Instituto Português da Qualidade

PORTUGUESE INSTITUTE FOR QUALITY

Final Report

Bilateral comparison of a 500 μl micropipette

EUROMET Project no. 1004

IPQ – Coordinator of the comparison

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Contents

1. Introduction

During the EUROMET meeting in Istanbul on March 2007, the Bureau of Measurements and Precious Metals of Serbia (ZMDM) manifested the interest to participate in a bilateral comparison in calibration of micropipettes with the Portuguese NMI, IPQ.
The main purpose of this project was to compare the results and uncertainties of a calibration of a 500 μl micropipette 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 EUROMET project 1004

Country	Laboratory	Periods	Responsible	Contact
Portugal	IPQ	July/August	Elsa Batista	Tel: +351212948167 Email: ebatista@mail.ipq.pt
Serbia	ZMDM	August	Branislav Tanasic	Tel : + 381112024465 Email: tanasic@szmdm.sv.gov.yu

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].

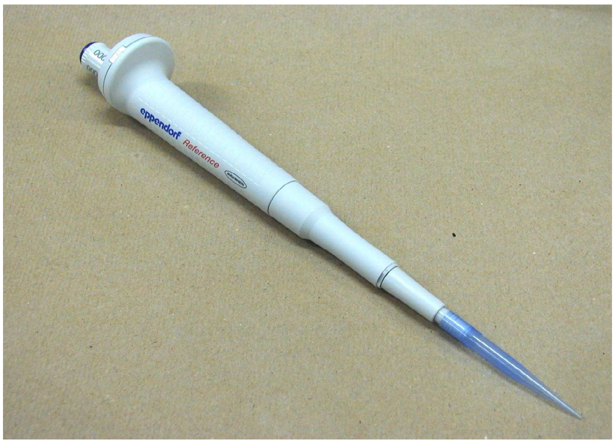

Figure 1- Fixed micropipette

3. The method

The used method and model for both laboratories was the one described in ISO 8655-2.

4. The experimental procedure

The experimental procedure was also for both laboratories the one described in ISO 8655-2, using 10 repeated measurements, at a reference temperature of 20 ºC.

4.1. 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
ZMDM	Electronic	(0 - 210) g	0,01 mg
Water thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(-30 to +150) ºC	0,01 ºC
ZMDM	Glass	(+20 to +25) ºC	0,01 ºC
Air Thermometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 to + 50)	0,1 ºC
ZMDM	Digital	(+10 to +30) ºC	0,01 ºC
Barometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(800 - 1 150) hpa	0,01 hpa
ZMDM	Digital	(800 - 1 100) hpa	0,01 hpa
Hygrometer	**Type**	**Range**	**Resolution**
IPQ	Digital	(0 - 100) %	0,1%
ZMDM	Digital	(0 - 100) %	0,1%

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
ZMDM	Distilled	Tanaka	< 0,5

The used water was similar for both participants

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	7 960 - 8 600
ZMDM	E2	7 950

The mass standards were of the same type for both laboratories.

5. Ambient conditions

The ambient conditions were described by the participants and they were also very similar:

Table 5 - Ambient conditions

	Air Temperature (ºC)	Pressure (hPa)	Humidity (%)	Air density (g/ml)
IPQ	20,5	1 010,15	55,0	0,001 2
ZMDM	20,90	1 001,135	56,3	0,001 18
IPQ	21,5	1 002,20	56,4	0,001 2

6. Measurement results

6.1 Volume measurements

Table 6 – Volume measurement results

Laboratory	Volume (μl)	U_{exp} (μl)
IPQ-1	497,56	0,51
ZMDM	497,99	0,50
IPQ-2	497,26	0,48

