperature did not vary more than 0.5 ºC during the 10 measurements.
It can also be seen from Table 4 that there are some differences in atmospheric pressure due to altitude.

5. Measurement results

5.1 Stability of the micropipettes

During the comparison two different measurements of both micropipettes were performed by the pilot laboratory in order to verify the stability of the standards, one in the beginning and one at the end of the comparison. The first measurement was chosen to represent the IPQ results, according to the following table:

Table 5 - Stability of the instruments

Micropipette	Measurement	Date	Volume/µL	Uncertainty/µL	ΔV/µL
354828Z	**1**	January 2015	100.43	0.15	0.07
	2	April 2016	100.50	0.10	
354853Z	**1**	January 2015	100.38	0.14	0.09
	2	April 2016	100.47	0.10	

ΔV refers to the difference between both IPQ measurements and it is smaller than the presented uncertainty. The two results obtained by IPQ, for both micropipettes, are therefore consistent with each other. We conclude that the micropipettes had a stable volume during the entire comparison.

5.2 Results of participants NMIs

The results for the two micropipettes reported by the participating NMIs are presented in Table 6.

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Table 6 – Volume measurement results

NMI	354828Z		354853Z	
	V/μL	U/μL	V/μL	U/μL
IPQ	100.43	0.15	100.38	0.14
BEV	100.35	0.37	100.28	0.37
DMDM	100.58	0.20	100.46	0.16
INM	99.97	0.14	100.063	0.14
GUM	100.735	0.054	100.738	0.044
SP	100.22	0.10	100.29	0.13
DPM	100.45	0.48	101.00	0.50
BIM	100.00	0.25	99.76	0.24
CMI	100.11	0.22	100.07	0.30
MIRS	100.35	0.12	100.46	0.12
EIM	100.36	0.16	100.49	0.16
FORCE	100.46	0.15	100.47	0.15
MBM	100.43	0.20	100.56	0.20
BOM	100.37	0.35	100.43	0.34

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 location X2 (with $p_{L,X2}$ atmospheric pressure at location X2) compared to a location X1 (with $p_{L,X1}$ atmospheric pressure at location X1) can be calculated using the following formula [5]:

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

Where,

ΔV/μL:	Volume change that results in the calibration at location X1 versus 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 location X2

Table 7 presents the corrected values of the participants for a standard atmospheric pressure of 1013.25 hPa using equation 2. These values will be used for the determination of the reference value and consistency of the results.

h_w/m = 0.030 and V_t/ µL = 437 were the values given by the manufacturer [6] for these micropipettes for air pressure correction.

Table 7 – Volume measurement results corrected for atmospheric pressure

NMI	354828Z		354853Z	
	V/µL	*U*/µL	*V*/µL	*U*/µL
IPQ	100.42	0.15	100.37	0.14
BEV	100.40	0.37	100.33	0.37
DMDM	100.59	0.20	100.47	0.16
INM	99.97	0.14	100.07	0.14
GUM	100.74	0.054	100.76	0.044
SP	100.24	0.10	100.31	0.13
DPM	100.45	0.48	101.01	0.50
BIM	100.08	0.25	99.84	0.24
CMI	100.16	0.22	100.12	0.30
MIRS	100.38	0.12	100.49	0.12
EIM	100.35	0.16	100.48	0.16
FORCE	100.46	0.15	100.47	0.15
MBM	100.44	0.20	100.57	0.20
BOM	100.41	0.35	100.47	0.34

The volume correction for the micropipette 354828Z is presented in Table 8 and for the micropipette 354853Z in Table 9.

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Table 8 – Volume change determination for micropipette 354828Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/μL	V/μL	V_{corr}/μL
IPQ	998.688	101914	0.007	100.43	100.42
BEV	998.207	97200	-0.054	100.35	100.40
DMDM	998.020	100566	-0.010	100.58	100.59
INM	998.081	101400	0.001	99.97	99.97
GUM	997.962	100607	-0.009	100.74	100.74
SP	998.230	99538	-0.023	100.22	100.24
DPM	998.232	100930	-0.005	100.45	100.45
BIM	996.929	95445	-0.078	100.00	100.08
CMI	998.096	97441	-0.051	100.11	100.16
MIRS	998.270	99102	-0.029	100.35	100.38
EIM	998.237	102492	0.014	100.36	100.35
FORCE	997.659	101415	0.001	100.46	100.46
MBM	998.350	100215	-0.014	100.43	100.44
BOM	998.098	97870	-0.045	100.37	100.41

