
Piston-operated volumetric apparatus —
Part 2:
Piston pipettes

Appareils volumétriques à piston —

Partie 2: Pipettes à piston



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Contents

	Page
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle of operation	2
5 Design	2
6 Type, designation	5
7 Metrological performance requirements	5
8 Marking	8

Annexes

A Information to be provided by the supplier for the user of piston pipettes and tips	9
B Possible sources of error for piston pipettes with air interface	10
Bibliography.....	11

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 8655 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 8655-2 was prepared by Technical Committee ISO/TC 48, *Laboratory glassware and related apparatus*, Subcommittee SC 1, *Volumetric instruments*.

ISO 8655 consists of the following parts, under the general title *Piston-operated volumetric apparatus*:

- *Part 1: Terminology, general requirements and user recommendations*
- *Part 2: Piston pipettes*
- *Part 3: Piston burettes*
- *Part 4: Dilutors*
- *Part 5: Dispensers*
- *Part 6: Gravimetric methods for the determination of measurement error*

The following part is under preparation:

- *Part 7: Non-gravimetric methods for the determination of measurement error*

Annex A forms a normative part of this part of ISO 8655. Annex B is for information only.

Introduction

ISO 8655 addresses the needs of:

- suppliers, as a basis for quality control including, where appropriate, the issuance of supplier's declarations;
- test houses and other bodies, as a basis for independent certification;
- users of the equipment, to enable routine checking of accuracy.

The tests specified should be carried out by trained personnel.

Piston-operated volumetric apparatus —

Part 2: Piston pipettes

1 Scope

This part of ISO 8655 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 piston pipettes, complete with their selected tip(s) and any other essential, consumable parts, designed to deliver their specified nominal volume (Ex).

NOTE General requirements and definitions of terms of piston-operated volumetric apparatus are given in ISO 8655-1. Conformity testing (type evaluation) of piston-operated volumetric apparatus is given in ISO 8655-6. Alternative test methods such as photometric and titrimetric methods will be the subject of a future Part 7 to ISO 8655. For all other tests (e.g. quality assurance by the supplier, analytical and measuring equipment quality assurance by the user) see ISO 8655-6 or alternative test methods. For safety requirements of electrically powered piston pipettes, see IEC 61010-1.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 8655. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 8655 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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

ISO 8655-1:2002, *Piston-operated volumetric apparatus — Part 1: Terminology, general requirements and user recommendations*

ISO 8655-6:2002, *Piston-operated volumetric apparatus — Part 6: Gravimetric methods for the determination of measurement error*

3 Terms and definitions

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

3.1

nominal volume

⟨variable-volume piston pipette⟩ greatest volume selectable by the user and specified by the manufacturer

NOTE This means that a variable-volume piston pipette with a useful volume range (see ISO 8655-1) of 10 µl to 100 µl has the nominal volume of 100 µl.

3.2

nominal volume

⟨multi-channel piston pipette⟩ greatest volume selectable by the user and specified by the manufacturer per channel

4 Principle of operation

The tip made of plastic or glass is attached to the piston pipette. With the piston at the lower aspiration limit, the tip is dipped into the liquid to be dispensed as a measured volume. 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 piston pipettes (see 5.1, type A) have an extra air volume which can be used to expel the last drop of liquid.

See also Figure 1.

5 Design

5.1 Types of piston pipette

A piston pipette may be designed as follows:

- fixed volume, designed by the manufacturer to dispense only its nominal volume, for example 100 µl;
- variable volume, designed by the manufacturer to dispense volumes selectable by the user within its specified useful volume range, for example between 10 µl and 100 µl.

