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Piston-operated volumetric apparatus —

Part 2: **Pipettes**

Appareils volumétriques à piston —
Partie 2: Pipettes

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Contents

Page

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 8655-2:2002), which has been technically revised. It also incorporates the Technical Corrigendum ISO 8655-2:2002/Cor.1:2008.

The main changes are as follows:

— ISO 8655-7 and ISO 8655-8 have been added as normative references;

— metrological performance requirements for pipette tips have been further specified;

— Tables 1 and 2 have been revised;

— a new Table 3 has been introduced;

— a new informative Annex B for motorised pipettes has been introduced;

— former Annex A has been added as new Clause 10.

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 2: **Pipettes**

1 Scope

This document specifies

— metrological requirements,

— maximum permissible errors,

— requirements for marking and

— information to be provided for users,

for air-displacement (type A) and positive displacement (type D) single-channel and multi-channel pipettes, complete with their selected tip(s) and any other essential, consumable parts, designed to deliver the selected volume (Ex).

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

ISO 8655-6:2022, *Piston-operated volumetric apparatus — Part 6: Gravimetric reference measurement procedure for the determination of volume*

ISO 8655-7:2022, *Piston operated volumetric apparatus — Part 7: Alternative measurement procedures for the determination of volume*

ISO 8655-8:2022, *Piston-operated volumetric apparatus — Part 8: Photometric reference measurement procedure for the determination of volume*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8655-1 apply.

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Principle of operation

Pipettes are used to accurately handle preselected volumes. A tip is attached to the pipette. Pipettes are typically operated using forward pipetting. Using forward pipetting, with the piston positioned at the lower aspiration limit, the tip is dipped into the liquid to be dispensed. When moved to the upper aspiration limit, the piston aspirates the liquid. The liquid volume to be dispensed is then expelled by depressing or sliding the piston between the volume-defining limits. Some air-displacement pipettes (see 6.1, Type A) have an extra air volume that can be used to expel the last drop of liquid.

See also Figure 1.

Manufacturers' instruction manuals should contain detailed and specific information about the proper operation of pipettes.

5 Adjustment

5.1 Basis of adjustment

A pipette shall be adjusted for the delivery (Ex) of its nominal volume (or selected volume, in the case of a variable-volume model).

For countries that have adopted the standard reference temperature of 20 °C, the adjustment shall be for the temperature of 20 °C, a relative air humidity of 50 % and a barometric pressure of 101,3 kPa, when handling grade 3 water as specified in ISO 3696:1987.

For those countries that have adopted a standard reference temperature of 27 °C, the adjustment shall be for the temperature 27 °C, a relative air humidity of 50 % and a barometric pressure of 101,3 kPa, when handling grade 3 water as specified in ISO 3696:1987.

5.2 Initial adjustment

A pipette shall be provided with an initial adjustment.

5.3 Subsequent adjustment

Some pipettes have provision for adjustment when, for example, it is found in a routine check that the volume delivered is not within specification. Such adjustment shall be made in accordance with the manufacturer's instructions and by reference to a measurement procedure in accordance with ISO 8655-6, ISO 8655-7 or ISO 8655-8.

Any pipette so adjusted shall have clear, visible evidence that the initial adjustment has been modified. This information shall also be recorded.

5.4 Adjustment for other liquid properties

Some pipettes are designed to have their factory pre-set adjustment altered by the user so that they will dispense their specified volume when used with liquids with physical properties differing from those of water (see Annex A for details). In such cases, the design shall prevent unintentional readjustment. Such adjustment shall be made in accordance with the manufacturer's instructions or by reference to the selected test procedure from ISO 8655-7 and the modifications made.

If the pipette is readjusted, it shall be clearly and unequivocally indicated on the outside of the pipette that readjustment has been affected. The outside of the pipette shall be marked with the name of the liquid and the adjusted volume range. This information shall be documented appropriately.

