भारतीय मानक

Indian Standard

IS 17094 (Part 7) : 2023
ISO 8655-7 : 2022

पिस्टन-चालित आयतनी के उपस्कर

भाग 7 आयतन के निर्धारण के लिए वैकल्पिक माप प्रक्रियाएं

(पहला पुनरीक्षण)

Piston-Operated Volumetric Apparatus

Part 7 Alternative Measurement Procedures for the Determination of Volume

(First Revision)

ICS 17.060

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Glass, Glassware and Laboratoryware Sectional Committee, CHD 10

NATIONAL FOREWORD

This Indian Standard (Part 7) (First Revision) which is identical with ISO 8655 (Part 7) : 2022 ‘Piston-operated volumetric apparatus — Part 7: Alternative measurement procedures for the determination of volume’ issued by the International Organization for Standardization (ISO), was adopted by the Bureau of Indian Standards on the recommendation of the Glass, Glassware and Laboratoryware Sectional Committee and the approval of the Chemical Division Council.

This document specifies alternative measurement procedures for the determination of volume of piston-operated volumetric apparatus.

The procedures are 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 process (Ex). Methods described in this document are suitable for various maximum nominal volumes of piston-operated volumetric apparatus. It is the responsibility of the user to select the appropriate method.

This standard was originally published in 2008 through adoption of ISO 8655 (Part 7) : 2005 under single numbering. The first revision of this standard has been undertaken to adopt the revised version i.e. ISO 8655 (Part 7) : 2022. This time the committee decided to adopt this standard under dual numbering. The major differences in this revision as compared to the previous edition are as follows:

a) A gravimetric test method was added (*see* 8.2);
b) A photometric/gravimetric hybrid test method was added (*see* 8.5);
c) A batch testing method was added (*see* 8.7);
d) Measurement procedures for all methods are given in normative Annexes A to E;
e) Standard dispense procedures for POVA described in ISO 8655−2, ISO 8655−3, ISO 8655−4, ISO 8655−5, and ISO 8655−9 were added (*see* Clause 9);
f) Requirements for operator qualification have been added (*see* 4.3);
g) Requirements for testing of multi-channel POVA is described in more detail, with specific procedures given for these apparatus (*see* 8.5, and Annex D);
h) Annexes A, B, and C of the first edition have been deleted and replaced.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words ‘International Standard’ appears referring to this standard, they should be read as ‘Indian Standard’.
b) Comma (,) has been used as a decimal marker, while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to certain International Standards for which Indian Standards also exit. The corresponding Indian Standards, which are to be substituted in their places, are listed below along with their degree of equivalence for editions indicated:

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 648 : 2008 Laboratory glassware — One-mark pipettes	IS 1117 : 2018 Laboratory glassware — Single — Volume pipettes (second revision)	Identical
ISO 1042 : 1998 Laboratory glassware — One-mark volumetric flasks	IS 915 : 2012 Laboratory glassware — One — Mark volumetric flasks (*third revision*)	Identical

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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.

Indian Standard

PISTON-OPERATED VOLUMETRIC APPARATUS

PART 7 ALTERNATIVE MEASUREMENT PROCEDURES FOR THE DETERMINATION OF VOLUME

(First Revision)

1 Scope

This document specifies alternative measurement procedures for the determination of volume of piston-operated volumetric apparatus.

The procedures are 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 process (Ex). Methods described in this document are suitable for various maximum nominal volumes of piston-operated volumetric apparatus. It is the responsibility of the user to select the appropriate method.

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 1042, *Laboratory glassware — One-mark volumetric flasks*

ISO 2859-1, *Sampling procedures for inspection by attributes — Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection*

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

ISO 3951-1, *Sampling procedures for inspection by variables — Part 1: Specification for single sampling plans indexed by acceptance quality limit (AQL) for lot-by-lot inspection for a single quality characteristic and a single AQL*

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

ISO 8655-2, *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 8655-6, *Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume*

ISO 8655-8, *Piston-operated volumetric apparatus — Part 8: Photometric reference measurement procedure for the determination of volume*

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, ISO/IEC Guide 99 and the following 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/

3.1
acceptance quality limit
AQL
<acceptance sampling> worst tolerable quality level

Note 1 to entry: This concept only applies when a sampling scheme with rules for switching and for discontinuation, such as in ISO 2859-1, ISO 3951-1 or ISO 3951-5 is used.

Note 2 to entry: Although individual lots with quality as bad as the acceptance quality limit may be accepted with fairly high probability, the designation of an acceptance quality limit does not suggest that this is a desirable quality level. Sampling schemes found in International Standards such as ISO 2859-1, ISO 3951-1 or ISO 3951-5, with their rules for switching and for discontinuation of sampling inspection, are designed to encourage suppliers to have process averages consistently better than the AQL.

4 General requirements

4.1 Metrological confirmation

Metrological confirmation of all POVA shall be performed on a regular basis to ensure the apparatus conforms to requirements for its intended use. The requirements of the methods and procedures described in this document are suitable to be used in the metrological confirmation of POVA. For calibrations and testing, no less than ten replicate measurements per selected volume shall be performed and the measurement procedures in this document shall be validated by comparison to one of the reference measurement procedures described in ISO 8655-6 or ISO 8655-8.

4.2 Uncertainty of measurement

When performing calibrations (ISO 8655-1:2022, 6.4) according to measurement procedures described in this document, the expanded measurement uncertainty of the mean delivered volume for each selected volume shall be estimated and reported (see Clause 10 (m)).

When performing testing (ISO 8655-1:2022, 6.4) or routine tests (ISO 8655-1:2022, 6.5), it is optional to estimate and report the expanded measurement uncertainty.

NOTE For further information on uncertainty for the photometric and gravimetric methods, refer to ISO/TR 16153[1] and ISO/TR 20461[2] respectively.

4.3 Operator qualification

An operator who uses POVA for volumetric transfers, performs metrological confirmation or routine tests of POVA shall be adequately trained on the use of the type of POVA under test. Operator training and competence should be documented.

NOTE 1 Previously calibrated POVA can be used for the qualification of operators.

NOTE 2 Training and qualification requirements for operators of POVA are intended to be included in ISO 8655-10.

5 Performance requirements

5.1 Performance tolerances

Calibration, testing, and routine test results may be reported without comparison to performance tolerances. If the results are verified against performance tolerances, these tolerances shall be stated on the test report/certificate.

Performance tolerances may be based on the user's liquid handling process tolerances or the product tolerances given in the part of ISO 8655 corresponding to the type of POVA under test or the tolerances specified by the manufacturer, subject to them being fit for purpose.

5.2 Operator impact

Measurement of volumetric performance includes random and systematic errors of the POVA, as well as errors introduced by the device's operator. The performance of a hand-held pipette is inseparable from the performance of its operator.

NOTE More information about operator impact is given in ISO 8655-10.

6 Test conditions

6.1 General

Test conditions described in this clause shall be validated for their suitability for the selected test method and procedure. Test conditions, together with the test equipment and detailed test procedure, impact the uncertainty of measurement. Examples for the calculation of the expanded uncertainty of the mean volume and of the uncertainty in use of a single delivered volume are given in ISO/TR 16153[1] and ISO/TR 20461[2].

6.2 Test equipment

All equipment used for the testing of POVA, including for the preparation of test solutions, shall be chosen such that the required uncertainty of measurement can be obtained.

All test equipment used shall be of suitable readability, accuracy, reproducibility and stability, consistent with the required expanded uncertainty of measurement.

Deviations from the test equipment given in this document shall be taken into account when calculating the expanded measurement uncertainty and shall be proven to yield measurement results fit for the intended purpose.

6.3 Test room, environmental conditions

The following applies:

a) The test room should be kept at a steady temperature throughout the entirety of the equilibration time for the test equipment and POVA (±1 °C), and throughout the POVA testing time (±0,5 °C). All test equipment, POVA, exchangeable parts (e. g. pipette tips), and reagents used shall be equilibrated to the test room temperature.

b) The air temperature, relative humidity, and barometric pressure at the time of the test shall be recorded. At the start and at the end of the n replicate measurements, the temperature of the test liquid shall be recorded.

NOTE 1 Air temperature and barometric pressure are necessary for the conversion of liquid mass to volume (see Annex F); the relative humidity is necessary for the stability of the room conditions and is necessary for documentation in the test report.

c) To aid evaluation of a POVA's fitness for purpose, the test room conditions (temperature, relative humidity, and barometric pressure) should reflect the environmental conditions under which the POVA is used, within the constraints mentioned in a). This can be achieved when a POVA is tested within the laboratory in which it is used. Other environmental and non-environmental factors can influence a POVA's fitness for purpose.

d) The test environment should be draft free.

e) Prior to the test, the apparatus to be tested, all test equipment, and test solutions shall have stood in the test room conditions for a sufficient time to reach equilibrium with the test room conditions.

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

NOTE 3 Calibration laboratories at test houses or pipette manufacturer's quality control laboratories can often precisely control environmental conditions to achieve a desired standard condition. It can be very challenging to reproduce such results under different environmental conditions.

6.4 Test volumes

6.4.1 Fixed volume POVA

In the case of a fixed-volume POVA, the selected volume V_S is the nominal volume V_{Nom} and is the only test volume.

6.4.2 Adjustable volume POVA

a) For calibrations and testing, adjustable volume POVA shall be tested at least at three volumes:

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

Measurement of further volumes is optional.

b) For routine tests, fewer than three volumes may be tested.

In case the POVA is to be tested at only two volumes, the nominal volume and the lower limit of the useable volume range, or 10 % of the nominal volume (whichever is the greater), shall be tested.

NOTE The linearity of delivered volumes between these two test points is unknown and is likely to increase the risk for volumetric errors as compared to a test at three volumes.

In case the POVA is tested only at one volume, it shall be tested at its nominal volume, or at the volume at which it will be used.

6.5 Number of measurements per test volume

The confidence of metrological confirmation increases with the number of replicate measurements for each test volume. A minimum of 10 measurements per volume is required by the reference measurement procedures specified in ISO 8655-6 and ISO 8655-8. For routine tests, 10 measurements are recommended, but fewer replicate measurements may be made if the expanded uncertainty of measurement for the POVA is fit for the intended purpose. The number of replicates shall not be less than 4.

After repair or adjustment of the POVA, a minimum of 10 measurements shall be performed.

The replicate volume measurements shall be used to calculate the systematic and the random errors of measurement in accordance with Clause 8. When applicable, the reported uncertainty shall be based on the number of replicates.

6.6 Test liquids

POVA are typically supplied with adjustments using water. Calibrations or routine tests may be performed using other liquids or solutions. For the purpose of this document, the term “test liquid” is used for pure solvents, as well as for prepared chromophore or other solutions. The test liquid used shall be described in sufficient detail to allow replication of the test and interpretation of the results.

The following characteristics of the test liquid shall be taken into account when determining the measured volume: *Z*-factor when weighing, absorbances of the chromophore when using photometric methods, and conductivity and reactivity when performing potentiometric titration.

Depending on the type of POVA, the following parameters can influence the amount of liquid aspirated and/or dispensed: viscosity, density, chemical composition, and surface tension.

The stability of each test liquid shall be known if it is to be stored for any length of time. Refer to the specific procedure for the preparation and storage of reagent solutions.

The test liquid used shall be reported. The influence of the test liquid on the expanded uncertainty of measurement shall be accounted for during calibrations.