Table 9 – Volume change determination for micropipette 354853Z

NMI	ρ_w/(kg/m^3)	$p_{L,X2}$/Pa	ΔV/μL	V/μL	V_{corr}/μL
IPQ	998.709	101949	0.008	100.38	100.37
BEV	998.207	97200	-0.054	100.28	100.33
DMDM	998.030	100494	-0.011	100.46	100.47
INM	998.123	100770	-0.007	100.06	100.07
GUM	998.049	99233	-0.027	100.74	100.76
SP	998.227	99540	-0.023	100.29	100.31
DPM	998.154	100830	-0.006	101.00	101.01
BIM	996.947	95442	-0.078	99.76	99.84
CMI	998.047	97457	-0.051	100.07	100.12
MIRS	998.238	99068	-0.029	100.46	100.49
EIM	998.193	102417	0.014	100.49	100.48
FORCE	997.659	101415	0.001	100.47	100.47
MBM	998.258	100560	-0.010	100.56	100.57
BOM	997.746	98420	-0.038	100.43	100.47

5.4 Uncertainty correction

The “process-related handling contribution” uncertainty should always be included in the determination of the measurement uncertainty according to the Guideline DKD-R 8-1[7]. This contribution value encompasses the influences on the dispensed volume which occur due to handling of the devices during the calibration of micropipettes. The DKD – R 8-1 guideline recommends to include a value of 0.07 % of the nominal volume of the micropipettes as the standard uncertainty for "process-related handling contribution". This value was added to the uncertainty budget of all participants in order to have a more realistic uncertainty result. The final results with all corrections applied are presented in Table 10.

Table 10 – Final volume measurement results (corrected)

NMI	354828Z		354853Z	
	V/µL	*U*/µL	*V*/µL	*U*/µL
IPQ	100.42	0.19	100.37	0.19
BEV	100.40	0.38	100.33	0.38
DMDM	100.59	0.24	100.47	0.21
INM	99.97	0.20	100.07	0.20
GUM	100.74	0.15	100.76	0.15
SP	100.24	0.17	100.31	0.19
DPM	100.45	0.50	101.01	0.52
BIM	100.08	0.29	99.84	0.28
CMI	100.16	0.26	100.12	0.33
MIRS	100.38	0.18	100.49	0.18
EIM	100.35	0.21	100.48	0.21
FORCE	100.46	0.21	100.47	0.21
MBM	100.44	0.24	100.57	0.24
BOM	100.41	0.38	100.47	0.37

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

To determine the reference value of this regional key comparison (RV) the weighted mean (3) was selected, using the inverses of the squares of the associated standard uncertainties as the weights [8], according to the instructions given by the BIPM:

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

To calculate the standard uncertainty *u(y)* associated with the volume *y* [8] equation (4) was used:

$$u(y) = \sqrt{\frac{1}{1/u^2(x_1) + \ldots + 1/u^2(x_n)}} \quad (4)$$

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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 [8].

$$\chi^2_{obs} = \frac{(x_1 - y)^2}{u^2(x_1)} + \ldots + \frac{(x_n - y)^2}{u^2(x_n)} \qquad (5)$$

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 RV x_{ref} and $U(x_{ref})$ was accepted as the expanded uncertainty of the RV.

If the consistency check failed then the NMI 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 NMI. When the consistency check passes, for each NMI results, x_i the degree of equivalence d_i between each NMI and the RV (x_{ref}) is calculated using the following formulas [8]:

$$d_i = x_I - x_{ref} \qquad (6)$$

$$U(d_i) = 2 \times u(d_i) \qquad (7)$$

where $u(d_i)$ is calculated from

$$u^2(d_i) = u^2(x_i) - u^2(x_{ref}) \qquad (8)$$

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

To calculate the degrees of equivalence d_{ij} between the NMIs the following formulas are used [8]:

$$d_{i,j} = x_i - x_j \qquad (9)$$

$$U(d_{i,j}) = 2 \times u(d_{i,j}) \qquad (10)$$

where $u(d_{i,j})$ is calculated from

$$u^2(d_{i,j}) = u^2(x_i) + u^2(x_j) \qquad (11)$$

The factor 2 in equation (7 and 10) corresponds to 95 % coverage under the assumption of normality.

The normalized error $E_{n,i}$ describes the degree of equivalence of a laboratory related to the RV.

