
Volumetrične naprave, delujoče na bat - 7. del: Nadomestni merilni postopki za določanje prostornine (ISO/DIS 8655-7:2020)

Piston-operated volumetric apparatus - Part 7: Alternative measurement procedures for the determination of volume (ISO/DIS 8655-7:2020)

Volumenmessgeräte mit Hubkolben - Teil 7: Alternatives Prüfverfahren zur Bestimmung des Volumens (ISO/DIS 8655-7:2020)

Appareils volumétriques à piston - Partie 7: Méthodes non gravimétriques pour l'estimation de la performance d'équipement (ISO/DIS 8655-7:2020)

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Piston-operated volumetric apparatus —

Part 7: Alternative measurement procedures for the determination of volume

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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 on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 48, *Laboratory Equipment*, Working Group WG 04, *Piston-operated instruments*.

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

The main changes compared to the previous edition are as follows:

- a gravimetric test method was added ([Clause 9.2](#));
- a photometric/gravimetric hybrid test method was added ([Clause 9.5](#));
- a batch testing method was added ([Clause 9.7](#));
- measurement procedures for all methods are given in normative [Annexes A](#) to [E](#);
- standard dispense procedures for POVA described in ISO 8655-2, -3, -4, -5, and -9 were added ([Clause 10](#));
- requirements for operator qualification have been added ([Clause 4.2](#));
- requirements for testing of multi-channel POVA is described in more detail, with specific procedures given for these apparatus ([Clauses 5](#), [9.5](#), 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.

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Introduction

ISO 8655 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, certification, and routine checking.

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

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Piston-operated volumetric apparatus —

Part 7:

Alternative measurement procedures for the determination of volume

1 Scope

This part of ISO 8655 specifies alternative measurement procedures for the determination of volume of piston-operated volumetric apparatus.

The tests 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 part of ISO 8655 are suitable for various maximum nominal volumes of piston-operated volumetric apparatus. The user of this standard shall ensure that the selected method is suitable for its intended purpose.

NOTE General requirements and definitions of terms for piston-operated volumetric apparatus are given in ISO 8655-1. For the metrological requirements, maximum permissible errors, requirements for marking and information to be provided for users for piston-operated volumetric apparatus, see ISO 8655-2 for pipettes, see ISO 8655-3 for burettes, see ISO 8655-4 for dilutors, see ISO 8655-5 for dispensers, and see ISO 8655-9 for manually operated precision laboratory syringes. The gravimetric reference measurement procedure for the determination of volume is given in ISO 8655-6. The photometric reference measurement procedure for the determination of volume is given in ISO 8655-8.

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2 Normative references

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

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

ISO/DIS 8655-1:2020, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

ISO/DIS 8655-2, 2022, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

ISO/DIS 8655-3:2020, *Piston-operated volumetric apparatus — Part 3: Piston burettes*

ISO/DIS 8655-4:2020, *Piston-operated volumetric apparatus — Part 4: Dilutors*

ISO/DIS 8655-5:2020, *Piston-operated volumetric apparatus — Part 5: Dispensers*

ISO/DIS 8655-6:2020, *Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume*

ISO/DIS 8655-8:2020, *Piston-operated volumetric apparatus — Part 8: Photometric reference measurement procedure for the determination of volume*

ISO/DIS 8655-9:2020, *Piston-operated volumetric apparatus — Part 9: Manually operated precision laboratory syringes*

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

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

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

ISO/IEC Guide 98-3:2008, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*

ISO/IEC Guide 98-4:2012, *Uncertainty of measurement — Part 4: Role of measurement uncertainty in conformity assessment*

ISO/IEC Guide 99:2007, *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/DIS 8655-1:2020, ISO/IEC Guide 2, and ISO/IEC Guide 99 apply.

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

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

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.

[SOURCE: ISO 3951-5:2006, 3.6 – modified: shortened Note 2 to entry.]

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 International Standard are suitable to be used for metrological confirmation of POVA. For calibrations, the procedure of this International Standard shall be validated by comparison to one of the reference measurement procedures described in ISO 8655-6 or ISO 8655-8.

4.2 Operator qualification

An operator who uses, calibrates, or tests POVA shall be adequately trained on the use of the type of POVA under test. Operator training and competence should be documented.

NOTE It is worth considering the qualification of operators with previously calibrated pipettes.

5 Requirements for testing multi-channel POVA

Metrological confirmation of multi-channel POVA shall test the performance of each channel of a multi-channel POVA by calibration and routine tests on a regular basis. Not testing each channel can leave the user exposed to significant risk of error.

