	Guidance Document on Calibration of Volume	**Copy No.** **Page 1 of 32** **Document No. GD07 /11** **Revision no. 0** **Effective Date. 2021- 05 -17**

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1. Classification for calibration of volumetric apparatus

Table 1

S. No.	Description	Relevant standard	Permanent facility	Onsite calibration	Mobile facility
1.	Burettes (manufactured as per ISO 385)	ISO 4787:2010	Yes	No	Yes *
2.	Single-volume (one mark) pipettes (manufactured as per ISO 648)	ISO 4787:2010	Yes	No	Yes *
3.	Graduated pipettes (manufactured as per ISO 835)	ISO 4787:2010	Yes	No	Yes *
4.	One-mark volumetric flasks (manufactured as per ISO 1042)	ISO 4787:2010	Yes	No	Yes *
5.	Graduated measuring cylinders(manufactured as per ISO 4788)	ISO 4787:2010	Yes	No	Yes *
6.	Piston operated volumetric apparatus like; a) Single – channel piston pipettes with air interface (as per ISO 8655-2) b) Multi-channel piston pipettes (as per ISO 8655-2) c) Positive – displacement pipettes (as per ISO 8655-	ISO 8655-6 &	Yes	No	Yes *

	2) d) piston burettes (as per ISO 8655-3) e) Diluters (as per ISO 8655-4) f) Dispensers (as per ISO8655-5)				
7.	Guidelines on the determination of uncertainty in gravimetric volume calibration	Euramet calibration Guide No.19 Version 3.0 (09/2018)			

Note 1: Since the validity of calibration of a weighing balance will be no more valid if its place is disturbed, because of change in 'g' value, environmental changes and effect of transportation, onsite calibration of volumetric apparatus is not recommended.

****Note 2:*** In case of mobile calibration, laboratory has to demonstrate competency in respect of weighing balance calibration every time, control of change of temperature and vibration at the place of calibration.

Note 3:The above standards are not applicable for medical syringes of the type used for giving injections.

Note 4: This technical requirement is based on the above-mentioned guideline. Lab may follow any relevant standard; however, care shall be taken to follow the requirements in totality.

2. Reference

2.1 Balance(for laboratory glassware's volumetric apparatus), with a resolution and standard deviation appropriate to the selected volume of the apparatus under calibration (see Table 2). The resolution of the display, the standard deviation and the linearity of the balance will be a limiting factor in the accuracy of the measurements. The balance shall be calibrated with adequate accuracy.

The balance used for calibration of volumetric apparatus shall have a readability / resolution of the order of 1/3rd of accuracy specified for volumetric apparatus.

Recommended weighing balances for calibration of volumetric apparatus as per ISO 4787:2010

Table2

Selected volume of the calibration liquid [a] V	**Resolution mg**	**Standard deviation (repeatability) mg**	**Linearity mg**
100 µl<*V* ≤ 10 ml	0,1	0,2	0,2
10 ml <*V* < 1 000 ml	1	1	2
1 000 ml ≤*V* ≤ 2000 ml	10	10	20
V > 2000 ml	100	100	200
a-For practical purposes, the nominal volume may be used to choose the balance			

2.2 Analytical balance or equivalent weighing device (for piston operated volume apparatus), with a resolution appropriate to the selected volume of the apparatus under test (see table 3)

Minimum requirements of balances for micro pipette calibration as per ISO 8655-6

Table 3

Selected volume of the calibration liquid [a] V	**Resolution mg**	**Repeatability and linearity mg**	**Standard uncertainty of measurement mg**

1 µl<*V* ≤ 10 µl	0,001	0,002	0,002
10 µl<*V* ≤ 100 µl	0,01	0,02	0,02
100 µl<*V* ≤ 1000 µl	0,1	0,2	0,2
1 ml <*V* ≤ 10 ml	0,1	0,2	0,2
10 ml <*V* ≤ 200 ml	1	2	2
a-For practical purposes, the nominal volume may be used to choose the balance			

If the standard uncertainty of measurement of the balance is known (e.g. from the balance calibration certificate), this may be used instead of repeatability and linearity. The standard uncertainty of measurement shall not be more than two to three times the resolution.

2.3 Other apparatus required

2.3.1 For volumetric instruments as per ISO 4787:2010

2.3.1.1 Thermometer, to measure the temperature of the calibration liquid (water) with a measurement error of maximum 0,2 °C for liquid volumes □□1 000 ml and with a measurement error of maximum 0,1 °C for liquid
volumes W 1 000 ml.

2.3.1.2 Hygrometer, to measure the humidity in the calibration laboratory with a measurement error of maximum 5 % within the humidity range of 35 % to 85 %.

2.3.1.3 Barometer, to measure the atmospheric pressure in the calibration laboratory with a measurement error of maximum 1 kPa.

2.3.1.4 Calibration liquid, distilled or deionized water complying with ISO 3696, Grade 3 should be used for calibration.

2.3.1.5 Receiving vessel, conical flask with ground joint, manufactured from glass, e.g. in accordance with ISO 4797. The nominal volume of the conical flask shall correspond to the volume of liquid to be measured.

2.3.2 For piston operated volumetric apparatus as per ISO 8655-6:2002

2.3.2.1 Liquid reservoir, with sufficient capacity for all the test liquid likely to be required for the complete series of tests.

2.3.2.2 Weighing vessel, (required as per ISO 8655-6), suitable for the test procedure selected as per clause no 6 Care shall be taken regarding the loss of water by evaporation during the dispensing and weighing procedure.
It is recommended that, especially for testing apparatus of the lowest volume, the height-to-diameter ratio of the weighing vessel be at least 3:1 or that a weighing vessel with a lid be used.

