Instituto Português da Qualidade

PORTUGUESE INSTITUTE FOR QUALITY

Final Report

Volume Calibration of 1000 μl micropipettes

EUROMET Project no. 865

IPQ – Coordinator of the comparison

Elsa Batista

March 2007

Contents

1. Introduction

The project for the comparison of the volume of 1000 μl micropipettes was proposed initially in the Euromet Flow meeting of 2005 in Thessaloniki. The project details were sent to all the members and 6 NMIs agreed to participate. The project officially started in February 2006 and was concluded in October 2006.

Each country took 3 weeks to perform the calibration of two micropipettes.

Table 1 - Participants in the EUROMET project 865

Country	Laboratory	Periods	Responsible
Portugal	IPQ	February	Elsa Batista
Norway	JV	March	Arne Lind/Gunn Svendsen
Denmark	FORCE	April	Lene S. Kristensen
Turkey	UME	June	Umit Akcadag
Czech Republic	CMI	July	Tomas Valenta
Spain	CEM	July	Noelia Herrero
Portugal	IPQ	August	Elsa Batista

The purpose of this comparison is to allow the participating laboratories to test the agreement of their results and uncertainties in the calibration of micropipettes despite the different used equipment and calibration method. 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. Travelling standards

There are several types of micropipettes, single channel or multichannel. The type suggested for this comparison is the single-channel piston pipette, which is the most common used in laboratories and easy to handle. The micropipette needs to have attached a removable plastic tip in order to aspirate the liquid. IPQ act as the pilot laboratory supplying two micropipettes and respective tips.

Micropipettes may be factory-preset to deliver a given volume, or have selectable volumes within a volume range [1]. In the following figures are described the variable and fixed micropipettes used in this comparison. These instruments are manufactured of plastic with a thermal expansion coefficient of $2{,}4 \times 10^{-4}$ ºC $^{-1}$ [2].

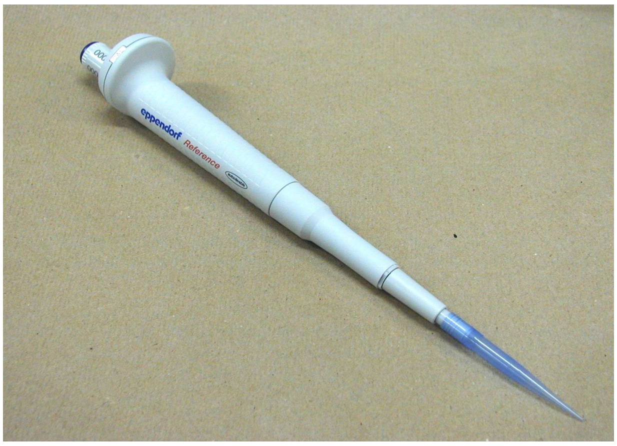

Figure 1- Fixed Micropipette

Figure 2- Variable micropipette

3. The experimental tests

Calibration tests

- Calibration of the fixed volume micropipette of 1000 μl (10 measurements).
- Calibration of the variable micropipette in 5 points, according to the following sequence: 1000 μl, 750 μl, 500 μl, 250 μl and 100 μl (10 measurements performed for each point).

Behaviour test

- To perform 100 measurements continually in the fixed micropipette of 1000 μl in order to verify the differences between the volume calibration and the nominal volume when used in real conditions of repeated additions.

4. The method

All laboratories used gravimetric methods to determine the volume of the micropipettes but with different models/equations.

4.1. Equipment

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

The equipment used had the following resolution:

Table 2 – Equipment resolution

Laboratory	Weighing instrument (mg)	Thermometer (ºC)	Barometer (hPa)	Hygrometer (%)
IPQ	0,001	0,01	0,1	0,1
JV	0,01	0,01	0,1	0,1
FORCE	0,01	0,1	0,5	0,1
UME	0,01	0,01	0,001	0,01
CMI	0,01	0,001	1	1
CEM	0,01	0,01	0,01	100 mK

The various equipments are described in more detail in Annex 2.

4.2. Type of water

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

Table 3 – Water characteristics

Laboratory	Type	Density reference	Conductivity (μS/cm)
IPQ	Distilled	Tanaka	0,046
JV	Air free and ion exchange	OIML R49 - 2	
FORCE	Distilled	Spieweck	1,14
UME	Distilled	Kell	0,43
CMI	Distilled	Tanaka	
CEM	Electro deionised	Tanaka	

The majority of the participants used distilled water; the countries that presented conductivity values are according to the ISO 3696 [3] < 5μS/cm.

4.3. Mass standards

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

Table 4 – Mass Standards

Laboratory	OIML Accuracy Class	Density (kg/m^3)
IPQ	E2	7960-8600
JV	F1	8000
FORCE	F1	8000
UME	E1	7996-8013
CMI	E2	8000
CEM	F1	7960

There are a great variety of weights used, that had influences in the determination of the mass uncertainty component.

5. Ambient conditions

The ambient conditions were described by all participants; in the following tables it is shown the values for the fixed micropipette and for the variable micropipette at the point of 1000 μl.

Table 5 - Ambient conditions - fixed micropipette

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ-1	20,3	1009,3	54,7	0,00120
JV	21,1	981	58,3	0,00115
FORCE	21,25-21,35	1018	30-50	0,00120
UME	20,7	987,96	59,8	0,00117
CMI	19,8	988	54	0,00117
CEM	19,86	933,249	49,5	0,00110
IPQ-2	20,5	1002,1	67	0,00120

Table 6 – Ambient conditions – variable micropipette (1000 μl)

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ-1	20,3	1009,7	55,3	0,00120
JV	20,8	993,68	57,5	0,00117
FORCE	21,35	1018	30-50	0,00120
UME	20,5	987	54	0,00117
CMI	20,7	999,81	58,7	0,00117
CEM	19,79	933,049	49,3	0,00110
IPQ-2	20,5	1003,1	69	0,00120

6. Measurement results

6.1. Volume measurements

Two micropipettes of 1000 µl were calibrated, a micropipette with fixed volume and a variable micropipette in 5 points, CMI only performed the calibration at 1000 µl. IPQ performed 2 measurements: at the beginning and at the end of the comparison.

6.1.1. Fixed micropipette

Table 7 – Volume measurement results - fixed micropipette

Laboratory	Volume (µl)	U_{exp} (µl)
IPQ-1	1000,55	0,80
JV	1001,3	0,16
FORCE	999,33	1,1
UME	1004,94	0,11
CMI	999,10	0,91
CEM	989,296	1,5
IPQ-2	1000,69	0,82

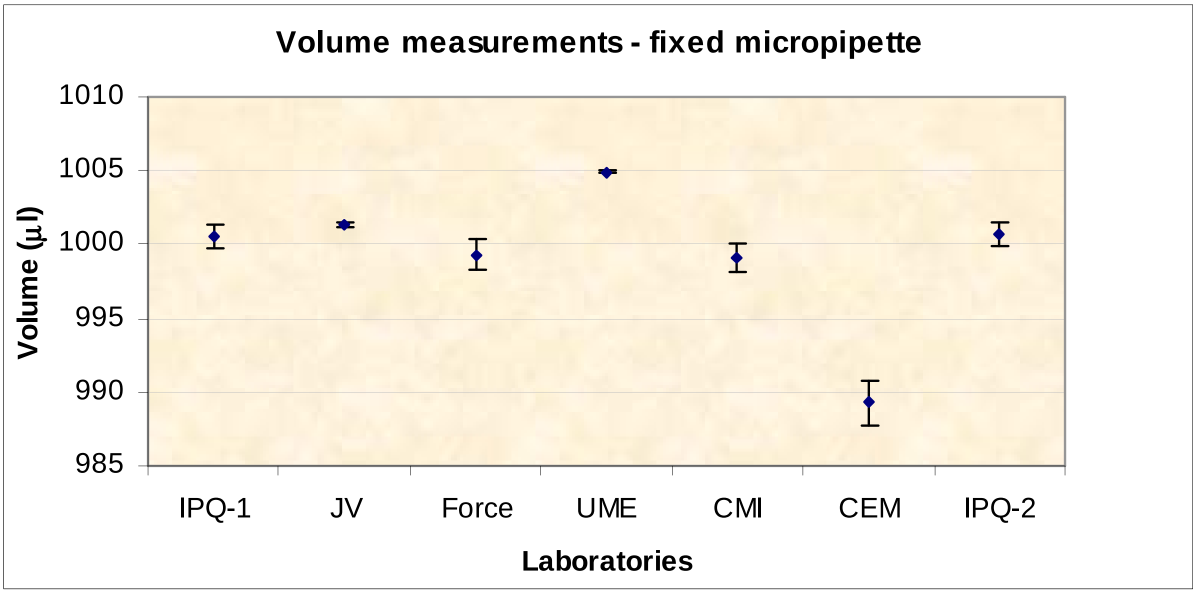

Figure 3 – Volume measurements - fixed micropipette

6.1.2. Variable micropipette

Table 8 – Volume measurement results - variable micropipette – 1000 μl

Laboratory	Volume (μl)	U_{exp} (μl)
IPQ-1	995,77	0,95
JV	992,36	1,19
FORCE	997,69	1,1
UME	1000,75	0,44
CMI	997,39	0,91
CEM	993,159	1,5
IPQ-2	995,97	0,91

