INTERNATIONAL STANDARD

ISO 8655-2

> Second edition 2022-04

Piston-operated volumetric apparatus —

Part 2: **Pipettes**

Appareils volumétriques à piston — Partie 2: Pipettes





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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 8655-2:2002), 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;
- <u>Tables 1</u> and <u>2</u> have been revised;
- a new <u>Table 3</u> has been introduced;
- a new informative <u>Annex B</u> 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 <u>6.1</u>, 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

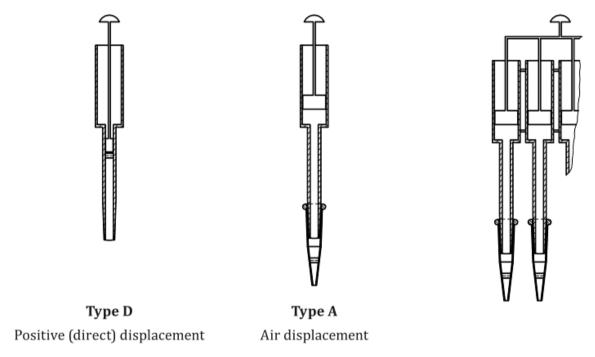
A pipette may be designed as follows:

- 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 $10 \mu l$ and $100 \mu l$.

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 <u>Figure 1</u> 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 <u>Clause 10</u>) 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 $100~\mu l$, ± 1.0 mm for $100~\mu l$ up to $350~\mu l$ and ± 1.5 mm for nominal volumes exceeding $350~\mu l$ (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 <u>7.1.2</u>.

8 Type, designation

Designation of a Type D1 single-channel pipette (positive displacement with reusable plunger) with a fixed volume of $100 \, \mu l$:

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 μ l to 200 μ l:

Pipette ISO 8655 - D2 - 20-200

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

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 <u>Tables 1</u>, <u>2</u> and <u>3</u> 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 <u>Annex B</u> 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 <u>Table 3</u> apply.

9.4 Variable-volume pipettes of types A, D1 and D2

In the case of variable-volume pipettes, the nominal volume is the greatest possible user-selectable volume which is specified by the manufacturer. For example, a pipette with a usable volume range from $10 \, \mu l$ to $100 \, \mu l$ has a nominal volume of $100 \, \mu l$.

The maximum permissible errors in Tables 1 (Type A and D1) and 3 (Type D2) apply.

Maximum permissible errors for intermediate volumes not given in <u>Tables 1</u> and <u>3</u> shall be determined according to 9.6.

NOTE 1 When not limited to 25 %, the maximum permissible errors in <u>Tables 1</u> and $\underline{3}$ are constant over the entire usable volume range. It is recognized that the errors of measurement of intermediate volumes in the usable volume range of a variable-volume pipette can be considerably smaller than those specified in <u>Tables 1</u> and $\underline{3}$.

NOTE 2 The errors in <u>Tables 1</u> and <u>3</u> apply to the usable volume range of the pipette. Volumes below 10 % of the nominal volume do not conform to these tables.

9.5 Multi-channel pipettes

The maximum permissible errors in <u>Table 2</u> apply. Each channel of the multi-channel pipette, considered independently, shall meet these specifications.

NOTE The errors in <u>Table 2</u> apply to the usable volume range of the pipette. Volumes below 10 % do not conform to this Table.

9.6 Calculation of maximum permissible errors for volumes not listed in <u>Tables 1</u>, <u>2</u> and <u>3</u>

The calculation of maximum permissible systematic and random errors in the usable volume range, not included in <u>Tables 1</u>, <u>2</u> and <u>3</u>, shall be made by dividing the nominal volume by the selected volume and multiplying the result by the maximum permissible error at nominal volume. This calculation does not apply to volumes below 10 % of the nominal volume.

Formula (1) shall be applied for the calculation:

$$e_{V_S} = \frac{V_{\text{nom}}}{V_S} \times e_{V_{\text{nom}}}$$
 (1)

where

 V_{nom} is the nominal volume;

Vs is the selected volume:

 $e_{V_{nom}}$ is the maximum permissible error (either systematic or random) at nominal volume;

e_{Ve} is the maximum permissible error (either systematic or random) at the selected volume.

If the calculated value e_{Vs} exceeds 25 %, then the value of 25 % shall be applied as the maximum permissible error.

EXAMPLE

Variable volume pipette Type A with a nominal volume of 2 μl and a usable volume range of 0,2 μl to 2 μl.

