and

Instituto Português da Qualidade

Final Report
Inter-laboratory calibration comparison of a 100 microliter micropipette

European Association of National Metrology Institutes

EURAMET Project no. 1159

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Elsa Batista

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Contents

1. Introduction

In 2007 the EUROMET comparison 865 regarding the calibration of 1000 µl micropipette was performed. This EUROMET comparison was the first one to be performed in the field of volume determination at the microliter (µl) level.
The calibration of micropipettes was at that time a relatively recent capability for the majority of National Metrology Institutes (NMI), so this lack of long term experience contributed to the observed dispersion in the results. The number of participating laboratories (6) was small and provided a small population for the statistical significance of the results. This was not very helpful for the needed conclusions.
Some lessons were learned from the experience gained and it was recommended at the time to repeat this comparison in the near future with more participants, in order to gather more information and experience about the calibration procedures.

During the EURAMET TC "Flow" meeting, held in Scotland in March 2010, it was agreed to start a comparison of a micropipette in order to verify if this experience were gained by the participants and it was proposed to use a more rigorous and detailed protocol in order to reduce possible errors in liquid handling.

The Portuguese Institute for Quality (IPQ) and Force Technology, acting as the pilots laboratories performed the initial and final measurements of the micropipette.

The project details were sent to all the members and 10 NMIs agreed to participate. The circulation of the micropipette started in June 2010 and was concluded in May 2011.
Each country took 3 weeks to perform the calibration of the micropipette. The participants are presented in table 1, by alphabetic order.

Table 1 – Participants of the EURAMET Project 1159

Country	Laboratory	Responsible
Austria	BEV	Michael Matus
Denmark	FORCE	Lene S. Kristensen
Finland	MIKES	Kari Riski
France	BNM-LNE	Paul-André Meury/ T. Madec
Fyr Macedonia	BoM	Anastazija Sarevska
Greece	EIM	Zoe Metaxiotou
Portugal	IPQ	Elsa Batista
Serbia	DMDM	Ljiljana Micic
Turkey	UME	Ȗmit Akcadag
United Kingdom	NMO	John Pain

Participants presented a report of their measurements before the end of the comparison according to a spreadsheet supplied by the pilot laboratory, Annex 1.

2. The instrument

The chosen instrument is single channel fixed micropipette of low nominal value, 100 µl. The micropipette needs to have attached a removable plastic tip in order to aspirate the liquid. IPQ acting as the pilot laboratory supplied the tips.

In the following figure is described the fixed micropipette used for this comparison made essentially of plastic with a coefficient of thermal expansion of 2,4 $\times 10^{-4}$ /ºC [1].

Figure 1- Eppendorf fixed micropipette of 100 µl

3. Volume definition

Calibration of the micropipette consisted of the determination of the amount of water that the micropipette delivers at reference temperature of 20 ºC, using the gravimetric method and the following equation described in ISO standard 4787 [2].

$$V_0 = (I_\mathrm{I} - I_\mathrm{E}) \times \frac{1}{\rho_\mathrm{W} - \rho_\mathrm{A}} \times \left(1 - \frac{\rho_\mathrm{A}}{\rho_\mathrm{B}}\right) \times [1 - \gamma(t - t_0)] \qquad (1)$$

Where:

V_0 volume, at the reference temperature t_0, in mL

I_I weighing result of the recipient full of liquid, in g

I_E weighing result of the empty recipient, in g

ρ_W liquid density, in g/mL, at the calibration temperature t, in ºC

ρ_A air density, in g/mL

ρ_B density of masses used during measurement (substitution) or during calibration of the balance, assumed to be 8,0 g/mL

γ cubic thermal expansion coefficient of the material of the instrument under calibration, in °C^{-1}

t liquid temperature used in the calibration, in °C

t_0 reference temperature, in ºC

4. The method

In the protocol several details were given in order to minimize experimental errors.

4.1 Getting the micropipettes ready for volume measurements

For temperature uniformity, it was requested to bring the micropipettes, the tips and the water that was used in these tests into the measurement laboratory at least 24 hours before any measurement was performed, at a temperature near 20 ºC.

4.2 Ambient conditions of the measurements

The ambient conditions of the laboratory room during the measurements suggested in the protocol were following:

Humidity higher than 50 %.

Ambient temperature between 17 ºC and 23 ºC.

The water temperature must be near the air temperature and shall not vary more than 0,5 ºC during the tests.

4.3 Calibration procedure – important details according to ISO Standard 8655

Some important details described in ISO 8655 [3] were suggested:

The weighing vessel should have a film of water (3 mm) before starting the measurements. The use of a lid or an evaporation trap was suggested.

Deliver the water from the micropipette to the weighing vessel touching the recipient in an angle between 30º to 60º and adding the drop retained at the end of the tip of the micropipette.

Change tip and wetted before each measurement.

Each participant laboratory should perform 10 consecutives measurements.

The volume results should be presented for a reference temperature of 20 ºC.

4.4 Equipment

Each laboratory described the equipment used in the calibration and the respective traceability.

The equipment used by each country is described in Annex 2.

4.5 Type of water

The water should have the quality suitable for the purpose of the calibration.

Table 2 – Water characteristics

Laboratory	Type	Density reference	Conductivity (µS/cm)
IPQ	Ultra pure	Tanaka	0,054
FORCE	Distilled	Spieweck	1,2
BEV	Ultra pure	-	-
MIKES	Distilled	Metrologia 38, 2001	1 – 2
EIM	Distilled	-	-
NMO	Distilled	0,998207 kg/m^3	-
BNM-LNE	Bi-distilled	0,99997 kg/m^3	0,1
BoM	Distilled	-	1,35
UME	Distilled	Tanaka	0,79
DMDM	Distilled	Tanaka	0,3

All participants used at least distilled water; The countries who presented conductivity values are all according to the ISO 3696 [4] < 5µS/cm.

4.6 Mass standards

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

Table 3 – Mass characteristics

Laboratory	OIML Class	Density (kg/m^3)
IPQ	E2	8000
FORCE	F1	8000
BEV	E2	8000
MIKES	F1	7960
EIM	F1	7950
NMO	-	-
BNM-LNE	F1	7980
BoM	E1	-
UME	E2	8000
DMDM	E2	7050

4.7 Balance

Some information about the type of balance used was also requested:

Table 4 - Balance

Laboratory	Type	Range	Resolution
IPQ	Mettler AX 26	0-22 g	0,001 mg
FORCE	Mettler-Toledo AX205	80/220 g	0,01 mg
BEV	Sartorius CC100	70 g	0,001 mg
MIKES	Sartorius-MC 210S	210 g	0,01 mg
EIM	Mettler-Toledo XP205	220 g	0,01 mg
NMO	-	-	-
BNM-LNE	Mettler XP26PC	22 g	0,001 mg
BoM	Sartorius-ME 235S	0 - 230	0,01 mg
UME	Sartorius-ME 235S	0 - 230	0,01 mg
DMDM	GPC 225 - CW	0 – 220 g	0,01 mg

5. Ambient conditions

The ambient conditions were described by all participants.

