EURAMET
European Association of National Metrology Institutes

Instituto Português da Qualidade
PORTUGUESE INSTITUTE FOR QUALITY

Final Report

Bilateral comparison of a 1000 μl micropipette

EURAMET Project no. 1136

IPQ – Coordinator of the comparison

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1. Introduction

During the EURAMET meeting held in Copenhagen of March 2009, the Hellenic Institute of Metrology (EIM) expressed the interest to participate in a bilateral comparison with the Portuguese NMI, IPQ on calibration of micropipettes.

The main purpose of this project was to compare the results and uncertainties of a 1000 μl micropipette calibration, despite the different used equipment and calibration method.

IPQ, acting as the pilot laboratory performed two measurements, one in the beginning and another in the end of the comparison.

Table 1 - Participants in the EURAMET project 1136

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	December 2009 /January 2010	Elsa Batista	Tel: +351212948167 Email: ebatista@mail.ipq.pt
Greece	EIM	December 2009	Zoe Metaxiotou	Tel : +302310569999 Email: zoe@eim.gr

2. The instrument

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

Micropipettes may be factory-preset to deliver a given volume, or have selectable volumes within a useful volume range (1). 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 $^{-1}$ (2).

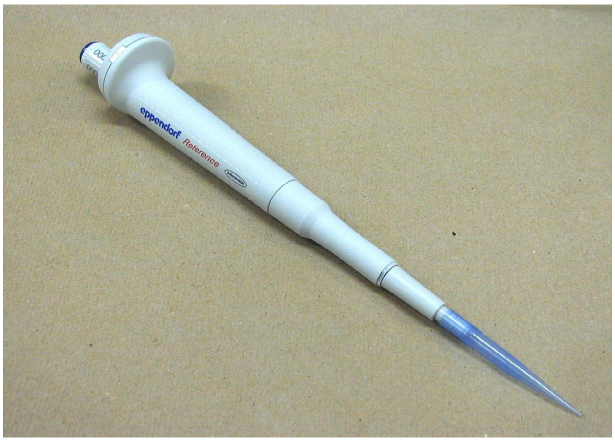

Figure 1- Fixed micropipette

3. Calibration procedure and mathematical model

The used calibration procedure of the pilot laboratory is based on ISO 8655-2 (direct weighing) and the mathematical model is based on ISO 4787.
EIM used there internal calibration procedure (double substitution) and the model based also on ISO 4787. Both laboratories performed 10 repeated measurements, at a reference temperature of 20 ºC.

3.1. Equipment

Each laboratory described the equipment used in the calibration.

Table 2 – Equipment characteristics

Balance	**Type**	**Range**	**Resolution**
IPQ	Electronic	(0-22) g	0,001 mg
EIM	Electronic	(0-220) g	0,01 mg
Water thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(-30 to 150) ºC	0,01 ºC
EIM	Digital	-	0,001 Ω
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to 50) ºC	0,1 ºC
EIM	Digital	(-50 to 200) ºC	0,1 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(800 - 1150) hPa	0,01 hPa
EIM	Digital	(870 – 1050) hPa	0,5 hPa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0-100) %	0,1%
EIM	Digital	(0-100) %	0,1%

3.2. Type of water

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

Table 3 – Water characteristics

Laboratory	**Type**	**Density reference**	**Conductivity (μS/cm)**
IPQ	Ultra-pure	Tanaka	0,046
EIM	Bi-distilled	Anton Paar density meter	-

IPQ used a density value described on the literature; while EIM determines the density of water using a density meter. Both laboratories used water of adequate purity.

3.3. Mass standards

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

Table 4 – Mass Standards

Laboratory	OIML Accuracy Class	Density (kg/m^3)
IPQ	E2	7960-8600
EIM	F1	7950

4. Ambient conditions

Both laboratories described the ambient conditions which the calibration was performed.

Table 5 - Ambient conditions

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ	20,5	1011,10	61,1	0,0012
EIM	16,9	1002,0	51,6	0,0012
IPQ	21,1	1012,32	58,3	0,0012

In the calibration of micropipettes the most important ambient conditions is the humidity that should have a value above 50 %. Both laboratories complied with this requirement.

