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**Piston-operated volumetric  
apparatus —**

**Part 6:  
Gravimetric reference measurement  
procedure for the determination of  
volume**

*Appareils volumétriques à piston —*

*Partie 6: Mode opératoire de mesure gravimétrique de référence pour  
la détermination de volumes*



Reference number  
ISO 8655-6:2022(E)

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 48, *Laboratory equipment*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 332, *Laboratory equipment*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 8655-6:2002), which has been technically revised. It also incorporates the Technical Corrigendum ISO 8655-6:2002/Cor.1:2008, which has been technically revised.

The main changes are as follows:

- expanded uncertainty of the test equipment in [Table 1](#) and [2](#) has been revised in conjunction with ISO/TR 20461;
- Annex B has been deleted;
- new [Clause 4](#) "General requirements" has been added;
- [Formula \(2\)](#) has been added based on ISO 4787<sup>[13]</sup>.

A list of all parts in the ISO 8655 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## **Introduction**

The ISO 8655 series addresses the needs of:

- manufacturers, as a basis for quality control including, where appropriate, the issuance of manufacturer's declarations;
- calibration laboratories, test houses, users of the equipment and other bodies as a basis for independent calibration, testing, verification and routine tests.

The tests specified in the ISO 8655 series are intended to be carried out by trained personnel.

## Piston-operated volumetric apparatus —

### Part 6: Gravimetric reference measurement procedure for the determination of volume

#### 1 Scope

This document specifies a gravimetric reference measurement procedure for the determination of volume of piston-operated volumetric apparatus (POVA). The procedure is applicable to complete systems comprising the basic apparatus and all parts selected for use with the apparatus, disposable or reusable, involved in the measurement by delivery (Ex) or contained (In).

#### 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 8655-1:2022, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendation*

ISO 8655-2:2022, *Piston-operated volumetric apparatus — Part 2: Pipettes*

ISO 8655-3, *Piston-operated volumetric apparatus — Part 3: Burettes*

ISO 8655-4, *Piston-operated volumetric apparatus — Part 4: Dilutors*

ISO 8655-5, *Piston-operated volumetric apparatus — Part 5: Dispensers*

ISO 8655-9, *Piston-operated volumetric apparatus — Part 9: Manually operated precision laboratory syringes*

ISO/IEC Guide 2, *Standardization and related activities — General vocabulary*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

#### 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8655-1, ISO/IEC Guide 2 and ISO/IEC Guide 99 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

## 4 General requirements

When performing calibrations according to the reference measurement procedure described in this document, all provisions and requirements of this document shall be followed or exceeded (e. g. performing 30 instead of 10 replicates per volume). If one or more of those requirements are not followed, conformity to this document shall not be claimed.

## 5 Test equipment

### 5.1 General

Measurements by the following equipment (balance, thermometers, hygrometer, barometer) shall be traceable to the International System of Units (SI) and shall meet the uncertainty requirements of this document.

NOTE An example of the calculation of the expanded uncertainty of the gravimetric reference procedure is given in ISO/TR 20461<sup>[10]</sup>.

### 5.2 Balance

The balance used for testing shall be chosen according to the minimum requirements specified in Table 1, depending on the nominal volume of the apparatus under test. The balance parameters are defined so that the expanded uncertainty in use is less than one-fourth of the maximum permissible systematic error of the apparatus.

Table 1 — Minimum requirements for balances

Nominal volume of apparatus under test ( $V$ )	Resolution ( $d$ )	Repeatability ( $s$ ) <sup>a</sup>	Expanded uncertainty in use $U(k=2)$ <sup>a, b</sup>
	mg	mg	mg
$0,5 \mu\text{l} \leq V < 20 \mu\text{l}$	0,001 <sup>c</sup> 0,01 <sup>d</sup>	0,006 <sup>c, e</sup> 0,03 <sup>d</sup>	0,012 <sup>c, e</sup> 0,06 <sup>d</sup>
$20 \mu\text{l} \leq V < 200 \mu\text{l}$	0,01	0,025	0,05
$200 \mu\text{l} \leq V \leq 10 \text{ ml}$	0,1	0,2	0,4
$10 \text{ ml} < V \leq 1\,000 \text{ ml}$	1	2	4
$1\,000 \text{ ml} < V \leq 2\,000 \text{ ml}$	10	10	40

<sup>a</sup> The repeatability and expanded uncertainty in use value, in this table, apply in the volume determination of a single channel apparatus. When a single-channel balance is used exclusively for volume determination of multichannel pipettes the repeatability and expanded uncertainty in use values are double the values of this table. See also Footnote d.

