中国计量科学研究院
National Institute of Metrology,China

National Institute of Metrology (NIM), China

Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,

Tel +86-10-64524627 *E-mail:tonglin@nim.ac.cn*

APMP key comparison APMP.M.FF-K4.2.2021
Volume comparison at 100 µL – Calibration of micropipettes

Technical Protocol
Final Version

Volume and Density Lab
National Institute of Metrology

March, 2021

中国计量科学研究院
National Institute of Metrology,China

National Institute of Metrology (NIM), China

Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,

Tel +86-10-64524627 *E-mail:tonglin@nim.ac.cn*

Contents

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 *E-mail:tonglin@nim.ac.cn*

1. Introduction

BIPM/CIPM key comparison CCM.FF-K4.2.2011 Volume comparison at 100 μL – Calibration of micropipettes (piston pipettes) was finished in 2013, after which each RMO should organize a new comparison. Five fixed 100μL pipettes will be used in this APMP comparison, which will be tested by the pilot laboratory three times to ensure the stability of the transfer standards. As the participant in CCM.FF-K4.2.2011, the volume and density lab in National Institute of Metrology China will be the pilot laboratory, and the Volume Laboratory of the Portuguese Institute for Quality (IPQ) will be invited to be the co-pilot. This technical protocol refers to the technical protocol of CCM.FF.K4.2.2011 in form.

2. Participants

Country	Laborotary	Contact person	Address and Postcode	Email	Telephone
China	National Insitute of Metrology(NIM), Volume Lab	Tong lin/Wang Jintao	National Insitute of Metrology, No.18, Bei San Huan Dong Lu,Chaoyang Dist, Beijing, P.R.China, 100013	tonglin@nim.ac.cn/wangjt@nim.ac.cn	+86-10-64524627
Portugal	Portuguese Institute for Quality(IPQ)	Elsa Batista	Portuguese Institute for Quality, Rua António Gião, 2,2829-513 Caparica, PORTUGAL	ebatista@mail.ipq.pt	+35 12 12 94 81 67
Hong Kong, China	The Standards and Calibration Laboratory (SCL)	Mr. Raymond Leung / Mr Henry Chiu	35/F., Immigration Tower, 7 Gloucester Road, Wanchai, Hong Kong	wmleung@itc.gov.hk / hklchiu@itc.gov.hk	2798 7347 / 2829 4839
India	NPLI	Mr. Goutam Mandal	CSIR-National Physical Laboratory of India (NPLI), Dr. K. S. Krishnan Marg, New Delhi - 110012, India	goutam@nplindia.org	+91-11-47091139
Indonesia	SNSU BSN	Renanta Hayu/Feizar Mahendra	Laboratorium SNSU BSN, Kompleks PUSPIPTEK Gd 420, Setu, Tangsel, Banten 15314, INDONESIA	renanta@bsn.go.id/feizar.mahendra@bsn.go.id	+6281519037120
Malaysia	NATIONAL METROLOGY INSTITUTE OF MALAYSIA (NMIM),Volume Laboratory	Kamarudin Mohamad Nor / Norhaslily Shamri	National Metrology Institute of Malaysia, SIRIM Berhad, PT 4803, Bandar Baru Salak Tinggi, 43900 Sepang, Selangor	kddin@sirim.my / lily@sirim.my	+603-87781600
Mongolia	Volume Standard Laboratory	Amartuvshin Batsuuri	Ulaanbaatar city, 13343, Bayanzurkh district, Peace avenue 46A, mailbox-48	amartuvshin@masm.gov.mn	+976 88014361
	Volume Calibration Laboratory	Sergelen Nohoijav		sergelen@masm.gov.mn	+976 88006969

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan Dong Lu,Chaoyang Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