5. Measurement results

5.1 Volume measurements

The volume measurements obtained by IPQ in the beginning of the comparison (IPQ-1) and in the end of the comparison (IPQ-2) and by EIM are presented in the following table and figure:

Table 6 – Volume measurement results

Laboratory	Volume (µl)	U_{exp} (µl)
IPQ-1	1001,25	0,60
EIM	1000,75	0,54
IPQ-2	1000,85	0,64

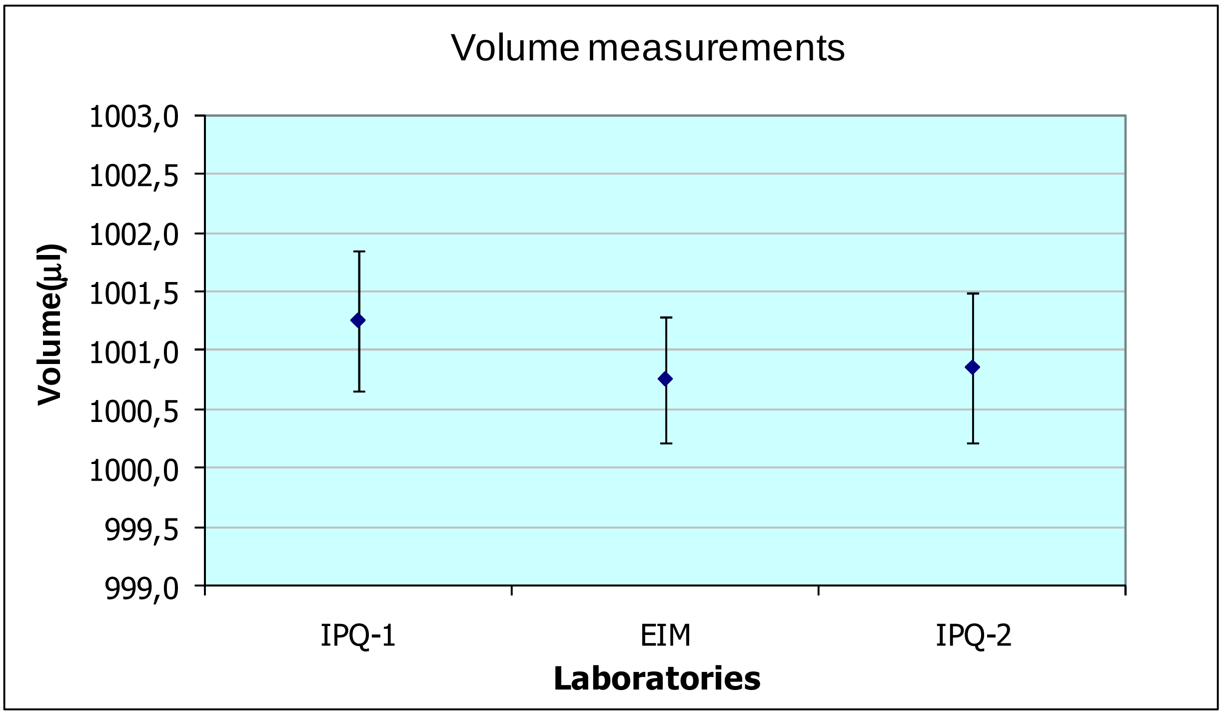

Figure 2 – Volume measurements

5.2. Determination of the reference value

The reference value was determined based on the mean average of both results of IPQ and the uncertainty of the reference value is the larger uncertainty value obtained by IPQ.

The determined values are ***y* = 1001,05 µl and *U(y)* = 0,64 µl**

In the next figure it is shown the measurement results with reference value and associated uncertainty.