7 Evaluation

7.1 Mean volume

Add together the n test volumes delivered $V_\mathrm{T}(i)$ (where $i = 1$ to n) and divide the sum by n to provide the mean volume $\overline{V}$ delivered at the test temperature, as shown in Formula (1). This value can be expressed in microlitres or millilitres:

$$\overline{V} = \frac{1}{n} \sum_{i=1}^{n} V_T(i) \tag{1}$$

where

$\overline{V}$ is the mean volume;

n is the number of replicate deliveries of the test volume;

$V_\mathrm{T}(i)$ is the volume of test liquid delivered by each replicate, $i = 1$ to n.

7.2 Systematic error of measurement

Calculate the systematic error of measurement e_S of the piston-operated volumetric apparatus using Formula (2):

$$e_\mathrm{s} = \overline{V} - V_\mathrm{s} \tag{2}$$

where

e_S is the absolute systematic error of measurement, expressed in units of volume;

V_S is the selected test volume at the POVA under test.

This systematic error of measurement may be expressed in percent using Formula (3):

$$\eta_S = \frac{(\overline{V} - V_S)}{V_S} \times 100\ \% \tag{3}$$

where η_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 guide 99:2007, 2.17, for more information see ISO 8655-1:2022, 6.2.

7.3 Random error of measurement

Calculate the random error of the POVA as repeatability or standard deviation s_r using Formula (4):

$$s_r = \sqrt{\frac{\sum_{i=1}^{n} (V_T(i) - \overline{V})^2}{n-1}} \tag{4}$$

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

This random error may also be expressed as a percentage by the coefficient of variation, C_V, using Formula (5).

$$C_V = \frac{s_r}{\overline{V}} \times 100\ \% \tag{5}$$

where C_V is the coefficient of variation, expressed in percent.

8 Test methods

8.1 General

This document describes five test methods and the corresponding test procedures: gravimetry, dual-dye ratiometric photometry, single dye photometry, hybrid method using photometry and gravimetry, and titration.

The test liquids and receiving vessels depend on the selected method. Test procedures corresponding to the selected methods are described in Annex A to Annex E, respectively, and shall be followed for the preparation of test liquids. Receiving vessels for the test liquid shall conform to those specified in the respective test procedure.

Chemicals used in the preparation of the test liquids and their corresponding CAS registration numbers are listed in Table 1.

Table 1 — CAS registration numbers

Chemical	CAS No.
copper(II) chloride dihydrate	10125–13–0

Table 1 *(continued)*

Chemical	CAS No.
disodium hydrogen phosphate dihydrate	10028–24–7
hydrochloric acid	7647–01–0
nitric acid	7697–37–2
4-nitrophenol	100–02–7
Orange G	1936–15–8
Ponceau S	6226–79–5
potassium chloride	7447–40–7
potassium hydrogen phthalate	877–24–7
potassium nitrate	7757–79–1
silver nitrate	7761–88–8
sodium chloride	7647–14–5
sodium hydroxide	1310–73–2
sulfuric acid	7664–93–9
Tartrazine	1934–21–0
tetrasodium ethylenediaminetetraacetic acid dihydrate (EDTA)	10378–23–1
water	7732–18–5

8.2 Gravimetric method

This method uses a balance to measure the mass of the delivered test volume. It can be used to evaluate the volumetric performance of a POVA for a variety of test liquids, provided that the specific density of the test liquid is known.

The gravimetric procedure is described in Annex A.

This method may be adapted for the use of multi-channel balances, which allow the simultaneous delivery of test liquid from multi-channel POVA.

When following the gravimetric reference measurement procedure specified in ISO 8655-6 but deviating from any of its requirements, A.2 applies.

8.3 Dual-dye ratiometric photometric method

This method uses two chromophore solutions: the test solution containing Ponceau S is delivered into copper(II) chloride solution, and the degree of dilution of both chromophores is calculated from photometric measurements at 520 nm and 730 nm.

The ratiometric photometric procedure described in Annex B is suitable for test volumes between 0,1 µl and 5 000 µl.

An adaptation of this method is suitable for testing multi-channel POVA by delivering the chromophore solutions from all channels simultaneously into 96-well or 384-well micro plates (see Reference [3] 5.2.1 and Annex B).

When following the photometric reference measurement procedure specified in ISO 8655-8 but deviating from any of its requirements, B.2 applies.

8.4 Single dye photometric method

In this method, the dilution of a chromophore solution (test solution) is calculated from the measured absorbance of the delivered test solution. The following chromophores may be used for this method: Ponceau S, Orange G, Tartrazine, and 4-nitrophenol.

The photometric procedure described in Annex C is based on the use of Ponceau S as chromophore and is suitable for test volumes between 2 µl and 200 µl.

8.5 Hybrid photometric/gravimetric method for multichannel POVA

This method allows the evaluation of the volumetric performance of multichannel POVA by combining a gravimetric measurement with subsequent photometric measurement. Test liquid containing either Tartrazine, Orange G, or 4-nitrophenol as chromophore is delivered by all channels in parallel into 96-well or 384-well microplates.

The procedure described in Annex D is generally suitable for test volumes between 1 µl and 100 µl in 96-well plates, and between 1 µl and 50 µl in 384-well plates.

8.6 Titration method

This method is suitable for testing volumes larger than 500 µl, using sodium chloride (NaCl) solution as test liquid, which is titrated with silver nitrate ($AgNO_3$) solution. The equivalence point is determined by potentiometric detection, e. g. with a silver electrode.

The potentiometric titration procedure is described in Annex E.

8.7 Batch testing

Statistical approaches based on random testing and sample inspection may be used to aid in batch testing of POVA if all provisions of this subclause are fulfilled. Those utilising this analysis shall maintain full control over all volume determining components of the POVA, including the tolerances and assembly process. The production of these components shall have series maturity.

The complete performance and production history of a full batch of POVA of a product line with the same volumetric characteristics (e.g. same nominal volume, fixed or variable volume device, number of channels) shall be maintained.

Additionally, the performance homogeneity of the POVA shall be ensured within the specified performance limits. The performance of volume-determining components shall be determined through measurements according to one of the reference measurement procedures specified in ISO 8655-6 or ISO 8655-8 in a technically competent laboratory[4].

POVA subject to batch testing according to this document shall be manufactured by an organization with a quality management system such as ISO 9001[5] or ISO 13485[6]. The sampling plan for this method shall follow ISO 2859-1 or ISO 3951-1, with an appropriate acceptance quality limit (AQL).

Details of the selected sampling plan and AQL, including reference to the corresponding ISO standard, shall be reported.

9 Dispense procedures

9.1 General

The test liquid shall be delivered into the receiving vessel following the specific procedures described in 9.2 to 9.9 unless the POVA manufacturer's instructions specify a different volume delivery procedure, in which case this procedure (manufacturer's instructions) may be used. If the manufacturer's instructions are used, this procedure shall be documented in the test report in sufficient detail to allow the test to be replicated.

9.2 Preparation

Prepare the test equipment, test liquid, and test liquid receiving vessel according to the selected procedure.

Leave the POVA under test, test equipment, exchangeable parts, and test liquids to reach thermal equilibrium.

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, in such a manner that delivery of the test liquid directly into the receiving vessel is possible. Prime the POVA under test according to the 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 by the manufacturer.

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

9.3.1 General

In the case of electronic motorised pipettes, the aspiration and delivery of test liquid are automatic. The remainder of the procedure is carried out following the steps described in 9.3.2. The user should refer to the 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.

9.3.2 Test cycle

Perform the test cycle as follows:

a) Fit the selected tip on the piston pipette;

b) Pre-wet pipette tip five times by aspirating the test solution and expelling to waste to reach a humidity equilibrium in the dead air volume of the air-displacement piston pipette;

c) Depress plunger;

d) Holding the pipette in a vertical position, immerse the tip in the test solution to the appropriate depth below the surface of the test solution (see Table 2).

Table 2 — 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 2);

g) Withdraw tip vertically and carefully from the test solution;

h) Touch the tip on the inside of the receiving vessel at an angle of approximately 30° to 45°;

i) Depress the plunger and deliver the contents of the pipette into the receiving vessel;

j) Where applicable, use the blow-out feature of the piston pipette (second stop, based on pipette type) to expel the last drop of liquid;

k) Draw the tip approximately 8 mm to 10 mm along the inner wall of the receiving vessel to remove any droplets at or around the tip orifice;

l) Remove the tip from the receiving vessel;

m) Release the plunger;

n) Close the lid of the receiving vessel, if appropriate;

o) Follow the selected measurement procedure to measure the delivered volume of test solution;

p) Repeat the test cycle described in 9.3.2 until the desired number (n) of measurements have been recorded.

During the replicate measurements, the pipette tip shall be changed at least once, e. g. after $n/2$ measurements, 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 every 5 measurements. When replacing the tip, start test cycle at step a).

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

The effect of barometric pressure on accuracy should be considered for air displacement pipettes. See ISO/TR 16153 or ISO/TR 20461 for further details.

The values obtained shall be evaluated according to Clause 7.

9.4 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 pipette and tested and reported as such according to 9.3. The obtained values shall be evaluated according to Clause 7.

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 four possible methods for testing multi-channel pipettes:

a) The aspirated test volume can be delivered from all channels simultaneously into a microplate. The test method described in 8.5 uses a hybrid gravimetric/photometric approach, which is suitable of testing the simultaneous delivery from up to 384 channels of a multi-channel POVA (Annex D). The maximum test volume is limited by the useable well volume of the microtitre plate used. Similarly, the ratiometric photometric test method described in 8.3 can be adapted to measure simultaneous test liquid deliveries from up to 384 channels in a microplate in accordance with ISO 23783-2:—, 5.2.1[3][1].

b) 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 8.2 can be adapted accordingly. The

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[1] Under preparation. Stage at the time of publication: ISO/FDIS 23783-2:2022.

maximum number of channels, which can be tested in parallel depends on the type of multi-channel balance.

For a) and b): 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.

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

c) Each channel can be tested individually, one after another, by aspirating test liquid into all channels and collecting liquid from one channel at a time. For the measurement of channel 1, for example, the volume of channel 1 is then delivered into the receiving vessel, while the volumes from all other channels are discarded.

NOTE One way to accomplish this delivery method is by providing a zero-retention flow connection to the receiving vessel which passes through a dish such that the liquid from the channel under test is delivered into the receiving vessel while the liquid from all other channels is collected in the dish. Alternatively, a zero-retention transfer vessel can be used to quantitatively transfer the test liquid from the channel under test to the receiving vessel.

d) Each channel can be tested individually, one after another, by aspirating test liquid into only one channel at a time and delivering the liquid from that channel into the receiving vessel. If this delivery method is used, the effects shall be included in the uncertainty budget, if applicable, and the delivery method shall be stated in the test report.

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

Positive displacement pipettes shall be tested according to 9.3.2. The fivefold pre-wetting of the pipette tip prior to the test only needs to be performed if required by the manufacturer. Only change the pipette tips according to 9.3.2 when testing positive displacement pipettes of type D2 with disposable tips (see ISO 8655-2). Follow the manufacturer’s instructions for filling the pipette tip without air bubbles.

The values obtained shall be evaluated according to Clause 7.

9.6 Burettes (in accordance with ISO 8655-3)

Perform the following test cycle:

a) Load the burette, bubble free, in accordance with the manufacturer's instructions;

b) Take a zero reading of the measurement equipment, depending on the selected procedure;

c) Deliver the test liquid from the burette into the receiving 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) Measure the dispensed volume of test solution following the required steps according to the selected method and its corresponding test procedure and record the measurement result.

e) Repeat the test cycle until the desired number (n) of replicate measurement results have been 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 according to Clause 7.

