INTERNATIONAL STANDARD

ISO 8655-7

Second edition
2022-04

Piston-operated volumetric apparatus —

Part 7: Alternative measurement procedures for the determination of volume

Appareils volumétriques à piston —

Partie 7: Modes opératoires de mesure alternatifs pour la détermination de volumes

Reference number
ISO 8655-7:2022(E)

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Published in Switzerland

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Foreword

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

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

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

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

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

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

This second edition cancels and replaces the first edition (ISO 8655-7:2005), which has been technically revised. It also incorporates the Technical Corrigendum ISO 8655-7:2005/Cor.1:2008.

The main changes are as follows:

— a gravimetric test method was added (see 8.2);

— a photometric/gravimetric hybrid test method was added (see 8.5);

— a batch testing method was added (see 8.7);

— measurement procedures for all methods are given in normative Annexes A to 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);

— requirements for operator qualification have been added (see 4.3);

— 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);

— Annexes A, B, and C of the first edition have been deleted and replaced.

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

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

Introduction

The ISO 8655 series addresses the needs of:

— manufacturers, as a basis for quality control including, where appropriate, the issuance of manufacturer's declarations;

— calibration laboratories, test houses, users of the equipment and other bodies as a basis for independent calibration, testing, verification, and routine tests.

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

Piston-operated volumetric apparatus —

Part 7: Alternative measurement procedures for the determination of volume

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 $V_T(i)$ (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;

$V_T(i)$ 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):

$$e_s = \overline{V} - V_s \tag{2}$$

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):

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

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):

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

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).

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

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;