Table 5 - Ambient conditions

Laboratory	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/cm^3)
IPQ-1	20	997	73,8	0,0012
FORCE-1	22,7	1005,5	59,8	0,001178
BEV	20,21	992,6	50	0,00116
MIKES	21,3	1009	70	0,001189
EIM	22,4	1015,5	42,7	0,001192231
NMO	18,2	1001,05	45,6	-
BNM-LNE	22,12	1018	65,6	0,0011937
BoM	21	983,6	70	0,001157457
UME	19,8	992,26	58,9	0,001174
DMDM	18,94	1017	54,09	0,0012
FORCE-2	21,1	1012,7	28,9	0,001194
IPQ-2	22,5	1005	61,7	0,0012

There were some problems with the low humidity that causes static electricity, and may have raised the uncertainty of the measurement in some laboratories, this low humidity can also cause some problems with evaporation.

6. Measurement results

6.1 Stability of the TS

FORCE acting as the pivot laboratory made a calibration of the TS in the beginning and at the end of the comparison. The first measurement result obtained was considered to be the official results of FORCE. Also IPQ as the pilot who supplied the artefact performed measurements before the start and after the end of the comparison. The results are presented in the following table:

Table 6 - Stability of the TS

NMI	Measurement	Date	Volume (ml)	Uncertainty (ml)	Δ*V* (ml)
FORCE	**Initial**	July 2010	99,94	0,18	0,04
	Final	April 2011	99,98	0,20	
IPQ	**Initial**	June 2010	100,12	0,13	0,03
	Final	April 2011	100,09	0,12	

The initial and final results obtained by both IPQ and FORCE are consistent with each other. The difference in measured volume is considerably smaller than the stated uncertainty. This demonstrates that the TS had a stable volume during the entire comparison.

The measurement results presented by each participant are collected in table 7.

Table 7 – Volume measurements

Laboratory	Volume (µl)	U_{exp}(µl)
IPQ-1	100,12	0,13
FORCE-1	99,94	0,18
BEV	100,26	0,16
MIKES	99,86	0,15
EIM	100,004	0,15
NMO	97,43	0,3
BNM-LNE	100,229	0,2
BoM	99,91	0,10
UME	100,28	0,20
DMDM	99,85	0,10
FORCE-2	99,98	0,20
IPQ-2	100,09	0,12

There are a total of 12 measurements of 10 laboratories. FORCE and IPQ performed 2 measurements: at the beginning and at the end of the comparison, but only the first results was taken into account for the determination of the reference value.

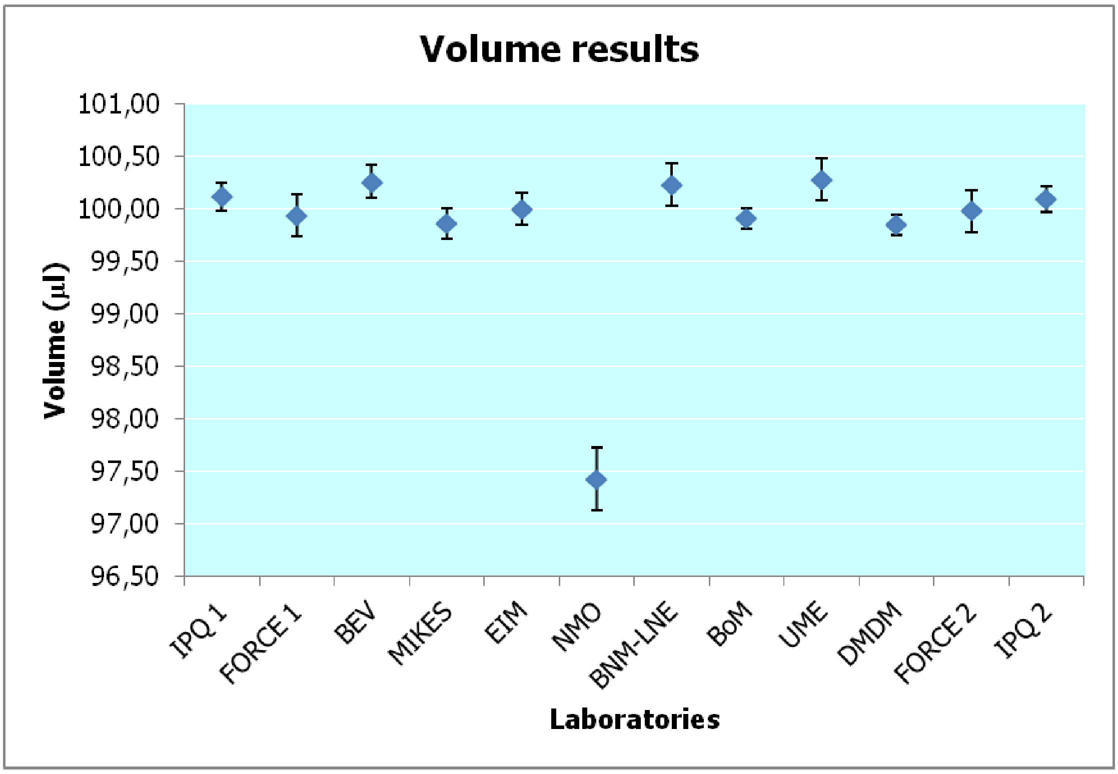


Figure 2 – Volume measurements

It's clear that the result of lab 4 is an outlier and therefore it will not be taken in to account for the determination of the reference value.

6.2 Determination of the reference value

To calculate the reference value (RV) three different approaches were tested:

- Mean [5]:

$$\bar{x} = \frac{1}{n}\sum_{j=1}^{n} x_j$$

- Median [6]:

$$M = \begin{cases} x_{\frac{n+1}{2}:n} & \text{, for n odd} \\ \dfrac{x_{\frac{n}{2}:n} + x_{\frac{n}{2}+1:n}}{2} & \text{, for n even} \end{cases}$$

The median [6] presented in figure 3 was calculated using the Monte Carlo Simulation, procedure B of reference [7], for one million trials.

- Weighted mean [7]:

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

In the following figure it is shown the measurement results and all the determined reference values. The reference values were calculated using only one value (the first) from IPQ and FORCE.