The piston may

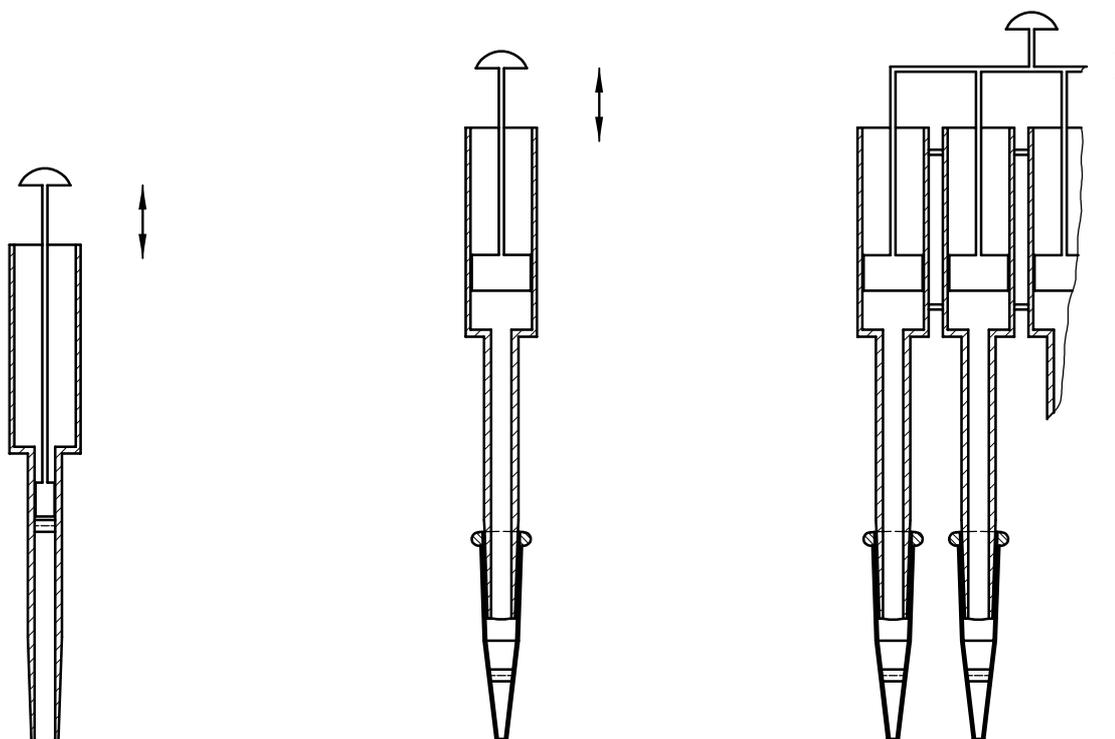
- either have a body of air contained between the piston and the surface of the liquid (air displacement – type A);
- or be in direct contact with the surface of the liquid (positive or direct displacement – type D).

In the case of the type D piston pipette, either the plunger or the capillary, or both may be reusable (type D1) or disposable (type D2).

See Figure 1 for details.

5.2 Adjustment

5.2.1 A piston pipette shall be adjusted by its manufacturer for the delivery (Ex) of its nominal volume (or selected volume, in the case of a variable-volume model), for the standard reference temperature of 20 °C, a relative air humidity of 50 % and a barometric pressure of 101 kPa (1,01 bar), using grade 3 water as specified in ISO 3696.

**Type D**

Positive (direct) displacement

Type A

Air displacement

a) Single-channel piston pipettes**b) Multi-channel piston pipette****Figure 1 — Piston pipettes**

5.2.2 Some piston pipettes have provision for adjustment by the user when, for example, it is found in routine calibration that the volume delivered is not within specification. Such user adjustment shall be made according to the manufacturer's instructions and by reference to the gravimetric test method specified in ISO 8655-6.

Any piston pipette so adjusted shall have clear, visible evidence that the initial adjustment has been modified. This information shall also be recorded on any certificate of conformity and the procedure should be included in the user's quality manual.

5.2.3 Some piston pipettes are designed to have their factory-preset 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 B for details). In such cases, the design shall prevent unintentional readjustment. Such user adjustment shall be made according to the manufacturer's instructions and by reference to the gravimetric test method specified in ISO 8655-6, but using the selected liquid instead of water.

If the user readjusts the piston pipette, it shall be clearly and unequivocally indicated on the outside of the piston pipette that readjustment has been effected. The user shall mark the outside of the piston pipette with the name of the liquid for which the nominal volume now applies. This information shall also be recorded on any certificate of conformity. The procedure should also be included in the user's quality manual.

5.3 Transfer of hand warmth

The constructional design of piston 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 or of test in accordance with ISO 8655-6 may be ignored.

NOTE Transfer of hand warmth will appear as a systematic drift of results during the series of tests to ISO 8655-6.

5.4 Pipette tips

5.4.1 General

5.4.1.1 The dispensing orifice of the tip shall be shaped in such a way that consistent dispensing of the liquid to be measured is ascertained. 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.

5.4.1.2 In the case of sterilizable pipette tips the sterilization procedures indicated as appropriate by the manufacturer in user information or on packaging (see annex A) 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 sterilized.