6 Design

6.1 Types of pipette

A pipette may be designed as follows:

— fixed volume, designed by the manufacturer to dispense only its nominal volume, e.g. 100 µl;

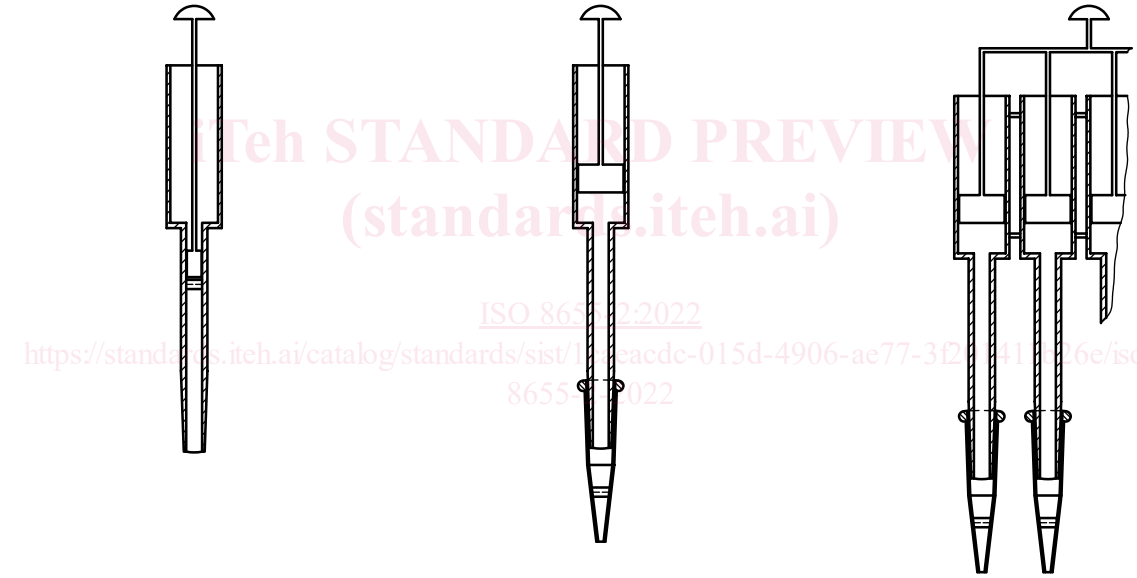
— variable volume, designed by the manufacturer to dispense volumes selectable by the user within its specified usable volume range, e.g. between 10 µl and 100 µl.

The piston may:

— either have a body of air contained between the piston and the surface of the liquid (air displacement – Type A); or

— be in direct contact with the surface of the liquid (positive or direct displacement – Type D).

In the case of the Type D pipette, either the plunger or the capillary, or both may be reusable (Type D1) or disposable (Type D2). See Figure 1 for details.



a) Single-channel pipettes **b) Multi-channel pipette**

Figure 1 — Pipettes

6.2 Transfer of hand warmth

The construction of pipettes and the materials used for their manufacture shall be chosen in such a way that any heat transmitted from the user's hand to the apparatus during periods of use is minimized.

NOTE Transfer of hand warmth will appear as a systematic drift of results (the delivered volume decreases over time) during a series of deliveries.

7 Pipette tips

7.1 General

7.1.1 The dispensing orifice of the tip shall be shaped in such a way that consistent dispensing of the liquid to be measured is achieved. When the tip is touched against the wall of a vessel in successive operations, any amount of liquid remaining in or around the dispensing orifice of the tip shall be consistent.

7.1.2 In the case of sterilisable pipette tips, the sterilisation procedures indicated as appropriate by the manufacturer in user information or on packaging (see Clause 10) shall not negatively affect the metrological characteristics of the tips such as shape, seal and wettability.

NOTE This requirement can be assessed by comparing errors of measurement using tips which have and have not been sterilised.