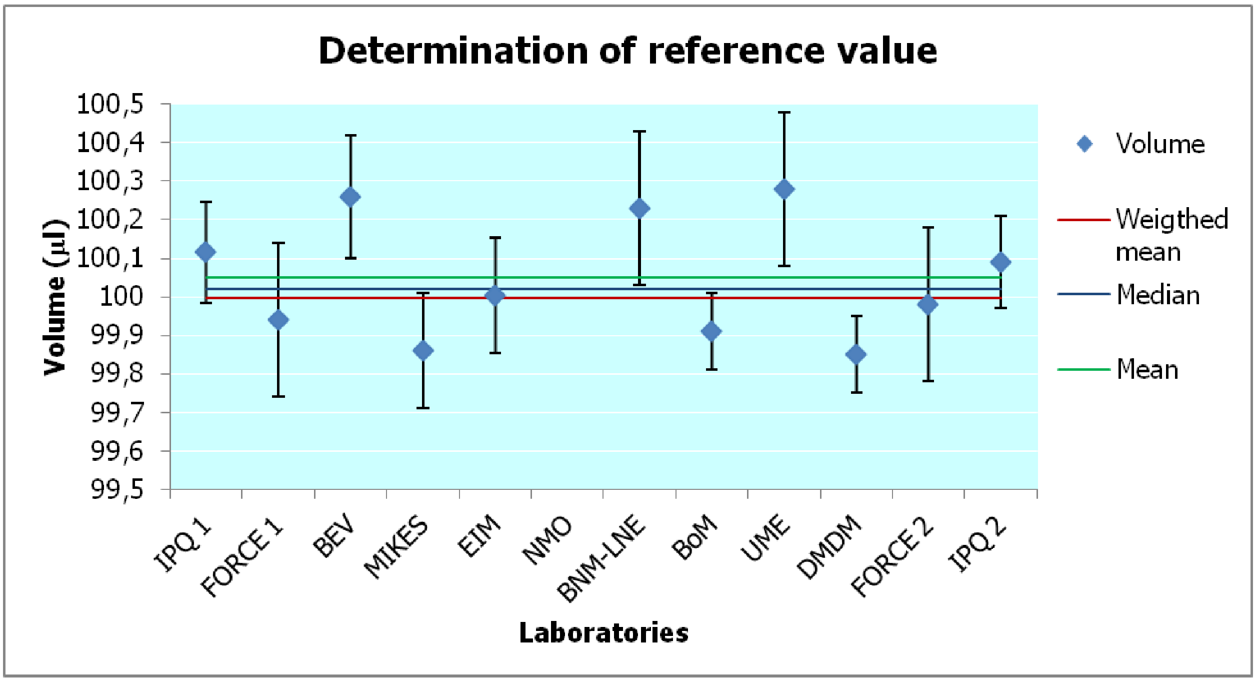


Figure 3 – Reference values - fixed micropipette

Table 9 – Reference value

	Mean (µl)	**Weighted mean (µl)**	**Median (µl)**
Reference value	100,05	99,999	100,02
Expanded Uncertainty	0,11	0,047	0,11

The values are very similar but since the dispersion is large the median allows us to calculate a reference value that is insensitive to the outliers, also, if the weighted mean was used the consistency of results would only be achieved for 5 laboratories which is not acceptable taken in to account the total number of participants, 10.

The results of the volume measurements for all laboratories, the reference value and its uncertainty (calculated according to the Monte Carlo Simulation with 10^8 runs) are presented in figure 4.

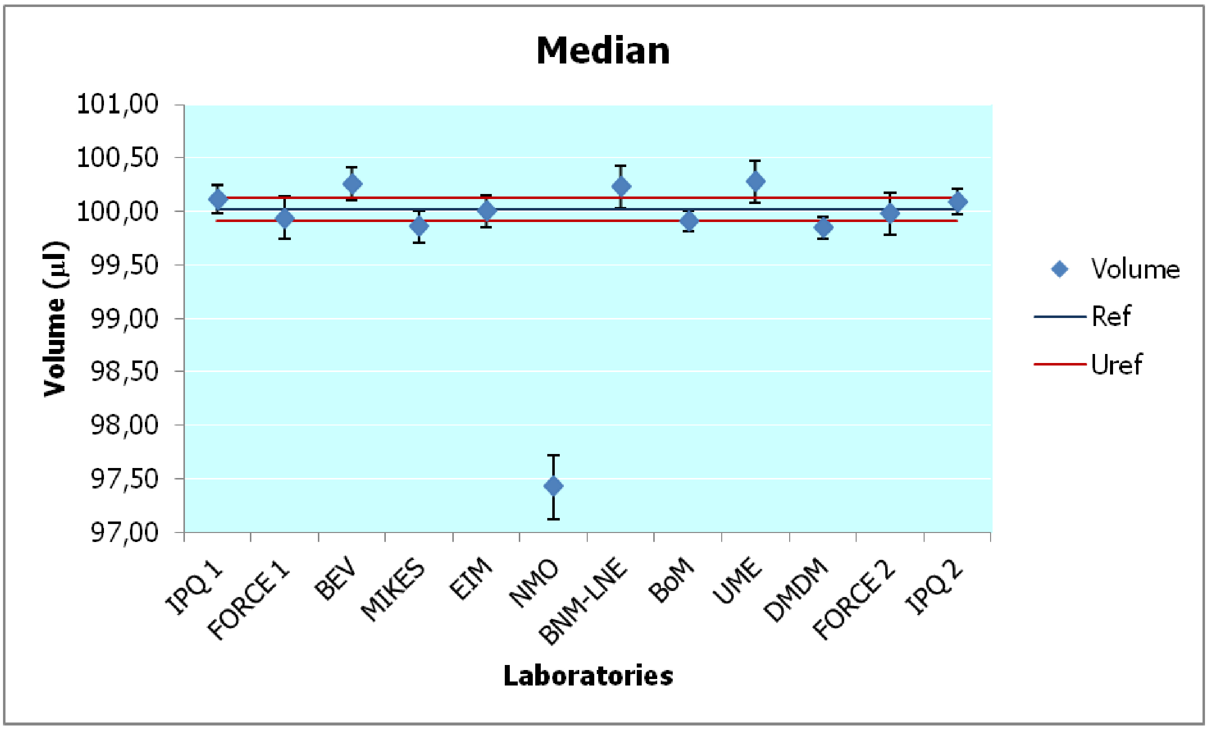


Figure 4 – Reference value and uncertainty

All the participants, except for lab 4, have presented results that are overlapping with the reference value uncertainty.

6.3. Degrees of Equivalence

To calculate the degrees of equivalence between the reference value (RV) and the laboratories de following formula is used [6]:

$d_i = x_I - x_M$

The corresponding uncertainties, $u(d_i)$ are calculated from Monte Carlo simulation.

In procedure B of reference [6] the concept of "expanded uncertainty" can not be applied in general since the corresponding probability density functions are usually not Gaussian. The analogous term is the shortest coverage interval at the 95 % level of confidence.
A specific result is deemed discrepant when the zero is outside of the respective coverage interval of the DoE.

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

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

In the following table it is showed the degree of equivalence, d_i, and between the RV and each individual national institute measurement.

