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AFRIMETS
Intra-Africa Metrology System
Système Intra-Africain de Métrologie

AFRIMETS key comparison AFRIMETS.FF-K4.2.2015

Volume comparison at 100 μL – Calibration of micropipettes)

Technical Protocol

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Volume and Fluid Flow Laboratory

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1- Introduction

During the **TCM meeting in Addis Ababa 2014**, it was agreed to start a Key Comparison (KC) concerning volume measurements in the range of the microliter. This Key Comparison has the main purpose of comparing the results and methods of calibration for 100 µL micropipette and will allow the participating laboratories to test the agreement of their results and uncertainties despite the different equipment and calibration methods.

This protocol describes the volume instruments, the methods and equations for volume determination, the calibration procedure, the experimental conditions and the presentation of measurement results with the associated uncertainties. The National Institute of Standards NIS-Egypt acting as the pilot laboratory, will perform the initial and final measurements of the micropipettes, collect the data from the participant laboratories, estimate the reference value and issue the comparison report.

The Pilot Laboratory NIS-Egypt will supply one 100 micro-liter fixed micropipettes, Eppendorf the serial numbers is 3563380A in addition, The Volume Laboratory of Portuguese Institute for Quality (IPQ) - Central Laboratory of Metrology (LCM), will supply two 100 micro-liter fixed micropipettes, Eppendorf the serial numbers are: 354568Z and 35472Z to be the comparison artifact, Technical protocol and the calculation sheet have been supplied by the IPQ. The necessary changes on the IPQ technical protocol have been done by NIS-Egypt to make it more suitable for the comparison.

IPQ- Portugal has performed initial measurement to the two micropipettes Eppendorf, the serial numbers are: 354568Z and 35472Z before sending them to NIS to start the comparison.

2. Participants

The following table contains the 12 participants' countries of this comparison:

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NIS	Egypt	AFRIMETS	M.Elsayed	**E-mail** : Elsayed.mohamed@gmail.com **Address :** Tersa St., Elharam, Giza, P.O. 136 Giza - Code 12211- Egypt Volume and Fluid Flow Laboratory, **Tel :** + 20 2 3740 1116 ext 3106, **Fax:** + 202 33867452 **Mobile:** + 20 111 1328 449
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Table (1) the participant countries in the comparison

3. General instructions

Each laboratory will be responsible for receiving the Transfer Standards (TSs), to test it and send it to the next participant according to the time schedule. When the standards arrive at the participating laboratory, a visual inspection should be made and the results reported to the pilot laboratory NIS-Egypt. The participating laboratories shall determine the volume of water that each of the two micropipettes is able to deliver at a reference temperature of 20 °C. Measurements should be done after an appropriate acclimatization time (at least 24 h after the reception of the equipment). Each participating laboratory shall ensure suitable source of water in order to make use of any of the formulas or tables. The excel sheet, see –Form sheets Micropipettes.xls -, for the measurement results, data for ambient conditions and traceability of the reference standards should be filled in and returned to the pilot laboratory within one month after the measurements, **in both xls and pdf format.**
According to the schedule, every laboratory will have **4 weeks** to complete the following activities:

a) to receive the TSs,

b) to perform the measurements,

c) to send the TSs to the next participant.

The pilot laboratory will collect and analyze the results, and report these according to MRA procedures. Draft B report is intended to be a publication for the CIPM Key Comparison Data Base. The report and the paper will be presented at the closest AFRIMETS - TCM meeting after the comparison is done. After the agreement the paper will be then submitted to the KCDB.

The AFRIMETS.FF-K4.2.2015 will run during 2014 to February 2016.

4. The instrument

The chosen instruments are single channel fixed micropipettes of low nominal value, 100 µL (see figure 1). The micropipettes need to have attached a removable plastic tip in order to aspirate the liquid. NIS-Egypt will supply the tips for the pipette marked with the serial number 3563380A,IPQ-LCM will supply the tips for the two pipettes marked with the serial number 354568Z and 35472Z . Inside the transfer package there are plastic cases Labeled with the name of every participant institute contains the necessary tips to perform the measurements.

Figure (1) Fixed micropipettes of 100 µL

The fixed micropipettes used for this comparison are essentially of plastic material with a coefficient of thermal expansion of 2,4 × 10-4 / ºC [1]. The serial numbers of the 3 micropipettes used in this comparison are the following; 3563380A, 354568Z and 35472Z.

5. The measurement procedure

5.1 Getting the micropipettes ready for volume measurements A set of three 100 µL micropipettes comprise the transfer package; all artifacts have to be calibrated; the results have to be expressed at a reference temperature of 20 °C.

The micropipettes must be handled with care, i.e., only by qualified metrology personnel. Avoid any mechanical shock. The instruments must be stored at a place where they are protected from dust, aerosols and vapors.

Each participating laboratory shall make use of its own instruments and procedures in order to measure water temperature. The gravimetric is the suggested method but the photometric method can also be used.

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

5.2 Ambient conditions of the measurements

The ambient conditions of the laboratory room during the measurements should be the following:

Humidity higher than 50 %. Ambient temperature between 17 ºC and 23ºC.