Calculation of maximum permissible systematic error at a selected volume of 0,5 ul:

$$e_{Vnom}$$
 = 2,5 %

$$V_{\text{nom}} = 2 \,\mu l$$

$$V_{\rm S} = 0.5 \, \mu l$$

$$\mathbf{e}_{V\mathbf{s}} = \frac{V_{\text{nom}}}{V\mathbf{s}} \times \mathbf{e}_{V\text{nom}}$$

$$e_{Vs(0,5 \mu l)} = \frac{2 \mu l}{0.5 \mu l} \times 2.5 \%$$

$$e_{Vs\{0,5~\mu l\}} = 4 \times 2.5 \%$$

$$e_{Vs(0.5 \mu l)} = 10 \%$$

Table 1 — Maximum permissible errors for types A and D1 (single channel pipettes)

Pipettir	ng volume	Maximum permissible systematic error ^a	Maximum permissible random error ^a
Nominal volumes μl	Setting as a proportion of the nominal volume %	±%	%ь
1 to 3 ^c	100	2,5	2,0
	50	5,0	4,0
	10	25	20
> 3 to 5	100	2,5	1,5
	50	5,0	3,0
	10	25	15
> 5 to 10	100	1,2	0,8
	50	2,4	1,6
	10	12	8,0
> 10 to 50	100	1,0	0,5
	50	2,0	1,0
	10	10	5,0
> 50 to 5 000	100	0,80	0,30
	50	1,6	0,60
	10	8,0	3,0
> 5 000 to 20 000	100	0,60	0,30
	50	1,2	0,60
	10	6,0	3,0

^a To calculate errors in units of microlitres, multiply the maximum permissible errors by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6, ISO 8655-7, or ISO 8655-8.

^c Handling of such low volumes can be very challenging. For additional information refer to <u>Annex A</u>.

Table 2 — Maximum permissible errors for types A and D1 (multi channel pipettes)

Pipettir	ng volume	Maximum permissible systematic error ^a	Maximum permissible random error ^a
Nominal volumes μl	Setting as a proportion of	±%	%ь
	the nominal volume		
	%		
2 ^c	100	8,0	8,0
	50	16	16
	10	25	25
> 2 to 5	100	5,0	3,0
	50	10	6,0
	10	25	25
> 5 to 10	100	2,4	1,6
	50	4,8	3,2
	10	24	16
> 10 to 20	100	2,0	1,0
	50	4,0	2,0
	10	20	10
> 20 to 50	100	2,0	0,80
	50	4,0	1,6
	10	20	8,0
> 50 to 2 000	100	1,6	0,60
	50	3,2	1,2
	10	16	6,0

^a To calculate errors in units of microlitres, multiply the maximum permissible errors by the selected volume.

b Expressed as the coefficient of variation according to ISO 8655-6, ISO 8655-7, or ISO 8655-8.

^c Handling of such low volumes can be very challenging. For additional information refer to Annex A.

Table 3 — Maximum permissible errors for type D2

Pipettii	ng volume	Maximum permissible systematic error ^a	Maximum permissible random error ^a
Nominal volumes μl	Setting as a proportion of the nominal volume %	±%	%ь
5°	100	2,5	1,5
	50	5,0	3,0
	10	25	15
> 5 to 10	100	2,0	1,0
	50	4,0	2,0
	10	20	10
> 10 to 20	100	2,0	0,80
	50	4,0	1,6
	10	20	8,0
> 20 to 100	100	1,4	0,60
	50	2,8	1,2
	10	14	6,0
> 100 to 1 000	100	1,2	0,40
	50	2,4	0,80
	10	12	4,0

To calculate errors in units of microlitres, multiply the maximum permissible errors by the selected volume.

9.7 Pipette tips

The maximum permissible errors always apply to the total system of pipette and tip. Before the pipette tip is placed on the market, it shall be proven that the system, including the tips, fulfils the requirements and maximum permissible errors of this document. The different batches of tips as well as the different moulds/cavities used shall be taken into account. A set of measurements shall be performed in accordance with ISO 8655-6. ISO 8655-7 or ISO 8655-8.

10 User information

10.1 Pipettes

Information essential for the proper use of the pipette and its accessories (see ISO 8655-1) shall be provided when making a pipette available on the market and shall be as follows.

- a) The basis of adjustment (Ex) at reference conditions according to ISO 8655-1.
- b) The nominal volume or the volume range of the pipette.
- c) A list of tips and their reference numbers recommended for use with the pipette.
- d) The error limits of the systematic and random error of measurement at the nominal volume. In addition, for variable volume pipettes, the error limits of the systematic and random error of measurement at 50 % of the nominal volume and either at 10 % of the nominal volume or the smallest selectable volume, whichever is greater.
- e) Suggestions as to the basis on which a minimum routine testing protocol can be established.

b Expressed as the coefficient of variation according to ISO 8655-6, ISO 8655-7, or ISO 8655-8.

Handling of such low volumes can be very challenging. For additional information refer to Annex A.