5.4.2 Air-displacement pipette tips

5.4.2.1 Air-displacement pipette tips shall be disposable parts, usually made of plastic, which fit on the tip holder of the pipette and prevent the instrument from contact with the aspirated liquid. To assure their metrological performance they shall conform to 5.4.1.1, 5.4.1.2, 5.4.2.2 and 5.4.2.3.

5.4.2.2 Tips for piston pipettes with air interface 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 piston pipette.

NOTE Variability of amount of externally retained liquid or an incomplete seal will contribute to poor precision when testing to ISO 8655-6.

Pipette tips made of plastic for piston 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.

5.4.2.3 The form of the pipette tips to be used with a multi-channel piston pipette shall be so straight 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 microtiter plate. The bottoms of properly fitted tips shall not vary in spacing from their nominal axes, nor from their common plane by more than $\pm 1,0$ mm for nominal volumes up to 350 μl and $\pm 1,5$ mm for nominal volumes exceeding 350 μl .

5.4.3 Positive-displacement pipette tips

5.4.3.1 Positive-displacement pipette tips shall consist of a plunger and a capillary which fit on the tip holder of the pipette. Various material 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 or disposable (both plunger and capillary is changed at each sampling).

5.4.3.2 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.

5.4.3.3 Sterilizability shall be in accordance with 5.4.1.2.

5.5 Type evaluation

Type testing of the design requirements specified in 5.2 to 5.4 shall satisfy the metrological performance requirements specified in clause 7 when the pipette is tested in accordance with ISO 8655-6.

6 Type, designation

Designation of a type D1 single-channel piston pipette (positive displacement with reusable plunger) with a nominal volume of 100 μl :

Pipette ISO 8655 - D1 - 100

Designation of a type D2 variable-volume single-channel piston 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 piston pipette with air interface, volume range variable from 10 μl to 100 μl :

Pipette ISO 8655 - A - 10-100

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

Pipette ISO 8655 - A - 200 \times 8

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

Pipette ISO 8655 - A - 20-200 \times 12

7 Metrological performance requirements

7.1 General

7.1.1 Conformity tests

The conformity test specified in ISO 8655-6 evaluates the total system of piston pipette with its selected tip or tip and piston combination and its operator. The conformity test shall be carried out in accordance with ISO 8655-6 by a test house or other authorized body prior to the issuance of a certificate of conformity.

The type test shall be carried out by the supplier prior to the issuance of a supplier's declaration of conformity.

For conformity tests, the maximum permissible errors of Tables 1 and 2 shall apply.

7.1.2 User tests

Users should establish a regular testing routine (see ISO 8655-1:2002, 7.3) for their piston pipettes using either ISO 8655-6 or alternative test methods (see note to clause 1) and having regard to:

- accuracy requirements of the liquid delivery undertaken;
- frequency of use;
- number of operators using the piston pipette;
- number of dispensings performed on each occasion of use;
- nature of the liquid dispensed (corrosiveness, solvent power etc.);
- any recommendations made by the supplier.

Annex B lists parameters which influence the metrological performance of piston pipettes and recommendations for their handling.

7.2 Fixed-volume piston pipettes of types A and D1

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

For the maximum permissible errors of piston pipettes with intermediate nominal volumes between those given in Table 1, the absolute values for the next greater nominal volume shall apply.

NOTE This means that the maximum permissible systematic error of a piston pipette with a nominal volume of 25 μl is $\pm 0,5 \mu\text{l}$ and its maximum permissible random error is $\pm 0,2 \mu\text{l}$.

Table 1 — Maximum permissible errors for types A and D1

Nominal volume μl	Maximum permissible systematic error		Maximum permissible random error	
	$\pm \%$	$\pm \mu\text{l}^{\text{a}}$	$\pm \%^{\text{b}}$	$\pm \mu\text{l}^{\text{c}}$
1	5,0	0,05	5,0	0,05
2	4,0	0,08	2,0	0,04
5	2,5	0,125	1,5	0,075
10	1,2	0,12	0,8	0,08
20	1,0	0,2	0,5	0,1
50	1,0	0,5	0,4	0,2
100	0,8	0,8	0,3 ^d	0,3 ^d
200	0,8	1,6	0,3 ^d	0,6 ^d
500	0,8	4,0	0,3	1,5
1 000	0,8	8,0	0,3	3,0
2 000	0,8	16	0,3	6,0
5 000	0,8	40	0,3	15,0
10 000	0,6	60	0,3	30,0

^a Expressed as the deviation of the mean of a tenfold measurement from the nominal or selected volume (see ISO 8655-6:2002, 8.4).
^b Expressed as the coefficient of variation of a tenfold measurement (see ISO 8655-6:2002, 8.5).
^c Expressed as the repeatability standard deviation of a tenfold measurement (see ISO 8655-6:2002, 8.5).
^d For piston pipettes of type D1 the maximum permissible errors may be $\pm 0,4 \%$.

