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## Piston-operated volumetric apparatus —

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

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*Appareils volumétriques à piston —*

*Partie 6: Méthode gravimétrique de mesurage de référence pour la  
détermination du 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](http://www.iso.org/directives)).

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

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

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

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

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

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

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

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

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

## Introduction

The ISO 8655 series addresses the needs of:

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

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

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# Piston-operated volumetric apparatus —

## Part 6:

# Gravimetric reference measurement procedure for the determination of volume

## 1 Scope

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

## 2 Normative references

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

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

ISO 8655-1:—<sup>1)</sup>, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendation*

ISO 8655-2:—<sup>2)</sup>, *Piston-operated volumetric apparatus — Part 2: Pipettes*

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

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

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

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

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

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

## 3 Terms and definitions

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

ISO and IEC maintain terminological 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/>

1) Under preparation. Stage at the time of publication ISO/FDIS 8655-1:2021.

2) Under preparation. Stage at the time of publication ISO/FDIS 8655-2:2021.

## 4 General requirements

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

## 5 Test equipment

### 5.1 General

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

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

### 5.2 Balance

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

Table 1 — Minimum requirements for balances

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

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

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

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

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

<sup>e</sup> For single-channel pipettes of nominal volumes of less than 2  $\mu\text{l}$ , a balance with 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.



### 5.3 Liquid reservoir

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

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

### 5.4 Weighing vessel

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

### 5.5 Measuring devices

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

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

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

## 6 Test liquid

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

## 7 Test conditions

### 7.1 General

All equipment used to test the POVA shall be operated as specified in the operation manual.

### 7.2 Test room

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

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

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

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

### 7.3 Evaporation

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

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

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

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

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

### 7.4 Test cycle time

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

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

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

## 8 Procedure

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### 8.1 General

#### 8.1.1 Test volume

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

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

#### 8.1.2 Number of measurements

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

#### 8.1.3 Weighing procedure

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

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

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

#### 8.1.4 Test conditions during weighing procedure

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

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

#### 8.1.5 Dispensing of samples

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

### 8.2 Preparation

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

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

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

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

#### 8.3.1 General

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

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

Forward pipetting shall always be performed.

#### 8.3.2 Test cycle

Perform the test cycle as follows:

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