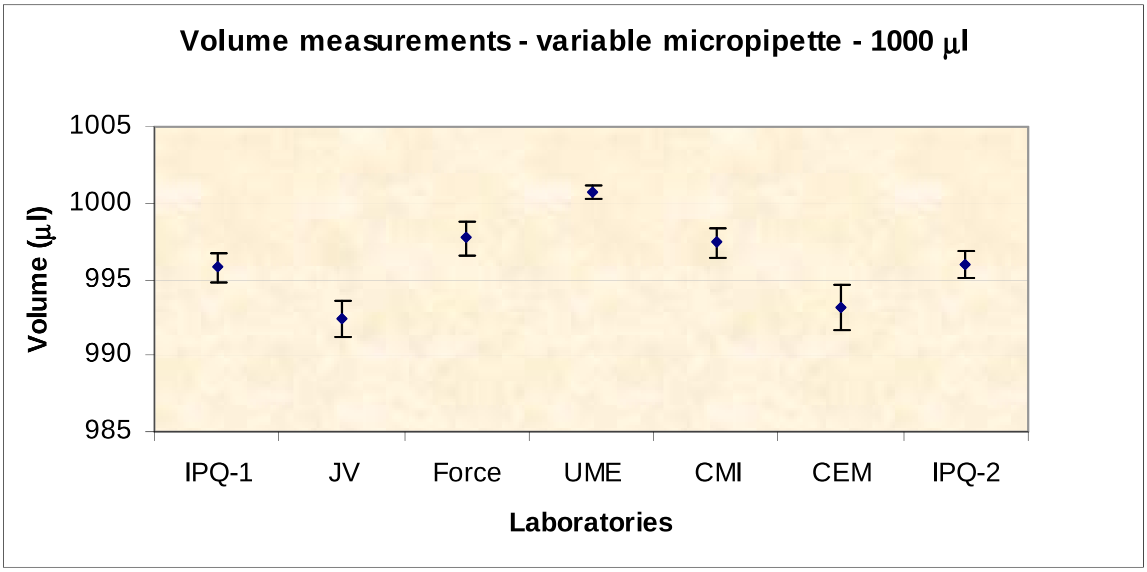

Figure 4 – Volume measurements - variable micropipette – 1000 μl

Table 9 – Volume measurement results - variable micropipette – 750 μl

Laboratory	Volume (μl)	U_{exp} (μl)
IPQ-1	745,63	0,40
JV	746,22	0,74
FORCE	747,46	0,71
UME	750,23	0,28
CEM	742,496	1,3
IPQ-2	745,95	0,44

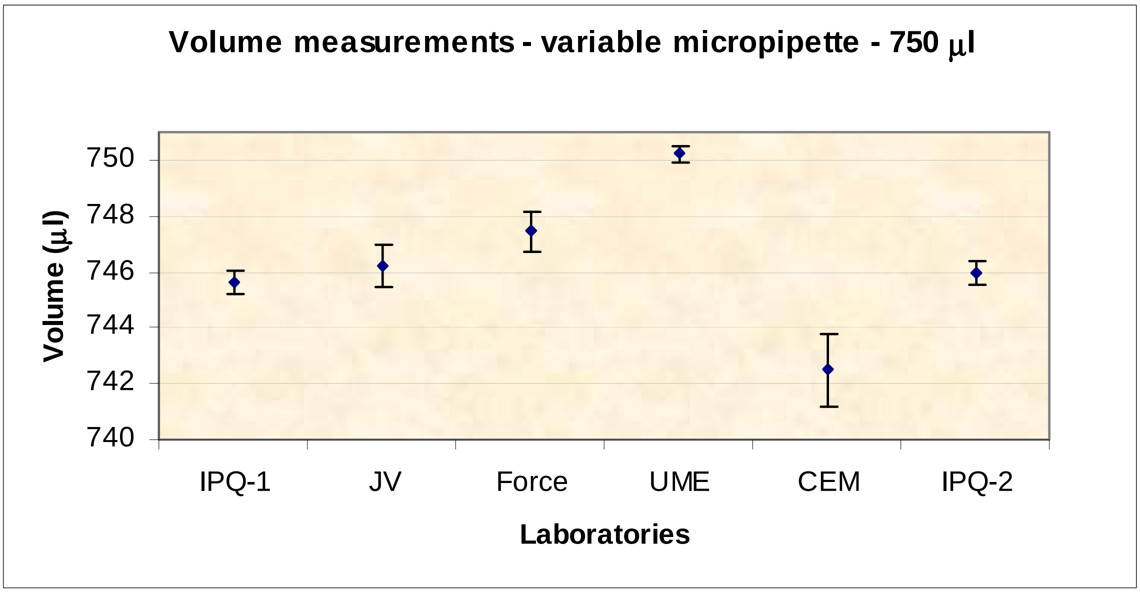

Figure 5 – Volume measurements - variable micropipette – 750 µl

Table 10 – Volume measurement results - variable micropipette – 500 µl

Laboratory	**Volume (µl)**	U_{exp} **(µl)**
IPQ-1	496,58	0,37
JV	496,60	0,39
FORCE	497,20	0,57
UME	499,91	0,27
CEM	498,721	0,99
IPQ-2	496,68	0,32

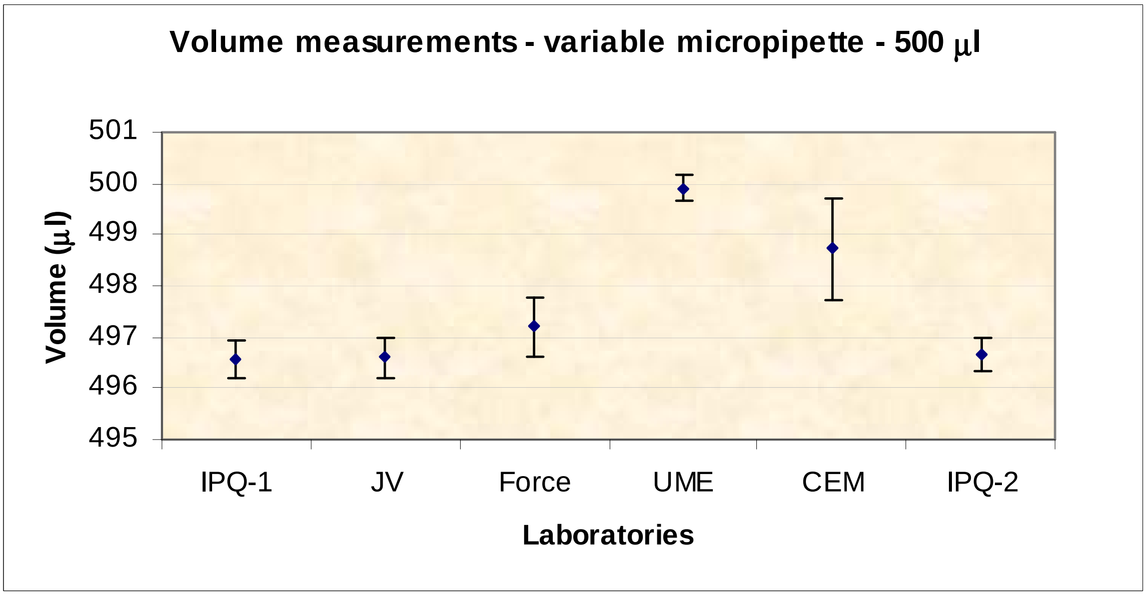

Figure 6 – Volume measurements - variable micropipette – 500 µl

Table 11 – Volume measurement results - variable micropipette – 250 μl

Laboratory	Volume (μl)	U_{exp} (μl)
IPQ-1	248,88	0,29
JV	248,31	0,65
FORCE	248,42	0,25
UME	251,30	0,14
CEM	249,765	0,6
IPQ-2	248,73	0,32