2.3.2.3 Timing device, with a standard uncertainty of ≤1s **with k=1.**

2.3.2.4 Thermometer, with a standard uncertainty of ≤0,2°C with k=1.

2.3.2.5 Hygrometer, with a standard uncertainty of ≤10% with k=1.

2.3.2.6 Barometer, with a standard uncertainty of ≤0,5kPa with k=1.

2.3.3 Use distilled or deionized water conforming grade3 as specified in ISO 3696 should be used for calibration. Water shall be at room temperature.

3. Environmental conditions: (at lab & at site)

3.1 Temperature: The calibration laboratory shall have a temperature between 15°C and 30 °C**.** The standard reference temperature, i.e.the temperature at which the volumetric instrument is intended to contain or deliver its volume (capacity), shall be 20 °C.

When the volumetric instrument is required for use in a country which has adopted a standard reference temperature of 27 °C (the alternative recommended in ISO 384 for tropical use), this figure shall be substituted for 20 °C.

3.1.1 **Temperature of Volumetric instrument** The capacity of the volumetric instruments varies with change of temperature. The particular temperature at which a volumetric instrument is intended to contain or deliver its nominal capacity is the “reference temperature” of the instrument (see 3.1).

3.1.2 A volumetric instrument which was adjusted at 20 °C, but used at 27 °C, would show an extra error of only 0,007 % if it is made of borosilicate glass having a coefficient of cubical thermal expansion of 9,9 x 10^{-6} $°C^{-1}$ and of 0,02 % if it is made of soda-lime glass having a coefficient of cubical thermal expansion of 27 x 10^{-6} $°C^{-1}$. These errors are smaller than the limits of error for most volumetric instruments. It follows, therefore, that the reference temperature is of minor importance in practical use. However, when performing calibrations, it is important to refer to the reference temperature.

3.2 Temperature of calibration liquid The temperature of the water used for the calibration shall be measured to ±0,1 °C. Corrections for differences in temperature from the reference temperature shall be applied in accordance with Annex B.

3.3 Cleanliness of glass surface

The volume contained in, or delivered by, a volumetric instrument depends on the cleanliness of the internal glass surface. Lack of cleanliness results in errors through a poorly shaped meniscus involving two defects:

a) incomplete wetting of the glass surface, i.e. the liquid surface meets the glass at an arbitrary angle instead of forming a curve such that it meets the glass tangentially;
b) a generally increased radius of curvature, due to contamination of the liquid surface reducing the surface tension.

The ascending or descending liquid meniscus shall not change shape (i.e. it shall not crinkle at its edges). To ascertain whether a piece of glass apparatus is satisfactorily clean, it shall be observed during filling and dispensing. Additionally, an experienced operator can recognize the shape of an uncontaminated meniscus, in relation to its diameter.

Lack of cleanliness causes additional errors with volumetric instruments used for delivery due to the film of liquid on the walls being irregularly distributed or incomplete, e.g. forming drops on the glass surface.

Furthermore, chemical residues can introduce an error in the analytical result by contamination. Therefore, where volumetric instruments are fitted with ground stoppers, special attention shall be paid to cleaning the ground zone.

3.4 Quality of used volumetric instruments

The glass surface shall be free from obvious damage, the graduations and inscriptions shall be clearly readable and especially with instruments adjusted to deliver the jet shall be free from damage and allow an unrestricted outflow of liquid.

3.5 Delivery time and waiting time

For volumetric instruments used for delivery of a liquid, the volume delivered is always less than the volume contained, due to the film of liquid left on the inner walls of the volumetric instrument. The volume of this film depends on the time taken to deliver the liquid, and the volume delivered decreases with decreasing delivery time. For example, the delivered volume of a pipette or burette will decrease if the jet is broken (shorter delivery time) or will increase if the jet is not clean and the outflow of liquid is restricted.

In view of the above, delivery times and waiting times have been specified in the International Standards on volumetric instruments; these times shall be observed.

3.6 a) **Humidity (for piston operated volumetric apparatus):** The calibration laboratory shall have relative humidity above 50% and a constant (± 0.5°C) temperature between 15°C and 30 °C.

b) **Humidity (for laboratory glassware):** to measure the humidity in the calibration laboratory with a measurement error of maximum 5 % within the humidity range of 35 % to 85 %.

3.7 Vibration: The calibration area shall be free from vibrations generated by central air-conditioning plants, vehicular traffic and other sources to ensure consistent and uniform operational conditions.

3.8 Acoustic noise: Noise level shall be maintained less than 60 dBA.

3.9 Illumination: The recommended level of illumination is 250-500 lux on the working table.

3.10 Mains power supply: The calibration laboratory shall make arrangements for regulated and uninterrupted power supply of proper rating. The recommended voltage regulation level is ±2% or better, and Frequency variation ± 2.5 Hz or better on the calibration bench.

3.11 Effective mains earthing shall be provided preferably earth resistance shall be less than 1 Ω.

4. Meteorological requirements

4.1 Volume definition is the quantity of three-dimensional space enclosed by a closed surface, for example, the space that a substance (solid, liquid, gas, or plasma) or shape occupies or contains. Volume is often quantified numerically using the SI derived unit, the cubic meter. The volume of a container is generally understood to be the capacity of the container; i. e., the amount of fluid (gas or liquid) that the container could hold, rather than the amount of space the container itself displaces**Volume measurement by gravimetric method:**

4.2 Calibration of volumetric instruments recommends the gravimetric method in which mass volume of the distilled water dispensed from a volumetric instrument is measured with a balance and then corrected to a dispensed quantity (volumetric value). No method can measure directly the physical quantity of a minute volume. Therefore, the most common and precise method is to measure the mass value of distilled water, whose physical properties are known using a balance and then convert the mass to a volumetric value.

