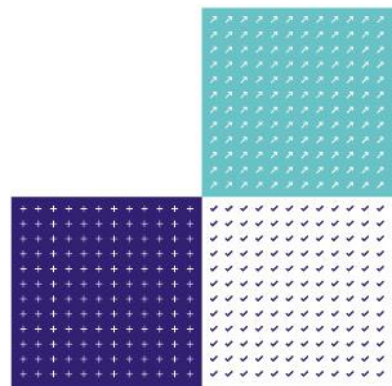


LAB 15

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Traceability: Volumetric Apparatus



Contents

1.	Introduction	3
2.	Glassware / Plasticware	3
2.1	Scope	3
2.2	Classification of Volumetric Glassware	3
2.3	Use of Calibrated Glassware	4
2.4	Marking Requirements	4
2.5	Calibration Intervals	5
2.6	Calibration Requirements	5
2.7	Calibration Procedures	7
2.8	Environmental Corrections to Volume	7
2.9	Specific Notes on the Use of Volumetric Glassware	8
2.10	Care of Volumetric Glassware	8
2.11	Marking and Segregation	9
2.12	Measuring Cylinders	9
3.	Related Apparatus	9
3.1	Scope	9
3.2	Apparatus	9
3.3	Operation	10
3.4	Calibration Intervals	10
3.5	Calibration Requirements	10
3.6	Care of Apparatus	11
3.7	Marking	11
3.8	Calibration Procedures	11
	Appendix A: Standards Relating to Volumetric Apparatus	12

Changes since last edition

The document has been updated to reflect the revised 2017 version of ISO/IEC 17025 and to cover volumetric plasticware and multi-channel pipettes. Some references have been updated and some other minor editorial alterations have been made.

1. Introduction

- 1.1 The requirements for equipment calibration and measurement traceability are given in ISO/IEC 17025:2017. Several guidance publications on the application of these requirements in the case of particular items of equipment and forms of measurement are listed on the UKAS website www.ukas.com.
- 1.2 This publication provides guidance for laboratories needing to meet the requirements for the traceability of volumetric measurement used in support of testing and calibration activities. By following this guidance laboratories will be able to demonstrate at assessment that they meet these requirements. Alternative methods may be used provided they are shown to give an equivalent outcome.
- 1.3 The first part of this publication details traceability requirements for volumetric glassware / plasticware. The second part refers to other volumetric apparatus, including electronic and mechanical hand single and multi-channel pipettes, dispensers, syringes, etc. whose calibration requirements are similar to volumetric glassware, but which may be constructed from plastic, metal or ceramic as well as glass.
- 1.4 The apparatus on which guidance is given includes syringes, where the volume delivered is frequently highly dependent on the application and operator technique, but excludes hydrometers and viscometers which are not strictly volumetric in nature although they are manufactured to high tolerances.

2. Glassware / Plasticware

(The word *glassware* beyond this point applies equally to volumetric plasticware certified to the same standards as glassware).

2.1 Scope

2.1.1 The following types of glassware are dealt with in this Section:

- (a) one-mark volumetric flasks
- (b) burettes
- (c) one-mark pipettes
- (d) graduated pipettes, including blow-out pipettes
- (e) graduated measuring cylinders
- (f) automatic pipettes

2.1.2 The Standards (BSI and, where applicable, ISO) that refer to these items are listed in Appendix A.

2.2 Classification of Volumetric Glassware

2.2.1 Volumetric glassware may either deliver or contain a stated volume. Each type may be further divided according to whether it is specified for single volumes or multiple volumes (one-mark or multi-mark).

- 2.2.2 Most items of volumetric glassware (with the exception of graduated cylinders) are available commercially in two classes, Class A and Class B (formerly Grade A and Grade B). The distinction between the two Classes is based principally on the tolerance limits of the nominal volume of the glassware as specified in the relevant British Standards. Normally, for a given volume, the tolerance for Class B is twice that for Class A.