<sup>b</sup> Expanded uncertainty in use can be estimated according to Reference [2] or Reference [11] at the value of the nominal volume. Expanded uncertainty in use shall include non-corrected errors as well as possible drift and environmental effects to balance sensitivity. Regular sensitivity adjustments are recommended to improve balance sensitivity. Expanded uncertainty in use may be taken from the balance calibration certificate or calculated separately (see example in ISO/TR 20461). Expanded uncertainty in use can be estimated from the expanded uncertainty of calibration by considering additional contributions as described above, where applicable

<sup>c</sup> Single-channel balance.

<sup>d</sup> Multi-channel balance, only valid for multi-channel pipettes. Multi-channel balances of 0,01 mg readability may be used to test multi-channel pipettes with nominal volumes below 20  $\mu\text{l}$  only if the expanded uncertainty in use is less than one-fourth of the maximum permissible systematic error of the apparatus.

<sup>e</sup> For single-channel pipettes of nominal volumes of less than 2  $\mu\text{l}$ , a balance with repeatability and an expanded uncertainty better than the values in the table shall be used so that the expanded uncertainty in use is less than one-fourth of the maximum permissible systematic error of the apparatus.



### 5.3 Liquid reservoir

The liquid reservoir shall have sufficient capacity for all the test liquid likely to be required for the complete series of tests.

NOTE The temperature difference between the test liquid and the room temperature can be minimized by the use of an appropriate liquid reservoir.

### 5.4 Weighing vessel

The weighing vessel should be chosen for the selected test procedure according to [Clause 8](#). Care shall be taken regarding the evaporation loss of water during the delivery and weighing procedure.

### 5.5 Measuring devices

The minimum requirements for each relevant measurement device are specified in [Table 2](#).

**Table 2 — Minimum requirements for the measuring devices**

Device	Resolution	Expanded uncertainty of measurement ( $k = 2$ )
Thermometer for liquids	0,1 °C	0,2 °C
Thermometer for room air	0,1 °C	0,3 °C
Hygrometer	1 % relative humidity	5 % relative humidity
Barometer	0,1 kPa	1 kPa
Timing device	1 s	not applicable

## 6 Test liquid

Use distilled or deionized water conforming to grade 3 or better as specified in ISO 3696:1987. The water temperature shall be within  $\pm 0,5$  °C of ambient air temperature (see [7.2](#)).

## 7 Test conditions

### 7.1 General

All equipment used to test the POVA shall be operated as specified in the manufacturer's instructions.

### 7.2 Test room

The test shall be carried out in a draught-free room with a stable environment. The test room shall have a relative humidity between 45 % and 80 % and a temperature of  $(20 \pm 3)$  °C with a maximum variation of  $\pm 0,5$  °C during the test. Prior to the test, the apparatus to be tested, all test equipment, and test liquid shall have reached equilibrium within the specified conditions. The temperature variation of the test room during this time should not be more than 0,5 °C per hour.

The environmental conditions, air temperature and air humidity, shall be within the specified limits for the test room for at least 2 h before starting the test (minimum equilibration time) and during the test itself.

NOTE It is unlikely that this minimum equilibration time will be less than 2 h and can be considerably longer.

When the POVA is required for use in a country which has adopted a standard reference temperature of 27 °C (the alternative temperature recommended in ISO 384 [\[1\]](#) for such use), this figure shall replace the reference to 20 °C.

### 7.3 Evaporation

Particularly for small tested volumes (<50 µl) errors due to evaporation of the test liquid during weighing should be taken into consideration. Apart from the geometry of the weighing vessel, the test cycle time (see 7.4) is important.

NOTE Especially for testing apparatus of the lowest volume possible, evaporation loss is an issue. A way to handle evaporation loss is, for example, careful selection of the geometry of the weighing vessel.

Any measures to minimise evaporation (e.g. the use of a weighing vessel with a lid) should be considered while the contribution to uncertainty due to evaporation should be estimated.

In order to keep the error due to evaporation as small as possible, the use of an evaporation trap may be considered.

The error due to evaporation for the measuring series shall be determined experimentally in the cycle (see 8.3.2) or in a separate study and corrected mathematically (see 9.1). The uncertainty of this correction shall be considered in the measurement uncertainty.

### 7.4 Test cycle time

The test cycle time is the time required to complete the weighing of one delivered volume and shall be kept to a minimum.

In the case of air-displacement pipettes, the test cycle time is the time between 8.3.2 h) and 8.3.2 r).

It is important that the test cycle time, as defined above, is regular from cycle to cycle, so that a reliable mathematical compensation of the error due to evaporation during the measuring series can be applied.

## 8 Procedure

### 8.1 General

#### 8.1.1 Test volume

In the case of a fixed-volume apparatus, the test volume is the nominal volume. In the case of variable-volume (user selectable volume) POVA, at least the following three volumes shall be tested:

- nominal volume;
- 50 % of the nominal volume or the closest possible (if equidistant, use the higher value);
- the lower limit of the usable volume range or 10 % of the nominal volume (whichever is greater).