4.3 Conversion from a mass value to a volumetric value involves the temperature of the distilled water and the barometric pressure as parameters. However, the variation in measured results due to barometric fluctuation is negligible and in practice it will be sufficient to set and use a representative value (fixed value) of the location of measurement. Consequently, the equipment at the time of volume calibration will be balance and the thermometer

5. Calibration method

5.1 Calibration of volumetric apparatus can be done either of the following two methods:

a) Gravimetric method

b) **Volumetric method**

a) **Gravimetric method**

This method consists on weighing the volumetric apparatus under calibration when empty and again when full. The procedure adopted the use of the reference line or marks to have the purpose to provide an exact measure of liquid volume and the draining or drying procedures shall be followed carefully because they all effect the measurements. The difference obtained in the weighing measurements gives the mass of contained or delivered liquid.

b) **Volumetric method**

In the volumetric method a certain amount of liquid is delivered into a container. The volume is determined at a reference value denoted by a graduation mark. When the standard capacity measure is equipped with an adjustable device or scale the volume can be adjusted to the nominal volume. The calibration can be performed adjusting the level exactly to the reference value, by adding or removing water until the level corresponds to the graduation mark, or calculating the actual volume at the reference value on the basis of the scale reading.

5.2.1 Gravimetric Method (For Laboratory Glassware and Piston Operated Volumetric Apparatus)

5.2.2 Based on ISO 4787:2010 for Laboratory glass ware - volumetric instruments. This is applicable for calibration of volumetric instruments made from glass for the range above 0.1ml to 10000 ml, in order to obtain the best accuracy in use.

5.2.3 Based on ISO 8655-6,2002 for piston operated volumetric apparatus:

This is applicable for ≤ 1µl to 200 ml volumetric apparatus

For calibration procedure of different types of apparatus, follow the clause references of the above Standard as given below:

a) Single –channel piston pipettes with air interface (as per ISO 8655-2) follow clause 7.2 of the standard.
b) Multi-channel piston pipettes (as per ISO 8655-2) follow clause 7.3 of the standard.
c) Positive –displacement pipettes (as per ISO 8655-2) follow clause 7.4 of the standard.
d) Piston burettes (as per ISO 8655-3) follow clause 7.5 of the standard.
e) Diluters (as per ISO 8655-4) follow clause 7.6 of the standard.
f) Dispensers (as per ISO8655-5) follow clause 7.6 of the standard.

6. Calibration procedure (based on gravimetric method)

6.1 General

A. Calibrationprocedure (Piston – operated volumetric apparatus)

6.1.1 In the case of a fixed-volume apparatus, the calibration volume is the nominal volume. In the case of a variable-volume (user-selectable volume) apparatus, at least three volumes shall be calibrated:

a) the nominal volume,
b) approximately 50 % of the nominal volume,
c) the lower limit of the useful volume range or of the nominal volume (whichever is the greater).

Measurement of further volumes is optional. The setting devices of the apparatus (e.g. dials, scales) shall be sufficient for the selection of the calibration volume.

6.1.2 **Number of measurements per test volume**

If the gravimetric methods of this part of ISO 8655 are used as conformity tests or type tests, e.g. prior to a declaration or certification of conformity, or if the gravimetric method is used as a reference method, 10 measurements for each test

volume shall be carried out. These measurements are used to calculate the systematic and the random error of measurement in accordance with clause 8 of ISO 8655-6:2002(E)

For re-establishing conformity, e.g. after repairs not performed by the supplier, 10 measurements at each volume shall also be performed.

If the gravimetric method is used for other purposes, such as supplier's quality control or supplier's after-sales service,

a) the number of test volumes (see 6.1.1),
b) the number of measurements per volume and,
c) where applicable, the number of channels tested

may be changed to an appropriate number. Alternative test methods may also be used for this purpose, provided that they can be proven to correlate with the reference method specified in this part of ISO 8655, in which case the user should choose a number of measurements for his metrological confirmation based on his accuracy requirements.

6.1.3 Weighing procedure

Weighing for apparatus designed to deliver (Ex) shall always involve dispensing of the test liquid into the weighing vessel. Weighing for apparatus designed to contain (In) shall always involve the removal of test liquid from the weighing vessel. An example of the latter is the sample uptake step in the use of a dilutor.

6.1.4 Calibration conditions during the weighing procedure

At the start and at the end of the weighing procedure, the temperature of the test liquid in its container shall be recorded to the nearest 0.2 °C. The barometric pressure in the calibration laboratory shall be recorded to the nearest 1 kPa and the relative humidity to the nearest 10%.

6.2 Single-channel piston pipettes with air interface (in accordance with ISO 8655-2)

6.2.1 In the case of power-driven piston pipettes, aspiration and delivery of liquid are automatic. The remainder of the procedure is carried out following the procedure described below.

6.2.2 Pour liquid from the water container in the weighing vessel to a depth of at least 3 mm. Record the temperature of the liquid and the barometric pressure and relative humidity in the calibration laboratory (see 6.1.4). If the weighing vessel has a lid, fit it.

Note; Temperature and barometric pressure are necessary for the choice of the correction factor (see 8.3 and annex A of ISO 8655-6:2002(E)); the relative humidity is not necessary for the evaluation as the correction factors Z in Annexure A apply to relative humidity ranging from 20%RH to 90%RH but are necessary for documentation in the calibration report [see clause 9, item d of ISO 8655-6:2002(E)].

6.2.3 If using a variable-volume piston pipette, select the test volume; this setting shall not be altered during the test cycle of 10 measurements.

6.2.4 Prepare the piston pipette and the test cycle as follows:

a) Fit the selected tip to the piston pipette.
b) Fill the tip with test liquid and expel to waste five times to reach a humidity equilibrium in the dead air volume (see ISO 8655-1:2002, 3.1.8) of the air-displacement piston pipette.
c) Place the weighing vessel with its added water on the balance pan.