$E_{n,i}$ was calculated for each reported value of the participant as follows,

$$E_{n,i} = d_i / U(d_i) \qquad (12)$$

If $|E_{n,i}| \leq 1$, the measurement is generally consider as acceptable and the measured values are consistent.

6.1 Micropipette 354828Z

The first RV obtained for micropipette 354828Z is 100.38 µL. The obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0.06 µL.
The calculated value $\chi^2(\nu)$ = 22.36 is smaller than χ^2_{obs} = 54.88 the observed value, therefore the results are not consistent with each other and with the reference value from a statistical point of view, so that the values with the larger E_i value and $\frac{(x_i - y)^2}{u^2(x_i)}$ will be deleted by magnitude until the values obtained are consistent. This deletion will be done one at the time.

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Table 11 – Degree of equivalence with RV 354828Z

NMI	d_i(µL)	Ud_i(µL)	E_i	Info
IPQ	0.04	0.18	0.21	
BEV	0.02	0.38	0.05	
DMDM	0.21	0.24	0.87	
INM	-0.42	0.19	-2.20	Excluded
GUM	0.36	0.14	2.61	Excluded
SP	-0.14	0.16	-0.88	
DPM	0.07	0.50	0.14	
BIM	-0.31	0.28	-1.09	
CMI	-0.22	0.25	-0.88	
MIRS	-0.01	0.17	-0.03	
EIM	-0.04	0.20	-0.19	
FORCE	0.07	0.20	0.38	
MBM	0.06	0.24	0.25	
BOM	0.04	0.18	0.21	

After removing first INM and second GUM the obtained RV for micropipette 354828Z is now 100.36 µL and the obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0.07 µL.
The calculated value $\chi^2(\nu) = 19.67$ is larger than $\chi^2_{obs} = 13.67$, the observed value, therefore the results are now 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 Figure 2:

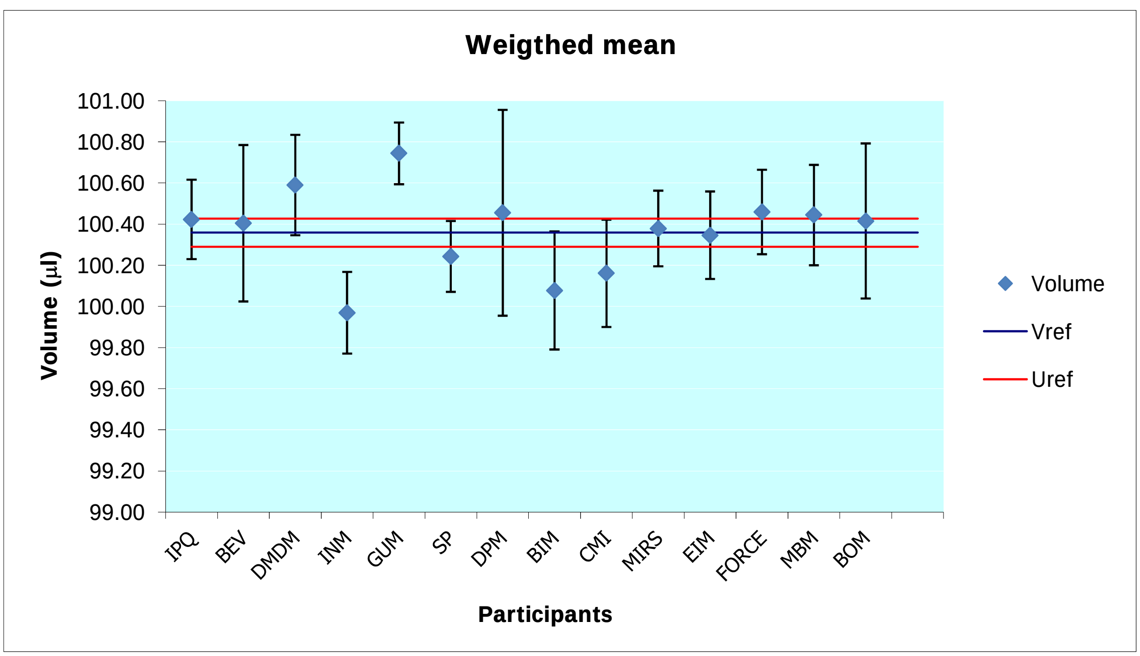

Figure 2- Measurement results of micropipette 354828Z with reference value and uncertainty