9.7 Dilutors (in accordance with ISO 8655-4)

9.7.1 General

With dilutors, only the gravimetric method (8.2) shall be used for testing.

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 until the desired number of replicate measurements (n) have been completed.

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.

9.7.2 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 obtained values shall be evaluated according to Clause 7.

9.8 Dispensers (in accordance with ISO 8655-5)

Perform the following test cycle:

a) Load the dispenser, bubble free, in accordance with the manufacturer's instructions.

b) Take a zero reading of the measurement equipment, depending on the selected procedure.

c) Deliver the test liquid from the dispenser into the receiving vessel.

d) Measure the dispensed volume according to the selected procedure.

e) Repeat the test cycle until the desired number (n) of replicate measurements have been recorded.

Due to the large effect of piston speed on the measuring result, any information contained in the operation manual regarding piston speed is particularly important (e. g. selection of the speed appropriate for the test liquid 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 the n test cycles to its initial position if there is sufficient test liquid remaining to deliver the next aliquot.

The obtained values shall be evaluated according to Clause 7.

9.9 Syringes (in accordance with ISO 8655-9)

9.9.1 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.

9.9.2 Test cycle

Perform the following test cycle after priming the syringe to remove all air bubbles:

a) Aspirate test liquid into the syringe until the fiducial line slightly exceeds the graduation line of the volume to be tested;

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 syringe tip;

c) Take a zero reading of the measurement equipment, depending on the selected procedure;

d) Deliver the contents of the syringe into the receiving vessel, touching the delivery end of the syringe tip against the inside wall of the vessel just above the liquid surface at an angle of approximately 30° to 45°;

e) Draw the syringe tip approximately 8 mm to 10 mm along the inner wall of the vessel to remove any droplets at or around the tip orifice;

f) Measure the dispensed volume according to the selected procedure;

g) Repeat the test cycle until the desired number (n) of replicate measurements have been recorded.

The obtained values shall be evaluated according to Clause 7.

10 Reporting of results

The results of each calibration or routine test shall be reported accurately, clearly, unambiguously and objectively, and in accordance with any specific instructions in the method. 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 the method used.

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 useable volume range;

b) Basis of test (In or Ex);

c) Reference temperature (20 °C or 27 °C) and cubic thermal expansion coefficient γ, if a correction for cubic thermal expansion of the POVA is made;

d) Identification of the tips or 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, as well as the employed test method and procedure (e. g. “ISO 8655-7:2022, D” if the photometric/gravimetric hybrid procedure according to Annex D was performed). In case batch testing according to 8.7 was performed, reference shall be made to ISO 8655-7:2022, 8.7 and shall include information about the used sampling plan and AQL;

g) Any deviation from the employed procedure described in this document;

h) Type of test liquid;

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) Expanded uncertainty of the mean delivered volume, for each selected volume and channel, if required (see 4.2);

n) Recommendation for the next test date (if agreed upon);

o) Date of test;

p) Identification of the operator performing the test;

q) If requested, pass or fail statement.

Annex A
(normative)

Gravimetric procedure

A.1 Outline

The gravimetric method uses a balance to measure the mass of the delivered test volume. This method can be used to evaluate the volumetric performance of a POVA for a variety of test liquids, provided that the specific density of the test liquid is known. Accommodations shall be made for stable and known environmental conditions (temperature, relative humidity, and barometric pressure), as these affect the conversion of the test liquid's mass to volume.

The gravimetric procedure according to A.3 to A.7 shall be followed unless the gravimetric reference measurement procedure according to A.2 is attempted.

A.2 Use of the gravimetric reference measurement procedure

It is permissible to use the gravimetric reference measurement procedure according to ISO 8655-6. If the reference measurement procedure according to ISO 8655-6 is attempted, all deviations from its requirements shall be recorded and reported according to Clause 10 g). Deviations may include, but are not limited to, using a different test liquid, using different numbers of test volumes, replicates, tip changes, or working at temperatures other than the reference conditions. Results shall be reported according to Clause 10 and shall reference (A.2) of this document as applicable procedure according to Clause 10 f).

A.3 Test equipment

A.3.1 General

All test equipment shall be chosen such that any required uncertainty of measurement can be achieved. An example for the calculation of the expanded uncertainty of measurement for the gravimetric measurement procedure is given in ISO/TR 20461[2].

A.3.2 Balance

The balance used for testing shall be chosen according to the minimum requirements specified in Table A.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 A.1 — Minimum requirements for balances

Nominal volume of apparatus under test (V)	Resolution (d) mg	Repeatability (s)[a] mg	Expanded uncertainty in use U (k = 2)[a, b] mg
0,5 µl ≤ V < 20 µl	0,001[c] 0,01[d]	0,006[c, e] 0,03[d]	0,012[c, e] 0,06[d]
20 µl ≤ V < 200 µl	0,01	0,025	0,05
200 µl ≤ V ≤ 10 ml	0,1	0,2	0,4
10 ml < V ≤ 1 000 ml	1	2	4
1 000 ml < V ≤ 2 000 ml	10	10	40

[a] 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.

[b] Expanded uncertainty in use can be estimated according to Reference [9] or [10] 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 (e.g., daily) 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.

[c] Single-channel balance

[d] 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 µl only if the expanded uncertainty in use is less than one-fourth of the maximum permissible systematic error of the apparatus.

[e] For single-channel pipettes of nominal volumes of less than 2 µl, a balance with a 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.

A.3.3 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.

A.3.4 Weighing vessel

Care shall be taken regarding the evaporation loss of test liquid during the delivery and weighing procedure.

Especially for testing apparatus of the lowest volume, possible measures to handle evaporation loss are, for example, that the height-to-diameter ratio of the weighing vessel be at least 3:1 or to use a weighing vessel with a lid. The height is measured from the liquid surface to the opening of the vessel.

An evaporation trap may be used in the balance to reduce evaporation.

A.3.5 Measuring devices

The thermometers, hygrometer, barometer, and timing device used for the gravimetric procedure shall conform to, or perform better than, the minimum requirements listed in Table A.2.

Table A.2 — Minimum requirements for measuring devices

Device	Resolution	Expanded uncertainty of measurement (k = 2)
Thermometer for liquids	0,1 °C	0,3 °C

Table A.2 *(continued)*

Device	Resolution	Expanded uncertainty of measurement ($k = 2$)
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

A.4 Test liquid

Water used for the gravimetric method shall be distilled or deionized and comply with Grade 3 as specified in ISO 3696:1987, degassed or air-equilibrated. The water shall be at room temperature.

Other test liquids may be used as long as its density and air buoyancy, or its *Z*-factor, is known; see 6.6 and Annex F.

A.5 Test procedure

A.5.1 Evaporation

Particularly for small tested volumes ($V_T \leq 50$ µl) measurement errors due to evaporation of the test liquid during weighing shall be taken into consideration. Apart from the geometry of the weighing vessel (see A.3.4), the test cycle time is important.

The error due to evaporation for the measuring series shall be determined experimentally (see A.5.5) and compensated mathematically (see A.6.1) if significant. The uncertainty of this compensation shall be added to the uncertainty of measurement.

A.5.2 Test cycle time

The test cycle time is the time required to complete the weighing of one delivered volume, from tare to the stable balance indication, and shall be kept to a minimum observing good technique. It is important that the test cycle time is regular, both within each cycle and as far as possible from cycle to cycle, so that a reliable mathematical compensation of the error due to evaporation during the measuring series can be applied.

A.5.3 Preparation of weighing vessel

The test liquid shall be placed in the weighing vessel with a minimum depth of approximately 3 mm. Record the temperature of the test liquid and the barometric pressure and relative humidity in the test room. If the weighing vessel has a lid, fit it.

NOTE Temperature and barometric pressure are necessary for the calculation of the *Z* correction factor (Annex F). The relative humidity is necessary for documentation in the test report but is not necessary for the conversion of mass to volume as the *Z* correction factors in Annex F apply to relative humidity ranging from 20 % to 90 %.

A.5.4 Test procedure

The procedure in the dispense requirements appropriate for the POVA under test (see Clause 9) shall be followed to dispense the volume into the receiving vessel on the balance pan.

Weighing for POVA designed to deliver (Ex) shall always involve delivering 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 this is the sample uptake step in the use of a dilutor.

A.5.5 Steps to test POVA

a) Place the prepared weighing vessel on the weighing pan.

b) Record the balance indication m_{00} of the weighing vessel or tare the balance to zero ($m_{00} = 0$). Start the timing device.

c) After the time interval of one cycle time, measure and record the balance indication of the weighing vessel as m_0. The difference between m_{00} and m_0 is the evaporation loss per cycle time.

d) Deliver the test volume V_T with the POVA under test to the weighing vessel.

e) Record the balance indication m_1 of the weighing vessel, or if tared after step c) the balance indication m_n of the quantity delivered.

f) Repeat the test cycle described in steps c) to e) until $n = 10$, or the desired number of replicate measurements have been recorded as a series of balance indications m_1 to m_n.

g) After the n^{th} delivery, wait for another cycle time to pass and record the balance indication m_{n+1} of the weighing vessel. The difference between m_n and m_{n+1} is the evaporative loss at the end of the test cycle.

h) Measure the temperature of the remaining test liquid and calculate and record the mean test temperature.

i) Measure the barometric pressure in the test room.

NOTE The conversion of the balance readings to volume of pure water is described in Annex F.

A.6 Calculation of dispensed volume by gravimetric method

A.6.1 Calculation of mass loss

Where a mass loss has been determined to enable a correction to be applied for evaporation of the test liquid from the weighing vessel during the test cycle, calculate the mass loss per cycle by using either Formula (A.1) or by another appropriate method or formula, e.g. specified by the supplier.

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

where

m_{evap} is the estimated evaporated mass within a delivery cycle;

m_{00} is the balance indication at the first tare reading;

m_0 is the balance indication after the cycle time;

m_n is the balance indication after the n^{th} replicate dispense of test liquid;

m_{n+1} is the balance indication after the n^{th} replicate dispense, after the cycle time.

A.6.2 Calculation of the corrected mass of each quantity delivered

If the tare facility of the balance has not been used, calculate the balance indication of each delivered quantity m_{n} by subtraction m_1- m_0, m_2 - m_1, ..., m_{10} - m_9. Add the mass value of the loss per cycle calculated in A.6.1 to each delivered quantity m_n.

A.6.3 Conversion of the corrected mass to volume

The values m_n obtained in accordance with A.5.2 are balance readings. A correction taking into account the test liquid's density and air buoyancy is necessary for the conversion of the balance readings m_n to volumes V_n. When using Grade 3 water as test liquid, determine the dispensed volume by Formula (F.1). Alternatively, the Z correction factors specified in Table F.1 may be applied for conversion, using Formula (F.4).

NOTE The Z correction factors given in Annex F take into account water density and air buoyancy during weighing at the corresponding test temperature.

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 thermal expansion correction factor γ of the POVA is known, the test volume may be expressed by Formula (A.2):

$$V_{\mathrm{T}}(i)_{\mathrm{corr}} = V_{\mathrm{T}}(i) \times [1 - \gamma \times (t_{\mathrm{test}} - t_{\mathrm{adj}})] \tag{A.2}$$

where

$V_{\mathrm{T}}(i)_{\mathrm{corr}}$	is the test liquid volume corrected with the POVA's thermal expansion factor;
$V_{\mathrm{T}}(i)$	is the test liquid volume converted from the measured mass according to Formula (F.1) or (F.4);
γ	is the cubic thermal expansion factor of the POVA;
Δt	is the temperature difference between the test temperature and adjustment temperature.