6.2.5 Perform the following test cycle (see Figure 1 and Figure 2):

a) Replace the disposable tip of the piston pipette.
b) Fill the piston pipette with test liquid, immersing its delivery orifice 2 mm to 3 mm below the surface of the water. Release the operating button slowly, if hand operated, and withdraw the pipette vertically and carefully from the surface of the water. Touch the delivery orifice against the side wall of the container with the test liquid.
c) Expel the water to waste in order to pre-wet the tip and refill the piston pipette as described in b)

d) Record the mass of the weighing vessel to the nearest readable graduation as in Table 1, or tare the balance to zero (m_0=0). Start the timing device (this may be omitted if using a weighing vessel with lid).

e) If the weighing vessel has a lid, remove it. Deliver the contents of the pipette into the weighing vessel, touching the delivery end of the pipette tip against the inside wall of the vessel just above the liquid surface at an angle of approximately 30° to 45° and draw it approximately 8 mm to 10 mm along the inner wall of the weighing vessel to remove any droplets at or around the tip orifice. Replace the lid if applicable.

Where applicable, use the blow-out feature of the piston pipette to expel the last drop of liquid before drawing the delivery end of the tip along the inner wall of the weighing vessel.

If it is necessary to remove the weighing vessel from the balance pan to permit delivery of the dispensed volume, avoid excessive handling and possible contamination by the use of lint-free gloves. Return the weighing vessel to the balance pan after delivery.

f) Record the mass of the weighing vessel, or if tared in step 6.2.4 c) the mass of the quantity delivered.

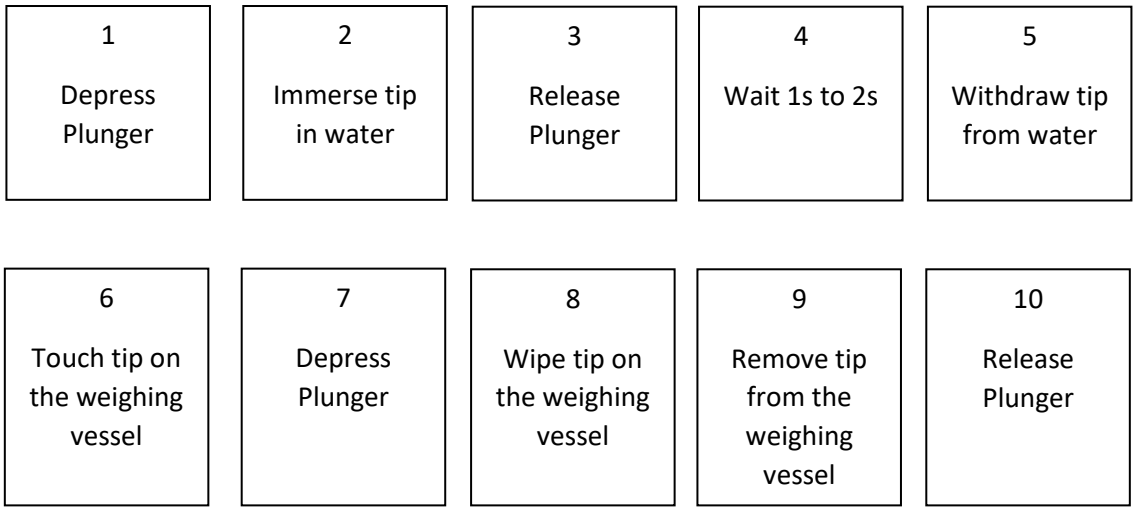

Figure 1- Pipetting of calibration volume into the weighing vessel

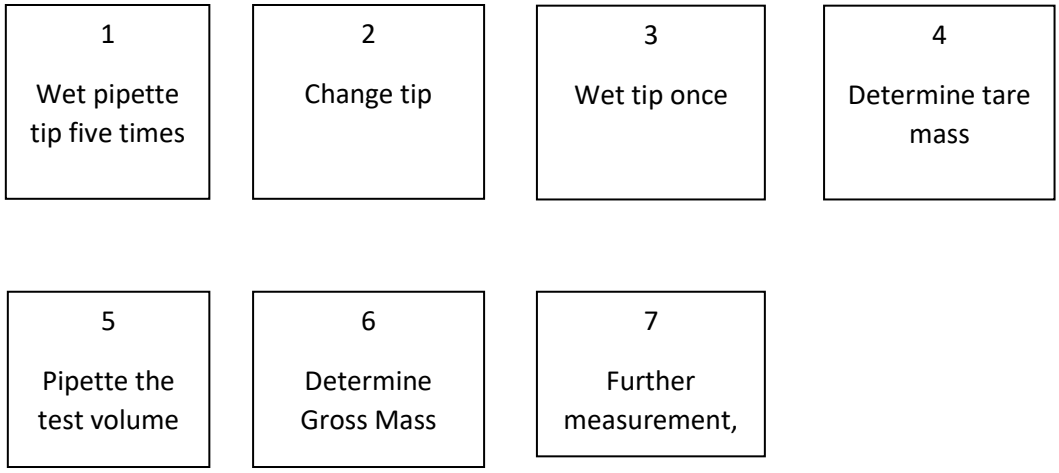

Figure 2- Scheme of calibration procedure for piston pipettes with air interface

6.2.6 Repeat the steps as described in 6.2.5 until 10 measurements have been recorded as a series of masses m_1 to m_{10}.

6.2.7 Note the time to the nearest second taken to complete the 10 test cycles.

6.2.8 After the last weighing of 6.2.6 leave the weighing vessel on the balance pan for the time measured in 6.2.7 and record its mass m_{11}.

If the weighing vessel was removed from the balance pan to enable delivery, leave it on the pan for half the time in 6.2.7 and then remove it from the balance and allow it to stand on the workbench for half the time measured in 6.2.7.

If the test volume is or if a weighing vessel with lid is used, omit steps 6.2.7 and 6.2.8, as a correction for evaporation is unnecessary. At and below, calculate the mass loss in accordance with the supplier's instructions.