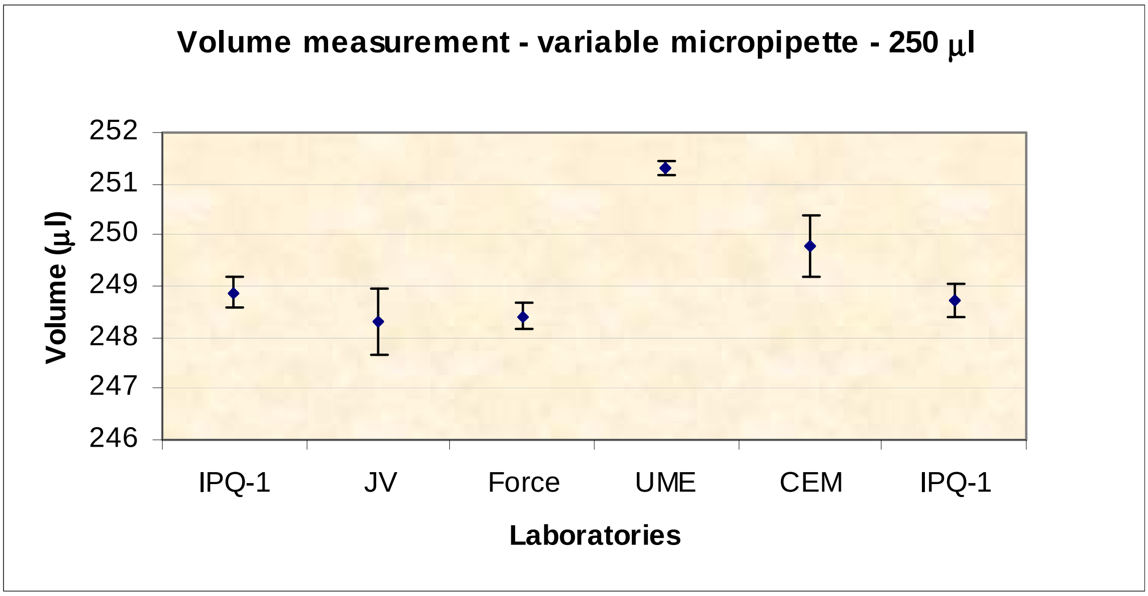

Figure 7 – Volume measurements - variable micropipette – 250 μl

Table 12 – Volume measurement results - variable micropipette – 100 μl

Laboratory	Volume (μl)	U_{exp} (μl)
IPQ-1	100,87	0,14
JV	99,98	0,69
FORCE	101,27	0,29
UME	102,37	0,13
CEM	101,867	0,3
IPQ-2	100,91	0,19

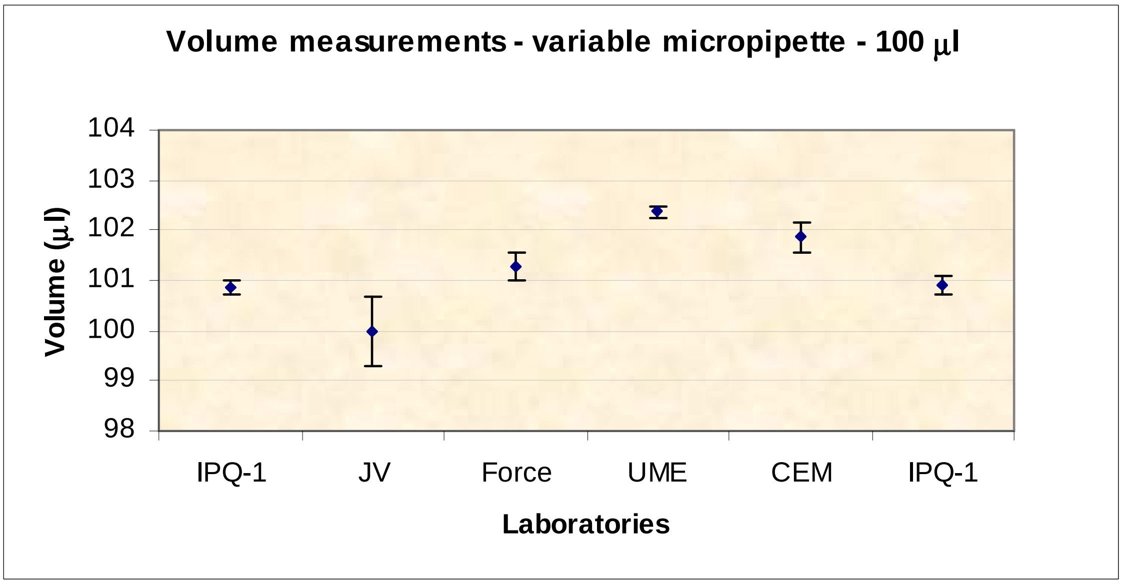

Figure 8 – Volume measurements - variable micropipette – 100 µl

6.2. Determination of the reference value

The reference values and its uncertainties was calculated using the Monte Carlo Simulation [4,5], for one million trials. In these cases where the dispersion is large the median allows us to calculate a reference value that is insensitive to the outliers.

All reference values were calculated using only one value (average) from the pilot laboratory (IPQ).

Table 13 – Reference values

Instrument	Nominal volume (µl)	Reference value (µl)	Uncertainty of reference value (µl)
Fixed micropipette	1000	1000,06	0,58
Variable micropipette	1000	996,54	0,63
	750	746,26	0,65
	500	497,22	0,55
	250	248,82	0,29
	100	101,29	0,29

The results of the volume measurements for all laboratories, the reference value and its uncertainty (*k*=2) and the maximum permissible error of the micropipettes of 1000 µl (ISO 8655) are presented in the following figures:

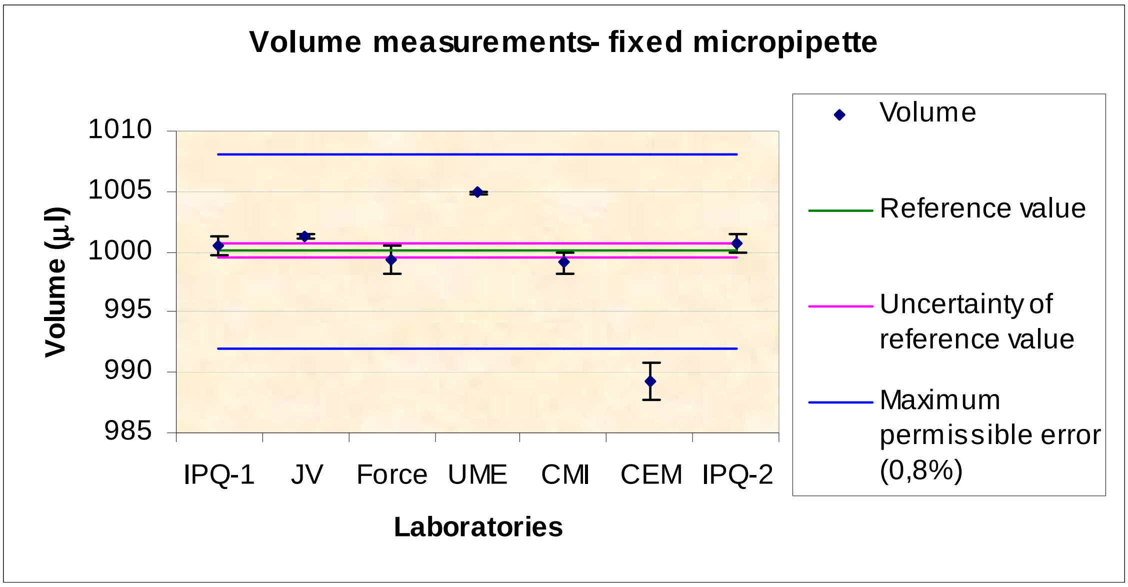

Figure 9 – Reference value and uncertainty - fixed micropipette

There are 4 results within the reference value uncertainty. The results of JV and UME are within the maximum permissible error of the micropipette and the result of CEM is outside this error.

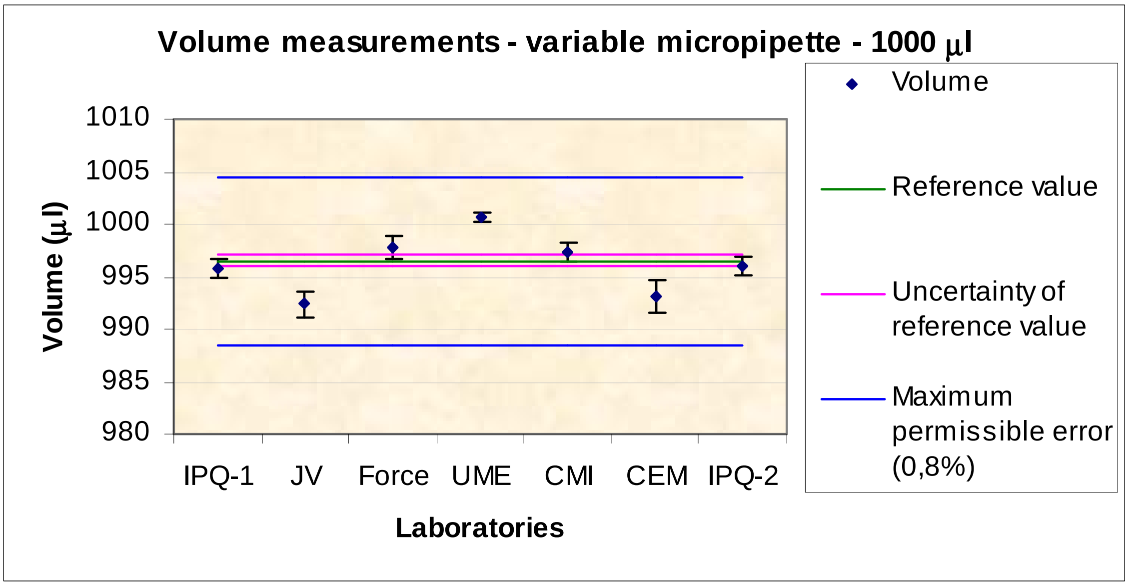

Figure 10 – Reference value and uncertainty - variable micropipette – 1000 μl

There are 4 results within the reference value uncertainty and three results corresponding to JV, UME and CEM within the maximum permissible error of the micropipette.