The degree of equivalence with the RV is presented in Figure 3:

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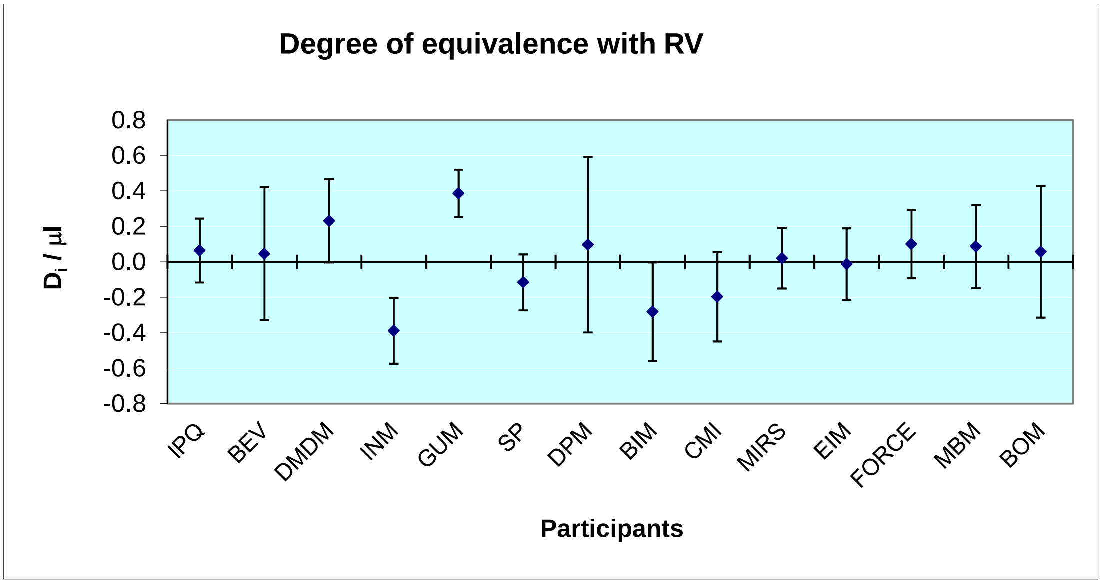

Figure 3- Degree of equivalence with reference value of micropipette 354828Z with reference value and uncertainty

Table 12 – Degree of equivalence with RV for micropipette 354828Z

NMI	d_i/µL	$U(d_i)$/µL	E_i
IPQ	0.06	0.18	0.35
BEV	0.05	0.37	0.12
DMDM	0.23	0.23	0.98
INM	-0.39	0.19	-2.10
GUM	0.39	0.13	2.89
SP	-0.12	0.16	-0.74
DPM	0.10	0.50	0.19
BIM	-0.28	0.28	-1.01
CMI	-0.20	0.25	-0.79
MIRS	0.02	0.17	0.11
EIM	-0.01	0.20	-0.07
FORCE	0.10	0.19	0.52
MBM	0.09	0.23	0.36
BOM	0.06	0.18	0.35

6.2 Micropipette 354853Z

The first RV obtained for micropipette 354853Z is 100.43 µL, the expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0.06 µL.
The calculated value $\chi^2(\nu)$ = 22.36 is smaller than χ^2_{obs}= 64.93, the observed value, therefore the results are not consistent with each other and with the reference value from a statistical point of view and so the values with the

larger E_i value and $\frac{(x_i - y)^2}{u^2(x_i)}$ will be deleted by magnitude order until the values are consistent. This deletion will be done one at the time.

Table 13 – Degree of equivalence with RV 354853Z

NMI	d_i(µL)	Ud_i(µL)	E_i	Info
IPQ	-0.06	0.18	-0.32	
BEV	-0.09	0.38	-0.25	
DMDM	0.04	0.20	0.20	
INM	-0.36	0.19	-1.90	Excluded
GUM	0.34	0.13	2.50	Excluded
SP	-0.12	0.18	-0.64	
DPM	0.58	0.52	1.12	
BIM	-0.59	0.27	-2.18	Excluded
CMI	-0.31	0.33	-0.94	
MIRS	0.06	0.17	0.34	
EIM	0.05	0.20	0.23	
FORCE	0.04	0.20	0.20	
MBM	0.14	0.24	0.59	
KMA	0.04	0.36	0.11	
BOM	-0.06	0.18	-0.32	

After removing INM, GUM and BIM, by that order, the obtained RV for micropipette 354853Z is now 100.44 µL and the obtained expanded uncertainty $U = 2 \times u(y)$ of the reference value is: 0.07 µL.
The calculated value $\chi^2(\nu)$ = 18.30 is larger than χ^2_{obs} = 12.76, the observed value, therefore the results are now consistent with each other and with the reference value from a statistical point of view.