2.3 Use of Calibrated Glassware

- 2.3.1 Each laboratory is responsible for ensuring that the volumetric glassware used is of a type and Class appropriate to the requirements of the measurement concerned.
- 2.3.2 Class A glassware need only be used where such volumetric accuracy is critical to the performance of the method and, where this is required, the glassware must be calibrated as conforming to Class A tolerances. Sub-Section 2.6 gives guidance on the circumstances where calibration is necessary.
- 2.3.3 Where an item of glassware has been calibrated, UKAS will require the laboratory to have a valid certificate of calibration or compliance for the item. If the item has been calibrated in-house, the laboratory shall have a documented record of the calibration data showing traceability to national standards. UKAS Policy on Metrological Traceability is defined in TPS 41, which also covers in-house calibrations.
- 2.3.4 Where a test method specifies accuracy and precision consistent with Class A tolerances but does not specify the use of calibrated Class A glassware, UKAS reserves the right to require calibrated Class A glassware to be used.

2.4 Marking Requirements

- 2.4.1 Commercially available volumetric glassware may be manufactured either from soda-lime glass, borosilicate glass or suitable thermally tolerant polymers. These are often distinguished by appropriate marking, either as the manufacturer's trade mark or a marking such as 'B', 'boro' or 'borosilicate' or accepted abbreviations such as PP or PMP.
- 2.4.2 Each item shall be marked in accordance with the British Standard (or other national/international standard) to which it is purchased. The following applies to glassware purchased to British Standards:
- (a) *tolerance*: Class A or B (not applicable to automatic pipettes);
 - (b) *capacity*: either 'ml', 'mL' or 'cm³' is permitted as the indicated unit of volume;
 - (c) *reference temperature, i.e. calibration temperature*: normally 20°C (27°C for tropical countries);
 - (d) *identification number*: all Class A glassware should bear a permanent identification number and shall bear such a number if the glassware is to be used for certification or verification purposes for legal metrology. Class B glassware may bear a number. Where volumetric apparatus has parts which are detachable but non-interchangeable, such as stoppers or stopcock keys, all parts should be numbered;
 - (e) *time of flow/delivery time*: Class A burettes used for verification or certification purposes for legal metrology shall be marked with a delivery time (other Class A and Class B burettes may be so marked). One-mark and multi-mark pipettes used for verification or certification purposes for legal metrology shall be marked with a delivery time (other Class A and Class B pipettes may be so marked). Multi-mark pipettes may be marked with a waiting time;

- (f) the glassware should bear:
 - (i) *colour code*: pipettes complying with BS 700 and BS 1583 may be colour coded, in which case the coding shall comply with BS 3996;
 - (ii) *maker's and/or vendor's name or mark*;
 - (iii) the letters *Ex* (*In* for volumetric flasks), to indicate that the glassware has been adjusted to deliver (or contain) its indicated volume;
- (g) multi-mark pipettes of the blow-out type shall be marked with an etched white ring and marked as *blow-out*.

2.4.3 If a particular item of glassware is not marked in accordance with the relevant British Standard, then it does not conform to that standard.

2.5 Calibration Intervals

2.5.1 The capacity of volumetric glassware may change with use. Particularly where the application warrants Class A glassware, the calibration should be verified at regular intervals. The interval will depend on the extent and nature of usage and should be determined by experiment so that re-calibration may be performed before the apparatus fails to perform to specified requirements. Initial calibration intervals should not normally exceed one year without documented justification to confirm stability.

2.6 Calibration Requirements

- 2.6.1 For most testing procedures, it is reasonable to expect that volumetric errors will be cumulative and thus dependent on the number of volumetric steps involved. If the testing procedure requires the measurement of a liquid volume to specified accuracy, then the volumetric glassware need not be calibrated if its specified maximum permissible uncorrected error does not exceed one fifth of the specified accuracy for the measurement. For example, if there is a requirement to measure a volume of 100 cm³ to ± 0.1 cm³, there is no need to use glassware which has been calibrated, if the glassware's maximum permissible error is $< 0.1 \text{ cm}^3 \div 5$, that is 0.02 cm³. This relaxation would not apply if other requirements are detailed in a relevant procedure or specification.
- 2.6.2 In Table 1, four error ranges have been assigned for one-mark volumetric glassware, based on the maximum overall error permissible in the analytical procedure. These do not apply to multi-mark volumetric glassware (such as graduated pipettes and burettes) for which tolerances are generally less strict. For these, two further error ranges have been defined (see Table 2).
- 2.6.3 Tables 1 and 2 also list glassware by type against the assigned error ranges, and indicate which sizes of glassware require specific calibration before use.