7.3 Fixed-volume piston pipettes of type D2

For positive-displacement piston pipettes with disposable plunger and capillary (type D2) and with fixed volume, the maximum permissible errors of Table 2 apply.

For the maximum permissible errors of piston pipettes with intermediate nominal volumes between those given in Table 2, the absolute values for the next greater nominal volume shall apply.

7.4 Variable-volume piston pipettes

In the case of variable-volume piston pipettes, the nominal volume is the greatest possible user-selectable volume which is specified by the manufacturer. For example, a piston pipette with a useful volume range from 10 μl to 100 μl has a nominal volume of 100 μl .

In the conformity test, the maximum permissible errors for the nominal volumes in Tables 1 and 2 apply to every selectable volume throughout the useful volume range of the piston pipette; i.e. the maximum permissible systematic errors of variable-volume piston pipette types A and D1 with a useful volume range of 10 μl to 100 μl are $\pm 0,8 \mu\text{l}$ and the maximum permissible random errors are $\pm 0,3 \mu\text{l}$ for every measured volume.

NOTE It is recognized that the errors of measurement of intermediate volumes in the useful volume range of a variable-volume piston pipette may be considerably better than those specified for the nominal volume of the piston pipette.

Table 2 — Maximum permissible errors for type D2

Nominal volume μl	Maximum permissible systematic error		Maximum permissible random error	
	$\pm \%$	$\pm \mu\text{l}^{\text{a}}$	$\pm \%^{\text{b}}$	$\pm \mu\text{l}^{\text{c}}$
5	2,5	0,13	1,5	0,08
10	2,0	0,2	1,0	0,1
20	2,0	0,4	0,8	0,16
50	1,4	0,7	0,6	0,3
100	1,5	1,5	0,6	0,6
200	1,5	3,0	0,4	0,8
500	1,2	6,0	0,4	2,0
1 000	1,2	12,0	0,4	4,0

^a Expressed as the deviation of the mean of a tenfold measurement from the nominal or selected volume (see ISO 8655-6:2002, 8.4).
^b Expressed as the coefficient of variation of a tenfold measurement (see ISO 8655-6:2002, 8.5).
^c Expressed as the repeatability standard deviation of a tenfold measurement (see ISO 8655-6:2002, 8.5).

Repetitive piston pipettes with a continuously selectable volume shall be considered as variable-volume piston pipettes of type D2 and the maximum permissible errors of Table 2 shall apply.

7.5 Multi-channel piston pipettes

The maximum permissible systematic and random errors of multi-channel piston pipettes shall be equal to twice the values specified in Table 1 for single-channel piston pipettes. Each channel of the multi-channel piston pipette, considered independently, shall meet these specifications.

In the case of variable-volume multi-channel piston pipettes, the maximum permissible errors for the nominal volume apply to every selectable volume throughout the useful volume range (see 7.4 for further details).

7.6 Pipette tips

The maximum permissible errors always apply to the total system of piston pipette and tip. When using pipette tips not delivered by the pipette supplier, the supplier's declaration or the certificate of conformity does not apply. In such cases, the evaluation of the maximum permissible errors shall be performed using the alternative tips with the method of test specified in ISO 8655-6.

Before the conformity evaluation of the piston pipette with the alternative tips, it shall be ascertained, that the piston pipette in combination with the original tips conforms to the metrological requirements specified in this part of ISO 8655.

Users may test the metrological performance for routine quality assurance procedures of the total system of

- piston pipette and tip (type A) and operator, or
- tip and piston combination (type D) and operator

in accordance with ISO 8655-6 or with alternative test methods (see note to clause 1).

8 Marking

8.1 Piston pipettes

The following data shall be permanently marked on each piston pipette:

- a) nominal volume or, for variable-volume piston pipettes, useful volume range;
- b) unit of measurement, e.g. μl or ml;
- c) supplier's name and/or trademark;
- d) supplier's apparatus name or type;
- e) apparatus identification serial number or an equivalent unique identifier.