Nepal	Nepal Bureau of Standards and Metrology	Ms. Maunta Manandhar	Balaju,Kathmandu,Nepal	mauntam@gmail.com	977-1-4356672
Philippines	National Metrology Laboratory of the Philippines	Jose Marco D. Latosa	Metrology Building, DOST Copmpound, General Santos Avenue, Bicutan, Taguig City, Philippines, 1631	marcolatosa@yahoo.com	+632 8837 2071 ext 2255
Russian Federation	VNIIM (D.I.Mendeleyev Institute for Metrology)	Victoria Bogdanova	Russia, St.Petersburg Moskovsky pr., 19，190005	vikvn9@yandex.ru	+7 903-098-95-01
Sri Lanka	Measurement Units, Standards and Services Department (MUSSD)	H L I S Sampath	Measurement Units, standards and services Department, Mahenawatta, Pitipana, Homagama, Sri Lanka	indika_sampathh@yahoo.com	Mobil: +94-718584947 Home: +94-342253431 Office: +94-112182266
Thailand	National Institute of Metrology (Thailand),Chemical metrology and biometry	Nongluck TANGPAI SARNKUL	National Institute of Metrology (Thailand) 3/4-5 Moo 3 Klong 5, Klong Luang, Pathumthani 12120	nongluck@nimt.or.th	+662 026 5400 Ext 5600
UNITED ARAB EMIRATES	EMIRATES METROLOGY INSTITUTE, Volume Laboratory	Mr. Nahyan Al Menhali	Krypto Labs, Masdar City Abu Dhabi, UAE PO Box 853	n.almenhali@qcc.gov.ae	Office: +971240665269 Mobile: +971509995298

3. General instructions

APMP.M.FF-K4.2 will be running in 2021 – 2022.The participants will be responsible for receiving the Transfer Standards (TSs), testing it and sending it to the next participant according to the time schedule.

Five single channel fixed micropipettes of 100µL volume are confirmed as the Transfer Standards (TSs). Each participating laboratory shall use the recommended tips by the manufacturer during measurements, and the pilot laboratory will supply these tips for the participants.

Based on experience and on reference data, see References given below[1], the pilot laboratory selects 2.4×10^{-4} $^{o}C^{-1}$ as the cubic coefficient of expansion for the plastic material used to make the TSs.

Figure 1- Fixed micropipette of 100 µL

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

When the standards arrive at the participating laboratory, a visual inspection should be made and the results should be reported to the pilot laboratory.

The participants shall determine the volume of water that each of the five micropipettes delivers at a reference temperature of 20 °C.

Each participating laboratory shall ensure suitable source of water in order to make any of the formulas, tables, and the ISO Standard 3696[2]can be referenced.

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) receive the TSs, b) perform the measurements, c) send the TSs to the next participant.

The pilot and co-pilot laboratories will organize the comparison, including receiving and analysing the data from all participants, then preparing the draft A, B and final report.

4. Schedule

Country	Laborotary	Contact person	Time
China	National Insitute of Metrology(NIM),Volume Lab	Tong lin/Wang Jintao	2021.03.01-03.31
Portugal	Portuguese Institute for Quality(IPQ)	Elsa Batista	2021.04.01-04.30
UNITED ARAB EMIRATES	EMIRATES METROLOGY INSTITUTE, Volume Laboratory	Mr. Nahyan Al Menhali	2021.05.01-05.31
Nepal	Nepal Bureau of Standards and Metrology	Ms. Maunta Manandhar	2021.06.01-06.30
India	NPLI	Mr. Goutam Mandal	2021.07.01-07.31
Sri Lanka	Measurement Units, Standards and Services Department (MUSSD)	H L I S Sampath	2021.08.01-08.31
Indonesia	SNSU BSN	Renanta Hayu/Feizar Mahendra	2021.09.01-09.30
Malaysia	NATIONAL METROLOGY INSTITUTE OF MALAYSIA (NMIM),Volume Laboratory	Kamarudin Mohamad Nor / Norhaslily Shamri	2021.10.01-10.31
Thailand	National Institute of Metrology (Thailand),Chemical metrology and biometry	Nongluck TANGPAISARNKUL	2021.11.01-11.30