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- f) Indication that volume variations may result from the measurement of liquids of different physical properties.
- g) Information regarding the care, cleaning and sterilisation of the pipette.
- h) Upon request, information regarding the interaction of the materials of the pipette with organic and inorganic solutions, solvents and caustic chemicals.
- i) Recommendations for the proper storage of the pipette.
- The correct method of use.

10.2 Pipette tips and accessories

Manufacturers of tips shall provide the following information on the packaging or in the instruction manual.

- a) The manufacturer and manufacturer's full apparatus name and volume for which the tips are proved to fulfil the requirements according to 9.7.
- b) The chemical compatibility information and sterilisation data.
- c) The nominal volume of the tip.

11 Marking

11.1 Pipettes

The following data shall be permanently marked on each pipette:

- a) nominal volume or, for variable-volume pipettes, usable volume range;
- b) unit of measurement, e.g. μl or ml;
- c) brand name and/or trademark;
- d) apparatus name or type;
- e) serial number of the pipette.

In addition, the following information should be marked:

- f) abbreviation "Ex" and the reference temperature "20 °C" or "27 °C";
- g) reference to this document, i.e. ISO 8655-2.

11.2 Pipette tips

The following information shall be printed on the packaging of each saleable unit of pipette tips:

- a) brand name and/or trademark;
- b) nominal volume of the tip:
- batch or lot number.

Annex A

(informative)

Possible sources of error for air displacement pipettes

Table A.1 gives possible sources of error for pipettes with an air interface.

Table A.1 — Influencing parameters, resulting errors in measurement and corrective measures

Influencing parameter	Effect ^a	Corrective measures	Determinable by
Variations in air pressure at adjustment versus use of the pipette	up to 0,2 %	Design of the pipette	Observing barometer at the measuring or working place
Difference in density of the liquid to be pipetted versus that of the liquid used for adjustment	up to 1,0 %	Observing user information	Comparing the density of the liquid to be pipetted to that of water
Difference in vapour pressure of the liquid to be pipetted versus that of the water used for adjustment	up to >50 %	Sufficient pre-wetting of pi- pette tip; observing ISO 8655- 6, ISO 8655-7 or ISO 8655-8	Dripping tip or drop hanging from the tip
Viscosity and/or flow characteristics and wetting characteristics of the liquid to be pipetted	Ь	Observing user information	Visual check for droplets or liquid trails during delivery of liquid, or incomplete liquid delivery
Leaky piston/cylinder system	up to >50 %	Regular check of pipette and the volumes aspirated	Dripping tip, maximum permitted errors are exceeded
Uneven piston movement	up to 0,5 %	Smooth operation of piston; observing user information	Observing one's own pipetting technique
Uneven rhythm and timing during pipetting	up to 1,5 %	Even pipetting technique; observing user information	Maximum permitted errors are exceeded
Immersion depth of the pi- pette tip and handling angle during pipetting	up to 1,0 %	Holding pipette in vertical position, observing user information or ISO 8655-6, ISO 8655-7 or ISO 8655-8	Visual control of immersion depth and handling angle
Variations in pipette temper- ature, ambient temperature and the temperature of the liquid to be pipetted	up to 0,3 %/K	To the extent possible, the temperature of the pipette, room and liquid to be pipetted should be the same	Measuring the temperature of air and liquid; measuring of pipette temperature, if possible
Changes in relative humidity of the ambient air	up to 3,0 %	Sufficient pre-wetting of pi- pette tip	Observing hygrometer
Failure to pre-wet pipette tip	up to 2,0 %	Pre-wetting of pipette tip	Maximum permitted errors are exceeded
Failure to wipe pipette tip on the vessel wall	up to 3,0 %	Wiping of the pipette tip on the vessel wall (wiping distance 8 mm to 10 mm), observing ISO 8655-6, ISO 8655-7 or ISO 8655-8	Maximum permitted errors are exceeded
Leaky/poorly seated pipette tips	0,5 % to 50 %	Using original or recommended pipette tips	Dripping tip or maximum permit- ted errors are exceeded

a Possible measurement errors are estimates and are specified in percent of the nominal volume.

b Indication of possible error of measurement is not realistic as it depends on the liquid to be pipetted.

Table A.1 (continued)

Influencing parameter	Effect ^a	Corrective measures	Determinable by	
Reuse of pipette tips	up to 4,0 %	Using pipette tips only once according to 7.2.2.	Maximum permitted errors are exceeded	
Straightness of pipette tips or worn tip cone on the pipette	up to 10 %	Using appropriate tips only	Visual check after positioning of the tips on the multi-channel pipette	
Possible measurement errors are estimates and are specified in percent of the nominal volume.				
Indication of possible error of measurement is not realistic as it depends on the liquid to be pinetted.				

Annex B

(informative)

Electronic motorised air displacement pipettes and method for testing in multi-dispensing mode

B.1 General

This annex describes the functions that electronic motorised air displacement pipettes offer compared to mechanical air displacement pipettes.