#### 8.1.2 Number of measurements

To determine the measurement error of a POVA according to this document, ten measurements or more for each volume to be tested shall be performed. These measurements are used to calculate the systematic and the random error of measurement in accordance with Clause 9.

#### 8.1.3 Weighing procedure

For apparatus designed to deliver (Ex), weighing shall always involve delivery of test liquid into the weighing vessel. Weighing for apparatus designed to contain (In) shall always involve removal of test liquid from the weighing vessel.

NOTE An example of contained (In) is the sample uptake step in the use of a dilutor.

The weighing vessel shall be clean and have enough liquid inside to cover the bottom of the vessel when the measurement procedure is started, to keep the relative humidity sufficiently high.

#### 8.1.4 Test conditions during weighing procedure

At the start and at the end of the measurements, the temperature of the test liquid shall be recorded. The air temperature, the barometric pressure and the relative humidity in the test room shall be recorded (see 7.2).

NOTE Air temperature and barometric pressure are necessary for the calculation of the correction factor Z (see 9.3 and Annex A); the relative humidity is necessary for the stability of the room conditions and is necessary for documentation in the test report [see Clause 10, item e)].

#### 8.1.5 Dispensing of samples

The test liquid shall be delivered into the weighing vessel following the specific procedures described in 8.2 to 8.9 unless the POVA manufacturer's instructions specify a different volume delivery procedure, in which case the procedure described by the manufacturer's instructions may be used. If the volume delivery procedure specified in the manufacturer's instructions is used, that procedure shall be documented in the test report in sufficient detail to allow the test to be replicated.

### 8.2 Preparation

Leave the POVA under test, the test equipment, exchangeable parts, and test liquid to reach thermal equilibrium (see 7.2).

If using a variable volume POVA, select the test volume; this setting shall not be altered during the test cycle of all replicate measurements.

If testing a burette, dilutor, or dispenser, place the POVA under test, with its reservoir already filled with test liquid, as close to the balance as possible. Prime the POVA under test according to manufacturer's instructions in order to remove any air bubbles inside the tubes and valves. Set the delivery velocity according to the manufacturer's instructions. The first drops of liquid might need to be discarded before starting the calibration, if indicated in the manufacturer's instructions.

### 8.3 Single-channel air displacement pipettes (in accordance with ISO 8655-2)

#### 8.3.1 General

In the case of electronic motorised pipettes, aspiration and delivery of test liquid are automatic. The remainder of the procedure is carried out following the steps described in 8.3.2. The user should refer to the manufacturer's instructions or operation manual for speed settings of aspiration and delivery.

NOTE More information regarding this type of piston pipette can be found in ISO 8655-2:2022, Annex B.

Forward pipetting shall always be performed.

#### 8.3.2 Test cycle

Perform the test cycle as follows:

- Fit the selected tip on the piston pipette.
- In order to reach humidity equilibrium in the air-displacement piston pipette, aspirate the test liquid five times.
- Depress plunger.
- Hold the pipette in a vertical position, immerse the tip in the test liquid to the appropriate depth below the surface of the test liquid (see Table 3).

**Table 3 — Immersion depths during aspiration and wait time after aspiration of test liquid [7, 8]**

Volume [μl]	Immersion depth [mm]	Wait time [s]
≤ 1	1 to 2	1
> 1 to 100	2 to 3	1
> 100 to 1 000	2 to 4	1
> 1 000 to 20 000	3 to 6	3

- e) Release the plunger slowly, if hand operated.
- f) Pause for the recommended wait time (see Table 3).
- g) Withdraw tip vertically and carefully from the test liquid.
- h) Record the balance indication  $m_0$  of the weighing vessel or tare the balance to zero ( $m_0 = 0$ ).
- i) Start the timing device; this may be omitted if using a weighing vessel with lid, or an evaporation trap.
- j) If the weighing vessel has a lid, remove it.
- k) Touch the tip on the inside of the weighing vessel at an angle of approximately 30° to 45°.
- l) Depress the plunger and deliver the test liquid into the weighing vessel.
- m) Where applicable, use the blow-out feature of the piston pipette (second stop, based on pipette type) to expel the last drop of liquid before drawing the delivery end of the tip along the inner wall of the weighing vessel.
- n) Draw the tip approximately 8 mm to 10 mm along the inner wall of the weighing vessel to remove any droplets at or around the tip orifice.
- o) Remove the tip from the weighing vessel.
- p) Release the plunger.
- q) Close the lid of the weighing vessel, if appropriate.
- r) Record the balance indication  $m_i$  ( $i = 1$  to  $n$ ) of the weighing vessel with the delivered test liquid (if tare is used  $m_i$  will be the delivered quantity of water) and calculate the weighing value as the difference between the balance indications.
- s) Repeat the test cycle starting at step c) until all measurements have been performed and values recorded.

