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Laboratory glassware — Graduated pipettes

Verrerie de laboratoire — Pipettes graduées

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 835 was prepared by Technical Committee ISO/TC 48, *Laboratory equipment*, Subcommittee SC 6, *Laboratory and volumetric ware*.

This first edition of ISO 835 cancels and replaces ISO 835-1:1981, ISO 835-2:1981, ISO 835-3:1981 and ISO 835-4:1981, which have been technically revised and combined into one document.

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Laboratory glassware — Graduated pipettes

1 Scope

This International Standard specifies metrological and constructional requirements for graduated pipettes, adequate for general laboratory purposes.

The details specified are in conformity with the principles of design and construction of volumetric glassware given in ISO 384.

NOTE For one-mark pipettes, see ISO 648. For piston-operated pipettes, see ISO 8655-2.

2 Normative references

The following referenced documents are indispensable for the application 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 384:1978, *Laboratory glassware — Principles of design and construction of volumetric glassware*

ISO 719, *Glass — Hydrolytic resistance of glass grains at 98 °C — Method of test and classification*

ISO 1769, *Laboratory glassware — Pipettes — Colour coding*

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

delivery volume

volume of liquid discharged from the pipette

NOTE Due to retention of liquid on the inner surface of the pipette, the volume of liquid delivered is not identical with the volume of liquid contained by the pipette.

3.2

delivery time

time required for the pipette to deliver its nominal volume

3.3

waiting time

time to be observed after apparent completion of the liquid delivery of the pipette and before the final reading of the delivered volume is taken

NOTE A waiting time applies for graduated pipettes of Class AS (see 5.1 and 7.6).

4 Basis of adjustment

4.1 Unit of volume

The unit of volume shall be the millilitre (ml) which is equivalent to the cubic centimetre (cm³).

4.2 Delivery volume

Graduated pipettes shall be adjusted with water according to ISO 3696, grade 3, to deliver their specified volume (Ex). The delivery should be under gravity and unrestricted. For details, see 7.5.

4.3 Reference temperature

The standard reference temperature, i.e. the temperature at which the pipette is intended to deliver its volume, shall be 20 °C.

When the pipette is required for use in a country which has adopted a standard reference temperature of 27 °C (the alternative recommended in ISO 384 for tropical use), this figure shall be substituted for 20 °C.

5 Types and classes of accuracy

5.1 Classes of accuracy

Two classes of accuracy are specified:

- Classes A and AS for the higher grade;
- Class B for the lower grade.

The maximum permissible errors for both classes are specified in Table 1.

The pipettes shall be adjusted as follows:

- Class A: without waiting time;
- Class AS: with a specified waiting time of 5 s (see 7.6);
- Class B: without waiting time.

5.2 Types of pipettes

5.2.1 Type 1: Partial delivery

Graduated pipettes adjusted for delivery of a liquid from zero line at the top to any graduation line; nominal capacity shall be represented by the lowest graduation line.

5.2.2 Type 2: Total delivery

Graduated pipettes adjusted for delivery of a liquid from any graduation line down to the jet; nominal capacity shall be represented by the highest graduation line.

5.2.3 Type 3: Total delivery

Graduated pipettes adjusted for delivery of a liquid from zero line at the top to any graduation line; nominal capacity shall be obtained by delivery down to the jet.

5.2.4 Type 4: Blow-out

Graduated pipettes for total delivery (see Type 3) where the last drop of liquid in the jet is expelled by blowing. Blow-out pipettes shall be adjusted to accuracy Class B.

6 Maximum permissible errors

Maximum permissible errors in the delivered volume shall not exceed the limits specified in Table 1. These limits represent the maximum permitted error at any point and also the maximum permissible difference between the errors at any two points.

Table 1 — Capacities, subdivisions and maximum permissible errors

| Nominal capacity ml | Smallest scale division ml | Maximum permissible error | |
|------------------------|----------------------------------|---------------------------|-----------------|
| | | Classes A and AS ± ml | Class B ± ml |
| 0,1 | 0,01 | 0,006 | 0,01 |
| 0,2 | 0,01 | 0,006 | 0,01 |
| 0,5 | 0,01 | 0,006 | 0,01 |
| 1 | 0,01 | 0,007 | 0,01 |
| 1 | 0,10 | 0,007 | 0,01 |
| 2 | 0,02 | 0,010 | 0,02 |
| 2 | 0,10 | 0,010 | 0,02 |
| 5 | 0,05 | 0,030 | 0,05 |
| 5 | 0,10 | 0,030 | 0,05 |
| 10 | 0,1 | 0,05 | 0,1 |
| 20 | 0,1 | 0,1 | 0,2 |
| 25 ^a | 0,1 | 0,1 | 0,2 |
| 25 | 0,2 | 0,1 | 0,2 |

^a Length 450 mm, see Table 2.