A method for testing the multi-dispensing mode is presented in this annex.

NOTE For safety requirements of motorised pipettes, see regional or national safety standards.

B.2 Setups for different operation modes

Electronic motorised pipettes are designed as variable volume pipettes.

The use of electronic motorised pipettes usually allows the user to perform different applications. The operation modes include:

- Pipetting mode: the selected volume is aspirated and dispensed in forward mode pipetting.
- Multi-dispensing mode: the aspirated volume is dispensed repeatedly in a predefined number of aliquots, which can be of equal or different volumes.
- Mixing mode: pipetting step is followed by a mixing phase, composed of repeated aspirating and dispensing.
- Reverse mode: aspiration of the selected volume, added with an extra amount of liquid that remains
 in the tip after the dispensing of the selected volume. The remaining volume can subsequently be
 discarded.
- Multi-aspiration: aspiration of a predefined number of equal or different volumes, followed by the dispense of the total aspirated volume.

For information regarding the different setups and operation modes offered by the pipette, refer to the manufacturer's instructions.

B.3 Adjustment of electronic motorised air displacement pipettes

Adjustment is usually performed internally during start up or by changing offsets in the internal set up.

The calibration of electronic motorised air displacement pipettes should be performed in pipetting mode unless testing specific requirements e.g. multi-dispensing mode.

B.4 Handling in multi-dispensing mode for electronic motorised air displacement pipettes

- a) Criteria to take into account to minimize volume delivery errors:
 - Regular movement during pipetting with wiping tips on weighing vessels is required.

NOTE Refer to manufacturer's instructions for correct techniques, if specified. If not specified, see below in item b).

- The speed of the piston movement during pipetting can have an impact on the results. The
 pipette should be tested at the speed specified by the manufacturer.
- b) Verification of dispensed volume in multi-dispensing mode with equal aliquots:

The verification of the dispensed volume in multi-dispensing mode may be performed in addition to the application of a measurement procedure in accordance with ISO 8655-6, ISO 8655-7, or ISO 8655-8.

The verification is based on the nominal volume, by performing 10 measurements that represent 10 % of the nominal volume of the pipette (e.g. for an electronic motorised pipette with a nominal volume of 200 μ l, 10 measurements of 20 μ l are performed).

The following amendments to the test cycles described in ISO 8655-6:2022, 8.4, ISO 8655-7:2022, 9.4, and ISO 8655-8:2022, 8.6 should be taken into account:

- Depending on predefined recommendations for the setup of the pipettes, it can be necessary to first dispense a discard volume after the test liquid has been aspirated (refer to the manufacturer's instruction).
- After the motor completed the first dispense, pause to allow complete dispensing (liquid stops moving). While removing the pipette, draw the tip along the inside surface of the receiving vessel to ensure that the last drop stays inside the receiving vessel.
- Repeat the operation for the second to tenth aliquots without changing the tip, or aspirating
 test liquid, as long as enough liquid for 10 replicates has been aspirated. When the amount of
 test liquid that can be aspirated is not enough for 10 replicates, the tip should be changed before
 aspirating the next amount of test liquid. In this case, pre-rinsing is required once again.
- Discard the residual liquid.

c) Specifications

For single channel pipettes, the results may be compared with <u>Table 1</u>, or other applicable specifications.

For multi-channel pipettes, the results may be compared with $\underline{\text{Table 2}}$, or other applicable specifications.

NOTE 1 The performance of electronic pipettes in modes such as multi-dispensing can be considerably lower than in pipetting mode. This is especially relevant at dispensed volumes below 50 μ l.

NOTE 2 In multi-dispensing mode the nominal volume remains the nominal volume of the pipetting mode and the specifications of the pipetting mode nominal volume apply.

d) Reporting of results

The following information shall additionally be noted in reports according to ISO 8655-6:2022, Clause 10, ISO 8655-7:2022, Clause 11, or ISO 8655-8:2022, Clause 10:

- operation mode (multi-dispensing mode);
- aspiration and dispense speed settings (if available).

Bibliography

- [1] ISO/TR 20461, Determination of uncertainty for volume measurements made using the gravimetric method
- [2] ISO/TR 16153, Piston-operated volumetric instruments Determination of uncertainty for volume measurements made using the photometric method
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- [4] MICHEL F. SOMMER K., SPIEWECK F., Untersuchungen zur Ermittlung der Meßunsicherheit von Kolbenhubpipetten mit Volumina von 1 μ l bis 50 μ l. PTB-Mitteilungen. 1995, **105** (6) pp. 437-444 [in German]
- [5] EURAMET cg-19 Version 2.1, Guidelines on the determination of uncertainty in gravimetric volume calibration