During the replicate measurements, the pipette tip shall be changed at least once in order to detect the use of damaged or incorrectly manufactured tips and assess the variability of the used tips. For  $n = 10$  replicates, at least two tips shall be used, and the tip shall be changed at minimum once every 5 measurements. When replacing the tips, they shall be pre-wetted, starting the test cycle at step a)

This tip change is also applicable to positive displacement pipettes with disposable tips (type D2).

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

NOTE Removing the weighing vessel for delivery will increase the measurement uncertainty of the procedure, further information can be found in ISO/TR 20461.

If a mathematical compensation of evaporation is performed, note the time to the nearest second taken to complete the 10 test cycles. Perform one evaporation trial at the beginning, and a second evaporation



trial at the end of the series, if relevant. Both evaporation trials should be a mimic of one dispense, but without delivering liquid. Follow the same details and timing as an actual delivery. Record the mass of the weighing vessel before and after each evaporation trial (see [Formula \(1\)](#)).

The values obtained shall be evaluated in accordance with [Clause 9](#).

The effect of barometric pressure on accuracy should be considered for air displacement pipettes. See ISO/TR 20461 for further details.

#### 8.4 Multi-channel pipettes (in accordance with ISO 8655-2)

Multi-channel pipettes are similar to single channel pipettes 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 volumetric performance test, each channel shall be regarded as a single-channel and tested and reported as such according to [8.3](#).

The values obtained shall be evaluated in accordance with [Clause 9](#).

All channels of a multi-channel pipette should be tested individually to account for the specific design and operational challenges of multi-channel pipettes.

There are generally two possible methods for testing multi-channel pipettes.

- a) A multi-channel balance can be used to measure the test volume delivered from all channels in parallel, by aspirating and emptying each channel at the same time and analysing the results of each channel individually. The test procedure described in [8.3.2](#) can be adapted accordingly. The following variation may be applied to calibrate multi-channel pipettes with more than 12 channels or with 4,5 mm cone distance: use every second cone of the pipette to calibrate the pipette in multiple sets. All channels shall be tested.

If the pipette has more than one row of channels, it may be tested one row at a time.

- b) Each channel can be measured individually, one after another, with a single-channel balance. For this purpose, test liquid shall be aspirated by all channels together and collected from one channel at a time. For the measurement of channel 1, for example, the volume of channel 1 is delivered into the weighing vessel, while the volumes from all other channels are discarded.

NOTE One way to accomplish this delivery method is by using a weighing vessel with a narrow neck which extends through a dish. The liquid from the channel under test is then delivered into the neck, while the liquid from all other channels collects in the dish.

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

Positive displacement pipettes shall be tested in accordance with [8.3](#). However, the conditioning to achieve humidity equilibrium prior to the test only needs to be performed if required by the manufacturer.

Only change the pipette tips according to [8.3.2](#) when testing positive displacement pipettes of Type D2 with disposable tips (see ISO 8655-2).

Manufacturer's instructions shall be followed for filling the pipette tip without air bubbles.

The values obtained shall be evaluated in accordance with [Clause 9](#).

#### 8.6 Burettes (in accordance with ISO 8655-3)

Perform the test cycle as follows:

- a) Load the burette, bubble free, in accordance with the manufacturer's instructions.
- b) Record the balance indication  $m_0$  of the weighing vessel or tare the balance to zero ( $m_0 = 0$ ).

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- c) 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 pre-set is reached and no further delivery occurs.
- d) Record the balance indication  $m_i$  ( $i = 1$  to  $n$ ) of the weighing vessel with the delivered test liquid (if tare is used  $m_i$  will be the delivered quantity of water) and calculate the weighing value as the difference between the balance indications.
- e) Repeat the test cycle until all measurements have been performed and values recorded.

When testing partial volumes of the nominal volume of the burette, the piston shall 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 burette, are not exceeded when dispensing a partial volume.

The values obtained shall be evaluated in accordance with [Clause 9](#).

### 8.7 Dilutors (in accordance with ISO 8655-4)

#### 8.7.1 General

Depending on the design of the dilutor to be tested, sample volume, diluent volume and/or total volume shall be tested. 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.

If the sample uptake is to be measured, the volume of liquid in the weighing vessel shall be at least 15 times the volume to be aspirated at each operation.

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  $n$  replicate measurements.

If testing diluent or total delivery, switch off the sample uptake system and set it as close to zero as possible. Set the diluent volume to either the nominal volume or an intermediate volume within the range. Do not change these settings during the series of  $n$  replicate measurements.

#### 8.7.2 Test cycle

Prior to performing the following test cycle, perform one complete cycle of aspiration and delivery (including delivery of test liquid from the diluent system, if necessary) and discharge the test liquid to waste. Follow standard weighing procedures throughout.