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

A.7 Evaluation

The measurement results shall be evaluated according to Clause 7 and, if applicable, 4.2.

Annex B
(normative)

Dual-dye ratiometric photometric procedure

B.1 Outline

This procedure is designed to be used with disposable measuring cuvettes, prefilled with copper(II) chloride solution, to which the unknown volume of test liquid is added (Ponceau S solution). After mixing, the absorbances of copper chloride and test liquid are measured in a spectrophotometer. For this method, cuvettes with an optical path length of 20 mm ± 2 mm are suggested, but cuvettes with another path length may be used.

The test procedure as described in this annex can be used for the measurement of test volumes between 0,1 µl and 5 000 µl, which are delivered by the POVA under test ("Ex"). The nominal volume and optical path length of the cuvette determine the test volumes which can be measured.

The dual-dye ratiometric photometric procedure according to B.3 to B.7 shall be followed unless the photometric reference measurement procedure according to B.2 is attempted.

B.2 Use of the photometric reference measurement procedure

It is permissible to use the photometric reference measurement procedure according to ISO 8655-8. If the reference measurement procedure according to ISO 8655-8 is attempted, all deviations from its requirements shall be recorded and reported according to Clause 10 g). Deviations may include, but are not limited to, using different test liquids, using different numbers of test volumes, replicates, tip changes, or working at temperatures other than the reference conditions. Results shall be reported according to Clause 10 and shall reference (B.2) of this document as applicable procedure according to Clause 10 f).

B.3 Test equipment

B.3.1 General

All test equipment shall be chosen such that any required uncertainty of measurement can be achieved. An example for the calculation of the expanded uncertainty of measurement for the photometric reference measurement procedure according to ISO 8655-8 is given in ISO/TR 16153[1].

B.3.2 Spectrophotometer

A spectrophotometer with adequate resolution, linearity, repeatability, and bandpass, over the range of absorbances utilized in the method shall be used. Bandpass filters with centre wavelengths of 520 nm ± 1 nm and 730 nm ± 2 nm shall be installed in the spectrophotometer.

Table B.1 lists the minimum performance requirements for the spectrophotometer.

Table B.1 — Minimum performance requirements of the spectrophotometer

Parameter	Requirement
Photometric resolution	0,000 1 AU
Photometric repeatability at A = 0,0 AU [a]	0,000 15 AU
Photometric repeatability at A = 0,5 AU [a]	0,000 15 AU
Photometric repeatability at A = 1,0 AU [a]	0,000 15 AU
Photometric repeatability at A = 1,5 AU [a]	0,000 15 AU
System linearity between 0 AU and 1,5 AU [b]	0,000 25 AU
Reproducibility of cuvette attenuance	0,000 1 AU
Cuvette pathlength uniformity	0,20 mm
Thermometer uncertainty (k = 2)	0,20 °C

[a] Bandpass and signal averaging time may be increased to meet the photometric repeatability requirement.

[b] Corrections may be applied to meet the system linearity requirement.

B.3.3 Cuvette

The ratiometric photometric procedure in this document is based on the use of cylindrical cuvettes with an optical pathlength of 20 mm ± 2 mm. Other cuvettes of adequate optical clarity and with an optical pathlength between 10 mm and 20 mm may be used.

NOTE The exact optical pathlength of the cuvette does not need to be known. The measurement of the second chromophore with known concentration and absorptivity enables ratiometric analysis without reference to the cuvette’s pathlength.

B.3.4 Cuvette holder

The cuvette holder shall allow for thorough mixing of the contents of the cuvette without the need for removing the cuvette from the light path of the spectrophotometer.

B.3.5 Measuring devices

The temperature of the chromophore solutions, ambient temperature, relative humidity, and barometric pressure prevailing in the test room shall be measured using test equipment consistent with the expanded uncertainty of the measurement. Table B.2 lists the minimum requirements for these measuring devices.

Table B.2 — Minimum requirements for measuring devices

Device	Resolution	Expanded uncertainty of measurement (k = 2)
Thermometer for liquids	0,01 °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

B.3.6 Equipment used for solution preparation

Known volumes of diluent or reagent solutions shall be prepared by volumetric or gravimetric means. In case of gravimetric preparation, the densities of the solutions shall be known and properly accounted for.

The liquid components of solutions may be weighed using balances, which shall meet the requirements of Table B.3.

For volumetric preparations, class A glassware meeting the maximum permissible errors for narrow neck flasks of ISO 1042 shall be used.

NOTE Specifications for one-mark volumetric pipettes can be found in ISO 648[11] and ASTM E969[12], and for one-mark volumetric flasks in ISO 1042 and ASTM E288[13].

B.3.7 Balances

Balances used for accurate weighing of dry reagents, preparation of calibrator solutions, and filling of cuvettes shall meet the requirements specified in Table B.3.

Table B.3 — Minimum requirements for balances

Minimum mass to be weighed	Resolution (d)	Repeatability (s)	Expanded uncertainty in use (k = 2) [a]
g	mg	mg	mg
1,0	0,1	0,2	0,4
10	1	2	4
100	10	20	40

[a] Expanded uncertainty in use is determined according to Reference [9] or [10] at the minimum mass listed in the table.

Weighing results for liquids shall be corrected for density, temperature and air buoyancy when determining volume (see Annex F).

B.3.8 Density meter

Densities of the chromophore solutions shall be measured for each lot of solutions using a temperature-controlled density meter with an uncertainty of 0,000 5 g/ml (k = 2) or better.

B.3.9 pH meter

The pH meter is used for the preparation of the reagents in B.4. It shall be calibrated with reference buffer solutions over a range from pH 4 to pH 7 including pH 6,00 according to the manufacturer's instructions. A reference material having a certified value in the range of pH 6,00 ± 0,05 and an uncertainty (k = 2) of 0,02 pH units, or better, is required for comparison.

B.4 Reagents

B.4.1 General requirements

All components of reagent solutions shall be of recognized analytical composition.

B.4.2 Water

Water used for chromophore solutions shall comply with Grade 1 in accordance with ISO 3696:1987.

B.4.3 Buffer solution

The buffer is prepared from 4,08 g/l of potassium hydrogen phthalate and 3,74 g/l of tetrasodium EDTA. Adjust to pH = 6,0 with either HCl or NaOH as needed. The solution is then filtered through a 0,2 µm filter.

B.4.4 Copper chloride solution

Copper(II) chloride solution is prepared by dissolving 1,12 g of $CuCl_2 \cdot 2H_2O$ per litre of buffer solution (B.4.3) and filtration through a 0,2 µm filter.

B.4.5 Ponceau S solution

Ponceau S solutions are prepared by dissolving the dye in water. Table B.4 gives the amount of Ponceau S dye to be dissolved per litre of water to produce a solution with the indicated nominal absorbance per 10 mm pathlength. Following preparation, the solutions are filtered through 0,2 µm filters.

Table B.4 — Ponceau S solutions

Ponceau S solution No.	**Test volume** µl	**Nominal absorbance** (10 mm pathlength) AU	**Ponceau S** g/l
1	200 ≤ V ≤ 5 000	1,2	0,024
2	50 ≤ V < 200	2,6	0,052
3	10 ≤ V < 50	8,2	0,165
4	2 ≤ V < 10	37	0,745
5	0,5 ≤ V < 2	185	3,72
6	0,1 ≤ V < 0,5	740	14,9

B.4.6 Calibrator solutions

Prepare a calibrator solution for each concentration of Ponceau S solution. The absorbance of a calibrator solution should be below the upper end of the linearity range of the spectrophotometer. Calibrator solutions shall be tightly capped to prevent changes in concentration due to evaporation. Calibrator solutions may be freshly prepared for each calibration or testing session, avoiding the issue of degradation in storage.

Table B.5 gives the necessary dilutions to make calibrator solutions. In Table B.5, calibrator solution No.1 is produced by diluting Ponceau S solution No. 1 from Table B.4, and so forth.

Table B.5 — Calibrator solutions

Calibrator solution No.	**First dilution**		**Second dilution**		**Dilution ratio** R
	Ponceau S solution ml	**$CuCl_2$ Solution** ml	**Dilution 1** ml	**$CuCl_2$ Solution** ml	
1	100	100	—	—	$5\,000 \times 10^{-1}$
2	50	200	—	—	$2\,000 \times 10^{-1}$
3	15	200	—	—	$6\,977 \times 10^{-2}$
4	20	100	20	200	$1\,515 \times 10^{-2}$
5	10	200	10	200	$2\,268 \times 10^{-3}$
6	5	200	5	200	$5\,949 \times 10^{-4}$

The dilution ratio R_1 for calibrator solutions No. 1 to 3 is calculated by Formula (B.1):

$$R_1 = \frac{V_{PS}}{V_{PS} + V_{C1}} \tag{B.1}$$

where

R_1	is the dilution ratio for calibrator solutions No. 1, 2, and 3;
V_{PS}	is the volume of Ponceau S solution;
V_{C1}	is the volume of $CuCl_2$ solution used in the first dilution.

The dilution ratio R_2 for calibrator solutions No. 4 to 6 is calculated by Formula (B.2):

$$R_2 = \frac{V_{\mathrm{PS}}}{V_{\mathrm{PS}} + V_{\mathrm{C1}}} \times \frac{V_{\mathrm{Dil1}}}{V_{\mathrm{Dil1}} + V_{\mathrm{C2}}} \tag{B.2}$$

where

R_2 is the dilution ratio for calibrator solutions No. 4, 5, and 6;

V_{Dil1} is the volume of the first dilution mixture used for the second dilution;

V_{C2} is the volume of $CuCl_2$ solution used in the second dilution.

B.4.7 Stability of solutions

If stock solutions are to be stored for any period of time, they shall be tested for chemical and absorbance stability. Preservatives may be added, if needed, to prevent microbiological growth. Such preservatives shall not alter the liquid properties or interfere with the absorbance properties and densities of the solutions. All solutions shall be stored protected from light.

B.5 Test procedure

B.5.1 System linearity

In case the linearity requirements given in Table B.1 are not met, linearity corrections shall be calculated.

B.5.2 Steps to test POVA

The steps to test POVA are as follows:

a) Accurately fill cuvettes with a known amount (V_{C0}) of copper chloride solution (B.4.4) and cap the cuvettes tightly. All sample cuvettes shall be taken from the same production lot to ensure cuvette-to-cuvette homogeneity of absorbance. The cuvette-to-cuvette reproducibility of results shall be tested and included in the uncertainty of measurement, if necessary.

Cuvettes with 20 mm ± 2 mm optical path length should be used. These cuvettes can be filled with 5 ml of copper chloride solution.

b) Zero the spectrophotometer with a cuvette containing buffer solution (B.4.3).

c) Measure the absorbances of calibrator solutions (B.4.6), corresponding to the Ponceau S solutions to be used, $A_{\mathrm{Std520},j}$ at 520 nm, where $j = 1$ to 6, corresponding to the number of the calibrator solution in Table B.5. Record the temperature of the calibrator solutions (t_{Std}).

d) Insert a cuvette containing copper chloride solution [prepared in step a)] and measure the absorbances A_{C520} and A_{C730} at 520 nm and 730 nm, respectively. Do not remove the cuvette from the spectrophotometer.

e) With the POVA under test, deliver the test volume V_{T} of the correct Ponceau S solution j ($j = 1$ to 6 corresponding to the number of the Ponceau S solution in Table B.4) into the cuvette while it is seated in the spectrophotometer. Follow the correct dispense procedure for the POVA under test according to Clause 9.

f) Mix the contents of the cuvette without removing it from the light path of the spectrophotometer.

g) Record the temperature of the contents in the cuvette (t_{M}).

h) Measure the absorbance A_{M520} at 520 nm and record it.

i) Repeat steps e) to h) until $n = 10$, or the desired number of replicate test volume deliveries have been measured.