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

Philippines	National Metrology Laboratory of the Philippines	Jose Marco D. Latosa	2021.12.01-12.31
Hong Kong, China	The Standards and Calibration Laboratory (SCL)	Mr. Raymond Leung / Mr Henry Chiu	2022.01.01-01.31
Mongolia	Volume Standard Laboratory/Volume Calibration Laboratory	Amartuvshin Batsuuri/Sergelen Nohoijav	2022.02.01-02.28
Russian Federation	VNIIM (D.I.Mendeleyev Institute for Metrology)	Victoria Bogdanova	2022.03.01-03.31
Portugal	Portuguese Institute for Quality(IPQ)	Elsa Batista	2022.04.01-04.30
China	National Insitute of Metrology(NIM),Volume Lab	Tong lin/Wang Jintao	2022.05.01-05.31

5. The measurement procedure

5.1 Technical requirements

• The 5 micropipettes should be carefully maintained by qualified metrology personnel. Avoid any mechanical shock. The instruments must be stored at a place where they are protected from dust, aerosols and vapours.

• For temperature uniformity, it is highly advisable to bring the five 100 µL 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 ℃.

• The air temperature in the laboratory should be kept at (20 ± 3) ºC, and the air temperature change should not be more than 1 ℃/h. The humidity should be higher than 50 %RH.

• The temperature drop between water temperature and laboratory should not be greater than 1℃.

• Each participating laboratory shall make use of its own instruments and procedures in order to measure water temperature. The gravimetry is the suggested method but the photometric method can also be used, but the measurement results of photometric method are for reference only.

5.2 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 [3] can be used:

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

Where:

V_{20}/µL: volume at reference temperature, 20 ºC;

I_I/mg: weighing result of the recipient full of liquid;

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

I_E /mg: weighing result of the empty recipient;

ρ_W/(mg/μL): water density at the calibration temperature t , using Tanaka density formula [4];

ρ_A/(mg/μL): air density;

ρ_B/(mg/μL): density of masses used during measurement (substitution) or during calibration of the balance;

γ/℃$^{-1}$: cubic thermal expansion coefficient of the material of the piston pipette, selects 2.4×10^{-4} °C^{-1} ;

t/℃: water temperature during the calibration process.

5.3 Procedures – important details

Based on experience and ISO Standard 8655 [5], there are some important details should be taken into account when the the laboratories use the gravimetric method to calibration.

- Correct tip immersion depth is especially important for micropipette calibration, before aspirating, immerse the tip below the meniscus 2-3 mm;
- When aspirating the water, hold the pipette vertically and pull the pipette straight out from the center of the container;
- When delivering the water from the micropipette, the tip should be at 30º to 60º angle to the weighing vessel in order to ensure good sample release;
- 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;
- Change tip before each measurement, and prewet tips at least twice before pipetting;
- Each participant laboratory must use the correct pipette tip which is supplied by the pilot laboratory;
- Each participant laboratory should perform 10 consecutive measurements;
- In order to avoid hand-warming the micropipette, the operator should not continually hold the pipette in your hand between pipetting cycles.

6. Pressure correction

The participating laboratories have the altitude difference between the geographical locations, and the altitude difference leads to a difference in atmospheric pressure. Piston stroke pipettes (air displacement) have an air-cushion which aspirates and dispenses the sample is influenced by the atmospheric pressure seriously. In order to avoid the dispersion in the dispensed volume of the micropipette by the atmospheric pressure, we should correct the results for a standard atmospheric pressure 1013.25 hPa using the following formula[6].

$$\Delta V = -V_t \times \rho_w \times g \times h_w \times \left(\frac{1}{P_L, X_2 - \rho_w \times g \times h_w} - \frac{1}{P_L, X_1 - \rho_w \times g \times h_w} \right) \quad (2)$$