Table 10 – Degree of equivalence

Laboratories	***d_i***	***Low95***	***High95***
IPQ	0,099	-0,001	0,255
FORCE	-0,081	-0,274	0,036
BEV	0,239	0,051	0,426
MIKES	-0,161	-0,315	0,000
EIM	-0,017	-0,154	0,083
NMO	-	-	-
LNE	0,208	0,000	0,401
BoM	-0,111	-0,234	0,000
UME	0,259	0,031	0,475
DMDM	-0,171	-0,314	-0,029

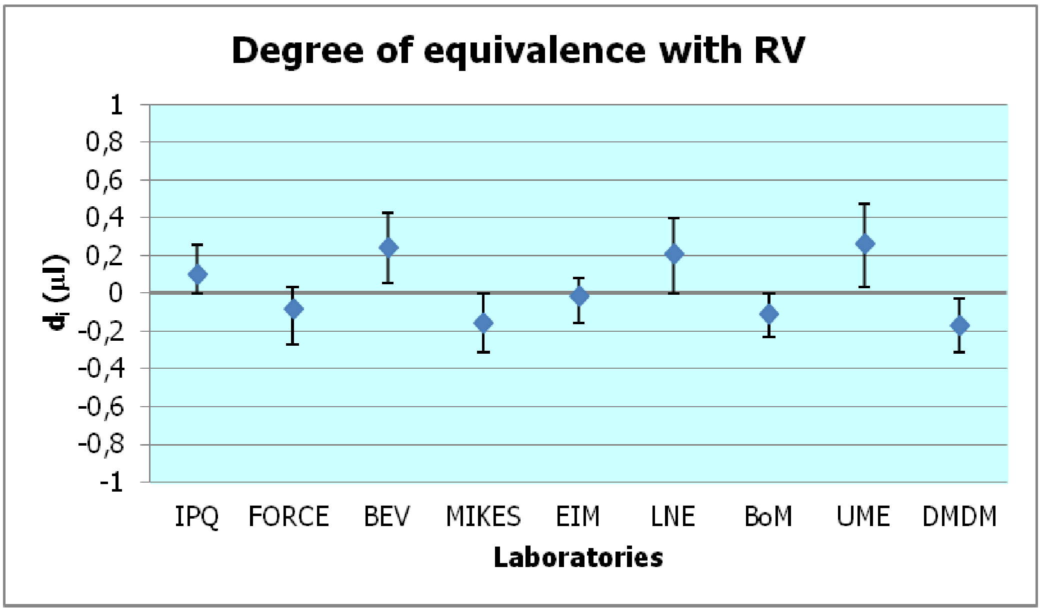


Figure 5 – Degree of equivalence with reference value

The laboratories, BEV, UME and DMDM do not include the zero value in the coverage interval for d_i, (which would be expected for "perfect" equivalence) and are therefore are consider discrepant. In the annex 3, plots for the probability density functions of each laboratory are presented.

The NMO value was already considered an outlier in figure 2.
The degree of equivalence, $d_{i,j}$, between different laboratories and the correspondent *En* are presented in annex 4.

7. Uncertainty presentation

It was requested that all participants present the type B and type A uncertainties.
In the figure 6 the difference between the systematic uncertainty / type B, the standard deviation of the mean / type A and the expanded uncertainty for all the participants are seen.

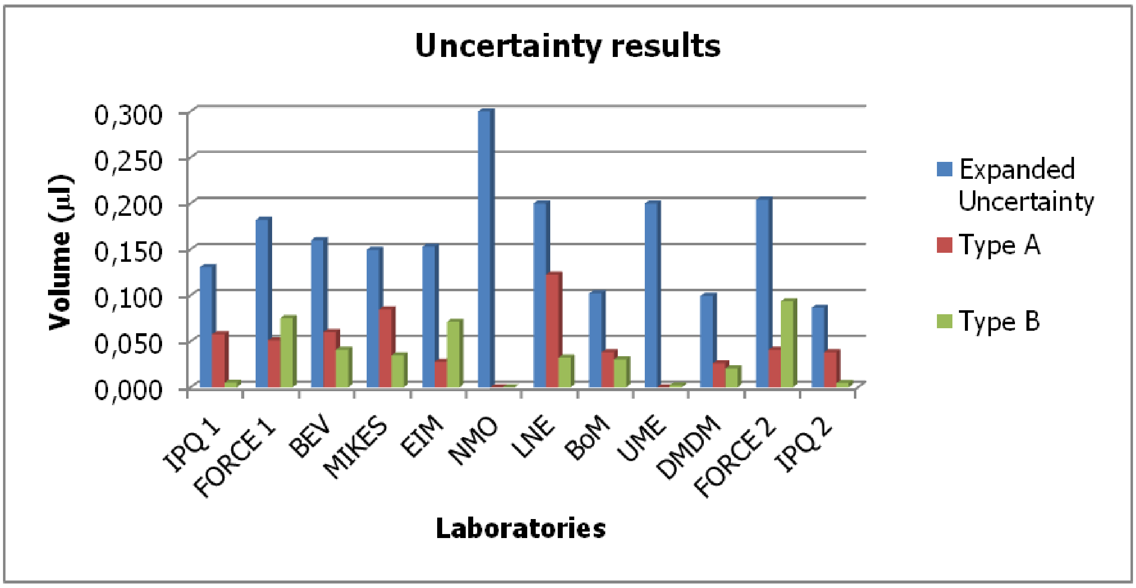


Figure 6 - Difference between type A and type B uncertainty

For the majority of the participants the type A uncertainty is larger than the type B uncertainty, as expected for this type of instrument.

7.1 Uncertainty components

A spreadsheet (Annex 1) with the proposed uncertainty components was presented to all participants and the majority of the laboratories replied according to this proposal, nevertheless some participants showed different components.
The proposed uncertainty components were: mass, mass pieces density, water density, air density, expansion coefficient and temperature.

7.2 Major source of uncertainty

Table 10 –Major source of uncertainty

Participant	Major source of uncertainty
IPQ-1	Repeatability
FORCE-1	Mass / balance
BEV	Repeatability
MIKES	Reproducibility
EIM	Mass
NMO	-
BNM-LNE	Operator effect
BoM	Repeatability
UME	Mass
DMDM	Repeatability

At it can be seen by this table the two major sources of uncertainty for almost every laboratory is the repeatability (reproducibility/ operator effect) and mass (for most labs includes balance).