6.2.9 Measure the temperature of the remaining test liquid to the nearest and calculate and record the mean test temperature (see 6.1.4).

6.2.10 The values obtained shall be evaluated in accordance with clause 8 of ISO 8655-6:2002(E).

6.3 Multi-channel piston pipettes (in accordance with ISO 8655-2)

Multi-channel piston pipettes are similar to single-channel in that they comprise a set of single-volume measuring and delivery units all operated simultaneously by a single operating mechanism. For the purposes of the test, each channel shall be regarded as a single channel and tested and reported as such.

Fill all channels of the multi-channel pipette by aspirating the test liquid. Expel only the test liquid aspirated by the channel being tested into the weighing vessel.

6.4 Positive-displacement pipettes (in accordance with ISO 8655-2)

Piston pipettes without an air interface shall be tested in accordance with 6.2. However, the five-fold prewetting of the pipette tip prior to the test and the single prewetting before each measurement only need be performed if required by the supplier. Only change the pipette tips when testing positive-displacement pipettes of type D2 (see ISO 8655-2). Wipe the pipette tip on the water container wall after aspiration of the test liquid and prior to expelling it into the weighing vessel in order to remove droplets from the outside of the tip. Without removing liquid from the inside of the pipette tip, further remove any droplets that may still be present after the wiping of the tip. Follow the supplier's instructions regarding air-bubble-free filling of the pipette tip.

Empty the contents of the pipette tip into the weighing vessel as specified in 6.2.5 e).

6.5 Piston burettes (in accordance with ISO 8655-3)

6.5.1 Preparation

Carry out the testing by delivery into the weighing vessel (see 6.1.3). Carefully clean the weighing vessel and add a small quantity of test liquid to it. Place the weighing vessel and its test liquid in the balance case. Then place the burette under test, with its reservoir already filled with test liquid, as close to the balance as possible. Leave both for at least to come to equilibrium.

6.5.2 Calibration procedure

Measure the mass of the weighing vessel and its test liquid and consider this value as the tare mass prior to the first measurement.

Load the piston burette, bubble free, with test liquid from the reservoir, in accordance with the supplier's instructions. Deliver the test liquid from the burette into the weighing vessel, until the selected volume is reached. If the burette is automatically controlled, deliver test liquid until the volume preset is reached and no further delivery occurs. Weigh the weighing vessel again and calculate the mass of liquid delivered.

When testing partial volumes (see 6.1.1) of the nominal volume of the piston burette, the piston need not be reset to the initial position (zero) prior to the next measurement. Ensure that the upper volume limit of the piston and thus the nominal volume of the piston burette is not exceeded when dispensing a partial volume.

When testing piston burettes — especially in the case of automated tests — wiping of the delivery jet on the vessel wall to remove droplets can be impossible due to the individual test setup. In such cases, ascertain that the weighing is carried out only after a complete drop has been delivered from the delivery jet into the weighing vessel.

The test liquid can, for example, be expelled through an extended tip in such a way that the stream breaks and no drops form.

The values obtained shall be evaluated in accordance with clause 6.8.

6.6 Dilutors (in accordance with ISO 8655-4)

6.6.1 General

Depending on the design of the dilutor to be tested, sample volume, diluent volume and/or total volume shall be tested by performing 10 measurements. If sample volume (In) or diluent volume (Ex) is to be tested independently, the cylinder not being tested shall be set to zero or switched off, if the design permits. If it does not, only sample volume and total volume can be tested by usual operation.

6.6.2 Preparation

Carefully clean the weighing vessel (see 2.3.2.2) and add a small quantity of test liquid. If the sample uptake is to be measured, the volume of liquid shall be at least 15 times the

volume to be aspirated at each operation. Consider the mass of the vessel including the liquid used for measurements prior to the first measurement as the tare mass (see 6.2.4c). Place the weighing vessel and its liquid in the balance case. Then place the dilutor, with its diluent system properly filled and air-bubble-free, as close to the balance as practicable and leave it for at least 2 h to equilibrate.

If testing sample uptake, set the dilutor sample volume to the desired volume for the test, which may be the maximum or an intermediate volume within the range, and switch off the diluent system, set it to zero or set it to the minimum as available. Do not change these settings for the duration of the series of 10 measurements.

If testing diluent or total delivery, switch off the sample uptake system, set it to zero or set it to any convenient volume as available. Set the diluent volume to either the nominal volume or an intermediate volume within the range. Do not change these settings for the duration of the series of 10 measurements.

When testing dilutors with test volume settings of less than 50 µl, pay special attention to the evaporation of the test liquid from the weighing vessel as this may lead to substantial errors in measurement of delivered (or residual, in the case of withdrawal by aspiration of test liquid) mass. Balances as shown in Table 1 equipped with special accessories (e.g. evaporation traps) may be used.

6.6.3 Calibration procedure

Before carrying out the test, perform one complete cycle of aspiration and delivery (if necessary, including delivery of test liquid from the diluent system) and discharge the test liquid to waste, in order to standardize the starting conditions. Touch the uptake and delivery probe against the side of the weighing vessel to remove droplets from around its orifice and weigh the weighing vessel to establish its starting mass. Measure the sample volume by aspirating the test liquid from the weighing vessel via the aspiration and delivery probe, and record the mass lost from the weighing vessel. Touch the end of the probe against the inside wall of the weighing vessel after aspiration to ensure that no random

droplets adhere round its orifice. Discharge the aspirated sample to waste, if necessary, with a quantity of “diluent” test liquid. Measure the diluent volume using the diluent delivery system as a dispenser, if possible. Otherwise, measure the total of the sample volume together with the diluent volume.

B. Calibration procedure (laboratory glassware)

6.7.1 **General**

Volumetric instruments other than disposable pipettes shall be thoroughly cleaned shortly before calibration (See 3.3). Volumetric instruments adjusted to contain shall be dried after cleaning.For volumetric instruments, adjusted to deliver, it is important that receiving vessels manufactured from glassware used. Capillary effects influencing the delivery time and the delivered volume depend considerably on the material on which the liquid runs down. In addition, the electrostatic charges of glass are minimal; this isimportant for the weighing procedure.