- a) 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.
- b) Measure the test volume by aspirating the first test liquid from the weighing vessel via the aspiration and delivery probe and record the difference of the balance indications.
- c) 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.
- d) Discharge the aspirated sample to waste, if necessary, with a quantity of diluent test liquid.
- e) 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.
- f) Repeat the test cycle Ex until all measurements have been performed and values recorded.

NOTE Many designs of dilutors permit use as dispensers by inactivation of the sample aspiration facility.

If the total volume is measured by aspiration of test liquid from the weighing vessel and then dispensing with 'diluent' test liquid back into the weighing vessel, the increase in mass will be accounted for only by 'diluent' test liquid. In either case, the increase in mass of the weighing vessel corresponds to a single delivery of 'diluent' test liquid.

During operation ensure that the piston does not hit the stroke limits too quickly which could cause a brief opening of the spring-loaded exhaust valve (spitting caused by the recoil force of the abruptly interrupted flow).

The values obtained shall be evaluated in accordance with [Clause 9](#).

## 8.8 Dispensers (in accordance with ISO 8655-5)

Perform the test cycle as follows:

- a) Load the dispenser, bubble free, in accordance with the manufacturer's instructions.
- b) Record the balance indication  $m_0$  of the weighing vessel or tare the balance to zero ( $m_0 = 0$ ).
- c) Deliver the test liquid from the dispenser into the weighing vessel.
- d) Record the balance indication  $m_i$  ( $i = 1$  to  $n$ ) of the weighing vessel with the delivered test liquid (if tare is used  $m_i$  will be the delivered quantity of water) and calculate the weighing value as the difference between the balance indications.
- e) Repeat the test cycle until all measurements have been performed and values recorded.

Due to the large effect of piston speed on the measuring result, any information contained in the manufacturer's instructions or operation manual regarding piston speed is particularly important (e.g. selection of the speed appropriate for water with electronic motorised apparatus).

During operation, ensure that the piston does not hit the stroke limits.

For multiple delivery dispensers (see ISO 8655-5), do not reset the piston between each of the  $n$  test cycles to its initial position if there is sufficient test liquid remaining to deliver the next aliquot.

The values obtained shall be evaluated in accordance with [Clause 9](#).

## 8.9 Syringes (in accordance with ISO 8655-9)

### 8.9.1 General

Syringes are instruments used for delivering liquids or gases and can be used for total or partial delivery. The procedure in this document applies to the delivery of liquids only.

### 8.9.2 Test cycle

Perform the test cycle as follows:

- a) Aspirate the test liquid into the syringe until the fiducial line slightly exceeds the graduation line of the volume to be delivered.
- b) Ensure that no air bubbles form when aspirating test liquid in step a). Adjust the plunger until the fiducial line corresponds to the graduation line of the selected volume to be tested, remove any droplet at the end of the syringe tip.
- c) Record the balance indication  $m_0$  of the weighing vessel or tare the balance to zero ( $m_0 = 0$ ).
- d) Deliver the contents of the syringe into the weighing vessel by touching the delivery end of the syringe tip on the inside wall of the vessel at an angle of approximately 30° to 45°.

- e) Draw the delivery end of the syringe tip approximately 8 mm to 10 mm along the inner wall of the vessel to remove any droplets at or around the syringe tip orifice.
- f) Record the balance indication  $m_i$  ( $i = 1$  to  $n$ ) of the weighing vessel with the delivered test liquid (if tare is used  $m_i$  will be the delivered quantity of water) and calculate the weighing value as the difference between the balance indications.
- e) Repeat the test cycle until all measurements have been performed and values recorded.

The measurement values obtained shall be evaluated in accordance with [Clause 9](#).

## 9 Evaluation

### 9.1 Calculation of evaporation loss

It is possible to determine the evaporation loss during each test cycle by calculating the difference between the starting mass ( $m_{00}$ ) and the mass after the first cycle time without making any delivery of liquid ( $m_0$ ). This can be repeated again at the end of the test, to obtain  $m_n$  and  $m_{n+1}$ .

The average evaporation loss per test cycle can be determined by using either [Formula \(1\)](#) or by another appropriate method or formula.

$$m_{\text{evap}} = \frac{(m_{00} - m_0) + (m_n - m_{n+1})}{2} \quad (1)$$

where

- $m_{\text{evap}}$  the estimated evaporated mass within a test cycle;
- $m_{00}$  is the balance indication at the first reading (starting mass);
- $m_0$  is the balance indication after the cycle time;
- $m_n$  is the balance indication after the  $n^{\text{th}}$  replicate delivered;
- $m_{n+1}$  is the balance indication after the  $n^{\text{th}}$  replicate delivered after the cycle time.