If graduated pipettes are required of capacities and/or subdivisions other than those listed in Table 1, they shall conform to the essential requirements of this International Standard.

The relationships between maximum permissible error and capacity, as well as between maximum permissible error and meniscus diameter as given in ISO 384:1978, Annex A and Annex B, shall be observed.

7 Construction

7.1 Material

Graduated pipettes shall be manufactured from glass of chemical resistance and thermal properties which are at least to class HGB3 in accordance with ISO 719, and shall be as free as possible from visible defects and shall be reasonably free from internal stress.

7.2 Dimensions

Graduated pipettes shall comply with the essential dimensional requirements shown in Table 2.

Table 2 — Dimensions

| Dimensions mm | | Nominal capacity ml | | | | | | | | | |
|--|--------------|------------------------|------------|------------|--------------|--------------|--------------|------------|------------|------------|------------|
| | | 0,1 | 0,2 | 0,5 | 1 | 2 | 5 | 10 | 20 | 25 | 25 |
| Smallest scale division | ml | 0,01 | 0,01 | 0,01 | 0,01 0,10 | 0,02 0,10 | 0,05 0,10 | 0,1 | 0,1 | 0,1 | 0,2 |
| Essential dimensions | | | | | | | | | | | |
| Distance from zero line to lowest graduation line for Type 1 pipettes | min. max. | 80 170 | 120 200 | 130 220 | 160 220 | 160 220 | 140 220 | 140 220 | 140 220 | 220 290 | 180 220 |
| Distance from highest to lowest figured graduation lines for Types 2, 3 and 4 pipettes | min. max. | 70 160 | 110 190 | 110 220 | 140 220 | 140 220 | 140 220 | 140 220 | 140 220 | 200 290 | 160 220 |
| Distance from highest graduation line to top of pipette | min. | 100 | | | | | | | | | |
| External diameter of suction tube | approx. | 5,25 to 9 | | | | 5,5 to 9 | | | | | |
| Length of tube of uniform bore below lowest graduation line | min. | 5 | | | | | | | | | |
| Recommended dimensions | | | | | | | | | | | |
| Overall length | approx. | 300 | 340 | 360 | 360 | 360 | 360 | 360 | 360 | 450 | 360 |
| Length of tapered portion forming jet | approx. | 15 | 15 | 20 | 20 | 20 | 25 | 25 | 30 | 30 | 30 |
| Wall thickness | approx. | 2 | 2 | 2 | 2 | 1,5 | 1 | 1 | 1 | 1 | 1 |

7.3 Top of pipette

The top of the graduated pipette shall lie in a plane at right angles to the axis of the pipette and shall be free from any blemishes which might interfere with the required accurate control by a mechanical device or by the finger, in setting the meniscus. The end may be lightly fire-polished or smoothly ground with a slight bevel on the outside.

The upper end of the pipette tube may be made from tubing of uniform bore or may have a constriction at about 25 mm below the top to enable the retention of a porous, air-permeable plug, such as a cotton plug.

7.4 Delivery jet

The lower end of the graduated pipette shall terminate either in a delivery jet having a smooth and gradual taper or in a capillary end, both without sudden constriction at the orifice which could give rise to turbulent outflow.

The end of the jet may be finished by grinding or machine tooling and may be fire polished.

7.5 Delivery time

The delivery time is defined as the time occupied by the free descent of the water meniscus (delivery under gravity) from the highest graduation line:

- to the lowest graduation line, in the case of Type 1 graduated pipettes;
- to the point at which the meniscus appears to come to rest in the jet, in the case of Type 2, Type 3 and Type 4 graduated pipettes.