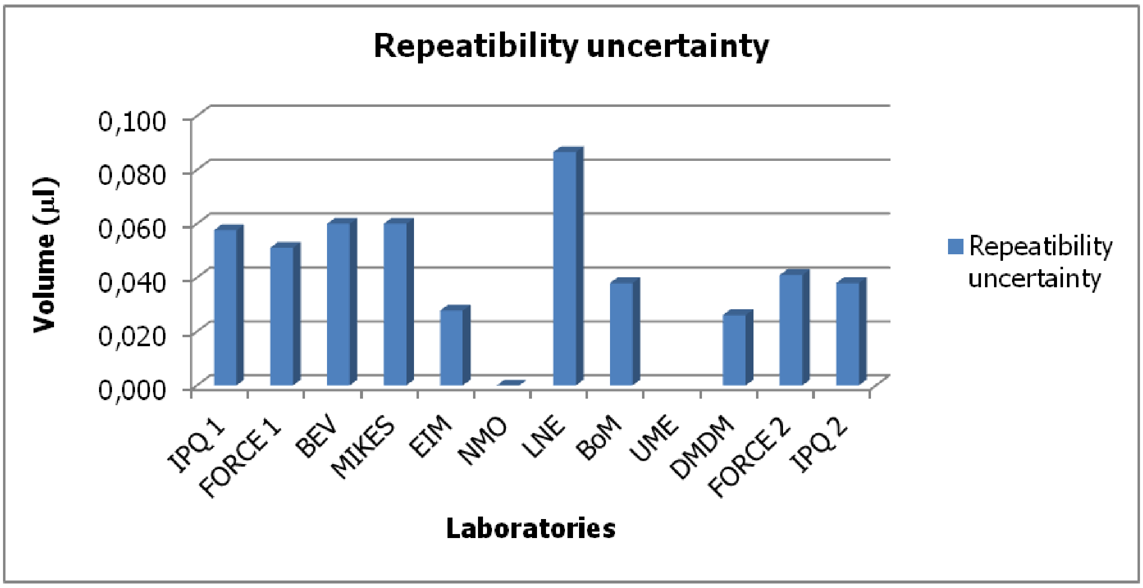


Figure 7 – Repeatability uncertainty

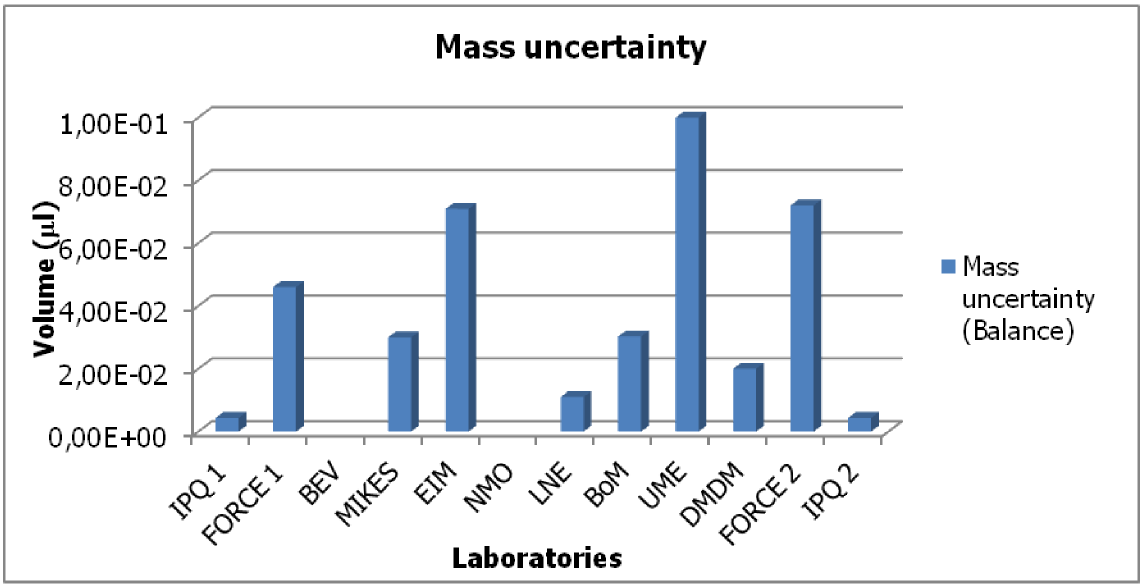


Figure 8 – Mass uncertainty

8. Conclusions

This comparison involved 10 laboratories at all, and lasted one year.

The initial and final results obtained by both IPQ and FORCE are consistent with each other. This demonstrates that the transfer standard had a stable volume during the entire comparison.

The values are very similar but since the dispersion is large the median was the chosen estimator to calculate the reference value.

Four laboratories don't have results consistent with the reference value.

Inconsistency in some laboratories could be attributed (at least partially) on lack of experience in handling the micropipette. The comparison could be repeated with a new very detailed technical protocol which includes handling instructions and techniques. Applying a detailed common and very detailed procedure could improve dramatically the performance of the laboratories.

The presented uncertainty budgets are very different but for the majority of the participants the two uncertainty components that have a major contribution to the final uncertainty, are the repeatability and the mass/balance.

9. References

1. ASTM E542: Standard Practice for Calibration of laboratory Volumetric Apparatus, 1st ed., American Standard, 1st ed., 2000.
2. ISO 4787-1984; Laboratory glassware – Volumetric glassware – Methods for use and testing of capacity.
3. ISO 8655-1/2/6, 2002, Piston-operated volumetric apparatus.
4. ISO 3696 – Water for analytical laboratory use: specification and test methods, 1st ed., Genève, International Organization for Standardization, 1987.
5. Pestana D. D., Velosa S. F., Introdução à probabilidade e à estatística, 1ª ed., Fundação Calouste Gulbenkian, Lisboa, 2000.
6. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595.
7. BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML; Guide to the expression of uncertainty in measurement (GUM), Geneva, 1995.

Annex 1 – Spreadsheet

EURAMET Project "Volume calibration of a 100 μl micropipette"

Data Form

General Information

Country		Laboratory	
Responsible		Date	

Equipment

	Type	Range	Resolution	Tracebility (when aplied)
Weighing instrument				
Thermometer				
Barometer				
Hygrometer				
Other equipment				

Other Informations

	Type	Density reference	Measured conductivity (if the liquid is water)
Calibration liquid			

	Type	Density (if the standard is a mass)	Tracebility (when aplied)
Mass standards			
Other standards			

Used volume calculation formula:

Calibration Procedure (short escription)

Comments:

Signature:

EURAMET Project "Volume calibration of 100 µl micropipette"

Results form calibration of 100 µl fixed micropipette

Ambient Conditions

Air temperature (ºC)	
Pressure (hPa)	
Humidity (%)	
Air Density (mg/µl)	

Measurement results

Test number	Volume (µl)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Mean value	
Standard deviation	

Uncertainty budget

Quantity (x_i)	Value	Distribution	Standard uncertainty $u(x_i)$	Sensitivity coefficient c_i	Uncertainty $u(y_i)$	Comment/ Explanation
Repetibility measurements						
Mass (mg)						
Air Density (mg/µl)						
Water Density (g/µl)						
Density of the mass pieces (mg/µl)						
Coefficient of expansion from the micropipette material (℃ $^{-1}$)						
Water temperature (ºC)						
Evaporation (µl)						
Other						

Combined Uncertainty (µl)	
Expanded uncertainty (µl) (*k*=2)	

Comments:

Signature:

Annex 2 - Equipment

Balance See page 7

Thermometer

Laboratory	Type	Range	Resolution
IPQ	Lufft PT100	-100 to 200 ºC	0,01 ºC
FORCE	Goldbrand,Hg	0 to 50°C	0,1 ºC
BEV	Vaisala PTU303		0,01 ºC
MIKES	Agilent 34970A + Pt 100	15 – 25°C	0,01 ºC
EIM	Agilent 34401T + Pt 100	0 – 50°C	0,01 ºC
NMO			
BNM-LNE	Testo AG	15 – 25°C	0,01 ºC
BoM	Testo type 650	-20 to 70 ºC	0,1 ºC
UME	Vaisala-HMI36	-40 to 180°C	0,1 ºC
DMDM	Testo 177 H1 1521, probe Pt-100 type 5618B-6	-50 to 150 ºC	0,001 ºC