Ensure that the absorbance of the mixture in the cuvette does not exceed 1,6 AU. Use additional cuvettes, according to step a), for completing the desired number of test liquid deliveries, starting at step d).

B.6 Calculation of dispensed volume

B.6.1 Calculation of dispensed volume

Each batch of Ponceau S solution shall be calibrated by comparison to the $CuCl_2$ solution. The calibration constant (K_j) is calculated using Formula (B.3), where $j = 1$ to 6 indicates the number of Ponceau S solution from Table B.4, and its corresponding calibrator solution (Table B.5). Absorbance values (A) are from the measurements in B.5.2. The dilution ratio (R_j) is taken from B.4.6. Each of these absorbance values is relative to buffer in the same standards cuvette.

$$K_j = \frac{1}{R_j}\left(\frac{A_{\mathrm{Std520},j} - A_{\mathrm{C520}}}{A_{\mathrm{C730}} - A_{\mathrm{C520}}}\right) \tag{B.3}$$

where

K_j	is the calibration constant for the corresponding Ponceau S and calibrator solutions ($j = 1$ to 6, according to Tables B.4 and B.5);
R_j	is the dilution ratio as defined in Formulae (B.1) and (B.2) for solution j ($j = 1$ to 6);
$A_{\mathrm{Std520},j}$	is the absorbance of the calibrator solution at 520 nm, see B.5.2 step c);
A_{C520}	is the absorbance of the $CuCl_2$ solution at 520 nm, see B.5.2 step d);
A_{C730}	is the absorbance of the $CuCl_2$ solution at 730 nm, see B.5.2 step d);

This calibration constant is used in subsequent calculations.

Using the measured absorbance values from B.5.2, calculate a differential absorbance of the copper chloride solution in the test cuvette and correct it for temperature as shown in Formula (B.4).

$$A_{\mathrm{C}} = (A_{\mathrm{C730}} - A_{\mathrm{C520}})\left[1 - K_{\mathrm{temp,C}}(t_{\mathrm{M}} - t_{\mathrm{Std}})\right] \tag{B.4}$$

where

A_{C}	is the temperature-corrected differential absorbance of the copper chloride solution in the test cuvette;
$K_{\mathrm{temp,C}}$	is the temperature coefficient of absorptivity of copper chloride solution (0,001 65 $°C^{-1}$);
t_{M}	is the temperature of the mixed chromophore solution in the cuvette;
t_{Std}	is the temperature of the calibrator solution.

The measured absorbance values $A_{\mathrm{M520,i}}$ from each test measurement [B.5.2 step h)] shall be corrected in a similar way, calculating the differential absorbance at 520 nm according to Formula (B.5).

$$A_{\mathrm{M}}(i) = (A_{\mathrm{M520}}(i) - A_{\mathrm{C520}})\left[1 - K_{\mathrm{temp,P}}(t_{\mathrm{M}}(i) - t_{\mathrm{Std}})\right] \tag{B.5}$$

where

$A_M(i)$ is the temperature-corrected differential absorbance at 520 nm of the mixture in the cuvette after the *i*-th delivery of Ponceau S test solution;

$A_{M520}(i)$ is the absorbance at 520 nm of the mixture in the cuvette after the *i*-th delivery of Ponceau S test solution;

$K_{temp,P}$ is the temperature coefficient of absorptivity of Ponceau S solution (0,000 5 °C^{-1});

$t_M(i)$ is the temperature of the mixed chromophore solution in the cuvette after the *i*-th delivery of Ponceau S test solution.

Calculate the total volume of delivered test solution after each delivery according to Formula (B.6) until the desired number of replicates have been delivered ($i = n$):

$$V_T(i) = V_{C0} \frac{\frac{A_M(i)}{A_C}}{K_j - \frac{A_M(i)}{A_C}} \tag{B.6}$$

where

$V_T(i)$ is the total volume of test solution which has been added to the test cuvette from the first delivery through the *i*-th delivery;

V_{C0} is the volume of copper chloride solution in the prepared test cuvette at the start of the test;

NOTE Formula (B.6) is derived from the Beer-Lambert Law. This formula is discussed in ISO/TR 16153.

Calculate the volume of the individual deliveries according to Formula (B.7):

$$V(i) = V_T(i) - V_T(i-1) \tag{B.7}$$

where $V(i)$ is the *i*-th delivered volume of Ponceau S test solution.

B.6.2 Correction for temperature

If the temperature during the test is different from the temperature of adjustment for the POVA under test (which is 20 °C or 27 °C, see ISO 8655-2, ISO 8655-3, ISO 8655-4, ISO 8655-5 and ISO 8655-9), and if the cubic thermal expansion coefficient γ of the POVA is known, delivered volumes calculated by Formula (B.6) may be corrected by Formula (B.8):

$$V_{tc}(i) = V(i) \times \left[1 - \gamma \times (t_{test} - t_{adj})\right] \tag{B.8}$$

where

$V_{tc}(i)$ is the temperature-corrected test liquid volume of the *i*-th dispense of test liquid;

$V(i)$ is the calculated volume of the *i*-th dispense of test liquid according to Formula (B.7);

γ is the cubic thermal expansion coefficient of the POVA under test;

t_{test} is the temperature at which the test was performed;

t_{adj} is the temperature of adjustment of the POVA.

The effect of barometric pressure on accuracy should be considered for air displacement pipettes. See ISO/TR 16153 for further details.

B.7 Evaluation

The measurement results shall be evaluated according to Clause 7 and, if applicable, 4.2.

Annex C
(normative)

Single dye photometric procedure

C.1 Outline

The test volume V_T of chromophore solution is delivered by the POVA under test into a cuvette, which contains a known volume of diluent with little or no absorbance at the wavelength at which the chromophore's absorbance is measured. The method is designed to be used with cuvettes of 10 mm optical pathlength in a spectrophotometer.

Suitable chromophores for this method are 4-nitrophenol, Orange G, Ponceau S, and Tartrazine. The procedure as described is suitable for test volumes from 2 µl to 200 µl, which are delivered by the POVA under test ("Ex").

C.2 Test equipment

C.2.1 General

All test equipment shall be chosen such that any required uncertainty of measurement can be achieved.

All requirements for the test equipment described in B.2 for the dual-dye ratiometric photometric procedure shall apply to this procedure as well, with the exception of the cuvettes (C.2.2) and filters (C.2.3) for the spectrophotometer.

C.2.2 Cuvette

Cuvettes of suitable optical clarity with 10 mm path length may be used. Accurate knowledge of the optical path length is critical and a single cuvette, or a matched set of high-quality cuvettes shall be used for best results.

C.2.3 Filters for single dye photometric methods

Depending on the chromophore used for this method, the bandpass filters listed in Table C.1 shall be used in the spectrophotometer.

Table C.1 — Bandpass filters for single-dye photometric methods

Chromophore	Bandpass filter #1	Bandpass filter #2
4-nitrophenol	405 nm	620 nm
Orange G	492 nm	620 nm
Ponceau S	520 nm	650 nm
Tartrazine	450 nm	620 nm

C.3 Reagents

C.3.1 General requirements

All components used in the preparation of reagent solutions shall be of at least 99 % analytical purity unless otherwise stated.

C.3.2 Water

Water used for chromophore solutions shall comply with Grade 1 in accordance with ISO 3696:1987.

C.3.3 Stability of solutions

If stock solutions are to be stored for any period of time, they shall be tested for chemical and absorbance stability. Preservatives may be added, if needed, to prevent microbiological growth. Such preservatives shall not alter the liquid properties or interfere with the absorbance properties of the solutions. All solutions shall be stored protected from light.

C.3.4 Reagents for Ponceau S procedure

C.3.4.1 Stock solutions

Ponceau S stock solutions are prepared by dissolving the chromophore in water. Table C.2 gives the approximate amount of Ponceau S to be dissolved in water to produce a solution with the indicated nominal absorbance per 10 mm pathlength. Following preparation, the stock solutions are filtered through 0,2 µm filters.

NOTE The purity of commercially available solid dyes can vary from lot to lot, and the amount of Ponceau S might need to be adjusted to yield the required nominal absorbances.

Table C.2 — Stock solutions for single-dye Ponceau S procedure

Stock solution No.	**Test volume** µl	**Nominal absorbance** per 10 mm pathlength	**Ponceau S dye** g/l
1	$50 \leq V \leq 200$	39	0,856
2	$10 \leq V < 50$	152	3,34
3	$2 \leq V < 10$	752	16,5

C.3.4.2 Diluent

Diluent can be a phthalate buffer solution prepared according to B.4.3.

C.3.4.3 Standard solutions

Prepare a standard solution for each concentration of stock solution. The absorbance of a standard should be below the upper end of the linearity range of the spectrophotometer. Standard solutions shall be tightly capped to prevent changes in concentration due to evaporation. Standard solutions may be freshly prepared for each calibration or testing session, avoiding the issue of degradation in storage.

Table C.3 gives the necessary dilutions to make standards. In Table C.3, standard solution No. 1 is produced by diluting stock solution No. 1 from Table C.2, and so forth.

Table C.3 — Standard solutions

Standard solution	**First dilution**		**Second dilution**		**Dilution ratio** *R*
No.	**Stock solution** ml	**Diluent** ml	**Dilution 1** ml	**Diluent** ml	
1	10	50	10	100	$1\,515 \times 10^{-2}$
2	20	300	20	300	$3\,906 \times 10^{-3}$
3	10	300	10	400	$7\,868 \times 10^{-4}$

The dilution ratio *R* is calculated according to Formula (B.2).

C.3.5 Reagents for 4-nitrophenol, Orange G, and Tartrazine

When using 4-nitrophenol, Orange G, or Tartrazine as chromophores in this procedure, prepare stock solutions and standard solutions following the procedure given in C.3.4. Stock solutions of these chromophores should have nominal absorbances as close to those listed in Table C.2 for Ponceau S.

C.4 Test procedure

C.4.1 General

The following procedure and absorbance measurements are based on using Ponceau S as chromophore. When using either 4-nitrophenol or Tartrazine as chromophore, measure at the absorbance wavelengths given in Table C.1.

C.4.2 System linearity

The combined linearity of the spectrophotometer and chromophore system shall be validated. Follow B.5.1.

C.4.3 Steps to test POVA

a) Accurately fill cuvettes with a known amount of diluent solution and cap the cuvettes tightly. If more than one cuvette is used, the cuvette-to-cuvette reproducibility of results shall be tested and included in the uncertainty of measurement, if necessary.

b) Zero the spectrophotometer with a cuvette containing diluent at 650 nm.

c) Measure the absorbance A_{Std520} at 520 nm of the Standard Solution (Table C.3) corresponding to the stock solution to be used as test liquid.

d) Place a cuvette with a known amount of diluent V_{Dil} in the spectrophotometer.

e) With the POVA under test, deliver the test volume of the correct stock solution into the cuvette. Follow the correct dispense procedure in Clause 10. Each cuvette can accommodate one delivery of test liquid.

f) Mix the contents of the cuvette. For best results, mix the contents without removing the cuvette from the optical path of the spectrophotometer. In case the cuvette is removed for mixing of the contents, the uncertainty introduced by removing and replacing the cuvette in the optical path shall be determined and considered in the uncertainty budget.

g) Record the temperature of the contents in the cuvette and the ambient temperature.

h) Measure the absorbance A_{M520} at 520 nm and record it.

i) Repeat steps d) to h) until $n = 10$, or the desired number of replicate test volume deliveries have been measured.