In addition, the following information should be marked:

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

Where appropriate, a conformity mark may be affixed to the piston pipette.

8.2 Pipette tips

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

- a) supplier's name and/or trademark;
- b) maximum volume to be dispensed with the tip;
- c) statement of whether or not the tips are suitable for sterilization.

The supplier should indicate, where appropriate, procedures for sterilization of the pipette tips.

Annex A (normative)

Information to be provided by the supplier for the user of piston pipettes and tips

A.1 Piston pipettes

The supplier shall provide information essential to the proper use of the apparatus and its accessories (see ISO 8655-1:2002, clause 6). This information shall be in the published specification on which the purchase contract is based, or in instructions that accompany the piston pipette, or in the certificate of conformity and shall be as follows.

- a) The basis of adjustment (Ex), reference temperature (20 °C), relative air humidity (50 %) and barometric pressure (101 kPa) shall be specified.
- b) The nominal volume or useful volume range of the apparatus shall be specified.
- c) A list of tips and their reference numbers, which the supplier recommends for use with the apparatus shall be specified.
- d) The correct method of use, especially of handling small liquid volumes $\leq 50 \mu\text{l}$ and avoiding introduction of air bubbles, or a reference to this part of ISO 8655 shall be specified.
- e) Any systematic and random error of measurement with reference to the procedures for their testing by the user in this part of ISO 8655 and in ISO 8655-6 shall be specified. For variable-volume piston pipettes, the systematic and random error of measurement shall be specified at the nominal volume, at 50 % of the nominal volume and either at 10 % of the nominal volume or the smallest selectable volume.
- f) Any suggestions as to the basis on which a minimum routine testing protocol should be established by the user or a reference to this part of ISO 8655 shall be specified.
- g) An indication that volume variations may result from the measurement of liquids of different physical properties shall be specified.
- h) Any information regarding the care, cleaning and sterilization of the piston pipette shall be specified.
- i) Upon request, information regarding the interaction of the materials of the piston pipette with organic and inorganic solutions and solvents shall be provided.
- j) Recommendations that piston pipettes are always stored vertically to avoid fluid ingress into the interior shall be specified.

A.2 Pipette tips and accessories

Suppliers of tips shall indicate the following information on the packaging or in the instruction manual:

- a) the supplier and supplier's apparatus name for which the tips are appropriate;
- b) the chemical compatibility information and sterilization data;
- c) the maximum volume to be measured with the tip.

Annex B (informative)

Possible sources of error for piston pipettes with air interface

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

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

Influencing parameter	Effect ^a	Influenceable by	Determinable by
Variations in air pressure at adjustment versus use of the pipette	up to 0,2 %	Constructive steps taken by manufacturer	Observing barometer at the measuring or working place
Difference in density of the liquid to be pipetted versus that of the water 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 2,0 %	Sufficient prewetting of pipette tip; observing ISO 8655-6	Dripping tip or drop hanging from the tip
Viscosity and/or flow characteristics and wetting characteristics of the liquid to be pipetted	^b	Observing user information	Visual check for droplets or liquid trails during delivery of liquid
Leaky piston/cylinder system	1 % 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 of 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
Depth of plunging of the pipette tip and handling angle during pipetting	up to 1,0 %	Holding pipette in vertical position, observing user information or ISO 8655-6	Visual control of plunging depth and handling angle
Variations in pipette temperature, 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 medium; measuring of pipette temperature impossible
Changes in relative humidity of the ambient air	up to 3,0 %	Sufficient prewetting of pipette tip	Observing hygrometer
Failure to prewet pipette tip	up to 2,0 %	Prewetting 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	Maximum permitted errors are exceeded
Leaky/poorly seated pipette tips	0,5 % to 50 %	Using original or recommended pipette tips	Dripping tip or maximum permitted errors are exceeded
Reuse of pipette tips	up to 4,0 %	Using pipette tips only once	Maximum permitted errors are exceeded
Straightness of pipette tips	up to 10 %	Using appropriate tips only	Visual check after positioning of the tips on the multi-channel pipette
^a Possible errors of measurement 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.			

Bibliography

For detailed information regarding possible errors of measurement refer to references [1], [3] or [4].

- [1] ISO/TR 20461:2000, *Determination of uncertainty for volume measurements made using the gravimetric method*
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