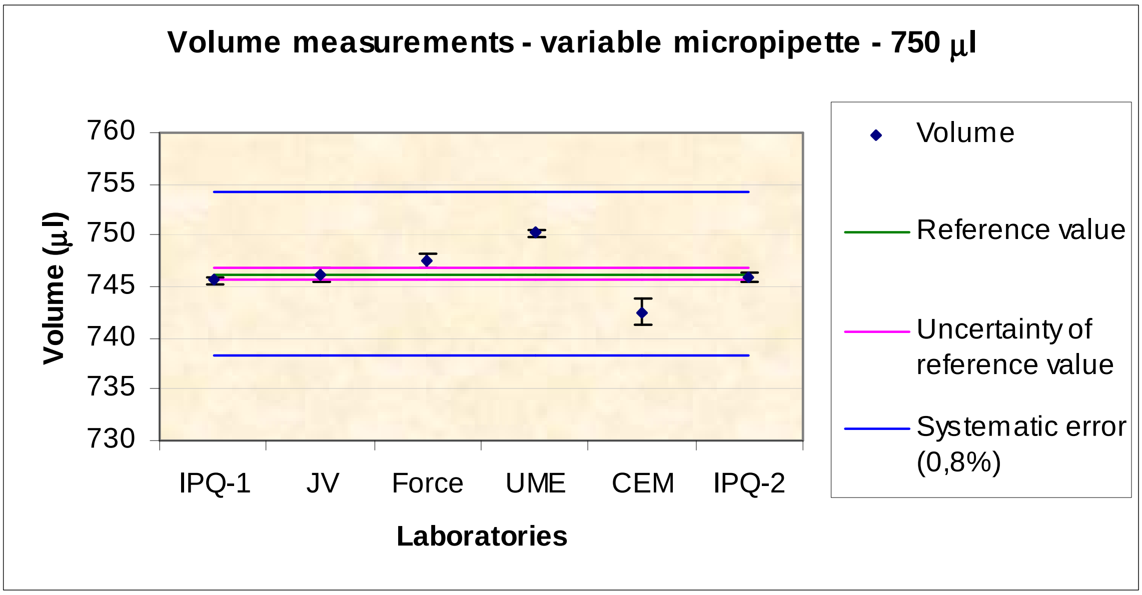

Figure 11 – Reference value and uncertainty - variable micropipette – 750 μl

There are 4 results within the reference value uncertainty and two results corresponding to, UME and CEM within the maximum permissible error of the micropipette.

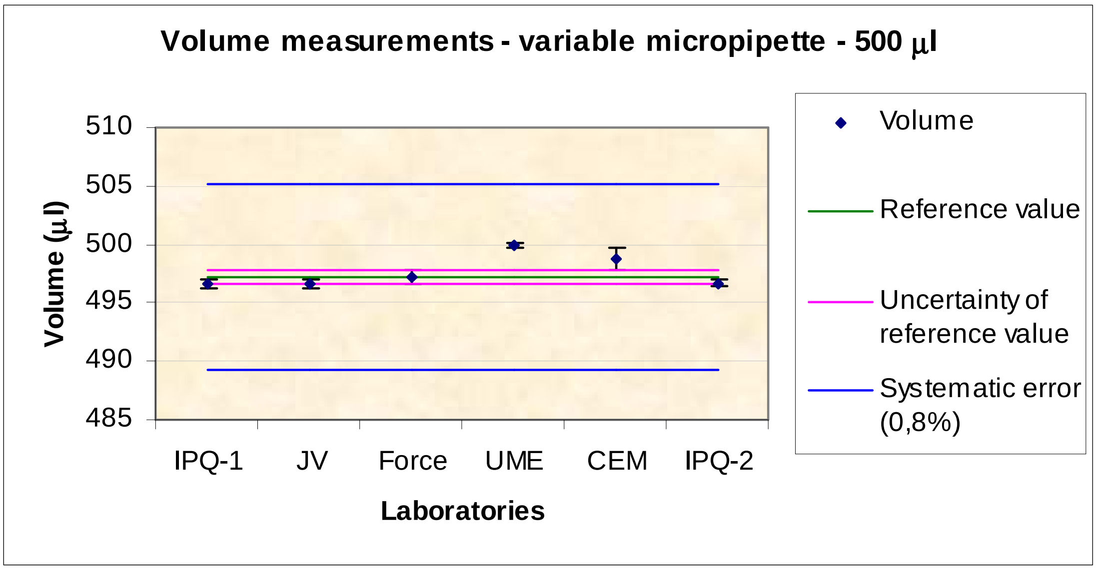

Figure 12 – Reference value and uncertainty - variable micropipette – 500 µl

There are 5 results within the reference value uncertainty and one result corresponding to UME within the maximum permissible error of the micropipette.

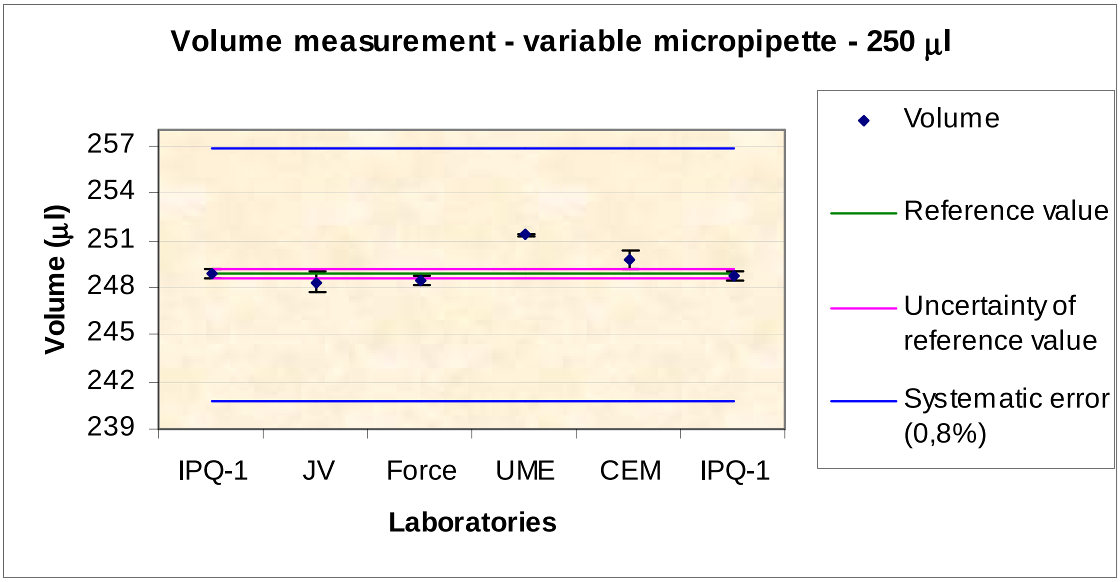

Figure 13 – Reference value and uncertainty - variable micropipette – 250 µl

There are 4 results within the reference value uncertainty and two results corresponding to UME and CEM within the maximum permissible error of the micropipette.