C.5 Calculation of dispensed volume by single dye photometric method

C.5.1 General

Calculations in this clause are given for use of Ponceau S as chromophore. When using 4-nitrophenol or Tartrazine as chromophores, follow the same steps but substitute the measured absorbances at the respective wavelengths according to Table C.1.

C.5.2 Temperature correction

If the temperature difference between the standard solutions and the mixture of diluent and added Ponceau S stock solution in the cuvette is larger than 0,2 °C, absorbances shall be corrected for the temperature difference as described in Formula (C.1).

$$A_{\mathrm{M520}}(t_{\mathrm{M}}) = A_{\mathrm{M520}}\left[1 - K_{\mathrm{temp,P}}(t_{\mathrm{M}} - t_{\mathrm{Std}})\right] \tag{C.1}$$

where

$A_{\mathrm{M520}}(t_{\mathrm{M}})$	is the temperature corrected absorbance of the mixture of Ponceau S stock solution and cuvette solution measured at 520 nm;
A_{M520}	is the absorbance of the mixture of Ponceau S stock solution and cuvette solution measured at 520 nm;
t_{M}	is the temperature of the mixed chromophore solution in the cuvette;
t_{Std}	is the temperature of the Ponceau S standard solution;
$K_{\mathrm{temp,P}}$	is the temperature coefficient of absorptivity of Ponceau S solution (0,000 5 °C^{-1}).

If the test temperature is different from the temperature of adjustment for the POVA under test (which is 20 °C or 27 °C, see ISO 8655-2, ISO 8655-3, ISO 8655-4, ISO 8655-5 and ISO 8655-9), and if the cubic thermal expansion coefficient γ of the POVA is known, delivered volumes calculated by Formula (C.2) may be corrected by Formula (B.8).

C.5.3 Volume calculation

The individual unknown volumes of test solution V_{T} dispensed with the POVA under test are calculated using Formula (C.2) derived from the Beer-Lambert law:

$$V_{\mathrm{T}} = V_{\mathrm{Dil}}\left(\frac{A_{\mathrm{M520}}R}{A_{\mathrm{Std520}} - A_{\mathrm{M520}}R}\right) \tag{C.2}$$

where

V_{T}	is the test volume of the delivered test liquid (stock solution) by the POVA under test;
V_{Dil}	is the volume of diluent used in the cuvette;
A_{M520}	is the absorbance at 520 nm after the contents of the cuvette have been mixed;
A_{Std520}	is the absorbance at 520 nm of the standard solution, corresponding to the stock solution used to deliver V_{T};
R	is the dilution ratio of stock solution to diluent, that was used in creating the standard solution, see Table C.3 and Formula (B.2).

C.6 Evaluation

The measurement results shall be evaluated according to Clause 7 and, if applicable, 4.2.

Annex D

(normative)

Photometric/gravimetric hybrid procedure

D.1 Outline

The photometric/gravimetric hybrid test method is suitable to measure liquid volumes delivered from multi-channel POVA into microplates. It involves two measurement steps, which are carried out successively: the first step involves a gravimetric measurement, which is followed by a photometric analysis of the dispensed volumes in the microplate. This method is suitable for POVA that deliver the test volume (“Ex”).

Suitable chromophores for this method are 4-nitrophenol, Orange G, and Tartrazine. The procedure as described is suitable for test volumes from 1 µl to 100 µl.

D.2 Test equipment

D.2.1 General

All test equipment shall be chosen such that any required uncertainty of measurement can be achieved.

The microplate absorption reader and the analytical balance shall be calibrated at regular intervals according to their manufacturers’ instructions.

D.2.2 Labware

The following labware is necessary for performing the photometric/gravimetric hybrid procedure:

- Micro centrifuge tubes of 1,5 ml capacity;
- High quality, transparent, optically clear, flat-bottom 96-well or 384-well plates, non-treated, made from polystyrene (PS);
- Reagent reservoirs for multi-channel POVA of 100 ml and 300 ml capacity.

D.2.3 Microplate reader

The microplate absorbance reader shall be capable of measuring the absorbance at 620 nm and the wavelength corresponding to the chromophore used for the photometric measurement in 96-well or 384-well plates. Table D.1 lists chromophores, which may be used for absorbance measurements and the corresponding absorbance wavelengths.

Table D.1 — Suitable chromophores for absorbance measurements in microplates

Chromophore	4-nitrophenol	Tartrazine	Orange G
Wavelength	405 nm	450 nm	492 nm

The microplate reader shall meet the requirements given in Table D.2.

Table D.2 — Microplate reader performance requirements

Systematic absorbance error [a] 0 AU – 2 AU	< ± (1 % + 10 mAU)
Systematic absorbance error [a] 2 AU – 3 AU	< ±2,5 %
Systematic wavelength error [a]	≤ ±1,5 nm

[a] Some plate reader manufacturers refer to this error as "accuracy."

D.2.4 Microplate shaker

An orbital motion microplate shaker should be used, with the performance characteristics given in Table D.3.

Table D.3 — Performance characteristics for the orbital motion microplate shaker

Orbit	0,9 mm – 1,1 mm
Speed	1 600 r/min - 1 800 r/min
Timer range	20 s – 2 min

D.2.5 Balance

A precision analytical balance with readability and repeatability of 0,1 mg or better, and linearity of ± 0,2 mg shall be used. The balance shall be verified with suitable weights before measurements are made.

NOTE For verification of the precision balance ASTM Class 2[14] or OIML E2[15] test weights are available.

D.2.6 Measuring devices

The thermometer, hygrometer, barometer, and timing device used for the hybrid procedure shall conform to, or perform better than, the minimum requirements listed in Table D.4.

Table D.4 — Minimum requirements for measuring devices

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

D.3 Reagents

D.3.1 General requirements

The stock solutions for dispensing test solutions are prepared from water according to B.4.2 and the respective chromophore. All reagents used shall be of recognized analytical composition and of 95 % or better purity.

Co-solvents or surfactants may be used to better reflect specific applications (e. g. dimethyl sulfoxide, DMSO). The influence of solvents other than pure water, co-solvents, or other additives on the liquid properties such as density, surface tension, and viscosity shall be considered when converting mass to volume.

Solutions shall be free of any solid particles, and chromophore solutions shall be passed through a 0,2 µm filter after preparation.

D.3.2 Chromophore solutions

D.3.2.1 General

The concentration of the chromophore solution should be adjusted so that the absorbance is measured in the optimal dynamic range of the microplate reader (usually between 0,1 AU and 2,0 AU).

D.3.2.2, D.3.2.3, and D.3.2.4 provide examples of recommended chromophore solutions based on Tartrazine, 4-nitrophenol, and Orange G for measuring different test volumes in 96-well and 384-well microplates.

The shelf life of these solutions can be up to 6 months when stored in a tightly capped container at 20 °C and protected from direct light. If stock solutions are to be stored for any length of time, they shall be tested for chemical and absorbance stability. Preservatives may be added, if needed, to prevent microbiological growth. Such preservatives shall not alter the liquid properties or interfere with the absorbance properties of the solutions. All solutions shall be stored protected from light.

Reservoirs filled with chromophore solutions shall be kept covered during the tests to prevent evaporation. The cover should be removed only during the aspiration cycle.

D.3.2.2 Tartrazine test solutions

Tartrazine has a molar mass of 534,3 g/mol. Recommended nominal concentrations are 96 µg/ml for measurements in 96-well plates and 24 µg/ml for measurements in 384-well plates. Water is used as solvent for the pre-fill volume. Table D.5 gives values for common test volumes with Tartrazine test solutions. Other volumes may be tested by adjusting the values from Table D.5 accordingly.

Table D.5 — Test solutions with Tartrazine as chromophore

	96-well microplates with 125 µl final volume/well			**384-well microplates with 50 µl final volume/well**		
test volume µl	**prefill volume or diluent** µl/well	**tartrazine concentration**		**prefill volume or diluent** µl/well	**tartrazine concentration**	
		mg/ml	mM		µg/ml	mM
100	—	0,12	0,225			
50	75	0,24	0,449	—	0,06	0,112
30	95	0,4	0,749	20	0,1	0,187
10	115	1,2	2,25	40	0,24	0,449
5	120	2,4	4,49	45	0,6	1,12
1	124	12	22,5	49	2,4	4,49

D.3.2.3 4-Nitrophenol test solutions

The molar mass of 4-nitrophenol is 139,1 g/mol. When using 4-nitrophenol as chromophore, 0,1 N NaOH is recommended as solvent and diluent to generate the optimal pH value of pH > 9,2 for the photometric measurement. The recommended nominal concentration is 16,7 mg/ml (0,12 mM) for measurements in 96 well plates and 384 well plates. The solvent for the pre-fill volume is 0,1 N NaOH. Table D.6 gives values for common test volumes with 4-nitrophenol test solutions. Other volumes may be tested by adjusting the values from Table D.6 accordingly.

Table D.6 — Test solutions with 4-nitrophenol as chromophore

	96-well microplates with 200 µl final volume/well			384-well microplates with 50 µl final volume/well		
test volume µl	prefill volume or diluent µl/well	4-nitrophenol concentration		prefill volume or diluent µl/well	4-nitrophenol concentration	
		mg/ml	mM		mg/ml	mM
100	100	0,033 4	0,24			
50	150	0,066 8	0,48	—	0,016 7	0,12
30	170	0,111	0,8	20	0,027 8	0,20
10	190	0,334	2,40	40	0,083 5	0,60
5	195	0,668	4,80	45	0,167	1,20
1	199	3,34	24,00	49	0,835	6,00

D.3.2.4 Orange G test solutions

Orange G has a molar mass of 452,36 g/mol. Recommended nominal concentrations are 55 µg/ml for measurements in 96 well plates and 11 µg/ml for measurements in 384 well plates. When Orange G is used as chromophore, a buffer solution of 17,8 g/l disodium hydrogen phosphate dihydrate and 1 ml/l of 1 M sodium hydroxide solution is recommended as solvent and diluent to generate the optimal pH value of 7 for the photometric measurement. The pH value shall be checked and recorded before each measurement. Water is used as solvent for the pre-fill volume. Table D.7 gives values for common test volumes with Orange G test solutions. Other volumes may be tested by adjusting the values from Table D.7 accordingly.

Table D.7 — Test solutions with Orange G as chromophore

	96-well microplates with 200 µl final volume/well			384-well microplates with 75 µl final volume/well		
test volume µl	prefill volume or diluent µl /well	Orange G concentration		prefill volume or diluent µl /well	Orange G concentration	
		mg/ml	mM		mg/ml	mM
100	100	0,11	0,243			
50	150	0,22	0,486	25	0,044	0,097 2
30	170	0,367	0,81	45	0,073	0,162
10	190	1,1	2,43	65	0,22	0,486
5	195	2,2	4,86	70	0,44	0,973
1	199	11	24,3	74	2,2	4,86

D.4 Test procedure to calibrate multichannel POVA

D.4.1 General

The volume of either a single channel, or the sum of all channels, is measured gravimetrically, which serves as reference for subsequent photometric measurements of individual channels.