6.7.2 **Calibration laboratory**

The calibration shall be carried out in a draught-free room with stable environment. The calibration laboratory shall have a relative humidity between 35 % and 85 % and shall provide a temperature locally constant to ±1 °C and temporally constant to ±0,5 °C between 15 °C and 30 °C. Prior to the calibration, the volumetric instrument to be calibrated and the calibration liquidshall have stood in the room for a sufficient time (1h to 2h) to reach equilibrium with the room conditions. Test water should be covered to avoid evaporation cooling. Temperatures (room and calibration liquid), atmospheric pressure and humidity should be recorded.

6.7.3 **Filling and delivery**

6.7.3.1 Volumetric flasks and measuring cylinders

Volumetric flasks in accordance with ISO 1042 and measuring cylinders in accordance with ISO 4788 shall be dried after cleaning. They shall be filled by means of a plastic tube

with tip to a distance of a few millimeters above the ring mark or the graduation line to be tested, so that the walls of the volumetric instrument considerably above the ring mark are not wetted. The final setting of the meniscus to the ring mark or graduation line shall be made by withdrawing the surplus water by means of a plastic tube drawn out to a jet. The movement of the meniscus when setting shall be downwards. If a little refilling is necessary or if the reading is delayed to the adjustment of the meniscus, careful swaying is necessary to refresh the meniscus shape.

6.7.3.2 Pipettes adjusted to deliver

Pipettes adjusted to deliver according to the specifications in ISO 648 and ISO 835 shall be clamped in a vertical position and filled through the jet to a few millimeters above the graduation line to be tested; any liquid remaining on the outside of the jet shall be removed. The final setting of the meniscus shall then be made by running out the surplus water through the jet. Any drop of liquid adhering to the jet shall be removed, for example by bringing a ground glass surface into contact with the tip of the jet at an angle of about 30°. Draw this ground glass surface downwards through a distance of about 10 mm to remove residual water. Delivery into the tared receiving vessel shall then be made with the flow unrestricted while the tip of the jet is in contact with the inner ground surface of the receiving vessel, finally drawing it over a distance of about 10 mm, with the receiving vessel held inclined at an angle of about 30°.

Other precautions which are necessary to obtain the correct delivered volume vary with different types of instruments and are described in the clause defining capacity in the appropriate International Standards.

Determine the delivery time while the tip of the jet is in contact with the inner surface of the receiving vessel, above the level of any collected liquid, but without movement of one against the other throughout the delivery period. The delivery time thus determined should be within the limits specified for the particular pipette. For further details, see ISO 648 and ISO 835.

A waiting time, if specified, shall be observed before making the final setting of the meniscus for delivery of a given volume. If the setting after delivery is done at a lower

graduation line, the liquid flow shall be nearly stopped a few millimeters above the graduation line. After observation of the waiting time, the final setting shall be completed quickly.

6.7.3.3 Pipettes adjusted to contain

Rinse the pipette with the reagent to be used to a few millimeters below the desired graduation line. Fill the pipette by suction to as close as possible above the selected graduation line. Remove any liquid remaining on the outside of the jet. Make the final setting of the meniscus to the line by withdrawing the surplus liquid by means of filter paper. For the discharge, rinse the pipette several times with the diluting medium.

6.7.3.4 Burettes adjusted to deliver

Burettes adjusted to deliver according to ISO 385 shall be clamped in a vertical position and filled through the jet to a few millimeters above the graduation line to be tested. The stopcock and jet shall be freed from air bubbles. Any liquid remaining on the outside of the jet shall be removed. The final setting of the meniscus shall then be made by running out the surplus water through the jet. Any drop of liquid adhering to the jet shall be removed by bringing a ground glass surface into contact with the tip of the jet at an angle of about 30°.Draw this ground glass surface downwards through a distance of about 10 mm.

Delivery into the tared receiving vessel shall then be made with the flow unrestricted until the meniscus has come to a few millimeters above the graduation line to be tested, while the stopcock is fully open and the jet is not in contact with the receiving vessel. After the final setting of the meniscus, any drop of liquid adhering to the jet is removed by bringing an inclined glass surface into contact with the tip of the jet at an angle of about30°, finally drawing it over a distance of about 10 mm.

Other precautions which are necessary to obtain the correct delivered volume vary with different types of burettes and are described in the appropriate International Standards in the clause defining capacity.

Determine the delivery time by the unrestricted outflow of the liquid from the zero mark to the lowest graduation mark with the stopcock fully open and the jet not being in contact

with the surface of the receiving vessel. The delivery time thus determined should be within the limits specified for the particular burette. For further details, see ISO 385.

A waiting time, if specified, shall be observed before making the final setting of the meniscus for delivery of a given volume. If the setting after delivery is done at a lower graduation line, the liquid flow shall be nearly stopped a few millimeters above the graduation line. After observation of the waiting time, the final setting shall be completed quickly.

6.7.4 Weighing

The volumetric instrument or the receiving vessel shall be tared and weighed using a balance in accordance with 6.1 and the temperature of the water shall be measured to ±0,1 °C.

Alternatively, two weighments can be performed, namely I_L, referring to the loaded vessel, and *I*E, referring to the empty vessel. Usually, I_E and I_L are observed under the same conditions, hence a precise zero adjustment of the balance is not necessary. Both of the required weighments shall be carried out in as short a time interval as convenient to ensure that they have been made at the same temperature. This temperature and the barometric pressure shall be recorded for use in the subsequent calculations.

The manufacturer's instructions shall be followed in making the requisite measurements. Weighings shall be made with care and made expeditiously to minimize evaporation losses which would constitute a source of error.