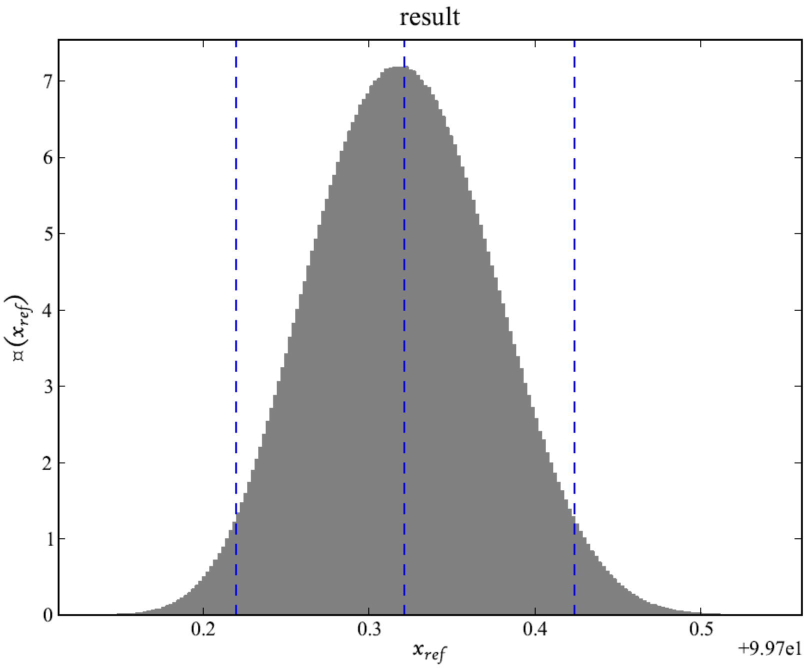
Barometer

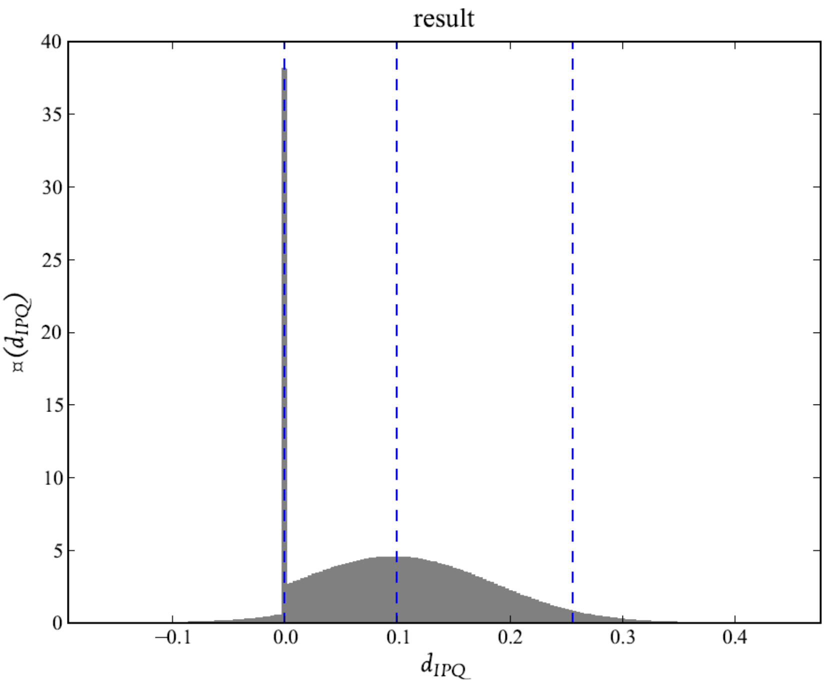
Laboratory	Type	Range	Resolution
IPQ	Druck DPI 142	750 - 1150 hpa	0,01 hpa
FORCE	Präzisions Aneroid Barometer	870 – 1050 mBar	1 mbar
BEV	Vaisala PTU303		1 Pa
MIKES	Vaisala-PTA427	600 – 1060 hPa	0,1 hPa
EIM	Lufft GmbH	870 – 1050 mbar	0,5 mbar
NMO			
BNM-LNE	Testo AG	950 – 1050 hPa	50 Pa
BoM	Testo type 650	120 – 200 hPa	0,1 hPa
UME	Setra-Digital	0 – 1,6 bar	0,01 mbar
DMDM	104	900 – 1060 hPa	1 Pa