When aspirating test solutions or pre-fill solutions, ensure that all tips of the multi-channel POVA are immersed to an equal depth below the liquid surface during the aspiration process (see Table 2 for recommended immersion depths when using air-displacement pipettes).

The final volume per well should be in the range of 100 µl to 250 µl for 96 well plates, and 40 µl to 80 µl for 384 well plates. Lower volumes can lead to higher measurement uncertainty.

D.4.2 Test environment

All measurements shall be performed under the environmental conditions specified in 6.3.

D.4.3 Evaporation

During method validation it shall be determined whether measurement results need to be corrected for evaporation. If evaporation needs to be taken into account, the effect shall be determined experimentally, and the measurement results corrected mathematically. The error due to evaporation shall also be reflected in the determination of the measurement uncertainty, when an uncertainty budget is prepared.

The error due to evaporation may be determined with a pre-filled microplate, which is subjected to the same timing and movements as a microplate with test solution during the actual volume measurement. This includes time periods while the plate is covered and uncovered, and while it is handled on the bench, inside and outside of the balance, and the plate reader.

D.4.4 System linearity

The combined linearity of the microplate reader and chromophore solution shall be validated. Within the absorbance range from 0,1 AU to 2,0 AU, the measured absorbance shall be proportional to the test volume. The optimum chromophore concentrations given in Tables D.5, D.6 and D.7 can thus be used to cover a volume range of 30 % to 150 % of each nominal test volume listed in these tables.

D.4.5 Gravimetric measurement step

D.4.5.1 Whole plate approach

This test shall be performed for the test volumes.

a) Tare the empty balance.

b) Weigh and record the tare balance indication (m_E) of the microplate with lid:

   1) For test volumes > 25 µl, weigh the empty microplate with lid;

   2) For test volumes ≤ 25 µl, weigh the microplate with pre-filled solvent volumes and its lid.

c) Aspirate the test liquid from the reservoir and deliver the test volume into the empty or pre-filled test plate, and immediately place the lid on the test plate.

d) Start the timer.

e) Weigh the test plate immediately to avoid evaporation.

f) The time interval between dispensing and weighing the test plate shall not exceed 15 s, otherwise the error due to evaporation shall be corrected.

g) Record the gross balance indication (m_G) of the test plate in grams.

h) Record the tare balance indication of the plate with its lid before dispensing the second replicate into the plate.

i) Repeat steps c) to h) until the desired number (n) of replicate dispenses have been measured.

j) Record the time between dispense and reading the balance indication for each dispense.

k) Calculate the mean balance indication for the volume dispensed per well ($\overline{m}$) for the test plate according to Formula (D.1):

$$\bar{m} = \frac{m_G - m_E}{N} \tag{D.1}$$

where

$\bar{m}$	is the mean dispensed balance indication per well;
m_G	is the gross balance indication of the plate with the lid after the dispense of test solution;
m_E	is the average balance indication of the empty plate with the lid before the dispense of test solution;
N	is the number of wells, which have been filled with test solution, e.g. $N = 8$ for a dispense with an 8-channel device;

Convert the mean dispensed mass($\bar{m}$) to the mean volume dispensed per well ($\bar{V}$) for the test plate according to Formula (F.1), or according to Formula (F.4) if using the Z conversion factor given in Table F.1.

D.4.5.2 Single channel approach

The single channel gravimetric measurement is usually performed on a corner channel for ease of handling. This channel will be considered the reference channel for all further measurements in this approach.

The micro centrifuge tubes shall be handled only with gloves to avoid the transfer, and weighing, of skin oils, as well as body warmth, which can increase the rate of evaporation.

a) Place a micro centrifuge tube on the balance and tare the balance.

b) Pre-wet the reference channel five times.

c) Aspirate test liquid from the reservoir and dispense the test volume into the micro centrifuge tube, which has been tared in step a), and close the tube's lid immediately after dispense, to avoid evaporation.

d) Place the micro centrifuge tube on the balance. Record the balance indication to tare the micro centrifuge tube for the next delivery, but do not count this first measurement towards the five replicate measurements.

e) Using the same tip, aspirate the test volume again from the reservoir.

f) Dispense into the micro centrifuge tube, touching the tip against the inside wall, and close the tube immediately after the dispense.

g) Weigh and record the balance indication in mg.

h) Repeat steps e) to g) until the desired number (n) of replicate dispenses have been measured.

i) The values obtained by balance readings are in milligrams. Convert each balance reading obtained in step g) to volume in µl according to Formula (F.1), or according to Formula (F.4) if using the Z conversion factor given in Table F.1.

j) Calculate the mean volume $\bar{V}_T$ at the test temperature as shown in Formula (D.2):

$$\bar{V}_T = \frac{1}{n}\sum_{i=1}^{n} V(i) \tag{D.2}$$

where

$\overline{V}_T$ is the mean test volume of n delivered volumes;

$V(i)$ is the delivered volume.

D.4.6 Photometric measurement step

D.4.6.1 General

After gravimetric testing, the photometric test shall be performed.

A reference measurement is performed at 620 nm for each plate, either empty or with the pre-filled volume of solvent, if applicable. The absorbances from the reference measurement are subtracted from the absorbance readings at the chromophore specific wavelength (450 nm for Tartrazine, 405 nm for 4-nitrophenol, 492 nm for Orange G) to eliminate the background absorbance and to compensate for reader and micro plate tolerances.

D.4.6.2 Conversion plate

A conversion plate needs to be created to correlate the absorbance as measured by the photometer to the delivered test liquid volume in microlitres:

a) Prefill volume (if needed): Pre-wet tips at least five times with the pre-fill volume. Aspirate specified volume of distilled water (or solvent of the chromophore solution).

b) Fill at least 8 wells per plate with the pre-fill volume. Be sure to cover the plate immediately to prevent evaporation.

c) If working with disposable tips: mount new tips to the pipetting head and pre-wet the tips five times with the test volume of the chromophore solution appropriate for the test volume.

d) Aspirate the test volume from the reservoir with chromophore solution and dispense into the wells of the micro plate.

e) Dispense to the entire plate in the case of the whole plate approach.

f) Using the reference channel in the case of the single channel approach, dispense 8 replicates into the wells containing the pre-fill volume of solvent (see D.4.6.2 a)).

g) Add the specified amount of diluent solvent (see Tables D.5, D.6, and D.7), in case it was not provided as pre-fill volume. Immediately cover the wells, which have been filled.

h) Place the microplate on the shaker, remove the lid, and shake it at 1 600 r/min to 1 800 r/min for 2 min. Confirm that no air bubbles are present in the wells.

i) Read the microplate with the plate reader to obtain the absorbance readings.

j) Subtract the reference readings at 620 nm from the absorbance readings. This step is sometimes performed automatically by the plate reader.

k) Calculate the mean absorbance according to Formula (D.3), where $N = 8$ in the case of the single channel approach, or $N = 96$ for a 96-well plate, or $N = 384$ for a 384-well plate in the case of the whole plate approach:

$$\overline{A} = \frac{1}{N}\sum_{i=1}^{N} A(i) \tag{D.3}$$

where

$\overline{A}$ is the mean absorbance,

$A(i)$ is the absorbance per well.

Calculate the conversion factor C according to Formula (D.4), using the mean volume from either the single channel or the whole plate gravimetric measurement.

$$C = \frac{\overline{V}}{\overline{A}} \tag{D.4}$$

where C is the conversion factor correlating gravimetric and photometric measurements.

D.4.6.3 Test plate

Dispense all plates to be measured successively:

a) Prefill volume (if needed):

   1) Pre-wet tips five times with the pre-fill volume.

   2) Aspirate the specified volume of dilluent from the reservoir and dispense the pre-fill volume into each well of the plate. Be sure to keep the plate covered to prevent evaporation.

b) If working with disposable tips: mount new tips to the pipetting head and pre-wet the tips five times with the test volume.

c) Aspirate and dispense the test volume into each well of the microplate, and immediately cover the wells, which have been filled.

d) Add the specified amount of diluent solvent (see Tables D.5, D.6, and D.7), in case it was not provided as pre-fill volume. Immediately cover the wells again.

e) Place the microplate on the shaker, remove the lid, and shake it at 1 600 r/min to 1 800 r/min for 2 min. Confirm that no air bubbles are present in the wells.

f) Read the absorbance of each well.

g) Subtract reference readings at 620 nm from the absorbance readings to obtain the corrected absorbance $A(i)$ of each well (i = 1 to 96 for 96-well plates, and i = 1 to 384 for 384-well plates). This step is sometimes performed automatically by the plate reader.

D.5 Calculation of dispensed volume

Using the conversion factor C, convert the absorbance readings obtained in D.4.6.3 step g) to volumes according to Formula (D.5).

$$V(i) = C \cdot A(i) \tag{D.5}$$

where $V(i)$ is the volume measured in an individual well i (i = 1 to 96 for 96 well plates, and i = 1 to 384 for 384 well plates).

D.6 Evaluation

The measurement results shall be evaluated according to Clause 7 and, if applicable, 4.2.

Annex E
(normative)

Titrimetric procedure

E.1 Outline

This method is suitable for test volumes of 500 µl and larger, which are delivered by the POVA under test (“Ex”). A sodium chloride solution of known concentration is prepared and dispensed by the POVA under test (see E.3.2.1, Method A). This solution is dispensed without any further diluting steps. Nitric acid or sulfuric acid is added to acidify the solution. Water is added so that the electrode remains covered by water while being stirred.

The titrator performs the titration in a dynamic manner; this means the titration steps are small in the area of the inflection point. The titration procedure shall take at least 3 min to 5 min to complete. It is possible to add a chloride-free surfactant for better precipitation and to maintain a cleaner electrode. The titrator calculates the inflection point and can calculate the “unknown” volume, which was dispensed by the piston-operated volumetric apparatus under test.

It is also possible that the POVA to be tested is part of the titration device, e.g. as a piston burette (see E.3.2.2, Method B). In this case, the titration solution ($AgNO_3$ solution) is delivered by the POVA to be tested, and the solution of the reference material (NaCl) or a precisely weighed amount of the reference material is presented. The amount of reference material is chosen so that the consumption of titrant at the equivalence point is within the range of the volume to be tested. The concentration of the titrant shall be known exactly, and the calculation shall be adapted accordingly.

E.2 Test equipment

E.2.1 General

All test equipment shall be chosen such that any required uncertainty of measurement can be achieved.

E.2.2 Titrator

The titrator shall be able to:

- perform dynamic titrations,
- control drift titrations,
- calculate an equivalence point,
- show or print the result,
- include a titration tip with minimal back-diffusion.

The burette cylinder volumes for the titration shall be selected so that refilling of the burette cylinder during the titration in the area of the inflection point can be avoided. Table E.1 lists recommended burette cylinder volumes depending on the equivalence point.

Table E.1 — Burette cylinder volumes

Volume for equivalence point ml	Recommended cylinder volume ml
0,5 to 3	5
3 to 8	10
8 to 18	20
18 to 48	50

The titration method shall be completely validated to ensure correct results.

E.2.3 Electrode

The electrode shall be a silver indication electrode and a reference electrode. The silver electrode can have a coating of AgCl. The reference electrode can be a glass or Ag/AgCl electrode. If an Ag/AgCl reference electrode is used, it shall be filled with a solution of KNO_3 (typically 2 mol/l). The reference electrolyte can contain trace amounts of KCl (maximum 0,001 mol/l).

NOTE 1 The AgCl coating provides a more stable electrode potential and a shorter response time.

NOTE 2 Usually, combined electrodes, an indication electrode and a reference electrode in one probe, are used.