### 9.2 Calculation of the corrected weighing value of each quantity delivered

If the tare of the balance has not been used, calculate the weighing value of each delivered quantity  $m_i$  by subtraction  $m_1 - m_0$ ,  $m_2 - m_1$ ,  $m_{10} - m_9$ . Add the evaporation loss per test cycle calculated in [9.1](#) to each delivered quantity  $m_i$ .

### 9.3 Conversion of the corrected weighing values to volume

#### 9.3.1 General

The corrected weighing values shall be converted to volume using one of the methods described in [9.3.2](#) or [9.3.3](#).

#### 9.3.2 Calculation of volume using the general formula

##### 9.3.2.1 General formula for volume

The general formula for calculation of the volume at the reference temperature of 20 °C,  $V_{20}$  (at a reference temperature of 27 °C,  $V_{27}$ ), from the weighing value of the delivered water is described in ISO 4787<sup>[13]</sup> and is the basis for [Formula \(2\)](#) in this document. The values  $m_i$  obtained in accordance with [9.2](#) are balance indications. A correction taking into account water density and air buoyancy is



necessary for the conversion of the weighing value of each delivered quantity  $m_i$  to volume  $V_i$ . If the cubic thermal expansion coefficient  $\gamma$  of POVA is known, as specified by the manufacturer or measured with sufficient reliability by other means,  $V_{\text{ref},i}$  can be calculated according to [Formula \(2\)](#).

$$V_{i,\text{ref}} = (m_L - m_E + m_{\text{evap}}) \times \frac{1}{\rho_W - \rho_A} \times \left( 1 - \frac{\rho_A}{\rho_B} \right) \times [1 - \gamma(t_W - t_{\text{ref}})] \quad (2)$$

where

- $V_{i,\text{ref}}$  is the calculated volume at the reference temperature, in ml;
- $m_L$  is the balance indication of the weighing vessel after liquid delivery, in g;
- $m_E$  is the balance indication of the weighing vessel before liquid delivery, in g ( $m_E = 0$  in case the balance was tared with the weighing vessel);
- $m_{\text{evap}}$  the estimated evaporated mass within a test cycle, in g;
- $\rho_A$  is the density of air, in g/ml, at the temperature, humidity and atmospheric pressure of the test, see [Formula \(3\)](#);
- $\rho_B$  is the reference density of the weights (typically 8 g/ml);
- $\rho_W$  is the density of water at the test temperature (in units of °C), in g/ml, calculated with the "Tanaka" [Formula \(4\)](#);
- $\gamma$  is the combined cubic thermal expansion coefficient of the POVA under test, in °C<sup>-1</sup>;
- $t_W$  temperature of the POVA, assumed to be equal to the temperature of the liquid used in the test, in °C;
- $t_{\text{ref}}$  is the reference temperature of the POVA (20 °C or 27 °C).

[Annex A](#) may be used to convert weighing values into volume but the use of [Formula \(2\)](#) is preferred. ISO/TR 20461 includes further information.

### 9.3.2.2 Calculation of air density

[Formula \(3\)](#) for the air density  $\rho_A$  can be used at temperatures between 15 °C and 27 °C, barometric pressure between 600 hPa and 1 100 hPa, and relative humidity between 20 % and 80 %:

$$\rho_A = \frac{1}{1\,000} \times \frac{0,348\,48 \times p - 0,009 \times h_r \times e^{(0,061 \times t_A)}}{t_A + 273,15} \quad (3)$$

where

- $\rho_A$  is the air density, in g/ml;
- $t_A$  is the ambient temperature, in °C;
- $p$  is the barometric pressure, in hPa;
- $h_r$  is the relative humidity, in %.

The uncertainty of this formula can be calculated according to OIML R111-1:2004<sup>[12]</sup>, section C.6.3.6. At other environmental conditions, [Formula \(3\)](#) shall be replaced with the current air density calculations.

NOTE At the time of publication of this document, the current CIPM air density calculations are described in Reference [\[5\]](#).

### 9.3.2.3 Calculation of water density

The density of pure water  $\rho_W$  is normally provided from formulae given in the literature. [Formula \(4\)](#) given by Tanaka [\[6\]](#) can be used:

$$\rho_W = a_5 \left[ 1 - \frac{(t_W + a_1)^2 (t_W + a_2)}{a_3 (t_W + a_4)} \right] \quad (4)$$

where

- $\rho_W$  is the density of water, in g/ml
- $t_W$  is the water temperature, in °C;
- $a_1$  = -3 983 035 °C;
- $a_2$  = 301 797 °C;
- $a_3$  = 522 528 9 (°C)<sup>2</sup>;
- $a_4$  = 69 348 81 °C;
- $a_5$  = 0,999 974 950 g/ml.

### 9.3.3 Calculation of volume using the Z correction factor

Another possible procedure for conversion of weighing values into volume is using the Z correction factors specified in [Table A.1](#). The Z correction factors given in [Table A.1](#) take into account water density and air pressure during weighing at the corresponding test temperature.