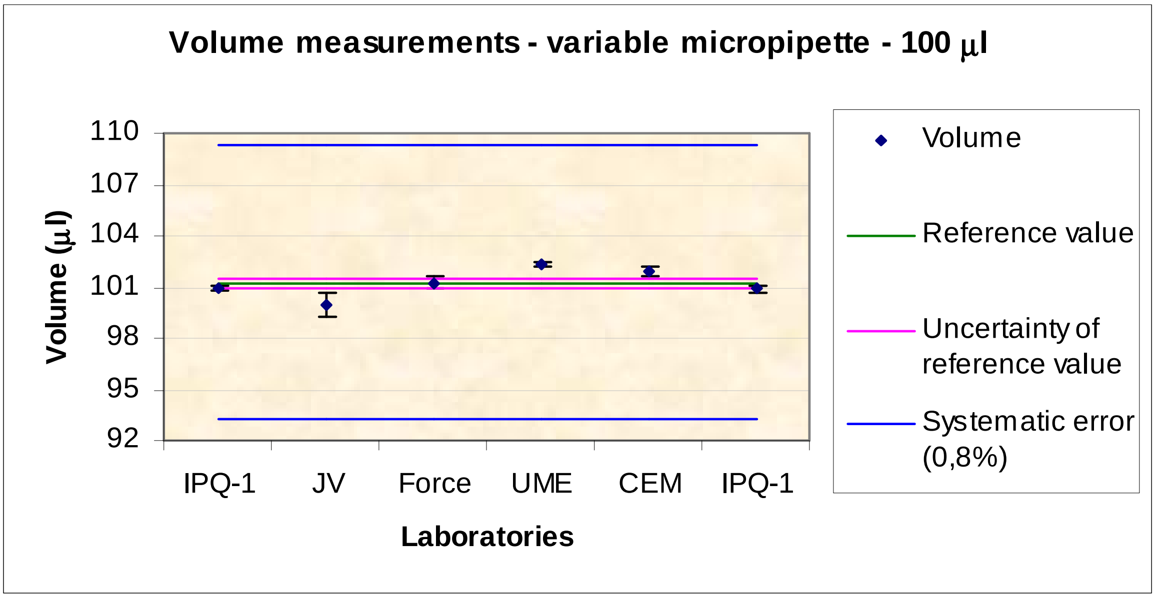

Figure 14 – Reference value and uncertainty - variable micropipette – 100 µl

There are 3 results within the reference value uncertainty and three results corresponding to JV, UME and CEM within the maximum permissible error of the micropipette.

6.3. Variable micropipette linearity

When all the reference values for each point are compared with the nominal value, the following figure can be obtained:

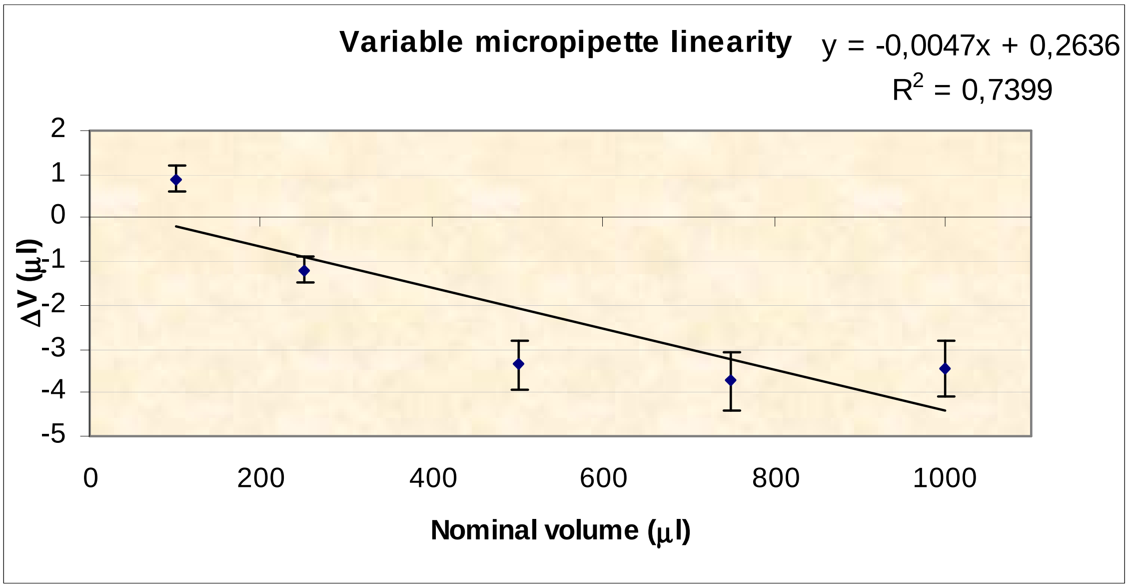

Figure 15 – Linearity of the variable micropipette

The variable micropipette does not present a linear behaviour. Its uncertainty increases with the measured volume because the lower the volume of the micropipette the better it's repeatability.

To verify if all the laboratories that perform the calibration of the variable micropipette, for all points, obtain such a non linear behaviour the following figure is presented.

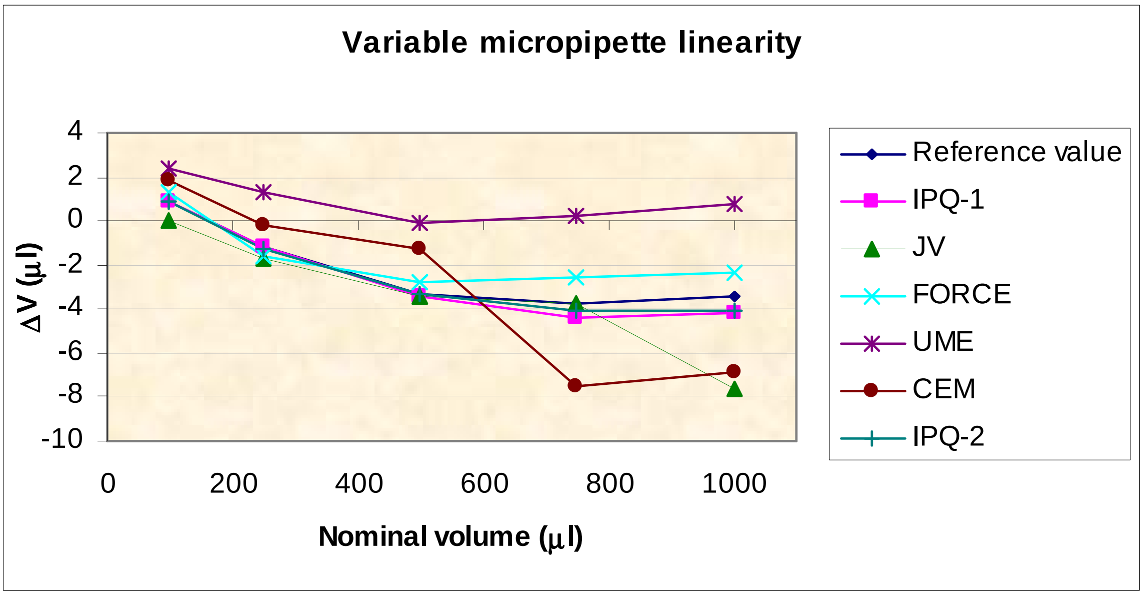

Figure 16 – Linearity for the variable micropipette – all the laboratories and reference values

There are 3 laboratories (UME, IPQ, FORCE) with all results having a similar behaviour that the one obtained from the reference values, but with UME having always higher values. JV has 4 results that follow the reference values behaviour, only the point 1000 μl has a deviation. CEM is the laboratory with values that have a major difference when compared with the reference.

6.4. Behaviour test on the fixed micropipette

Each laboratory performed 100 measurements continually in the fixed micropipette of 1000 μl in order to verify the differences between the volume calibration and the nominal volume when used in real conditions of repeated additions; the results are presented in table 14 and figure 17.

Table 14 – Behaviour test

	Continuous procedure		Normal procedure	
Laboratory	**Volume (μl)**	**Standard-deviation of the mean**	**Volume (μl)**	**Standard-deviation of the mean**
IPQ-1	1000,02	0,63	1000,55	0,40
JV	1001,40	0,11	1001,30	0,08
FORCE	1000,05	0,17	999,33	0,52
UME	1005,01	0,08	1004,94	0,05
CMI	998,88	0,33	999,10	0,43
CEM	989,78	0,55	989,30	0,72
IPQ-2	1000,00	0,61	1000,69	0,41

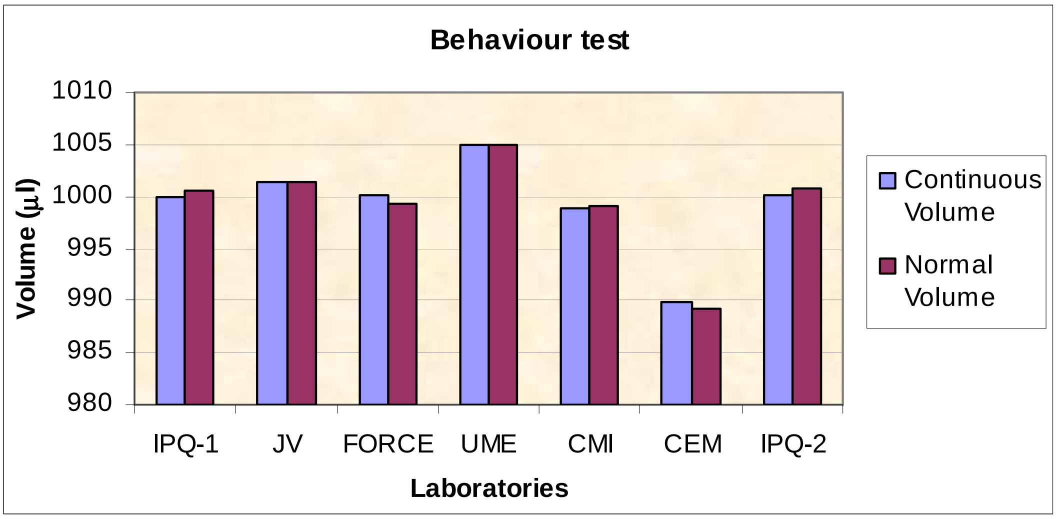

Figure 17 – Behaviour test

There is no significant difference in both procedures for all laboratories.