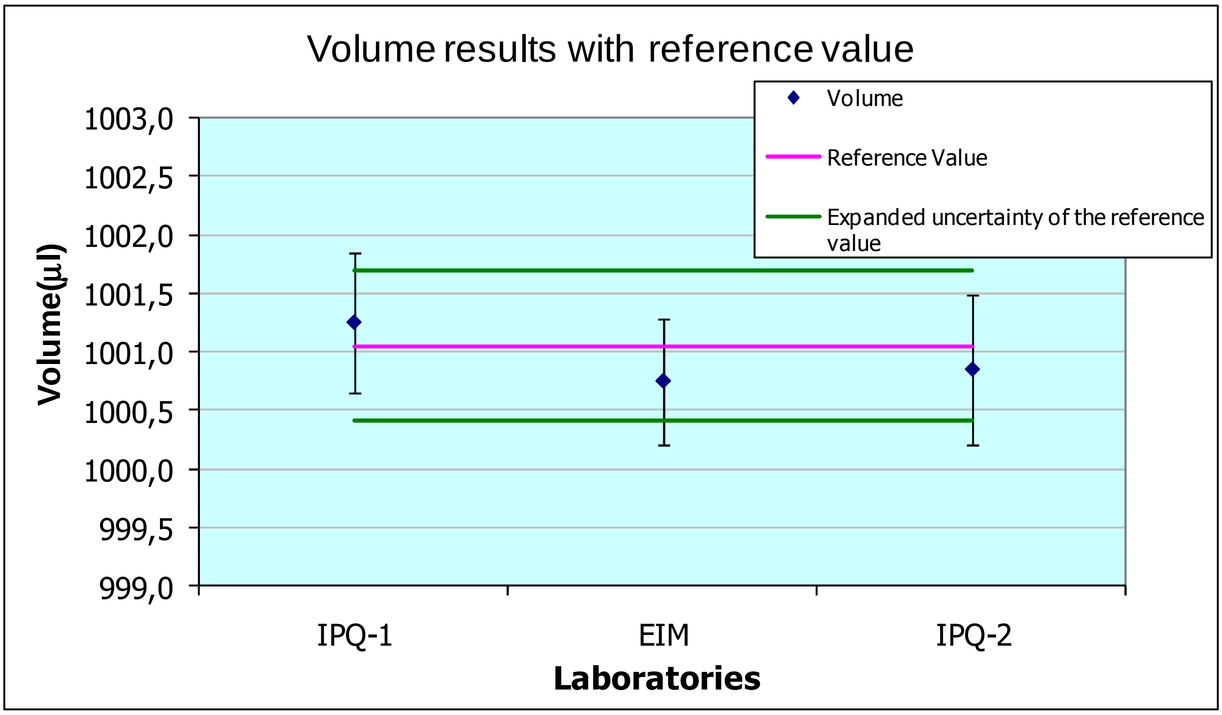

Figure 3 – Volume results with reference value

From this figure it can be observed that the volume result of EIM is consistent with the reference value.

6. Uncertainty calculation

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

The following figure shows the different approaches on the evaluation of measurement uncertainty (3). The standard deviation of the mean from the repeated measurements was taken as the "type A" contribution for the standard-uncertainty. The "type B" uncertainty component comprises the combination on the standard-uncertainties of the input variables, mass, air density, water density, mass standards density, expansion coefficient, water temperature and others components. The expanded uncertainty for each participant is also presented.