Convert each weighing value  $m_i$  obtained from [9.2](#) to the delivered volume  $V_i$  by applying the Z correction factors at the mean temperature and barometric pressure measured according to [8.1.4](#) and using [Formula \(5\)](#):

$$V_i = m_i \times Z \quad (5)$$

If the test temperature is different from the temperature of adjustment (which is 20 °C or 27 °C, see ISO 8655-2 to ISO 8655-5 and ISO 8655-9) and if the cubic thermal expansion coefficient  $\gamma$  of the piston-operated volumetric apparatus is known, as specified by the manufacturer or measured with sufficient reliability by other means [Formula \(5\)](#) may be replaced by [Formula \(6\)](#):

$$V_{i,\text{ref}} = m_i \times Z \times [1 - \gamma(t_W - t_{\text{ref}})] \quad (6)$$

### 9.3.4 Mean delivered volume

Calculate the mean delivered volume  $\bar{V}$  as shown in [Formula \(7\)](#).

$$\bar{V} = \frac{\sum_{i=1}^n V_{i,\text{ref}}}{n} \quad (7)$$

where

- $\bar{V}$  is the mean delivered volume;
- $V_{i,\text{ref}}$  is each delivered reference volume;
- $n$  is the number of replicates.

When the cubic thermal expansion coefficient is not applied,  $V_{i,\text{ref}}$  is replaced by  $V_i$  when using [Formula \(7\)](#).

#### 9.4 Systematic error of measurement

Calculate the systematic error of measurement  $e_s$  of the POVA using [Formula \(8\)](#):

$$e_s = \bar{V} - V_s \quad (8)$$

where

$e_s$  is the systematic error of measurement, expressed in units of volume;

$V_s$  is the selected test volume at the POVA under test.

or in percent using [Formula \(9\)](#):

$$\eta_s = 100 \% \times (\bar{V} - V_s) / V_s \quad (9)$$

where  $\eta_s$  is the relative systematic error of measurement, expressed in percent.

In the case of fixed volume POVA the selected test volume  $V_s$  is the nominal volume.

The systematic error of measurement in the ISO 8655 series is based on historic convention within the pipetting industry and is reversed in sign compared to the definition described in ISO/IEC guide 99:2007,2.17, for more information see ISO 8655-1:2022, 6.2.

#### 9.5 Random error of measurement

Calculate the random error of measurement of the POVA as repeatability or standard deviation  $s_r$  using [Formula \(10\)](#):

$$s_r = \sqrt{\frac{\sum_{i=1}^n (V_i - \bar{V})^2}{n-1}} \quad (10)$$

where  $s_r$  is the standard deviation, expressed in units of volume.

This random error of measurement may also be expressed as a percentage by the coefficient of variation,  $C_V$  using [Formula \(11\)](#):

$$C_V = 100 \% \frac{s_r}{\bar{V}} \quad (11)$$

where  $C_V$  is the coefficient of variation.

#### 9.6 Uncertainty of measurement

For calibrations (ISO 8655-1:2022, 6.4), the expanded measurement uncertainty of the mean delivered volume for each selected volume shall be estimated and reported. It shall include contributions from both the gravimetric measurement procedure of this document and the device under test. For further information see ISO/TR 20461<sup>[10]</sup>. When performing testing (ISO 8655-1:2022, 6.4) it is optional to estimate and report the expanded measurement uncertainty of the mean delivered volume for each selected volume.

Guidance can be found in ISO/IEC Guide 98-3<sup>[9]</sup>.

**NOTE** To determine the uncertainty of an uncorrected delivered volume, the uncertainty in use of a single delivered volume can be estimated. For further information, see ISO/TR 20461<sup>[10]</sup>.

## 10 Reporting of results

The results of each test, calibration, or series of tests or calibrations carried out shall be reported accurately, clearly, unambiguously and objectively and in accordance with any specific instructions in the test or calibration methods. The results shall be reported, usually in a test report or a calibration certificate (see NOTE) and shall include all the information necessary for the interpretation of the test or calibration results, and all information required by this document.

The test reports or calibration certificates may be issued as hard copy or by electronic means, provided that the requirements of this document are met.

NOTE Test reports and calibration certificates are sometimes called test certificates and calibration reports, respectively.