For testing of the delivery time, the clean graduated pipette shall be held in a vertical position, if appropriate with no porous, air-permeable plug fitted, and filled with water to a few millimetres above the uppermost graduation line; the falling meniscus shall then be set to this line. Any drop adhering to the jet of the pipette shall be removed by touching the tip of the jet against the inner surface of an inclined glass vessel.

Delivery shall then be made into another glass vessel (the receiving vessel), so that the tip of the jet is in contact with the inner surface of this receiving vessel, above the level of any collected liquid, but without movement of one against the other throughout the delivery period.

IMPORTANT — It is important that a glass receiving vessel is used. Capillary effects influencing the delivery time depend considerably on the material on which the liquid runs down.

The delivery time thus determined shall be within the limits specified for the particular pipette (see Annex A).

In the case of Type 4 (blow-out) graduated pipettes, the last drop of liquid shall be expelled by blowing after observing a delay time of approximately 3 s.

7.6 Waiting time

The waiting time of 5 s specified for Class AS graduated pipettes is defined as the period of time to be observed in order to ensure complete delivery after the meniscus appeared to come to rest in the jet, and before the tip of the jet is removed from the inner surface of the receiving vessel.

The waiting time of 5 s shall be marked on the pipette [see 10.1 e)] by the manufacturer.

8 Graduation, figuring and patterns

8.1 Graduation patterns

All graduated pipettes shall be graduated as detailed in ISO 384:1978, Clauses 8, 9 and 10, except that graduation lines should be confined to cylindrical positions and should preferably be situated not less than 5 mm from any change of diameter.

- Graduation pattern I applies to Class A graduated pipettes.
- Graduation pattern II applies to Class AS graduated pipettes.
- Graduation pattern III applies to Class B graduated pipettes.

8.2 Position of graduation lines

Position of graduation lines shall be according to ISO 384:1978, 9.4.

8.3 Figuring of graduation lines

Figuring of graduation lines shall be according to Table 3.

Table 3 — Figuring of graduation lines

| Nominal capacity ml | Smallest scale division ml | Figured at every ml |
|------------------------|-------------------------------|------------------------|
| 0,1 | 0,01 | 0,01 |
| 0,2 | 0,01 | 0,02 |
| 0,5 | 0,01 | 0,1 |
| 1 | 0,01 | 0,1 |
| 1 | 0,10 | 0,1 |
| 2 | 0,02 | 0,2 |
| 2 | 0,10 | 0,2 |
| 5 | 0,05 | 0,5 |
| 5 | 0,10 | 1,0 |
| 10 | 0,1 | 1,0 |
| 20 | 0,1 | 1,0 |
| 25 ^a | 0,1 | 1,0 |
| 25 | 0,2 | 2,0 |

^a Length 450 mm, see Table 2.

9 Setting of the meniscus

Setting of the meniscus shall be performed according to ISO 384:1978, 5.4.

10 Marking

10.1 The following shall be marked on each graduated pipette:

- the symbol “ml” or the symbol “cm³” to indicate the unit in terms of which the pipette is graduated;
- the inscription “20 °C” to indicate the reference temperature; where, exceptionally, the reference temperature is 27 °C, this value shall be substituted for 20 °C;
- the letters “Ex” to indicate that the pipette has been adjusted to deliver its indicated capacity;
- the inscription “A”, “AS” or “B” to indicate the class of accuracy for which the pipette has been adjusted;
- the waiting time, if appropriate, in the form: “Ex + 5 s”;
- the manufacturer's and/or supplier's name or mark.

It is recommended to mark graduated pipettes complying with this International Standard with the inscription “ISO 835”.

10.2 If required for certification purposes, the delivery time in seconds shall be marked on Class A and Class AS graduated pipettes intended for official verification or certification.

10.3 The maximum permissible error according to Table 1 may be marked on all pipettes, for example by the inscription “± ... ml”.

10.4 For identification, blow-out pipettes shall be provided with a narrow white band below any colour coding. Additionally, the graduated pipette may have an inscription indicating that the instrument is a blow-out pipette (for example, “blow-out”, “à souffler” or similar).

11 Visibility of graduation lines, figures and inscriptions

All figures, graduation lines and inscriptions shall be permanent, and of such size and form as to be clearly legible under usual conditions of use.

NOTE The permanence of marking can be assessed by the test methods specified in ISO 4794.