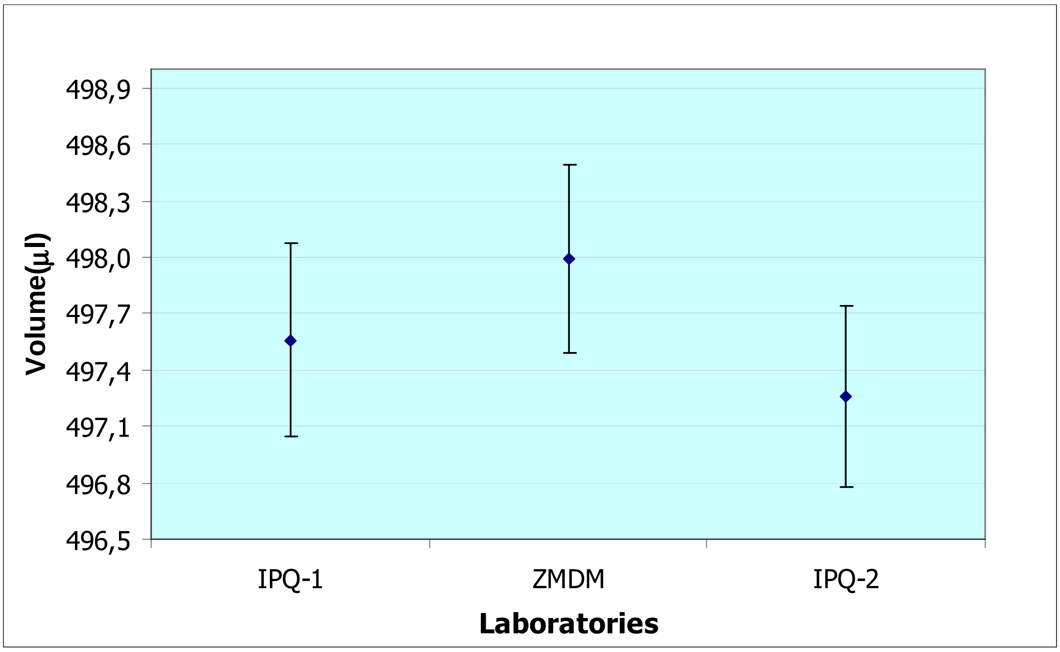

Figure 2 – Volume measurements

6.2. Determination of the reference value

To calculate the reference value of the three results the weighted mean and its uncertainty was used [(3)]:

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

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

The determined values are $\boldsymbol{y}$ **= 497,59 µl and** $\boldsymbol{u(y)}$ **= 0,29 µl**

In the next figure it is shown the measurement results with the weighted mean and associated uncertainty.