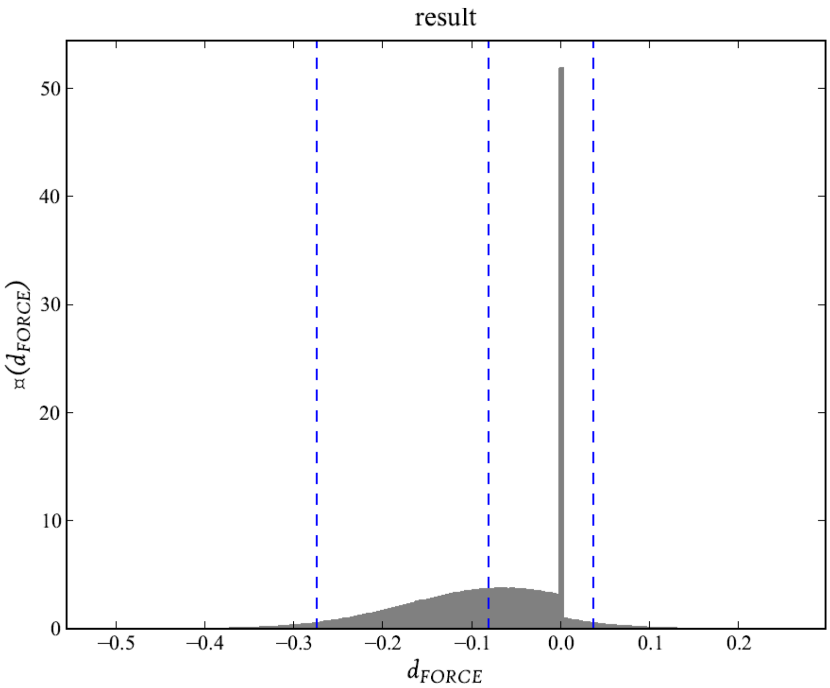
Hydrometer

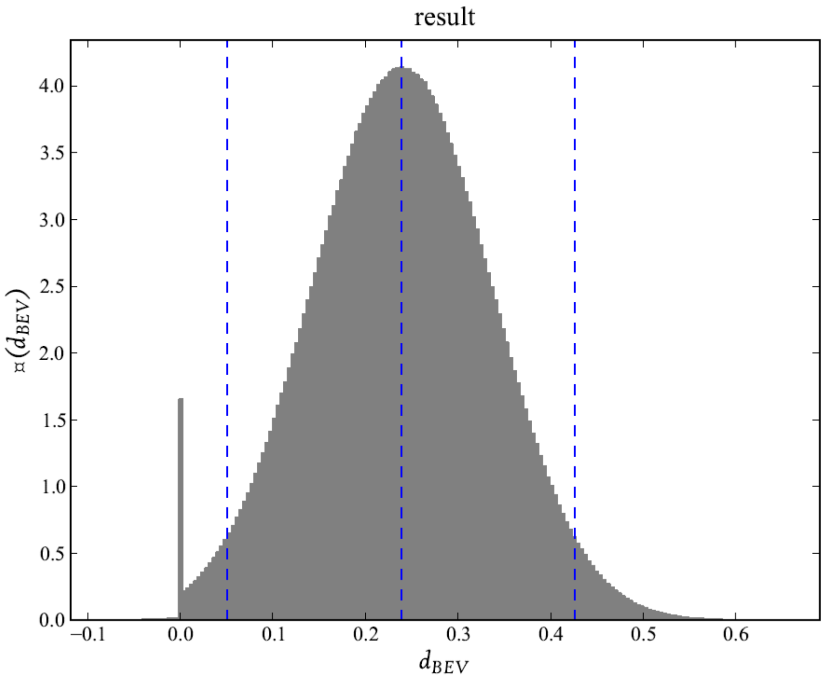
Laboratory	Type	Range	Resolution
IPQ	Hygroclip	0 - 100 %	0,1 %
FORCE	Almemo	5 - 98 %	0,1 %
BEV	Vaisala PTU303		0,01 %
MIKES	Vaisala HM34C	0 - 100 %	2 %
EIM	Rotronic A.G.	10 - 60 %	0,10 %
NMO			
BNM-LNE	Idem	20 - 80 %	1 %
BoM	Testo type 650	0 - 100 %	0,10 %
UME	Vaisala-HMI36	0 - 100 %	0,1 %
DMDM	Testo 177 H1	0 - 100 %	0,1 %