12 Colour coding

If colour coding is used on graduated pipettes, it shall comply with the requirements of ISO 1769.

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Annex A (normative)

Definition of capacities and delivery times

A.1 Type 1 graduated pipettes (partial delivery)

A.1.1 Definition of capacity

A.1.1.1 Class A and Class B pipettes

The capacity corresponding to any graduation line of a Type 1 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied from the zero line to that graduation line, the outflow being unrestricted until making the final setting of the meniscus on the graduation line.

NOTE It is assumed that the time taken for final setting of the meniscus will exceed the nominal delay of 3 s allowed for assurance of completeness of delivery.

A.1.1.2 Class AS pipettes

The capacity corresponding to any graduation line of a Type 1 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied from the zero line to that graduation line, the outflow being interrupted when the meniscus has come to a few millimetres above the graduation line. The final setting is made to the graduation line after the specified waiting time of 5 s has been observed.

A.1.2 Delivery times

The observed delivery time shall be within the limits specified in Table A.1. When the delivery time is marked on a graduated pipette, then the observed delivery time and the marked delivery time shall both be within the limits given in Table A.1 and shall not differ by more than 2 s.

Table A.1 — Delivery times of Type 1 pipettes

| Nominal capacity ml | Smallest scale division ml | Delivery time | | | | | |
|------------------------|-------------------------------|---------------|-----------|-----------|-----------|-----------|-----------|
| | | Class A | | Class AS | | Class B | |
| | | s min. | s max. | s min. | s max. | s min. | s max. |
| 0,1 | 0,01 | 2 | 3 | — | — | 2 | 3 |
| 0,2 | 0,01 | 2 | 4 | — | — | 2 | 4 |
| 0,5 | 0,01 | — | — | 4 | 10 | 2 | 11 |
| 1 | 0,01 | 7 | 10 | 4 | 10 | 2 | 11 |
| 1 | 0,10 | 2 | 10 | 4 | 10 | 2 | 11 |
| 2 | 0,02 | 8 | 12 | 4 | 10 | 2 | 12 |
| 2 | 0,10 | 2 | 12 | 4 | 10 | 2 | 12 |
| 5 | 0,05 | 10 | 14 | 7 | 13 | 5 | 14 |
| 5 | 0,10 | 4 | 14 | 7 | 13 | 5 | 14 |
| 10 | 0,1 | 13 | 17 | 7 | 13 | 5 | 17 |
| 20 | 0,1 | — | — | 11 | 17 | 9 | 21 |
| 25 ^a | 0,1 | 15 | 21 | 11 | 17 | 9 | 21 |
| 25 | 0,2 | 5 | 15 | — | — | 5 | 15 |

^a Length 450 mm, see Table 2.

A.2 Type 2 graduated pipettes (total delivery)

A.2.1 Definition of capacity

A.2.1.1 Class A and Class B pipettes

The capacity corresponding to any graduation line of a Type 2 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied from that graduation line to the jet, outflow being unrestricted until it is sure that the meniscus has come to rest in the jet before removing the pipette from the receiving vessel.

To ensure that delivery is complete, a nominal delay time of approximately 3 s should be observed before removing the pipette from the receiving vessel.

NOTE The nominal delay time of 3 s is specified only for the purpose of definition. In use, it is unnecessary to adhere closely to this period; it is sufficient to be certain that the meniscus has come to rest in the jet before removing the pipette from contact with the receiving vessel.

A.2.1.2 Class AS pipettes

The capacity corresponding to any graduation line of a Type 2 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied from that graduation line to the jet, the outflow being unrestricted and the specified waiting time of 5 s being observed before the tip of the jet is removed from contact with the inner surface of the receiving vessel.

A.2.2 Delivery times

The observed delivery time shall be within the limits specified in Table A.2. When the delivery time is marked on a graduated pipette, then the observed delivery time and the marked delivery time shall both be within the limits given in Table A.2 and shall not differ by more than 2 s.