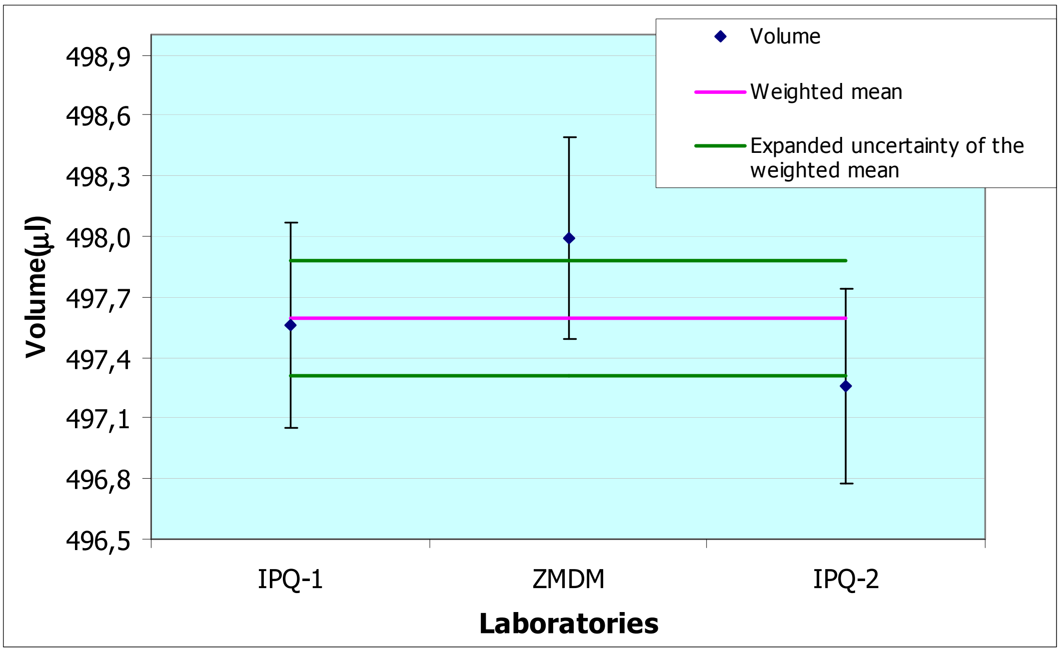

Figure 3 – Participants results with reference value

From this figure it can be observed that the volume results are quite close to each other and consistent with the reference value.

6.3. Degrees of Equivalence

To calculate the degrees of equivalence between the reference value and the laboratories de following formula is used [(3)]:

$$d_i = x_I - x_{ref}$$

and $U(d_i) = 2u(d_i)$, the factor 2 gives 95% coverage under the assumption of normality

were $u(d_i)$ is given by

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

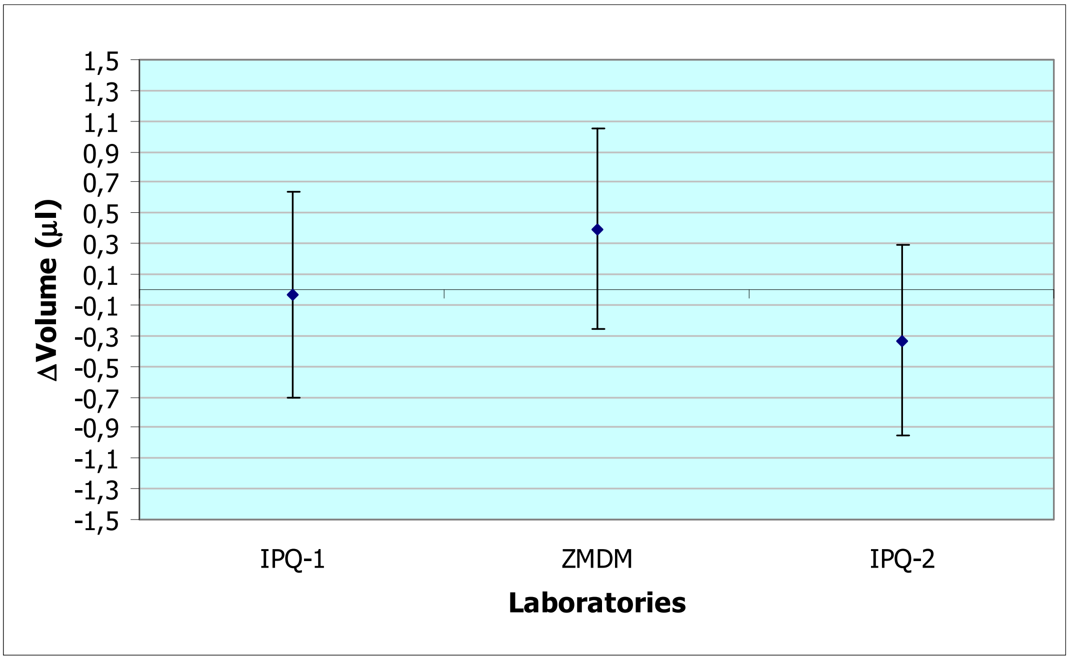

Figure 4 –Degree of equivalence between laboratories and reference value

The degree of equivalence between the laboratories and the reference value is quite good.