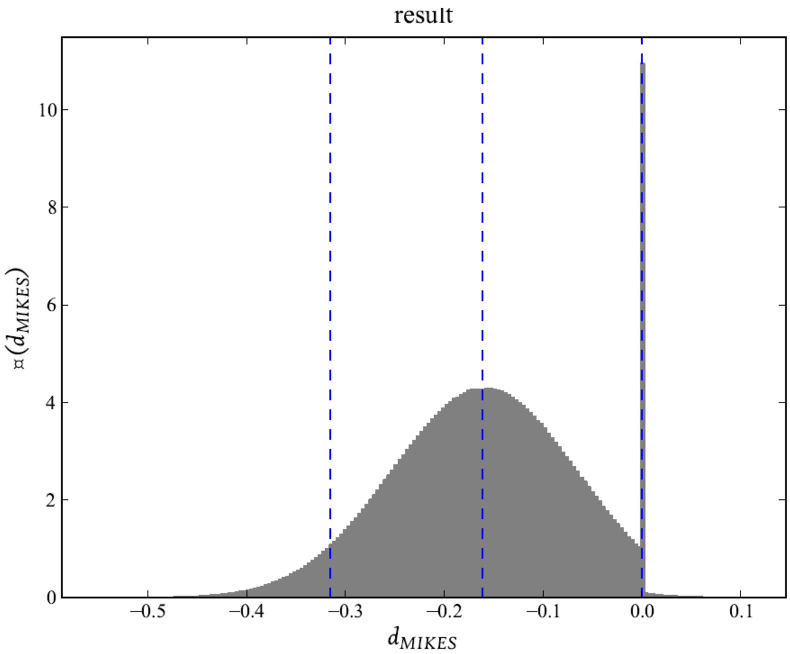
Annex 3 – PDF Functions

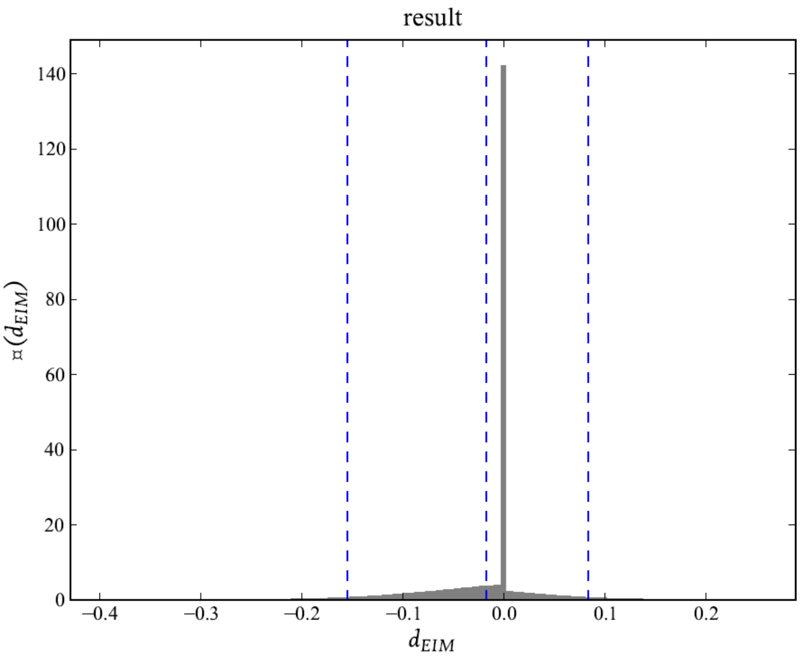


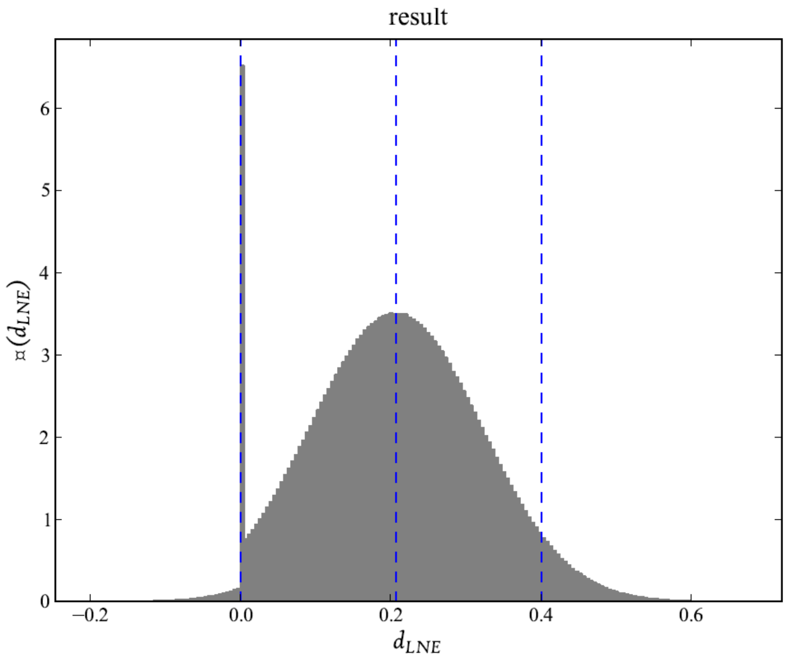


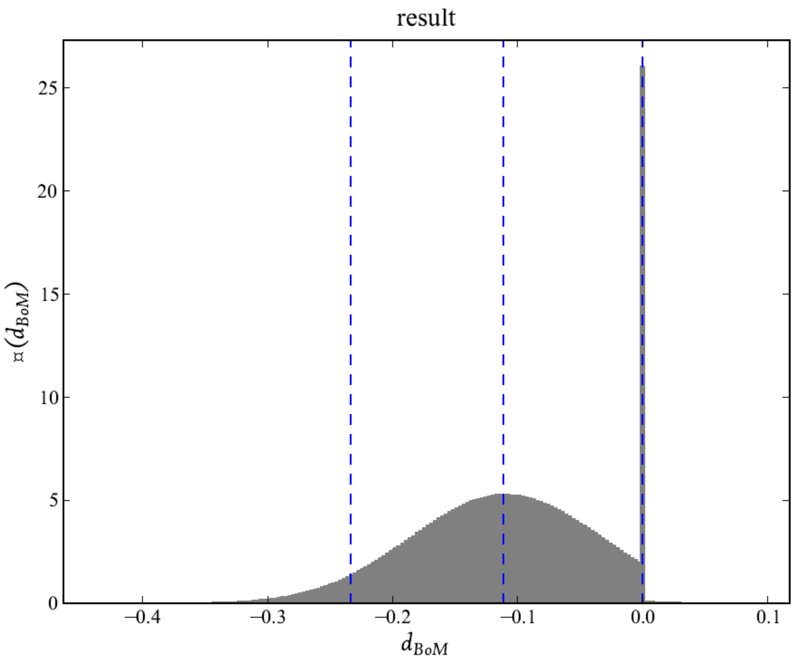

result
50
40
30
20
10
0
−0.5
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
d_{FORCE}

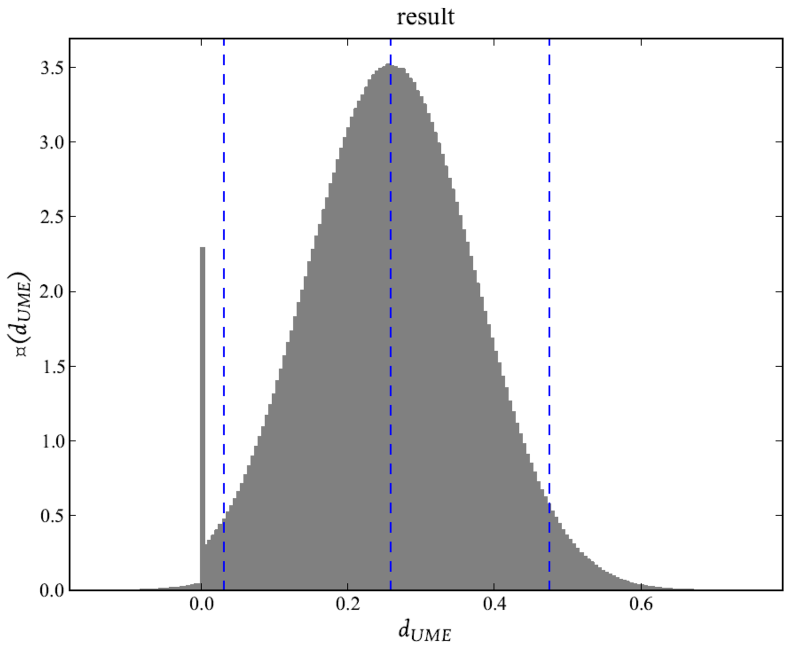

result
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
d_{BEV}

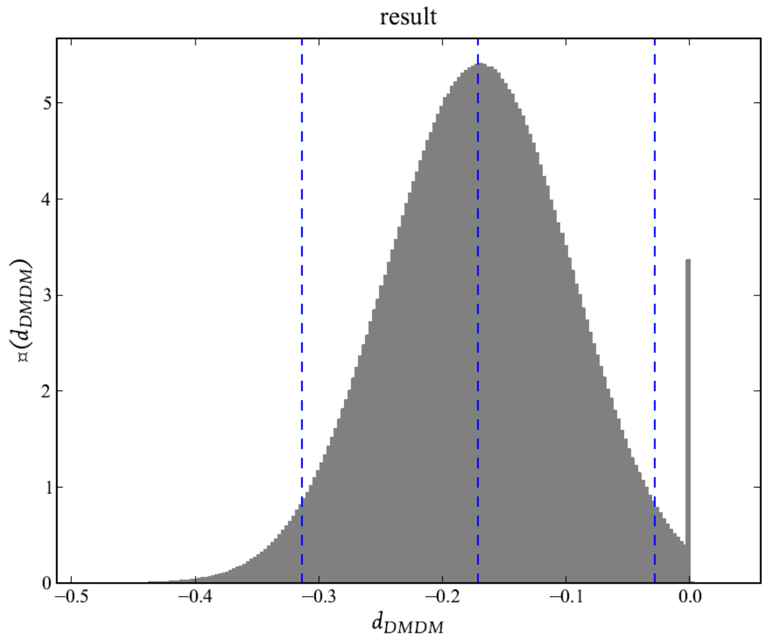

result
10
8
6
4
2
0
−0.5
−0.4
−0.3
−0.2
−0.1
0.0
0.1
¤(d_{MIKES})
d_{MIKES}


result
140
120
100
80
60
40
20
0
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0.2
¤(d_{EIM})
d_{EIM}


result
¤(d_LNE)
d_LNE
−0.2
0.0
0.2
0.4
0.6
0
1
2
3
4
5
6


result
¤(d_BoM)
d_BoM
−0.4
−0.3
−0.2
−0.1
0.0
0.1
0
5
10
15
20
25


result
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
d_{UME}


result
5
4
3
2
1
0
−0.5
−0.4
−0.3
−0.2
−0.1
0.0
d_{DMDM}

Annex 4 – Degree of equivalence between laboratories

	IPQ 1	FORCE 1	BEV	MIKES	EIM	NMO	LNE	BoM	UME	DMDM	FORCE 2
IPQ 1											
FORCE 1	-0,73614										
BEV	0,696368	1,24939									
MIKES	-1,28546	-0,32	-1,82384								
EIM	-0,56239	0,256	-1,16726	0,678823							
NMO	-8,21434	-6,96981	-8,33235	-7,2538	-7,68313						
LNE	0,472638	1,021769	-0,12103	1,476	0,9	7,77135					
BoM	-1,24995	-0,13416	-1,855	0,27735	-0,52142	7,85194	-1,42661				
UME	0,685952	1,202082	0,078087	1,68	1,104	7,9128	0,180312	1,65469			
DMDM	-1,61402	-0,40249	-2,173	-0,05547	-0,85424	7,6622	-1,69494	-0,42426	-1,92302		
FORCE 2	-0,56884	0,141421	-1,09322	0,48	-0,096	7,08075	-0,88035	0,31305	-1,06066	0,581378	
IPQ 2	-0,14635	0,64312	-0,85	1,197332	0,447698	8,24178	-0,59596	1,152332	-0,81462	1,536443	0,471621

There are several results not consistent with each other; these values are marked in red.