E.2.4 Volumetric glassware, Class A

Known volumes of diluent or reagent solutions shall be prepared by volumetric or gravimetric means. In the case of gravimetric preparation, the densities of the solutions shall be known and properly accounted for.

NOTE Specifications for one-mark volumetric pipettes can be found in ISO 648[11] and ASTM E969[12], and for one-mark volumetric flasks in ISO 1042 and ASTM E288[13].

E.3 Reagents

E.3.1 Test solutions

The sodium chloride test solutions shall be prepared as follows: For a solution of 0,1 mol/l NaCl, dissolve 5,844 2 g of NaCl in approximately 800 ml of water and make up to 1 l. In the case of a different amount of NaCl, the concentration of the solution shall be calculated according to Formula (E.1):

$$c(\text{NaCl}) = (\text{mass of NaCl, in grams}) / (58{,}442\ \text{g mol}^{-1} \times \text{volume in litres}) \qquad \text{(E.1)}$$

Table E.2 describes all solutions for performing the titrimetric method.

Table E.2 — Solutions for the titrimetric test method

Test volume	Test liquid	Receiver liquid for test liquid [a]		Titration solution
	$c(\text{NaCl})$	H_2O	$c(HNO_3)$ = 1 mol/l	$c(AgNO_3)$
ml	mol/l	ml	ml	mol/l
0,5 to 1	1	100	2	0,1
> 1 to 10	0,1	100	2	0,1
> 10 to 200	0,01	100	2	0,1

[a] See E.4.2.

E.3.2 Titration solution

E.3.2.1 Method A

The titration reagent is $AgNO_3$ solution, with a concentration of $c(AgNO_3)$ = 0,1 mol/l.

The concentration shall be checked by a titre determination with a solution of known concentration. This titre determination may be done either with the test solution or with a certified reference material. For this titre determination, three or more titrations shall be made. Volumes of test solutions should be chosen so that more than 50 % of the burette's nominal volume is delivered to minimize the random error due to the burette used. The standard deviation of these titrations shall be smaller than 0,25 %.

The solution shall be stored protected from light.

E.3.2.2 Method B

The titration reagent is $AgNO_3$ solution, with a concentration of $c(AgNO_3)$ = 0,1 mol/l.

The titre determination shall be performed with a separate, validated system or by using a certified reference solution. The concentration shall be checked by a titre determination with a certified reference material if no certified standard solution is used. For this titre determination, 3 to 10 titrations shall be made. Volumes of test solutions should be chosen so that more than 50 % of the burette's nominal volume is delivered to minimize the random error due to the burette used. The standard deviation of these titrations shall be smaller than 0,25 %.

The solution shall be stored protected from light.

E.3.3 Auxiliary solution

Prepare the auxiliary solution by dissolving polyvinyl alcohol (PVA) in deionized water to yield a 0,5 % (mass fraction) PVA solution.

NOTE A chloride-free surfactant will improve the titration accuracy, leading to cleaner electrodes and smaller precipitate particle size.

E.4 Test procedure

E.4.1 System linearity

The linearity of the titration system shall be ensured. The entire titration procedure shall be validated before use in order to ensure correct results. Follow the instructions provided by the manufacturer of the titration system.

E.4.2 Titration

With the POVA under test, dispense the test volume of sodium chloride solution into the titration vessel. Add 2 ml of 1 M nitric acid (HNO_3) or 0,5 M sulfuric acid (H_2SO_4) and make up to about 100 ml with deionized water. Add 1 ml to 2 ml of auxiliary solution (0,5 % PVA).

The titration tip and the electrode shall be immersed deep enough into the solution so that the diaphragm of the reference electrode and the titration tip remain covered while stirring.

After careful mixing, titrate with 0,1 M $AgNO_3$ solution, observing the manufacturer's instructions for the titration equipment.

E.4.3 Titration result

The equivalence point (E) of the titration is reached when an equal amount of silver ions has been added to the provided chloride ions in the solution. It is represented as the inflection point of the S-type titration curve and can be calculated from the first or second derivative of the titration curve. A smooth titration curve without noise is important for the clear determination of the equivalence point.

NOTE 1 Calculation of the equivalence point is often performed automatically by the titrator.

NOTE 2 As the total potential is not necessary for the calculation of the titration result, it is not important to always have the same start potential or end potential.

E.5 Calculation of dispensed volume by titrimetric method

The test volume (V_T) dispensed by the POVA under test shall be calculated according to Formula (E.2):

$$V_T = E\left(\frac{c(\mathrm{AgNO_3})}{c(\mathrm{NaCl})}\right) \tag{E.2}$$

where

V_T is the volume of delivered test liquid by the POVA under test, in ml;

E is the equivalence point, in ml;

$c(\mathrm{AgNO_3})$ is the concentration of $\mathrm{AgNO_3}$, in mol/l;

$c(\mathrm{NaCl})$ is the concentration of NaCl, in mol/l.

E.6 Evaluation

The measurement results shall be evaluated according to Clause 7 and, if applicable, 4.2.

Annex F

(normative)

Conversion of liquid mass to volume

F.1 Calculation of volume from the balance reading of liquid

For the conversion of the balance reading m to volume V at the test temperature, a correction for the liquid's density and air buoyancy is necessary. The calculation of the liquid volume at the test temperature is given by Formula (F.1).

$$V_L = (m_L - m_E + m_{evap}) \times \frac{1}{\rho_L - \rho_A} \times \left(1 - \frac{\rho_A}{\rho_B}\right) \tag{F.1}$$

where

V_L is the calculated volume at the test temperature, in ml;

m_L is the balance reading of the weighing vessel after liquid delivery, in g;

m_E is the balance reading of the weighing vessel before liquid delivery, in g (m_E = 0 in case the balance was tared with the weighing vessel);

m_{evap} is the mass lost to evaporation, see Formula (A.1);

ρ_A is the density of air, in g/ml, see Formula (F.2);

ρ_B is the actual or assumed density of the weights used to calibrate the balance, in g/ml;

ρ_L is the density of the liquid at the test temperature , in g/ml;

For water, ρ_L can be calculated with the "Tanaka" Formula (F.3), the density of other test liquids should be determined with sufficient accuracy.

NOTE 1 Stainless steel weights of density 8,0 g/ml are typically used for balance calibration.

Formula (F.2) for the air density ρ_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 %.

$$\rho_A = \frac{1}{1\ 000} \times \frac{0{,}348\ 48 \times p - 0{,}009 \times h_r \times e^{(0{,}061 \times t)}}{t + 273{,}15} \tag{F.2}$$

where

ρ_A is the air density, in mg/ml;

t is the ambient temperature, in °C;

p is the barometric pressure, in hPa;

h_r is the relative air humidity, in %.

The uncertainty of this formula can be calculated according to OIML R 111-1:2004[15], section C.6.3.6.

Under other environmental conditions, Formula (F.2) shall be replaced with the current air density calculations.

NOTE 2 At the time of publication of this document, the current CIPM air density calculations are described in Reference [16].

The density of pure water is normally provided from formulae given in the literature. Formula (F.3) by Tanaka[17] provides a good basis for standardization:

$$\rho_W = a_5 \times \left[1 - \frac{(t_W + a_1)^2 \times (t_W + a_2)}{a_3 \times (t_W + a_4)} \right] \tag{F.3}$$

where

ρ_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)^2;

a_4 = 69,348 81 °C;

a_5 = 0,999 974 950 g/ml.

F.2 Correction factors for the conversion of water balance readings to volume

Another possible procedure for conversion of the balance reading to volume is using the Z correction factors specified in Table F.1.

Convert each balance reading $m(i)$ obtained to volume by applying the Z correction factors at the mean temperature and barometric pressure measured during the measurement procedure, using Formula (F.4):

$$V(i) = m(i) \times Z \tag{F.4}$$

where

$V(i)$ is the delivered test volume, in µl;

$m(i)$ is the balance reading of the delivered test volume, in mg;

Z is the Z correction factor from Table F.1, in µl/mg.

Table F.1 — *Z* correction factors for distilled water as a function of test 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

Bibliography

[1] ISO/TR 16153, *Piston-operated volumetric instruments — Determination of uncertainty for volume measurements made using the photometric method*

[2] ISO/TR 20461, *Determination of uncertainty for volume measurements made using the gravimetric method*

[3] ISO 23783-2:—, *Automated liquid handling systems – Part 2: Measurement procedures for the determination of volumetric performance*

[4] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

[5] ISO 9001, *Quality management systems — Requirements*

[6] ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes*

[7] ASTM E1154-14, *Standard specification for piston or plunger operated volumetric apparatus*

[8] DKD-R 8-1, *Calibration of piston-operated pipettes with air cushion,* German Calibration Service, Physikalisch-Technische Bundesanstalt, Braunschweig, Germany.

[9] EURAMET cg-18, Version 4.0, *Guidelines on the calibration of non-automatic weighing instruments,* European Association of National Metrology Institutes, Braunschweig, Germany

[10] ASTM E898-20, *Standard Practice for Calibration of Non-Automatic Weighing Instruments*

[11] ISO 648, *Laboratory glassware — Single-volume pipettes*

[12] ASTM E969-02, *Standard Specification for Glass Volumetric (Transfer) Pipets*

[13] ASTM E288-17, *Standard Specification for Laboratory Glass Volumetric Flasks*

[14] ASTM E617-18, *Standard Specification for Laboratory Weights and Precision Mass Standards*

[15] OIML R 111-1:2004, *Weights of classes* E_1, E_2, F_1, F_2, M_1, M_{1-2}, M_2, M_{2-3}, *and* M_3. International Organization of Legal Metrology, Paris, France.

[16] PICARD A. et al., CIPM-2007, *Revised formula for the density of moist air*, Metrologia, 2008, **45**, 149-155

[17] TANAKA M. et al., Recommended table for the density of water between 0 °C and 40 °C based on recent experimental reports, Metrologia, 2001, **38**, 301-309

(*Continued from second cover*)

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 2859-1 : 1999 Sampling procedures for inspection by attributes — Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection	IS 2500 (Part 1) : 2000 Sampling procedures for inspection by attributes: Part 1 Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection (*third revision*)	Identical with ISO 2859-1 : 1999
ISO 3696 : 1987 Water for analytical laboratory use — Specification and test methods	IS 1070 : 1992 Reagent grade water —Specification (*third revision*)	Not Equivalent
ISO 8655-2 Piston-operated volumetric apparatus — Part 2: Pipettes	IS 17094 (Part 2) : 2019 Piston — Operated volumetric apparatus: Part 2 Piston pipettes	Identical with ISO 8655-2 : 2002

The technical committee responsible for the preparation of this standard has reviewed the provisions of the below mentioned ISO/IEC standards and has decided that they are acceptable for use in conjunction with this standard

International Standard	*Title*
ISO 3951-1	Sampling procedures for inspection by variables — Part 1: Specification for single sampling plans indexed by acceptance quality limit (AQL) for lot-by-lot inspection for a single quality characteristic and a single AQL
ISO 8655-1	Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations
ISO 8655-3 : 2021	Piston-operated volumetric apparatus — Part 3: Burettes
ISO 8655-4 : 2021	Piston-operated volumetric apparatus — Part 4: Dilutors
ISO 8655-5 : 2021	Piston-operated volumetric apparatus — Part 5: Dispensers
ISO 8655-6	Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume
ISO 8655-8	Piston-operated volumetric apparatus — Part 8: Photometric reference measurement procedure for the determination of volume
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)

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’.

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Review of Indian Standards

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Amendments Issued Since Publication

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