7.2 Air-displacement pipette tips

7.2.1 Air-displacement pipette tips shall be disposable parts, usually made of plastic, which fit on the tip cone of the pipette and prevent the instrument from contact with the aspirated liquid.

7.2.2 Tips for air-displacement shall be fitted in accordance with the pipette supplier's instructions to form a good seal between the tip and the tip cone of the pipette.

NOTE Variability of the amount of externally retained liquid or an incomplete seal will contribute to poor precision.

Pipette tips made of plastic for pipettes with air interface are designed for single use. They shall not be cleaned for reuse as their metrological characteristics will no longer be reliable.

Single use of a pipette tip means mounting the tip on the pipette only once, and then discarding it after use. While the tip is mounted on the pipette, it may be used to handle several replicate aspiration and delivery cycles, as long as a tight seal between the tip and pipette's tip cone is maintained.

7.2.3 The form of the pipette tips to be used with a multi-channel pipette shall be such that all tips fitted are positioned with parallel axes in the same plane in order to allow for even liquid dispensing in the target vessels, e.g. the adjacent wells of a microplate. The bottoms of properly fitted tips shall not vary in spacing from their nominal axes, nor from their common plane by more than ±0,5 mm for less than 100 µl, ± 1,0 mm for 100 µl up to 350 µl and ±1,5 mm for nominal volumes exceeding 350 µl (nominal volumes).

7.3 Positive-displacement pipette tips

7.3.1 Positive-displacement pipette tips shall consist of a plunger and a capillary which fit on the pipette. Various materials may be used for the plunger, such as metal or plastic, and the capillary, such as plastic or glass. These pipette tips may be reusable (D1) or disposable (D2).

7.3.2 The shape and material of the plunger and capillary shall confer a good seal of the tip, as well as a smooth action between the plunger and the capillary, to ensure consistent dispensing of the liquid.

7.3.3 Sterilisability shall be in accordance with 7.1.2.

8 Type, designation

Designation of a Type D1 single-channel pipette (positive displacement with reusable plunger) with a fixed volume of 100 µl:

Pipette ISO 8655 - D1-100

Designation of a Type D2 variable-volume single-channel pipette (positive displacement with disposable plunger/capillary), volume range variable from 20 µl to 200 µl:

Pipette ISO 8655 - D2 - 20-200

Designation of a Type A variable-volume single-channel pipette with air interface, volume range variable from 10 µl to 100 µl:

Pipette ISO 8655 - A - 10-100

Designation of an 8-channel pipette with air interface (A) and with a fixed volume of 200 µl:

Pipette ISO 8655 - A - 200 × 8

Designation of a 12-channel pipette with air interface (A), volume range variable from 20 µl to 200 µl:

Pipette ISO 8655 - A - 20-200 × 12

9 Metrological performance requirements

9.1 General

9.1.1 General. The metrological performance of POVA (especially pipettes of type A) can be affected in many ways. Annex A lists parameters which influence the metrological performance of pipettes and recommendations for their handling.

9.1.2 Reference test. In order to state the metrological trueness and precision of the POVA and thus determine its systematic and random errors, a reference measurement procedure as specified in ISO 8655-6 and ISO 8655-8 or a measurement procedure in accordance with ISO 8655-7 shall be used. The maximum permissible errors given in Tables 1, 2 and 3 shall apply.

9.1.3 Routine testing. Users shall establish routine testing of POVA in accordance with ISO 8655-1.

9.1.4 Further testing. In the case of electronic motorised pipettes, an additional set of measurements can be done using dispense mode. See Annex B for more information.

9.2 Fixed-volume pipettes of types A and D1

For air-displacement pipettes (Type A) with fixed volume and for positive-displacement pipettes with reusable plunger and capillary (Type D1) and with fixed volume, the maximum permissible errors given in Table 1 apply.

9.3 Fixed-volume pipettes of type D2

For positive-displacement pipettes with a disposable plunger and capillary (Type D2) and with fixed volume, the maximum permissible errors given in Table 3 apply.