6.7.5 Evaluation

The balance reading after tare or the difference of the results of the first and second weighing is the apparent
mass of the water contained in, or delivered by, the volumetric instrument tested.
NOTE The apparent mass, thus obtained, is the mass uncorrected for air buoyancy.

In order to obtain the volume contained in, or delivered by, the volumetric instrument under test at the
reference temperature from the apparent mass of water, the following factors shall be taken into account:

a) the density of water at the temperature of test;

b) the thermal expansion of the glass between the temperature of test and the reference temperature;

c) the effect of air buoyancy on the water and on the weights used.

Instructions for calculating the volume of the instrument and tables, in which these factors have been taken
into account for a reference temperature of 20 °C, are given in Annex B.

6.8 Equation for Calculation of Volume

6.8.1 Method -I

Calculation of Volume as per ISO 4787:2010

The general equation for calculation of the volume at the reference temperature of 20°C, V_{20}, from the apparent mass of the water, contained or delivered, is as follows:

$$V_{20} = (I_L - I_E) \times (\rho_W - \rho_A)^{-1}(1 - \rho_A/\rho_B) \times [1 - \gamma(t - 20)]$$

Where;

$\boldsymbol{I_L}$ is the balance reading of vessel with water, in grams;

$\boldsymbol{I_E}$ is the balance reading of empty vessel, in grams (zero in case the balance was tared with the volumetric instrument or receiving vessel);

$\boldsymbol{\rho_A}$ is the density of air, in g/ml, obtained from Table at the temperature and atmospheric pressure of the test;

$\boldsymbol{\rho_B}$ is either the actual density of the balance weights when these are adjusted to their nominal mass, or the reference density for which the weights have been adjusted (see the note below), in g/ml, or, when using an electronic balance without weights, the (reference) density of the weights with which it has been adjusted;

$\boldsymbol{\rho_W}$ is the density of water at t °C, in grams per milliliter calculated with the “Tanaka” formula (See table B-4 of IS/ISO 4787:2010).

γ is the coefficient of cubical thermal expansion of the material of which the volumetric instrument tested is made, in per °C (see Table below);

t is the temperature of the water used in calibration, in degrees Celsius.

6.8.1.1 Coefficient of Cubical Thermal Expansion 'γ'

Table 4

Material	**Coefficient of cubical thermal expansion, γ^a $°C^{-1} \times 10^{-6}$**
Borosilicate glass 3.3	9,9
Borosilicate glass 50	15
Soda- lime Glass	27

Note: Weights conforming to International Document OIML D 28 of the International Organization of Legal Metrology have been adjusted to give correct results when weighing in air as though the density of the weights was 8.0 g/ml. Electronic balances are usually adjusted by means of these weights.

6.8.1.2 In order to give an impression of the extent to which the various parameters influence the result, some parametric tolerances, with the corresponding error in the volume determined, are given in Table below. It is evident from these figures that the measurement of the water temperature is the most critical factor.

Table 5

Parameter	**Parametric Tolerance**	**Volumetric Error relative to the Volume[a]**
Water temperature	± 0,5 °C	$\pm 10^{-4}$
Air pressure	± 8 mbar (0,8 kPa)	$\pm 10^{-5}$
Air temperature	± 2,5 °C	$\pm 10^{-5}$
Relative humidity	± 10 %	$\pm 10^{-6}$
Density of weights	± 0,6 g/ml	$\pm 10^{-5}$
[a] example : a relative volumetric error of 10^{-4} to the measured volume of 100 ml would be 0,01 ml		

6.8.1.3 When the temperature at which the volumetric instrument is used (t2) differs from the reference temperature (t1), the volume of the volumetric instrument at (t2) can be calculated from the following equation:

$V_{t2}=V_{t1}[1+\gamma\ (t_2-t_1)]$

Where γ is the coefficient of cubical thermal expansion. For information on the effect of temperature differences.

6.8.2 Method –II

[This can be used for both calibration procedures either ISO 4787:2010 or ISO 8655-6 for calculation of volume]

Alternatively, the following equation can be used for easy calculations:

$V_{20}= (I_L - I_E)$ x Z

Where, *V* is volume in µl, $(I_L - I_E)$ is weight in mg, Z is conversion factor (mg/µl)

Note: The conversion factor used to calculate the volume of the distilled water from its mass is called the Z factor (Z conversion factor) . The volumetric value can be obtained by multiplying the measured mass value with the Z factor. Tables B-6, B-7 and B-8 of ISO 4787:2010 give the factor Z conversion values for different types of glass at common air pressure versus temperature in these tables, the combines effects of the density of the water, the thermal expansion of glass and the air buoyancy have been taken into account. The used balance weight is ρ_B =8.0 g/m

6.8.2.1 The Z conversion values is derived from the following equation

$$Z = (\rho_W - \rho_A)^{-1}(1 - \rho_A/\rho_B) \times [1 - \gamma(t - 20)]$$

If, the general equation is used for calculation of volume, the table B-3, B-4, B-5 list the necessary values for ρ_A, ρ_B and γ.