Test liquid should be aspirated simultaneously with all channels of a multi-channel POVA, as described in ISO 8655-2. If liquid is aspirated in one channel only, this fact shall be reported within the test report. Depending on the selected test method and available measurement equipment, it might be necessary to test each channel individually in successive tests.

NOTE 1 Using a test method based on volume measurements in a microplate allows for simultaneous dispensing of the test liquid from all channels into the microplate. [Annex D](#) provides a procedure for calibrating multi-channel pipettes.

NOTE 2 Consistent and even tip fitting is important and can impact performance.

NOTE 3 Channel to channel variation can be exaggerated by poor quality tips. Differences in dimensions will lead to inconsistent aspiration and delivery.

6 Performance requirements

6.1 Performance tolerances

Calibration and routine test results may be reported without comparison to performance tolerances. If the results are verified against performance tolerances, these tolerances shall be agreed upon with the user.

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 manufacturers tolerances, subject to them being fit for purpose.

6.2 Operator impact

Measurement of volumetric performance includes random and systematic errors of the POVA, as well as errors introduced by the device's operator. Performance tolerances for POVA shall include the operator error component as well, where applicable.

NOTE The performance of a hand-held pipette is inseparable from the performance of its operator.

7 Test conditions

7.1 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 and ISO/TR 20461.

7.2 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.

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

7.3 Test room, environmental conditions

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 ($\pm 0,5$ °C). All test equipment, POVA, exchangeable parts (e.g. pipette tips), and reagents used shall be equilibrated to the test room temperature.

The ambient temperature, relative humidity, and barometric pressure at the time of the test shall be recorded.

To evaluate a POVA's fitness for purpose, the test room conditions should reflect the environmental conditions under which the POVA is used. This can generally be achieved when a POVA is calibrated within the laboratory in which it is used.

NOTE 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.

7.4 Test volumes

7.4.1 Fixed volume POVA **iTeh STANDARD PREVIEW**

In the case of a fixed-volume POVA, the selected volume V_s is the nominal volume V_0 and is the only test volume.

7.4.2 Adjustable volume POVA oSIST prEN ISO 8655-7:2020 https://standards.iteh.ai/catalog/standards/sist/de613e86-c508-44d0-9b50-ac4046c4f64b/osist-pr-en-iso-8655-7-2020

- a) For calibrations, 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 leaves the user with increased 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.

7.5 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 ten measurements per volume are required by the reference measurement procedures of ISO 8655-6 and ISO 8655-8. For the test procedures of this International Standard, ten measurements are encouraged, 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.

The replicate volume measurements shall be used to calculate the systematic and the random errors of measurement in accordance with [Clause 8](#). The reported uncertainty shall be based on the number of replicates.

7.6 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 standard, 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 chromophores 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.

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8 Evaluation

8.1 Mean volume

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

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

where

\bar{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 .

8.2 Systematic error

Calculate the systematic error e_S of the piston-operated volumetric apparatus using [Formula \(2\)](#):

$$e_S = \bar{V} - V_S \quad (2)$$

where

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

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

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This systematic error may be expressed in percent using [Formula \(3\)](#):

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

where η_S is the relative systematic error, expressed in percent.

In the case of fixed volume POVA, the selected test volume V_S is the nominal volume V_0 and V_S can be replaced by V_0 .

8.3 Random error

Calculate the random error of the POVA as repeatability standard deviation s_r , using [Formula \(4\)](#):

$$s_r = \sqrt{\frac{\sum_{i=1}^n (V_{T,i} - \bar{V})^2}{n-1}} \quad (4)$$

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

This random error may also be expressed as a percentage by the coefficient of variation, CV , using [Formula \(5\)](#).

$$CV = \frac{s_r}{\bar{V}} \times 100\% \quad (5)$$

where CV is the coefficient of variation.

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9 Test methods

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9.1 General

This standard 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 [Annexes A](#) to [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
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

Table 1 (continued)

Chemical	CAS No.
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

9.2 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 of ISO 8655-6 but deviating from any of its requirements, Annex A.2 of this International Standard applies.

9.3 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.[4]

When following the photometric reference measurement procedure of ISO 8655-8 but deviating from any of its requirements, Annex B.2 of this International Standard applies.

9.4 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.

9.5 Hybrid photometric/gravimetric method for multichannel POVA

This method allows the evaluation of volumetric performance of multichannel POVA by combining a gravimetric measurement with subsequent photometric measurement. Test liquid containing either