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

5.3 Volume determination formula

Calibration of the micropipette will consist of the determination of the amount of water that the micropipette delivers at reference temperature of 20 ºC, using the gravimetric method the following equation described in ISO standard 4787 [2] can be used:

$$V_{20} = (I_I - I_E) \times \frac{1}{\rho_W - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \times [1 - \gamma(t - 20)]$$

Where:
V_0 volume, at the 20 ºC , in µL
I_I weighing result of the recipient full of liquid, in mg
I_E weighing result of the empty recipient, in mg
ρ_w water density, in mg/µL, at the calibration temperature *t* , in ºC, is advisable to use the Tanaka density formula [3]
ρ_A air density, in mg/µL
ρ_B density of masses used during measurement (substitution) or during calibration of the balance, in mg/µL
γ cubic thermal expansion coefficient of the material of the micropipette, in °C^{-1}
t water temperature used in the calibration, in °C

5.4 Calibration procedure – important details according to ISO Standard 8655 [4]

The laboratories may use its calibration procedure, but some important details should be taken into account if the gravimetric method is used in order to avoid large measurements errors:

- the weighing vessel should have a film of water (3 mm) before starting the measurements. The use of a lid or an evaporation trap is advisable;
- Deliver the water from the micropipette to the weighing vessel touching the recipient in an angle between 30º to 60 º and adding the drop retained at the end of the tip of the micropipette;
- Change tip and wet it before each measurement;
- Each participant laboratory should perform 10 consecutives measurements.

6. Uncertainty calculation

Each laboratory has to describe in excel sheet (see section 10), the uncertainty components in order that each laboratory results can be compared on a common basis. Both values, i.e. standard uncertainty and expanded uncertainty shall be stated, along with the relevant coverage factor $k = 2$.

For the evaluation of the measurement uncertainty, reference should be made to *the Guide to the Expression of Uncertainty in Measurement*. [5]

7. Time schedule

The time schedule of the comparison is as follows:

No	NMI / Country	Time
1	IPQ / Portugal	September – 2014
2	NIS / Egypt	February – 2015
3	LPEE/LNM / Morocco	March – 2015
4	GSA / Ghana	April – 2015
5	NMISA / South Africa	May – 2015
6	BOBS / Botswana	June – 2015
7	SIRDC-NMI / Zimbabwe	July – 2015
8	ZABS / Zambia	August – 2015
9	TBS/Tanzania	September – 2015
10	KEBS / Kenya	October – 2015
11	UNBS / UGANDA	November - 2015
12	NMIE / Ethiopia	December – 2015
13	NIS / Egypt	January -2016
14	IPQ / Portugal	February -2016

Table (2) shows the time schedule of the participant laboratories

The comparison will be completed in February 2016. The Draft A report will be present in May 2016. The Draft B report will be presented after the approval of the AFRIMETS –TCM in the closest meeting. then the paper will be submitted to the KCDB.

8. Transport and costs

Responsibility for transport rests with the preceding laboratory. The cost of shipping will be paid by the sender laboratory. The customs and any other cost will be paid by the receiving laboratory.

For the transport the micropipettes will be packed in a transportation box. IPQ will provide this box. The actual transport should be done with a well-known company (for example TNT; DHL). Such companies are used to handle ATA Carnets, which is need in some countries. Responsibility for complying with customs regulations rests with the participants. The value of the micropipettes for insurance purposes is: 500 €.

According to the Guidelines for CIPM Key Comparisons, each participating institute is responsible for its own cost for the measurements, transportation to the next laboratory and any extra customs charges, as well as any damage caused on the micropipettes during the permanence at the laboratory facilities.

In case of total equipment lost, the comparison will be interrupted and the report will be developed based on the results collect until that moment.

In case of some of the micropipettes are broken the comparisons will continue for the instruments that are still working properly. The report will be developed based upon presented results.

9. Receipt of the device

After arrival of the device, the participating institute shall inform the pilot institute NIS-Egypt by e-mail. Immediately after receipt a visual inspection should be made and the results be noted on the corresponding formats. The participating institute shall check the device for any damage. NIS-Egypt, as the pilot laboratory for this comparison, should be informed about the arrival and departure dates and about the results of the visual inspection as soon as possible, by e-mail using the appropriate form, in Annex 1.

NIS-Egypt will inform the IPQ-Portugal by the status of the artifact using email after each sending /receiving of the artifacts between the participant countries.

10. Reporting the results

The results are to be reported to the pilot institute at most one month after the measurements of the respective participants were completed. If this deadline was not met the participant will be removed from the comparison.

An excel spreadsheet will be supplied - see Form sheet Micropipettes.xls - for the presentation of the measurement results, uncertainty components, data for ambient conditions and traceability of the reference standard.

All observations which might be important for the interpretation of the results should be reported.

It is mandatory to send the results in xls and pdf format.

11. Determination of the reference value

To determine the reference value the formula of the weighted mean will be used, using the inverses of the squares of the associated standard uncertainty as the weights [6]:

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

To determine the standard deviation *u(y)* associated with *y*:

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

12. References

1. ASTM E 542:2000 - Standard practice for calibration of laboratory volumetric apparatus;

2. ISO 4787:2010; Laboratory glassware – Volumetric glassware – Methods for use and testing of capacity;

3. Tanaka, M., et. al; Recommended table for the density of water between 0 °C and 40 °C based on recent experimental reports, Metrologia, 2001, Vol.38, 301-309.

4. ISO 8655-1/2/6:2002, Piston-operated volumetric apparatus;

5. BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML; Guide to the expression of uncertainty in measurement (GUM), Geneva, 1995;

6. M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595.

7. Technical protocol CCM.FF.K4.2.2011 _micropipettes

Annex 1 - Reception form

Reception form			
Laboratory:		**Date:**	
Date of arrival of the transfer standards:		**From:**	
Condition of the standards/visual inspection:			
Other remarks:			
Name of the contact person:			
E-mail:			

Note: Fill and send it by e-mail to the pilot laboratory upon arrival of the standards.