All measurement results, reference value and its uncertainty are presented in Figure 4:

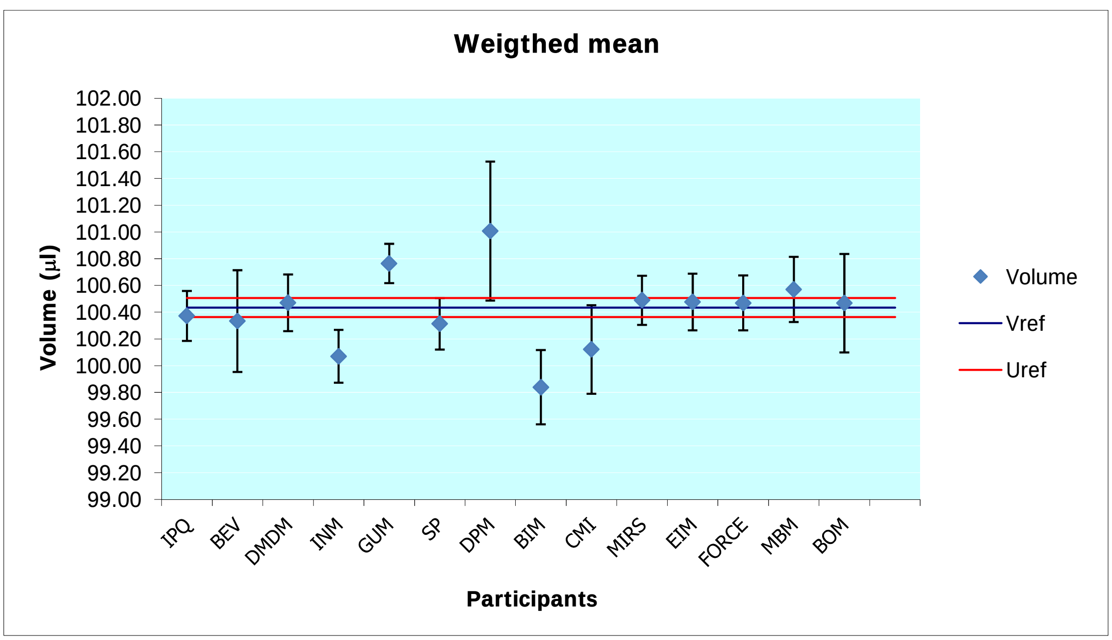

Figure 4- Measurement results of micropipette 354853Z with reference value and uncertainty

The degree of equivalence with the RV is presented in Figure 5:

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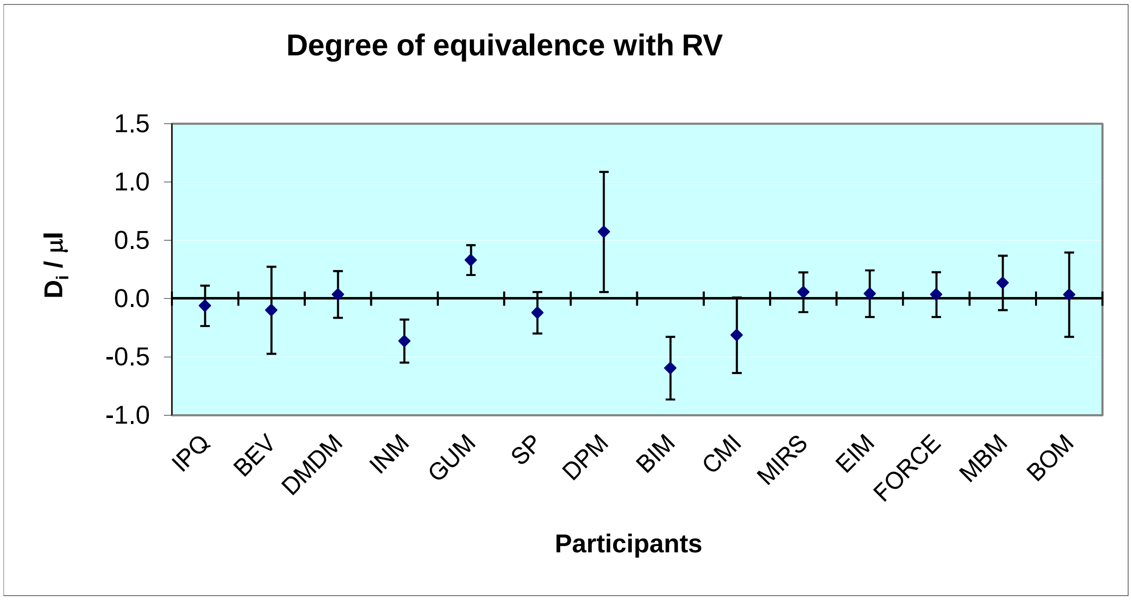