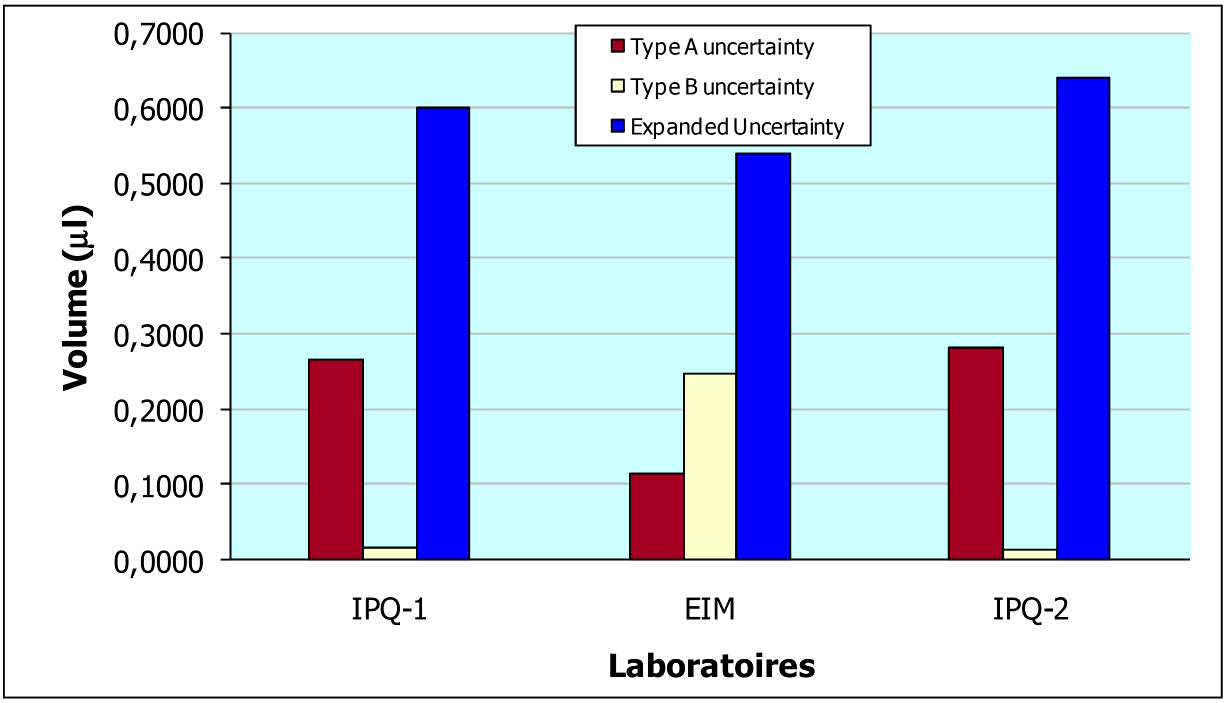

Figure 4 – Difference between the type A and type B uncertainty

The "type B" uncertainty is the larger source for EIM, while for IPQ the largest source of uncertainty is the "type A" uncertainty.

6.2. "Type B" uncertainty components

The presented "Type B" uncertainty components were the following:

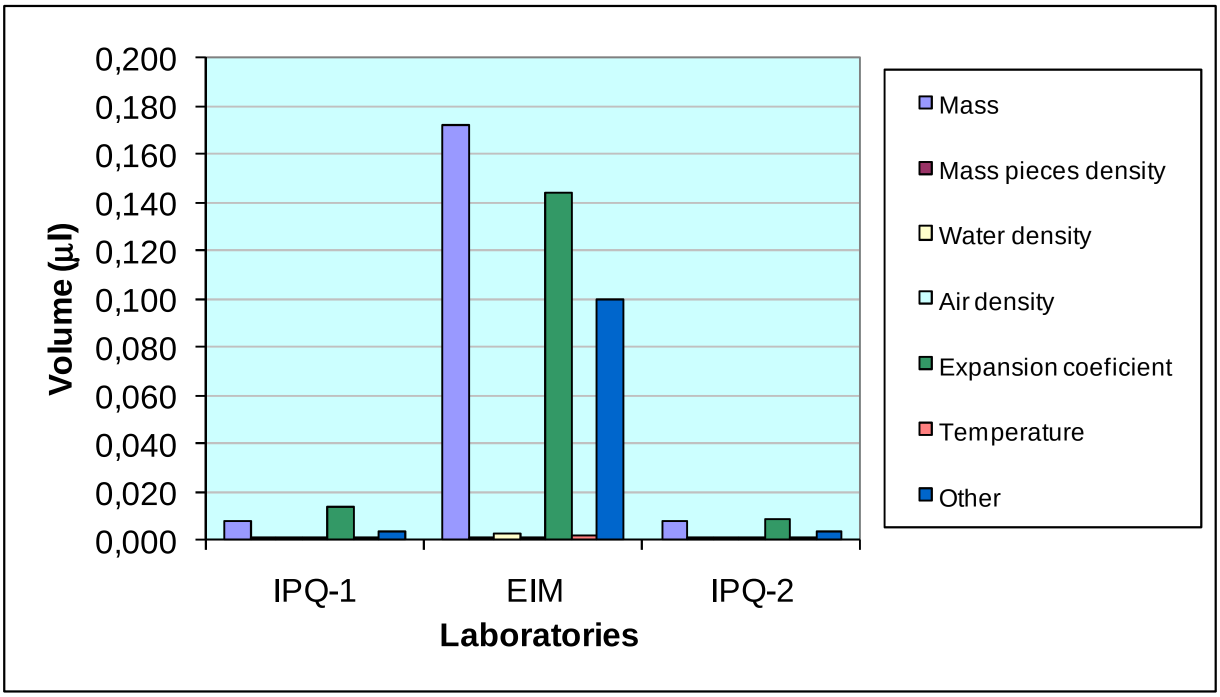

Figure 5 – "Type B" uncertainty components

The larger "type B" uncertainty at IPQ budget comes from the expansion coefficient of the micropipette and at EIM budget comes from mass. The difference of values between the laboratories is due to the calibration method: at EIM procedure it is used a double substitution method with F1 masses while at IPQ procedure uses a directed reading at a balance with a better resolution.

7. Comparison between EUROMET project 865 and EURAMET project 1136

This project followed the EUROMET Project 865 protocol, thus permitting EIM to test the agreement of their results despite the different used equipment.

The first step to the linking is to compare the result of IPQ in both projects by making the difference of the two values; in the case of this project 1136 the mean of IPQ values was used as reference value. From the difference of IPQ values it was obtained and represented at Fig. 6 the position of EIM value related to the reference value of project 865.

Table 7 – Linking of the results of EUROMET project 865 with EIM results in EURAMET project 1136

Laboratory/project	Volume (µl)	Uncertainty (µl)	Difference (µl)
IPQ / 865	1000,55	0,80	0,50
IPQ / 1136	1001,05	0,64	
EIM / 1136	1000,75	0,54	
EIM related to 865	1000,25	0,54	

The results can then be presented in the following figure:

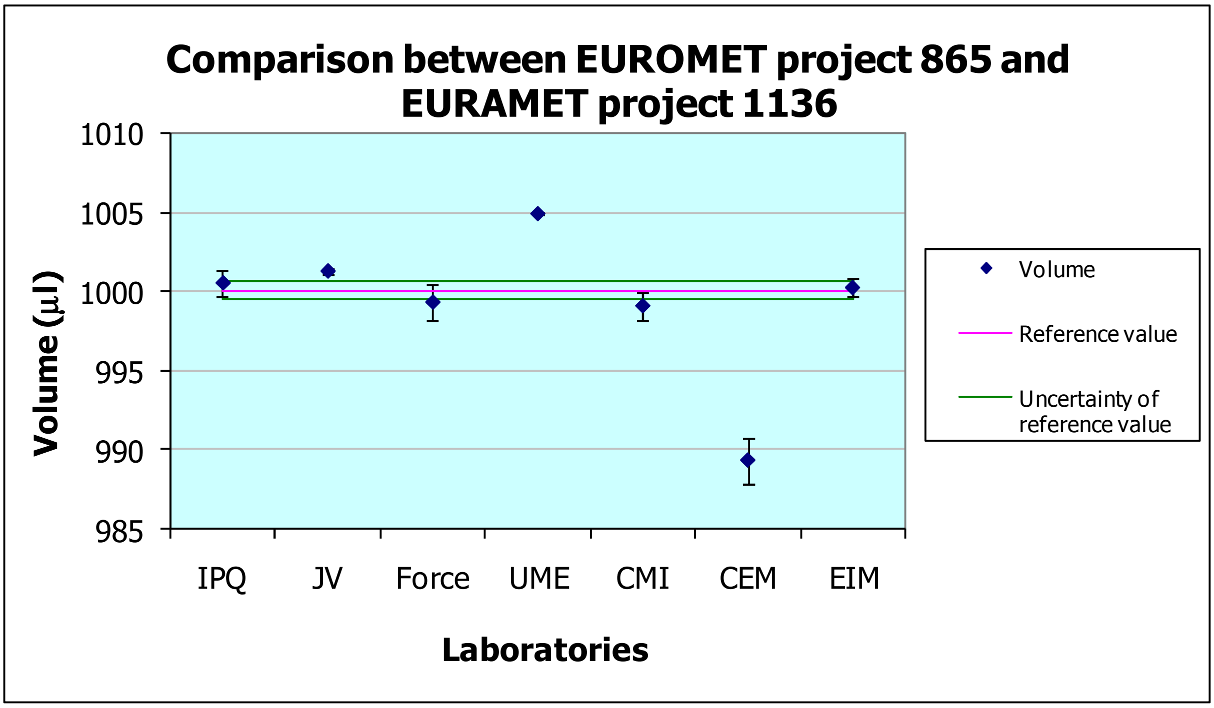

Figure 6 – Comparison between EUROMET project 865 and EURAMET project 1136

From analyse of Fig. 6 it can be stated that EIM is in agreement with the reference value of the EUROMET project 865.