7. Uncertainty calculation

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

The following figure shows the different approaches on the evaluation of measurement uncertainty[(4)]. 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 components 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 both participants is also presented.

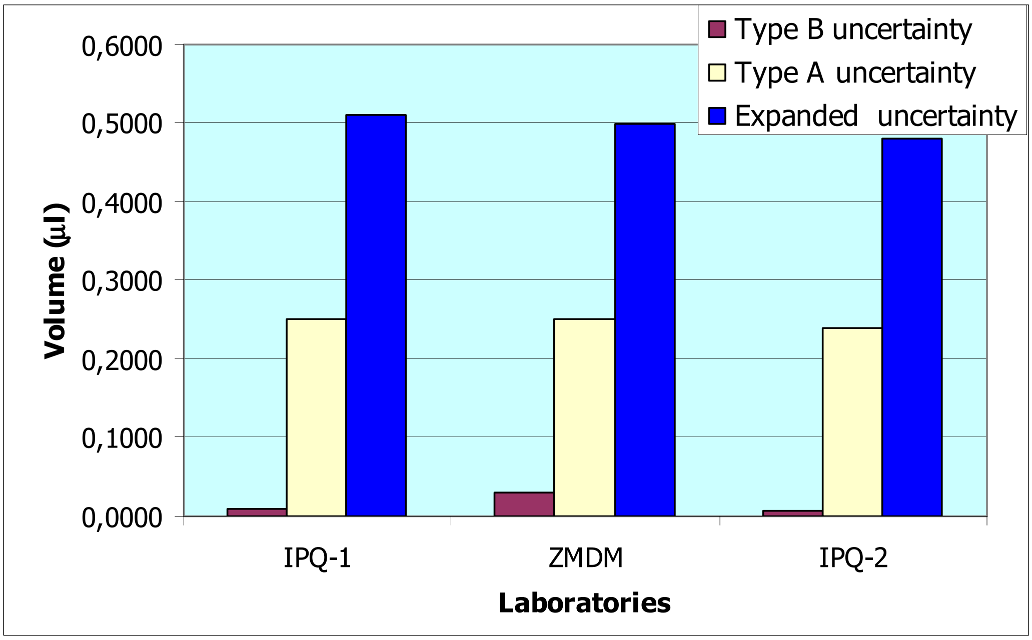

Figure 5 – Difference between the type A and type B uncertainty

In both laboratories the repeatability of the measurements is larger than the "type B" uncertainty.

7.2. "Type B" uncertainty components

A spreadsheet with the uncertainty components to be considered was supplied. The proposed uncertainty components were: mass, air density, water density, mass standards density, expansion coefficient, water temperature and evaporation.