Figure 5- Degree of equivalence with reference value of micropipette 354853Z with reference value and uncertainty

Table 14 – Degree of equivalence with RV for micropipette 354853Z

NMI	d_i/µL	$U(d_i)$/µL	E_i
IPQ	-0.06	0.17	-0.36
BEV	-0.10	0.37	-0.27
DMDM	0.04	0.20	0.18
INM	-0.37	0.18	-1.98
GUM	0.33	0.13	2.57
SP	-0.12	0.18	-0.69
DPM	0.57	0.51	1.11
BIM	-0.60	0.27	-2.22
CMI	-0.31	0.32	-0.97
MIRS	0.05	0.17	0.32
EIM	0.04	0.20	0.21
FORCE	0.03	0.19	0.18
MBM	0.13	0.23	0.58
KMA	-0.06	0.17	-0.36
BOM	-0.10	0.37	-0.27

After all statistical analyses were done and the chi-square test passes for both micropipettes there are still some laboratories with E_i values in the range $1 < |E_i| \leq 1.2$ which is the "warning level". In this case some actions to check are recommended to the laboratory.

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7. Uncertainty calculation

It was requested that all participants NMIs present their uncertainty budget according to a spreadsheet supplied by the pilot laboratory in the comparison protocol. The results for the micropipette 354828Z are presented in Table 15, the results for micropipette 354853Z are very similar, and all values are described as absolute values.

Table 15 – Uncertainty contributions for micropipette 354828Z

Uncertainty contributions (μL)	IPQ	BEV	DMDM	INM	GUM	SP	DPM	BIM
Repeatability	0.064		0.065	0.0384	0.010	0.04	0.040	5.51×10^{-2}
Balance	1.64×10^{-2}		0.073	0.057	6×10^{-3}	2.7×10^{-2}	0.24	2.87×10^{-2}
Air density	5.4×10^{-5}		2.55×10^{-8}	2.53×10^{-5}	2.4×10^{-5}	2.54×10^{-3}	0.000214	9.49×10^{-5}
Water density	1.4×10^{-4}		1.5×10^{-7}	2.06×10^{-4}	1.2×10^{-3}	2.89×10^{-3}	1.1×10^{-5}	-1.16×10^{-4}
Density of the mass pieces	6.67×10^{-5}		1.32×10^{-7}	5.59×10^{-5}	2.2×10^{-7}	1.04×10^{-4}	7.59×10^{-5}	7.07×10^{-5}
Expansion coefficient	1.73×10^{-3}		6.29×10^{-7}	8.31×10^{-4}	0.02	1.94×10^{-3}	0.004871	-2.62×10^{-2}
Water temperature	1.2×10^{-4}		2.74×10^{-7}	1.20×10^{-3}	-	2.08×10^{-4}	0.00139	-8.41×10^{-2}
Evaporation	1.9×10^{-3}		0.003	-	0.012	8.66×10^{-3}	0.003464	9.00×10^{-2}
Others			0.002	-	1.4×10^{-3}		0.039827	5.00×10^{-2}
Combined Uncertainty (μL)	0.066	0.18	0.098	0.0686	0.0268	0.05	0.24	0.12
Expanded uncertainty (μL)	**0.15**	**0.37**	**0.20**	**0.14**	**0.054**	**0.10**	**0.48**	**0.25**