7.1. Degrees of equivalence

To calculate the degrees of equivalence between the reference value of EUROMET project 865 and the results of EIM the following formula is used:

$d_i = x_I - x_{ref} = 1000{,}25 - 1000{,}06 = 0{,}19\ \mu l$

The results are presented in the following figure:

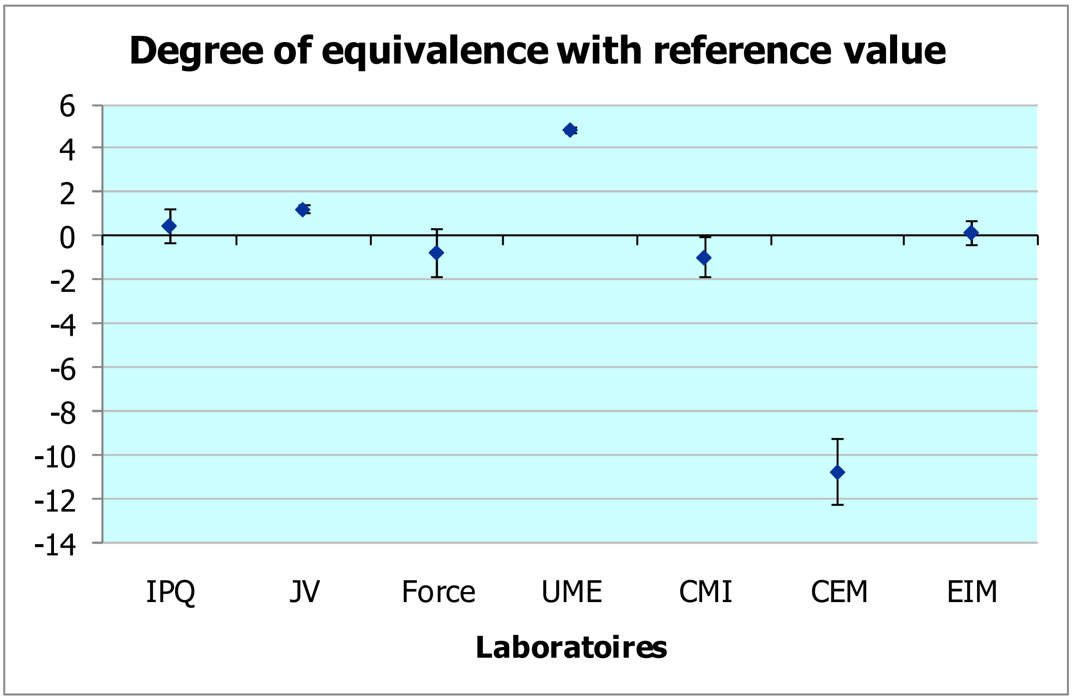

Figure 7 – Degree of equivalence between EIM and EUROMET project 865

8. Conclusions

This bilateral comparison of a 1000 µl micropipette involved IPQ and EIM. IPQ, acting as the pilot laboratory determined the reference value.

The volume results of EIM are consisted with the reference value despite the use of a different calibration method.

The value of the expanded uncertainty is quite similar but there are some differences in the values of the uncertainty components. The uncertainty component that has a major contribution to the final uncertainty was the repeatability of the measurements for IPQ and the mass for EIM.

In this report it is as also determined the equivalence of EIM results with the EUROMET comparison 865. EIM results showed a good agreement with the reference value of EUROMET project 865.

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. BIPM et al, Guide to the Expression of Uncertainty in Measurement (GUM), 2nd ed., International Organization for Standardization, Genève, 1995.