6.8.2.2 If the test temperature is different from the temperature of adjustment (which is 20°C, see ISO 8655-2, ISO 8655-3, ISO 8655-4, ISO 8655-5) and if the thermal expansion correction factor Y of the piston operate volumetric apparatus is known, the equation $\mathbf{V_i=m_i.Z}$ may be replaced by equation $\mathbf{V_i=m_i.Z.Y}$

6.8.3 Method-III

[Applicable for calibration as per ISO 4787:2010 using calibrated standard weight as reference]

6.8.3.1 Equation to calculate the mass of water (M_w) contained in the measure up to the mark is as given below:

$$M_w = \left(1 - \frac{\rho_a}{\rho_s}\right) \times [(M_1 - M_2) + (R_2 - R_1)]/(1 - \frac{\rho_a}{\rho_w})$$

Where,

$\boldsymbol{\rho_a}$ *is the* density of air during the measurement and calculated using BIPM formula or from the table using measured value of temperature, pressure and relative humidity of air

$\boldsymbol{\rho_s}$ *is the* density of the material of the standard weight

$\boldsymbol{\rho_w}$ *is the* density of water at temperature t_w= average temperature of the dispensed water [can be taken from water density table]

$(M_1 - M_2)$is the Weighed mass value of the water contained in the measure, upto the mark

$(R_2 - R_1)$Difference in balance reading

6.8.3.2 Equation to calculate volume is as given below:

$$V_w = M_w \rho_w = \left(1 - \frac{\rho_a}{\rho_s}\right) \times [(M_1 - M_2) + (R_2 - R_1)]/(\rho_w - \rho_a$$

6.8.3.3 Equation to convert the volume to the reference temperature:

$$V_{20} = V_m \times [1 - \gamma\,(t_w) - 20]$$

Where γ is the coefficient of cubical thermal expansion of the material of the measure.

6.8.3.4 Evaporation

Especially for small volumes below 50 µl, errors due to evaporation of the test liquid during weighing shall be taken in to consideration. Apart from the design of the weighing vessel, the measurement cycle time is important.

In order to keep the error due to evaporation as small as possible, the following additional items can be considered, if volumes below 50 µl are calibrated:

a) A balance with appropriate accessories such as an evaporation trap could be used ; or

b) the calibration liquid to be weighed could be delivered in to a capillary tube, although this method does not replicate the normal method of use and the user should verify for himself that correlation exists.

c)

Regardless of these items, the error due to evaporation during the measuring series can be determined experimentally (see 7.2.8 of the standard ISO 8655-6) and compensated mathematically (see 8.1 of the standard ISO 8655-6). The uncertainty of this compensation should be added to the uncertainty of measurement.

7. Recommended calibration interval

As per user requirement or legal metrology requirement

8. Measurement uncertainty

8.1 Parameters that may affect the measurement result and its associated uncertainty in gravimetric determination of volume

During the gravimetric calibration of volume instruments, the main parameters that can influence the quality of the result are the following.

8.1.1 Weighing

Weighing is the most important step in gravimetric calibration. The weighing results are influenced by several factors such as the resolution and sensitivity of the balance, the calibration of the balance eccentricity, linearity, and repeatability), the class and density of the reference weights used to calibrate an electronic scale or balance.

8.1.2 Water characteristics

Mass is converted into volume using the density of the calibration liquid. This value can be obtained from equation (2) or from the literature [5,6] or from direct measurements, if pure water is not available.

The water temperature influences the determination of the water density; thus, it should be carefully measured and recorded in each measurement. Methods for estimating the temperature of the water without affecting the volume have to be established. The viscosity of water at a specific temperature influences the residual volume in volume instruments used to deliver.

8.1.3 Ambient conditions

The ambient conditions (air temperature, humidity, barometric pressure) influence gravimetric measurement mainly through the air density determination, so those quantities must be measured and recorded during the measurements because of the possible fluctuations.

8.1.4 Volume instrument characteristics

The characteristics of the instrument (tank, volume measure, pipette, etc) under calibration, e.g. the scale or the expansion coefficient of the material, must also be considered.
The volume instrument temperature depends on the ambient temperature and on the water temperature. This variation is important for the volume conversion at the reference temperature.

8.1.5 Other parameters

There are other parameters that can directly affect the measurements, namely the evaporation or the operator skills and experience that have a direct impact on the accuracy of the calibration result since he or she has direct influence on several steps during calibrations (e.g. meniscus reading, filling and emptying procedure or in the handling of the equipment).

8.2 General procedure for the uncertainty calculation

As per Euramet Calibration Guide No. 19, the evaluation of measurement uncertainty follows the methods described in the Guide to the Expression of Uncertainty in Measurement (GUM) [1]. The
method consists of the following steps.

a) Expressing, in mathematical terms, the relationship between the measurand and its input quantities.
b) Determining the expectation value of each input quantity.
c) Determining the standard uncertainty of each input quantity.
d) Determining the degree of freedom for each input quantity.
e) Determining all covariance between the input quantities.
f) Calculating the expectation value for the measurand.
g) Calculating the sensitivity coefficient of each input quantity.
h) Calculating the combined standard uncertainty of the measurand.
i) Calculating the effective degrees of freedom of the combined standard uncertainty.
j) Choosing an appropriate coverage factor, *k*, to achieve the required confidence level.
k) Calculating the expanded uncertainty.

It should be noted that for steps (f) to (k) well suited computer programmes exist which can avoid the error-prone manual calculation. Step (a) is the most important part in the whole GUM procedure.

For further details refer (Guidelines on the Determination of Uncertainty in Gravimetric Volume Calibration EURAMET Calibration Guide No. 19)

9. Legal Aspects

Calibration of volumetric measures done by any accredited laboratories is meant for scientific and industrial purpose only. However, if used for commercial trading, additional recognition/ approval shall be complied as required by Dept. of Legal Metrology, Regulatory bodies.

10. Sample Scope

Sr. No.	Parameter/ Product name/ Device under Calibration /Class/Least Count	Standard used	Range	Calibration and Measurement Capability (CMC)			Remarks+ / Method Used
				Claimed by Laboratory	Observed by Assessor	Recommended by Assessor	
1	Laboratory – Glassware, Volume, Burette, Pipette,	Weighing balance with resolution 1 mg	10 - 1000 ml @ 27 °C	± 1ml	± 0.8 ml	± 1 ml	Gravimetric Method based on ISO 4787".
2	Piston Operated Volumetric apparatus, Volume, Micro-pipette	Weighing Balance with resolution 0.001 mg	1µl - 10 µl @ 27 °C	±0.05µl	±0.07µl	±0.07µl	ISO 8655 part 6

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