7. Uncertainty calculation

7.1. "Type A" and "type B" standard - uncertainties

The following figures show the different approaches on the evaluation of measurement uncertainty in both fixed and variable micropipette at 1000 μl. The standard deviation of the mean from the repeated measurements was taken as the "type A" contribution for the standard-uncertainty [6]. The "type B" uncertainty component comprise the combination on the standard-uncertainties of the input variables, mass, air density, water density, mass standards density, expansion coefficient, water temperature and evaporation. The expanded uncertainty for each participant is also presented.

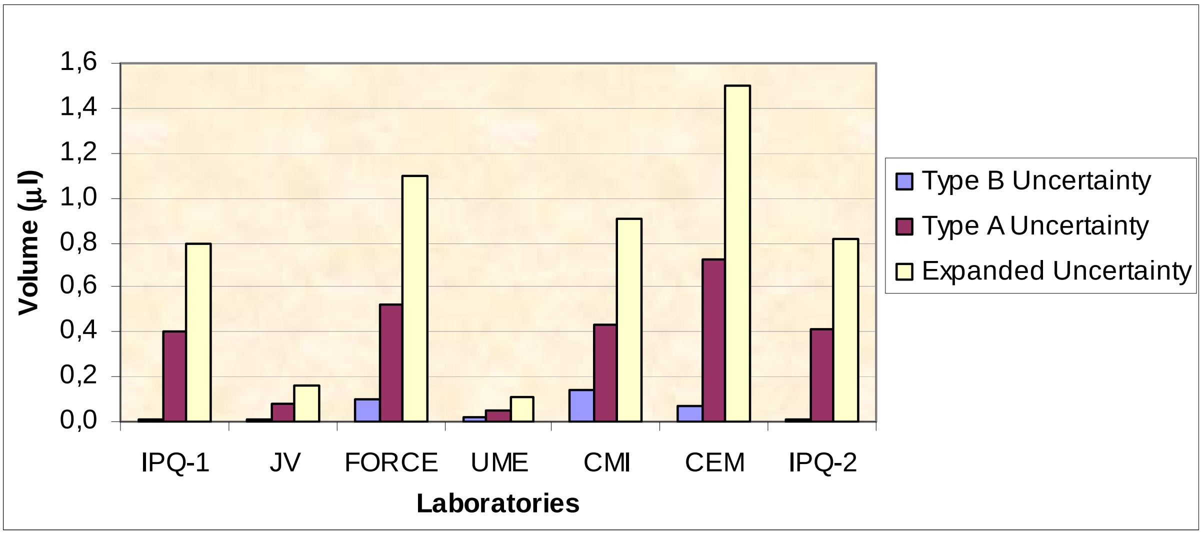

Figure 18 – Difference between the type A and type B uncertainty - fixed micropipette

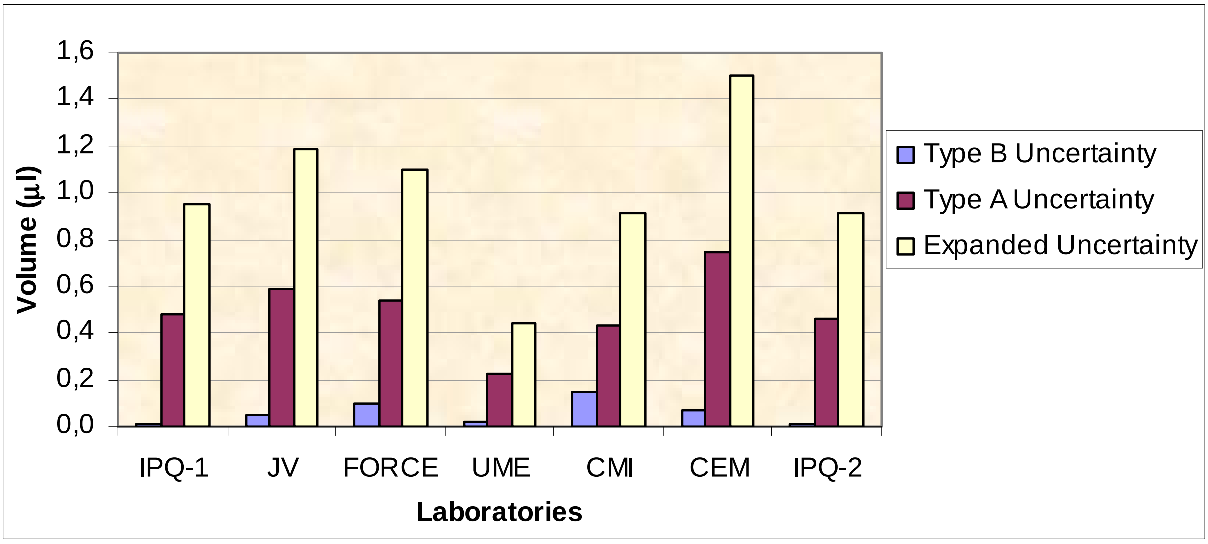

Figure 19 – Difference between the type A and type B uncertainty - variable micropipette at 1000 μl

In both micropipettes for the point 1000 µl and for all laboratories the repeatability of the measurements is larger than the "type B" uncertainty. For all the other points and in a general way the "type A" uncertainty decreased with the decrease of volume measure causing also a general decrease in the expanded uncertainty.

7.2. Uncertainty components

A spreadsheet (Annex 1) with the uncertainty components to be considered was presented to all participants. The majority of the laboratories replied according to this proposal.

The proposed uncertainty components were: mass, air density, water density, mass standards density, expansion coefficient, water temperature and evaporation.