Where,

ΔV/μL: volume change that results in the calibration at location X_1 over a location X_2

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

V_t/µL: volume of the air cushion

g/(m/s^2): acceleration of gravity

h_w/m: rising height of the liquid column in the pipette tip

$p_{L,X1}$/Pa: atmospheric pressure at location X_1

$p_{L,X2}$/Pa: atmospheric pressure at location X_2

ρ_w/(kg/m^3): water density at X_2

h_w/m = 0.0288 and V_t/µL = 550 were informed by the micropipettes manufacturer (eppendorf), for the air pressure correction.

Volume measurement results corrected for atmospheric pressure using equation 3.

$$V_{corr} = V_{20} - \Delta V \quad (3)$$

Where,

V_{corr}/µL: volume measurement results corrected for a standard atmospheric pressure 1013.25 hPa;

V_{20}/µL: volume at reference temperature, 20 ºC;

ΔV/µL: volume change that results in the calibration at location X_1 over a location X_2.

7. Uncertainty budget

Each laboratory has to describe in a word sheet (see section 9), 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.

For the evaluation of the measurement uncertainty, reference should be made to the Guide to the Expression of Uncertainty in Measurement [7].

8. Transport and costs

All participating laboratories are responsible for the international express to the next laboratory.

9. Receipt of the device

Each participating laboratory should make a visual inspection for the device and note the results on the corresponding form. The participating laboratory shall check the device for any damage. NIM, 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.

10. Reporting the results

The results are to be reported to the pilot laboratory at least 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.

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 E-mail:tonglin@nim.ac.cn

An excel spreadsheet will be supplied - see Form sheet Micropipettes.docx - 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 .docx and .pdf format.

11. Determination of the reference value

The comparison reference values (KCRV), which provide linkage to CCM.FF-K4.2.2011, will be obtained based on the method in C. Elster et al. (2010).

Then the degree of equivalence (DoE) between the KCRV and each of the participant, and DoE between each pair of the participants will be calculated.

NIM and IPQ will serve as the linking laboratories.

12. References

1) ASTM E 542:2000 - Standard practice for calibration of laboratory volumetric apparatus;
2) ISO 3696:1995; Water for analytical laboratory use - Specification and test methods;
3) ISO 4787:2010; Laboratory glassware – Volumetric glassware – Methods for use and testing of capacity;
4) 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;
5) ISO 8655-1/2/6:2002, Piston-operated volumetric apparatus;
6) Calibration guide DKD-R 8-1; calibration of piston pipettes, 2011;
7) BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, OIML; Guide to the expression of uncertainty in measurement (GUM), Geneva, 1995;
8) M.G. Cox, The evaluation of key comparison data, Metrologia, 2002, Vol. 39, 589-595;
9) C. Elster, A. G. Chunovkina and W. Wöger, 2010, “Linking of a RMO key comparison to a related CIPM key comparison using the degrees of equivalence of the linking laboratories”, Metrologia, vol. 47, pp. 96 – 102;
10) COOMET R/GM/14:2016, Guidelines for data evaluation of COOMET key comparison.

中国计量科学研究院
National Institute of Metrology,China

National Institute of Metrology (NIM), China
Address: No.18,Bei San Huan
Dong Lu,Chaoyang
Dist,Beijing,P.R.China,
Tel +86-10-64524627 *E-mail:tonglin@nim.ac.cn*

Annex 1 - Reception form

Reception form			
Laboratory:		Date:	
Date of arrival of the transfer standards:		From:	
Conditions of the transfer standards on arrival			
Carrying case:			
Conditions of the standard:			
Other remarks:			

Contact person of the laboratory	
Name:	
Full mailing address:	
Telephone:	
Telefax:	
e-mail:	

Note: Fill and send it by e-mail to the pilot laboratory upon arrival of the standards.
tonglin@nim.ac.cn