Table A.2 — Delivery times of Type 2 pipettes

| Nominal capacity ml | Smallest scale division ml | Delivery time | | | | | |
|------------------------|-------------------------------|---------------|-----------|-----------|-----------|-----------|-----------|
| | | Class A | | Class AS | | Class B | |
| | | s min. | s max. | s min. | s max. | s min. | s max. |
| 0,1 | 0,01 | 1 | 3 | — | — | 1 | 3 |
| 0,2 | 0,01 | 1 | 4 | — | — | 1 | 4 |
| 0,5 | 0,01 | — | — | 4 | 10 | 2 | 11 |
| 1 | 0,01 | 5 | 7 | 4 | 10 | 2 | 11 |
| 1 | 0,10 | 2 | 7 | 4 | 10 | 2 | 11 |
| 2 | 0,02 | 6 | 9 | 4 | 10 | 2 | 12 |
| 2 | 0,10 | 2 | 9 | 4 | 10 | 2 | 12 |
| 5 | 0,05 | 8 | 11 | 7 | 13 | 5 | 14 |
| 5 | 0,10 | 4 | 11 | 7 | 13 | 5 | 14 |
| 10 | 0,1 | 10 | 13 | 7 | 13 | 5 | 17 |
| 20 | 0,1 | — | — | 11 | 17 | 9 | 21 |
| 25 ^a | 0,1 | 11 | 16 | 11 | 17 | 9 | 21 |
| 25 | 0,2 | 11 | 16 | — | — | 9 | 21 |

^a Length 450 mm, see Table 2.

A.3 Type 3 graduated pipettes (total delivery)

A.3.1 Definition of capacity

A.3.1.1 Class A and Class B pipettes

The capacity corresponding to any graduation line of a Type 3 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied from the zero line to that graduation line – or, in the case of total capacity delivery, to the jet – the outflow being unrestricted until making the final setting of the meniscus on the graduation line.

To ensure that delivery is complete, a nominal delay time of approximately 3 s should be observed before removing the pipette from the receiving vessel.

NOTE The nominal delay of 3 s is specified only for the purpose of definition. In use, it is unnecessary to adhere closely to this period; it is sufficient to be certain that the meniscus has come to rest in the jet before removing the pipette from contact with the receiving vessel.

A.3.1.2 Class AS pipettes

The capacity corresponding to any graduation line of a Type 3 graduated pipette is defined as the volume of water at 20 °C, expressed in millilitres, delivered by the pipette at 20 °C when emptied under gravity from the zero line to that graduation line. The outflow shall be unrestricted until a few millimetres above the graduation line; when making final setting to the graduation line, the specified waiting time of 5 s shall be observed before removing the tip of the jet from contact with the inner surface of the receiving vessel.

In the case of delivery of the total capacity to the jet, the outflow shall also be unrestricted and a waiting time of 5 s shall be observed before removing the pipette from the receiving vessel.

A.3.2 Delivery times

The observed delivery time shall be within the limits specified in Table A.3. When the delivery time is marked on a pipette, then the observed delivery time and the marked delivery time shall both be within the limits given in Table A.3 and shall not differ by more than 2 s.

Table A.3 — Delivery times of Type 3 pipettes

| Nominal capacity ml | Smallest scale division ml | Delivery time | | | | | |
|------------------------|-------------------------------|---------------|--------|----------|--------|---------|--------|
| | | Class A | | Class AS | | Class B | |
| | | s min. | s max. | s min. | s max. | s min. | s max. |
| 0,1 | 0,01 | 1 | 3 | — | — | 1 | 3 |
| 0,2 | 0,01 | 1 | 4 | — | — | 1 | 4 |
| 0,5 | 0,01 | — | — | 4 | 10 | 2 | 11 |
| 1 | 0,01 | 5 | 7 | 4 | 10 | 2 | 11 |
| 1 | 0,10 | 5 | 7 | 4 | 10 | 2 | 11 |
| 2 | 0,02 | 6 | 9 | 4 | 10 | 2 | 12 |
| 2 | 0,10 | 6 | 9 | 4 | 10 | 2 | 12 |
| 5 | 0,05 | 8 | 11 | 7 | 13 | 5 | 14 |
| 5 | 0,10 | 8 | 11 | 7 | 13 | 5 | 14 |
| 10 | 0,1 | 10 | 13 | 7 | 13 | 5 | 17 |
| 20 | 0,1 | — | — | 11 | 17 | 9 | 21 |
| 25 ^a | 0,1 | 11 | 16 | 11 | 17 | 9 | 21 |
| 25 | 0,2 | 11 | 16 | — | — | 9 | 21 |

^a Length 450 mm, see Table 2.