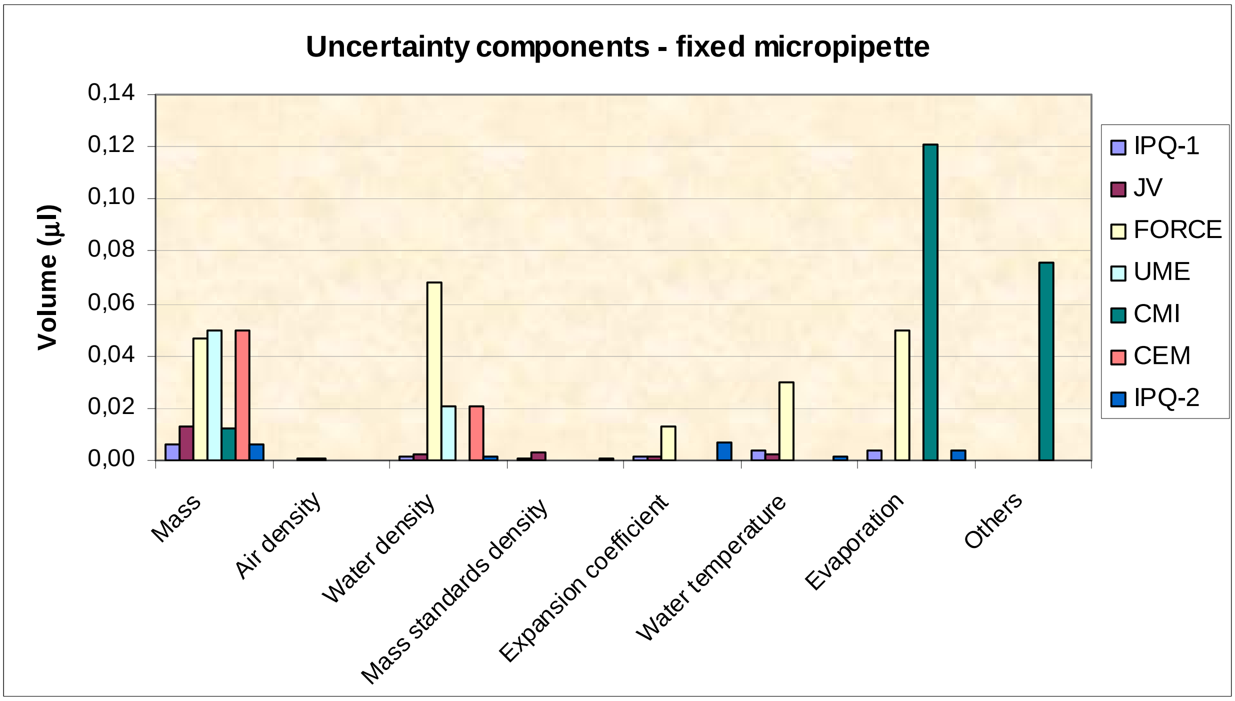

Figure 20 – Uncertainty components - fixed micropipette

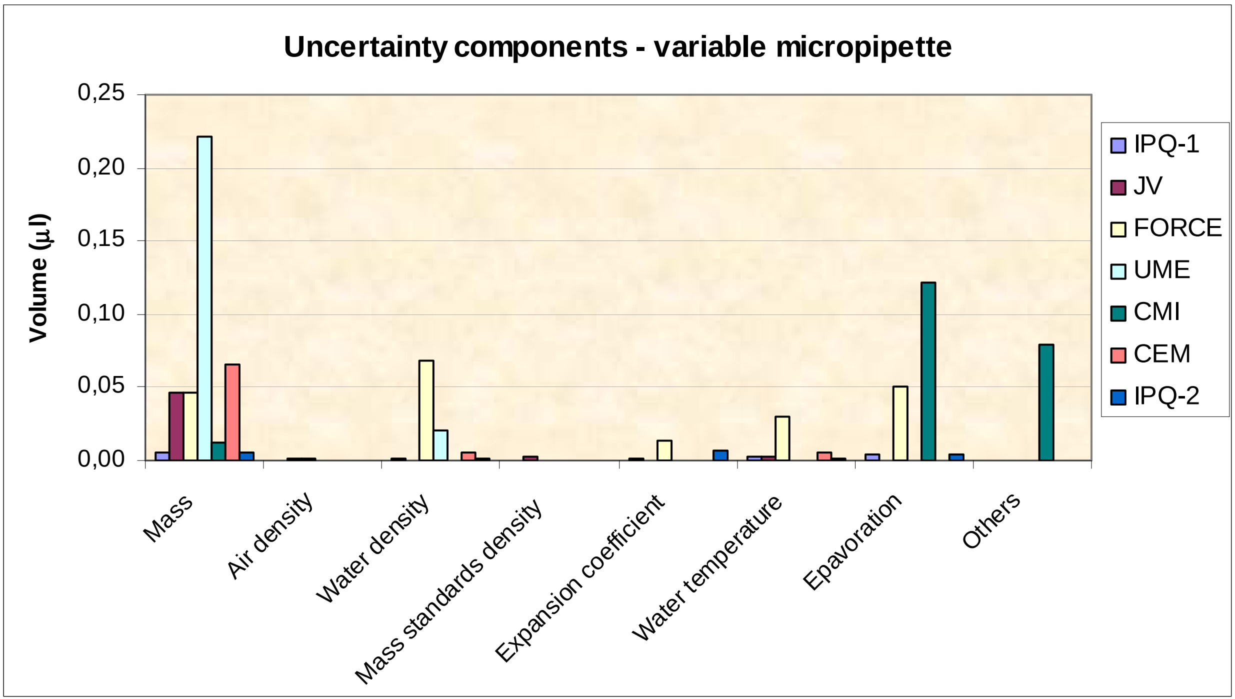

Figure 21 – Uncertainty components - variable micropipette

CMI presented uncertainties components different from the proposed in the spreadsheet by the pilot laboratory; these components were added in order to present a single result.

The uncertainty components for each laboratory for the fixed and variable micropipette at 1000 µl are defined in Annex 3.

The other points for the variable micropipette have the some uncertainty components has the ones presented in figure 21.

7.3. Major source of "type B" standard-uncertainty

For the calibration in both micropipettes the major source of "type B" standard-uncertainty is the following:

Table 15 – Major source of uncertainty

Participant	Major source of uncertainty
IPQ-1	Mass
JV	Mass
FORCE	Water density
CMI	Evaporation
UME	Mass
CEM	Mass
IPQ-2	Mass

As it can be seen by this table and the previous figures the major source of uncertainty is the quantity mass, for the majority of the laboratories.

8. Conclusions

More than one third of the laboratories have results consistent with the reference value and almost all results are within the maximum permissible error of the micropipettes. The uncertainty sources were comparable but some laboratories presented very low values for these components. The uncertainty component that has a major contribution to the final uncertainty was the repeatability of the measurements for all laboratories.

9. References

1. ISO 8655-1/2/6, Piston-operated volumetric apparatus, 1st ed., Genève, International Organization for Standardization, 2002.
2. ASTM E542: Standard Practice for Calibration of laboratory Volumetric Apparatus, 1st ed., American Standard, 1st ed., 2000.
3. ISO 3696 – Water for analytical laboratory use: specification and test methods, 1st ed., Genève, International Organization for Standardization, 1987.
4. BIPM et al, Guide to the Expression of Uncertainty in Measurement (GUM) – Supplement 1 – Numerical Methods for the Propagation of Distribution, International Organization for Standardization, Genève, 2004.
5. M.G. Cox, "The evaluation of key comparison data", *Metrologia*, 2002, Vol. 39, 589-595.
6. BIPM et al, Guide to the Expression of Uncertainty in Measurement (GUM), 2nd ed., International Organization for Standardization, Genève, 1995.

Annex 1 – Spreadsheet

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EUROMET Project "Volume calibration of 1000 μl micropipettes"

Data Form

General Information

Country		Laboratory	
Responsible		Date	

Equipment

	Type	Range	Resolution	Tracebility (when aplied)
Weighing instrument				
Thermometer				
Barometer				
Hydrometer				
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 description)

Comments:

Signature:

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EUROMET Project "Volume calibration of 1000 µl micropipettes"

Results form calibration of 1000 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 (ºC $^{-1}$)						
Water temperature (ºC)						
Evaporation (µl)						
Other						

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

Comments:

Signature:

The same type of sheet was supplied for presenting the results of the variable micropipette.

Annex 2 - Equipment

Weighing instrument

Laboratory	Type	Range	Resolution
IPQ	Mettler, AX 26	(0 - 22) g	0,001 mg
JV	Mettler-Toledo, AT 261	-	0,01 mg
FORCE	Mettler Toledo, AX 205	(80/221) g	0,01 mg
UME	Mettler-Toledo, AX 205	(0- 220) g	0,01 mg
CMI	Mettler-Toledo, AX 205	(0 - 220) g	0,01 mg
CEM	Mettler-Toledo, AX 205	(0 - 220) g	0,01 mg

Water Thermometer

Laboratory	Type	Range	Resolution
IPQ	Digital Luft	(-100 to 200) ºC	0,01 ºC
JV	Dostmann P650-EX		0,01 °C
FORCE			0,01 ºC
UME	Guildline/9540	(-40 to 180)°C	0,001°C
CMI	Digital ALS, F250	(-30 to 100) ºC	0,001 ºC
CEM	Labfacility/Tempmaster 100	(18 to 24) °C	0,010 ºC

Air Thermometer

Laboratory	Type	Range	Resolution
IPQ	Digital	(0 to 50) ºC	0,1 ºC
JV	JRI, Selene T/HYG-vol-01		0,1 ºC
FORCE	Goldbrand, Hg	(0 to 50) ºC	0,1 ºC
UME	Vaisala 38E	(-40 to 80) ºC	0,01 ºC
CMI			0,1 ºC
CEM	ASL/F250+SB250	(18 to 24) ºC	0,010 ºC

Barometer

Laboratory	Type	Range	Resolution
IPQ	Digital	(800 - 1150) hPa	0,1 hPa
JV	Druck LTd 1115/94-4		0,01 kPa
FORCE	Prazition Aneroid	(870 - 1055) mbar	0,5 mbar
UME	Desgranges & Huat DPM1	(0 – 1,6) bar	0,001 mbar
CMI	Digital COMET, THPZ	(80 - 105) kPa	0,1 kPa
CEM	DRUCK DPI-141	(800 - 1200) hPa	1 Pa

Hygrometer

Laboratory	Type	Range	Resolution
IPQ	Digital	(0 - 100) %	0,1 %
JV	JRI, Selene T/HYG-vol-01		0,1 %
FORCE	Almeno	(5 - 98) %	0,1 %
UME	VAISALA 38	(0 - 100) %	0,01 %
CMI	Digital COMET, THPZ	(5 - 95) %	1 %
CEM	MBW K12+PolyStart CC1	(1 - 15) ºC	100 mK

Annex 3 – Uncertainty components for each laboratory

IPQ-1

Fixed micropipette

Quantity (x_i)	Distribution	Standard uncertainty $u(x_i)$	Sensitivity coefficient c_i	Uncertainty $u(y_i)$
Repeatability measurements (µl)		0,401		0,401
Mass (mg)	Normal	0,006	1	0,006
Air Density (mg/µl)	Rectangular	2,89E-7	8,78E+2	2,53E-4
Water Density (mg/µl)	Rectangular	1,16E-6	-1E+3	-1,17E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	1,88E-2	6,53E-4
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	2,89E-6	-5,03E+2	-1,45E-3
Water temperature (ºC)	Normal	1,51E-2	-2,40E-1	-3,60E-3
Evaporation (µl)	Normal	0,004	1	0,004