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Uncertainty contributions (µL)	CMI	MIRS	EIM	FORCE	MBM	BOM
Repeatability	0.11	3.71×10^{-2}	0.05	0.0212	6.66×10^{-3}	0.085
Balance	0.0072	4.01×10^{-2}	0.011	0.0299	7.93×10^{-3}	0.02
Air density	2.54×10^{-8}	2.22×10^{-4}	6.95×10^{-5}	0.0058	2.54×10^{-8}	5.78268×10^{-5}
Water density	4.51×10^{-8}	1.09×10^{-3}	5.9×10^{-4}	0.0058	3.50×10^{-7}	0.00053
Density of the mass pieces	3.46×10^{-6}	1.30×10^{-4}	1.6×10^{-5}	0.0000	1.32×10^{-7}	7.3×10^{-5}
Expansion coefficient	0.001	4.82×10^{-3}	8×10^{-3}	0.0000	4.90×10^{-7}	0.00034
Water temperature	0.0001	1.28×10^{-3}	2.4×10^{-4}	0.0661	3.61×10^{-7}	0.0008
Evaporation	0	2.44×10^{-2}		0.0058		
Others			0.0254	0.0033	1.00×10^{-1}	0.15
Combined Uncertainty (µL)	0.11	0.06	0.08	0.076	0.10	0.17
Expanded uncertainty (µL)	**0.22**	**0.12**	**0.16**	**0.15**	**0.20**	**0.35**

From the previous table it can be seen that the variability of the expanded uncertainty is quite large, reaching more than 50 %. The expanded uncertainty varies from 0.05 µl to 0.48 µl. That means that the uncertainty determination procedure is not yet harmonized between laboratories.
In general the largest uncertainty component is the contribution of repeatability of the measurements.

After draft A report was issued several laboratories informed the pilot laboratory that the uncertainty values were underestimated, but according to the rules of BIPM it is not possible to change results at this stage, so the values were not corrected.

8. Comparison with CIPM KCRV

The two micropipettes that circulated within the European laboratories were two of five that were calibrated earlier by eight laboratories from three regional metrology organizations SIM (America), APMP (East Asia and Australia), EURAMET (Europe) and AFRIMETS (Africa) in the CIPM Key comparison – CCM-FF.K4. One laboratory from Europe IPQ (Portugal) re-measured the changed volume of these micropipettes. The linking procedure and its uncertainty analysis are based on the principles which are given in the papers of Elster et al, [9], Kharitonov et al, [10] and Decker et al, [11]. Considering IPQ as the linking laboratory, the results are presented in Figure 6 and Figure 7.

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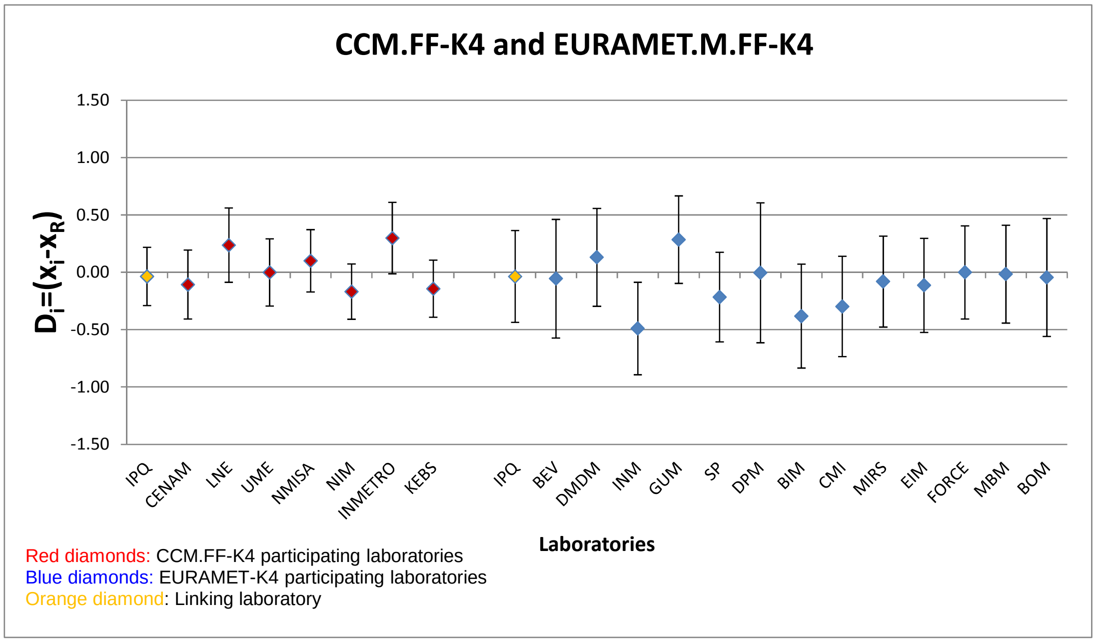