At least the following information shall be reported:

- a) identification of the POVA by
  - manufacturer's name;
  - type name or model number;
  - manufacturer's serial number or organization's unique asset tag number;
  - nominal volume or usable volume range.
- b) basis of the test (Ex) or (In);
- c) reference temperature (20 °C or 27 °C) and cubic thermal expansion coefficient  $\gamma$ , if a correction for cubic thermal expansion of the POVA is made;
- d) identification of the tips and other exchangeable parts used with the piston-operated volumetric apparatus for the test, e. g. make, model, and serial or lot number;
- e) test conditions under which the test was performed including air temperature, barometric pressure and relative humidity of the test room;
- f) reference to this document, i.e. ISO 8655-6;
- g) any variation from the reference measurement procedure specified in this document, see [Clause 4](#);
- h) reference to the formula used to convert weighing values into volume ("[Formula \(2\)](#)" or "[Formula \(5\)](#)");
- i) volumetric measurement results for each delivered volume;
- j) total number of replicate measurements made for each selected volume, and the number of measurement results used for statistical analysis;
- k) systematic and random measurement errors obtained for the selected volumes;
- l) tolerances to which the test results are compared, if applicable;
- m) the expanded uncertainty of the mean delivered volume, for each selected volume and channel, if required;
- n) date of the test;
- o) identification of the operator performing the test;
- p) if requested, pass or fail statements.

## Annex A (informative)

### Calculation of volumes from balance readings

**Table A.1 — Z correction factors for distilled water (see [Clause 6](#)) as a function of water temperature and air pressure**

Z values in microlitres per milligram

Temperature °C	Air pressure kPa						
	80	85	90	95	100	101,3	105
15,0	1 001 7	1 001 8	1 001 9	1 001 9	1 002 0	1 002 0	1 002 0
15,5	1 001 8	1 001 9	1 001 9	1 002 0	1 002 0	1 002 0	1 002 1
16,0	1 001 9	1 002 0	1 002 0	1 002 1	1 002 1	1 002 1	1 002 2
16,5	1 002 0	1 002 0	1 002 1	1 002 1	1 002 2	1 002 2	1 002 2
17,0	1 002 1	1 002 1	1 002 2	1 002 2	1 002 3	1 002 3	1 002 3
17,5	1 002 2	1 002 2	1 002 3	1 002 3	1 002 4	1 002 4	1 002 4
18,0	1 002 2	1 002 3	1 002 3	1 002 4	1 002 5	1 002 5	1 002 5
18,5	1 002 3	1 002 4	1 002 4	1 002 5	1 002 5	1 002 6	1 002 6
19,0	1 002 4	1 002 5	1 002 5	1 002 6	1 002 6	1 002 7	1 002 7
19,5	1 002 5	1 002 6	1 002 6	1 002 7	1 002 7	1 002 8	1 002 8
20,0	1 002 6	1 002 7	1 002 7	1 002 8	1 002 8	1 002 9	1 002 9
20,5	1 002 7	1 002 8	1 002 8	1 002 9	1 002 9	1 003 0	1 003 0
21,0	1 002 8	1 002 9	1 002 9	1 003 0	1 003 1	1 003 1	1 003 1
21,5	1 003 0	1 003 0	1 003 1	1 003 1	1 003 2	1 003 2	1 003 2
22,0	1 003 1	1 003 1	1 003 2	1 003 2	1 003 3	1 003 3	1 003 3
22,5	1 003 2	1 003 2	1 003 3	1 003 3	1 003 4	1 003 4	1 003 4
23,0	1 003 3	1 003 3	1 003 4	1 003 4	1 003 5	1 003 5	1 003 6
23,5	1 003 4	1 003 5	1 003 5	1 003 6	1 003 6	1 003 6	1 003 7
24,0	1 003 5	1 003 6	1 003 6	1 003 7	1 003 7	1 003 8	1 003 8
24,5	1 003 7	1 003 7	1 003 8	1 003 8	1 003 9	1 003 9	1 003 9
25,0	1 003 8	1 003 8	1 003 9	1 003 9	1 004 0	1 004 0	1 004 0
25,5	1 003 9	1 004 0	1 004 0	1 004 1	1 004 1	1 004 1	1 004 2
26,0	1 004 0	1 004 1	1 004 1	1 004 2	1 004 2	1 004 3	1 004 3
26,5	1 004 2	1 004 2	1 004 3	1 004 3	1 004 4	1 004 4	1 004 4
27,0	1 004 3	1 004 4	1 004 4	1 004 5	1 004 5	1 004 5	1 004 6
27,5	1 004 5	1 004 5	1 004 6	1 004 6	1 004 7	1 004 7	1 004 7
28,0	1 004 6	1 004 6	1 004 7	1 004 7	1 004 8	1 004 8	1 004 8
28,5	1 004 7	1 004 8	1 004 8	1 004 9	1 004 9	1 005 0	1 005 0
29,0	1 004 9	1 004 9	1 005 0	1 005 0	1 005 1	1 005 1	1 005 1
29,5	1 005 0	1 005 1	1 005 1	1 005 2	1 005 2	1 005 2	1 005 3
30,0	1 005 2	1 005 2	1 005 3	1 005 3	1 005 4	1 005 4	1 005 4

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