Variable micropipette (1000 µl)

Quantity (x_i)	Distribution	Standard uncertainty $u(x_i)$	Sensitivity coefficient c_i	Uncertainty $u(y_i)$
Repeatability measurements (µl)		0,477		0,477
Mass (mg)	Normal	0,006	1	0,006
Air Density (mg/µl)	Rectangular	2,89E-7	8,74E+2	2,52E-4
Water Density (mg/µl)	Rectangular	1,17E-6	-9,99E+2	-1,17E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	1,87E-2	6,49E-4
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	2,89E-6	-3,65E+2	-1,06E-3
Water temperature (ºC)	Normal	1,01E-2	-2,39E-1	-2,42E-3
Evaporation (µl)	Normal	0,004	1	0,004

JV

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (µl)	Normal	0,07900668	1,002802424	0,07922809
Mass (mg)	Normal	0,013	-1,002802424	-0,01303643
Air Density (mg/µl)	Normal	5,92305E-07	879,2205876	0,00052077
Water Density (mg/µl)	Normal	2,33467E-06	-1004,413954	-0,00234497
Density of the mass pieces (mg/µl)	Normal	0,15	0,018032019	0,0027048
Coefficient of expansion from the micropipette material (°C^{-1})	Normal	0,0000072	-250,3356077	-0,00180242
Water temperature (ºC)	Normal	0,01	-0,240322183	-0,00240322
Evaporation (µl)	Normal	0,000000005	-1	-5E-09

Variable micropipette (1000 µl)

Quantity (x_i)	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Uncertainty** $u(y_i)$
Repetibility measurements	Normal	0,59	1,002825589	5,91E-01
Mass (mg)	Normal	4,63E-02	-1,002825589	-4,64E-02
Air Density (mg/µl)	Normal	1,18461E-06	871,2541096	1,03E-03
Water Density mg/µl)	Normal	4,62506E-09	-995,3176684	-4,60E-06
Density of the mass pieces (mg/µl)	Normal	0,15	0,018148723	2,72E-03
Coefficient of expansion from the micropipette material (°C^{-1})	Normal	7,20E-06	-19,84716692	-1,43E-04
Water temperature (ºC)	Normal	0,01	-0,238166003	-2,38E-03
Scale (µl)	Rectangular	0,29	-	-
Evaporation (µl)	Normal	0,000000005	-1	-5E-09
Other: Reference temperature (ºC)	Normal	-	-	-

FORCE

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (ml)		0,52		0,519
Mass (mg)	1	0,0471	1,003	0,047
Air Density (mg/ml)	0,58	0,0025	1,003	0,001
Water Density (mg/ml)	0,50	0,14	1,001	0,068
Density of the mass pieces (mg/ml)	Included in mass			
Coefficient of expansion from the micropipette material (°C^{-1})	0,50	0,000020	1299,1	0,013
Water temperature (ºC)	0,50	0,27	0,22	0,030
Evaporation (ml)	0,50	0,010	10	0,050

Variable micropipette (1000 µl)

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (µl)		0,538		0,538
Mass (mg)	1	0,047	1,003	0,047
Air Density (mg/ml)	0,58	0,0025	1,001	0,001
Water Density (mg/ml)	0,50	0,14	0,9998	0,068
Density of the mass pieces (mg/ml)	Included in mass			
Coefficient of expansion from the micropipette material (°C^{-1})	0,50	0,000020	1321,9	0,013
Water temperature (ºC)	0,50	0,27	0,22	0,030
Evaporation (µl)	0,50	0,010	10	0,050

UME

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Mass (mg)	Normal	0,000050	1,00E+03	5,00E-02
Air Density (mg/µl)	Rectangular	6,04E-09	8,80E-01	5,32E-09
Water Density (g/µl)	Rectangular	2,06E-08	1,01E+03	2,07E-02
Density of the mass pieces (mg/µl)	Rectangular	0,0048	1,83E-05	8,78E-08
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	0,000080	-7,03E-02	5,62E-06
Water temperature (ºC)	Rectangular	0,000021	-2,41E-04	4,96E-09

Variable micropipette (1000 µl)

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Mass (mg)	Normal	0,000221	1,00E+03	2,21E-01
Air Density (mg/µl)	Rectangular	6,04E-09	8,77E-01	5,30E-09
Water Density (g/µl)	Rectangular	2,07E-08	1,00E+03	2,08E-02
Density of the mass pieces (mg/µl)	Rectangular	0,0048	1,84E-05	8,85E-08
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	0,000080	3,70E-02	2,96E-06
Water temperature (ºC)	Rectangular	0,000021	-2,40E-04	4,97E-09

CMI

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repetibility measurements	normal	0,43028	1,000E+00	0,43028
Mass (mg)	normal	0,01258	1,003E+00	1,262E-02
t_{water} [°C]	normal	0,02000	-1,000E-03	-2,001E-05
$t_{heating\ of\ water}$ [°C]	rectangular	0,08660	-1,000E-03	-8,663E-05
t_{air} [°C]	normal	0,10000	5,894E-03	5,894E-04
p_{air} [kPa]	normal	0,01300	3,062E-06	3,981E-08
rel.humidity$_{air}$ [%]	normal	2,50000	-3,008E-02	-7,519E-02
Evaporation[mg]	rectangular	0,12124	1,003E+00	1,216E-01

Variable micropipette (1000 µl)

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repetibility measurements	normal	0,42750	1,000E+00	4,275E-01
Mass (mg)	normal	0,01258	1,003E+00	1,262E-02
t_{water} [°C]	normal	0,02000	-1,000E-03	-2,001E-05
$t_{heating\ of\ water}$ [°C]	rectangular	0,08660	-1,000E-03	-8,663E-05
t_{air} [°C]	normal	0,10000	5,916E-03	5,916E-04
p_{air} [kPa]	normal	0,01300	3,062E-06	3,981E-08
rel.humidity$_{air}$ [%]	normal	2,50000	-3,141E-02	-7,852E-02
evaporation[mg]	rectangular	0,12124	1,003E+00	1,216E-01

CEM

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Mass (g)	normal	0,00066	1,00277	0,000066
Air Density (kg/m^3)	rectangular	0,00013	0,000868	1,12897E-07
Water Density (kg/m^3)	rectangular	0,0052	0,000993	5,16268E-06
Density of the mass pieces (kg/m^3)	rectangular	15	1,7262E-08	2,5893E-07
Coefficient of expansion from the micropipette material (°C^{-1})	rectangular	2,9E-06	0,029698	8,61251E-08
Water temperature (ºC)	normal	0,025	0,000237	5,93966E-06

Variable micropipette (1000 µl)

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Mass (g)	normal	0,000066	1,00277	0,000066
Air Density (kg/m^3)	rectangular	0,00013	0,000873	1,13489E-07
Water Density (kg/m^3)	rectangular	0,0052	0,000998	5,1897E-06
Density of the mass pieces (kg/m^3)	rectangular	15	1,73536E-08	2,60304E-07
Coefficient of expansion from the micropipette material (°C^{-1})	rectangular	2,90E-06	0,059708	1,73152E-07
Water temperature (ºC)	normal	0,025	0,000239	5,97076E-06

IPQ-2

Fixed micropipette

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (µl)		0,412		0,412
Mass (mg)	Normal	0,006	1	0,006
Air Density (mg/µl)	Rectangular	2,89E-7	8,78E+2	2,54E-4
Water Density (mg/µl)	Rectangular	1,33E-6	-1E+3	-1,34E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	1,85E-2	6,42E-4
Coefficient of expansion from the micropipette material ($°C^{-1}$)	Rectangular	2,89E-6	-2,35E+3	-6,78E-3
Water temperature (ºC)	Normal	5E-3	-2,40E-1	-1,20E-3
Evaporation (µl)	Normal	0,004	1	0,004

Variable micropipette (1000 µl)

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (µl)		0,457		0,457
Mass (mg)	Normal	0,006	1	0,006
Air Density (mg/µl)	Rectangular	2,89E-7	8,74E+2	2,52E-4
Water Density (mg/µl)	Rectangular	1,33E-6	-9,99E+2	-1,32E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	1,85E-2	6,41E-4
Coefficient of expansion from the micropipette material ($°C^{-1}$)	Rectangular	2,89E-6	-2,23E+3	-6,43E-3
Water temperature (ºC)	Normal	5,78E-3	-2,39E-1	-1,38E-3
Evaporation (µl)	Normal	0,004	1	0,004