Figure 6- Degree of equivalence with respective KCRV – 354828Z

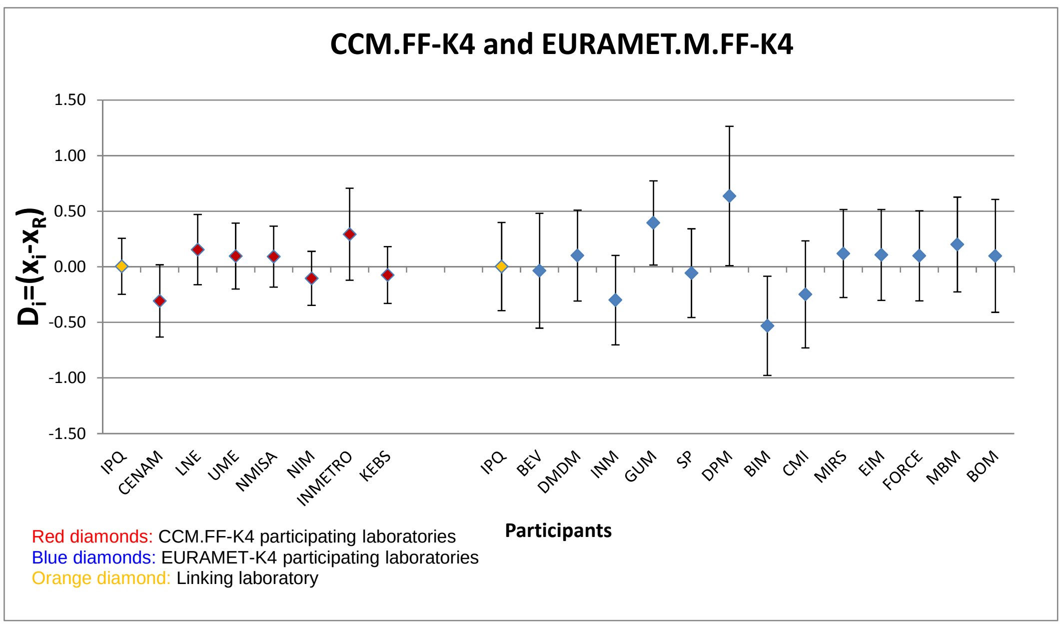

Figure 7- Degree of equivalence with respective KCRV – 354853Z

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9. CMC

The following table summarizes the uncertainty claims as published in the KCDB and those given by the participants of this comparison.

Table 16 - Expanded standard uncertainty claims as stated by the participants for this comparison and as published in the KCDB (CMCs).

NMI	*U*/%	*U* CMC/%
IPQ	0.15	0.3 – 0.1
BEV	0.37	0.4 – 0.01
DMDM	0.20	0.6 – 0.1
INM	0.14	-
GUM	0.054	-
SP	0.10	Only glassware
DPM	0.48	-
BIM	0.25	-
CMI	0.22	2
MIRS	0.12	Only glassware
EIM	0.16	0.2 – 0.05
FORCE	0.15	0.24 - 0.84
MBM	0.20	-
BOM	0.35	0.3 – 0.2

There are several NMIs that don't have CMCs in the field of micropipettes. The majority of laboratories that have CMCs presented uncertainties values according to the ones stated in the KCDB. Only FORCE presented a value lower that the claim uncertainty.

10.Conclusions

The micropipettes showed a stable volume during the whole comparison, which was confirmed by the results from IPQ, the pilot laboratory.

The original results of all participant NMIs were corrected to the standard atmospheric pressure in order to compare results under the same calibration conditions, and the contribution of the "process-related handling contribution" was added to the uncertainty budget of each participant.

For the micropipette 354828Z, two laboratories had inconsistent results. For micropipette 354853Z, three laboratories had inconsistent results.

There is a large variability in the uncertainty values presented by the participating laboratories, which means that the uncertainty procedure is not yet harmonized, considering that for micropipettes the largest source of uncertainty arises from the repeatability and not from the calibration method.

In general the declared CMC are according to the KCDB.

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It is recommended that all laboratories add the process-related handling contribution uncertainty to their uncertainty budget and to the declared CMCs.

11. References

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