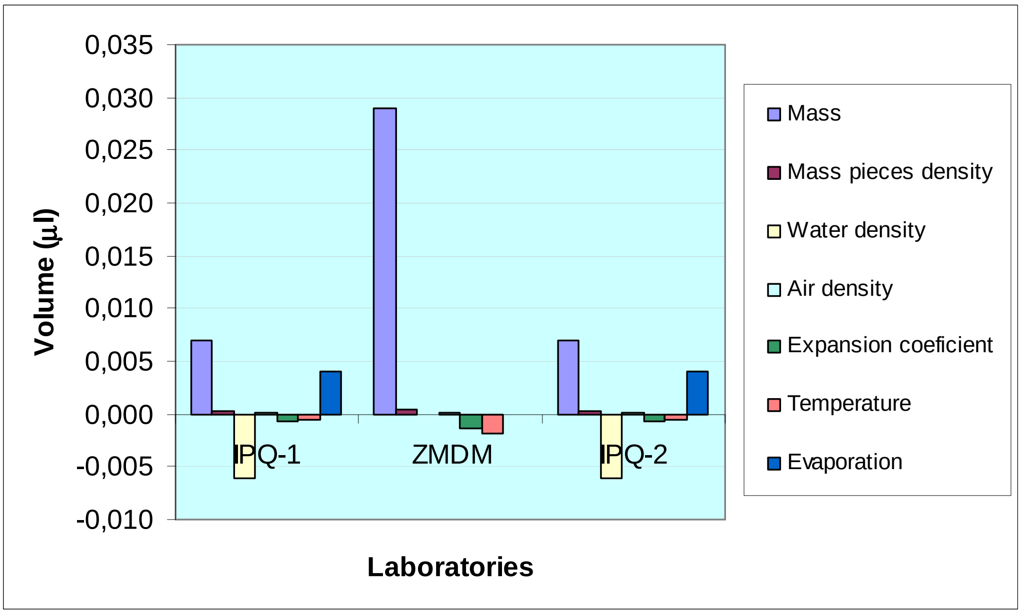

Figure 6 – "Type B" uncertainty components

The larger type B uncertainty for IPQ and ZMDM was the mass.

8. Conclusions

This bilateral comparison of a 500 μl micropipette involved IPQ and ZMDM. The volume results are quite similar and consisted with each other and with the reference value.

The presented uncertainties are also quite close, with some small differences in the type B components. The uncertainty component that has a major contribution to the final uncertainty was the repeatability of the measurements for both 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. M.G. Cox, "The evaluation of key comparison data", *Metrologia*, 2002, Vol. 39, 589-595.
4. BIPM et al, Guide to the Expression of Uncertainty in Measurement (GUM), 2nd ed., International Organization for Standardization, Genève, 1995.

Annex 1 – Uncertainty components for each laboratory

IPQ -1

Quantity *(x_i)*	**Distribution**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repeatability measurements (µl)		0,25		0,25
Mass (mg)	Normal	0,007	1	0,007
Air Density (mg/µl)	Rectangular	2,89E-7	4,37+2	1,26E-4
Water Density (mg/µl)	Rectangular	1,22E-5E-6	-4,99E+2	-6,10E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	9,37E-3	3,24E-4
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	2,89E-6	-2,49E+2	-7,14E-4
Water temperature (ºC)	Normal	5E-3	-1,19E-1	-5,97E-4
Evaporation (µl)	Normal	0,004	1	0,004

ZMDM

Quantity *(x_i)*	**Distributio n**	**Standard uncertainty** *$u(x_i)$*	**Sensitivity coefficient** *c_i*	**Uncertainty** *$u(y_i)$*
Repetibility measurements		0,250		0,250
Mass (mg)	Normal	0,029	1	0,029
Air Density (mg/µl)	Rectangular	1,43E-07	4,37E+02	6,26E-05
Water Density (mg/µl)	Rectangular	1,20E-07	-5,00E+02	-5,99E-05
Density of the mass pieces (mg/µl)	Rectangular	4,0E-02	9,30E-03	3,76E-04
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	2,89E-06	-4,96E+02	-1,43E-03
Water temperature (ºC)	Normal	1,58E-02	-1,20E-01	-1,89E-03

IPQ-2

Quantity (x_i)	**Distribution**	**Standard uncertainty** $u(x_i)$	**Sensitivity coefficient** c_i	**Uncertainty** $u(y_i)$
Repeatability measurements (µl)		0,24		0,24
Mass (mg)	Normal	0,007	1	0,007
Air Density (mg/µl)	Rectangular	2,89E-7	4,36+2	1,26E-4
Water Density (mg/µl)	Rectangular	1,22E-5	-4,99E+2	-6,10E-3
Density of the mass pieces (mg/µl)	Rectangular	3,46E-2	9,25E-3	3,20E-4
Coefficient of expansion from the micropipette material (°C^{-1})	Rectangular	2,89E-6	-2,49E+2	-7,18E-4
Water temperature (ºC)	Normal	5E-3	-1,19E-1	-5,97E-4
Evaporation (µl)	Normal	0,004	1	0,004