Table 1 Error ranges for one-mark volumetric glassware

Error Range	Permissible overall error in analytical procedure*	Maximum specified tolerance for volumetric glassware*	Glassware usable without prior calibration
1	0.1%	(±) 0.02%	None
2	0.1-0.5%, mean 0.3%	(±) 0.06%	Certified volumetric flasks Class A, capacity at least 250 cm ³ Uncertified volumetric flasks Class A, capacity at least 2000 cm ³ Certified bulb-pipettes Class A, capacity at least 200 cm ³
3	0.5-1.5%, mean 1.0%	(±) 0.2%	Certified volumetric flasks Class A, capacity at least 25 cm ³ Uncertified volumetric flasks Class A, capacity at least 100 cm ³ Certified bulb-pipettes Class A, capacity at least 10 cm ³ Uncertified bulb-pipettes Class A, capacity at least 50 cm ³
4	> 1.5%	eg > (±) 0.3%	Certified volumetric flasks Class A, capacity at least 10 cm ³ Uncertified volumetric flasks Class A, capacity at least 25 cm ³ Certified bulb-pipettes Class A, capacity at least 5 cm ³ Uncertified bulb-pipettes Class A, capacity at least 20 cm ³

* This data has been adapted from procedures outlined in an MOD MQAD Calibration Bulletin (#11), published in January 1978 and amended in June 1978. This Bulletin was published as guidance for conformity to DEF STAN 05-33, AQAP 6 (formerly DEF STAN 05 26).

Note that Error Range 4 is open-ended and, as the permissible error increases, it becomes possible to use Class B glassware throughout.

Table 2 Error ranges for multi-mark volumetric glassware

Error Range	Permissible overall error in analytical procedure*	Maximum specified tolerance for volumetric glassware*	Glassware usable without prior calibration
A	< 1%	0.2%	Certified burettes Class A, capacity at least 5 cm ³ Uncertified burettes Class A, capacity at least 25 cm ³
B	> 1%	eg > 0.2%	Certified graduated pipettes Uncertified graduated pipettes Class A, all capacities Class B, all capacities Certified graduated burettes Uncertified graduated burettes Class A, all capacities Class B, all capacities

2.7 Calibration Procedures

- 2.7.1 Calibration of capacity must be carried out using a suitable, documented procedure. Detailed procedures for the calibration of volumetric glassware are given in ISO 4787 (see Appendix A). The calibration of glassware is a skilled process, which should only be carried out by experienced or trained personnel. Where there is only an occasional need for calibration of glassware, consideration should be given to the sub-contracting of this work to a UKAS accredited calibration laboratory.
- 2.7.2 Volumetric capacity is normally determined gravimetrically, using water conforming to ISO 3696. Before starting, scrupulous care must be taken to ensure that the glassware is clean and, in particular, grease free. The amount of water that the vessel contains, or delivers at a measured temperature, is accurately weighed, and the volume calculated in cubic centimetres at standard temperature and pressure. Tables to facilitate this calculation are available in ISO 4787 (see Appendix A).
- 2.7.3 The balance used must be calibrated using an approved method (see LAB 14, Calibration of Weighing Machines) with weights traceable to national standards. Similarly, any temperature measurements shall be traceable to national standards (see LAB 11, Traceability of Temperature Measurement). Suitability of the balance used should be based on the resolution, standard deviation and linearity recommendations in Table 2.

Selected volume under test ⁽¹⁾ V	Resolution mg	Standard deviation (repeatability) mg	Linearity mg
100 µl < V ≤ 10 ml	0,1	0,2	0,2
10 ml < V < 1 000 ml	1	1	2
1 000 ml ≤ V ≤ 2 000 ml	10	10	20
V > 2 000 ml	100	100	200

⁽¹⁾ For practical purposes, the nominal volume may be used to choose the balance.

- 2.7.4 For burettes, and all types of pipettes normally encountered, a 200 g capacity balance reading to 0.1 mg can be used. The calibration of volumetric glassware larger than 100 cm³ capacity will require a balance of adequate sensitivity with a capacity of 2 kg or more.
- 2.7.5 Further detail on the equipment needed is given in ISO 4787.

2.8 Environmental Corrections to Volume

- 2.8.1 The correct calibration and subsequent use of volumetric glassware is critically dependent on temperature. Volumetric glassware is normally calibrated at 20°C (27°C in tropical countries). Errors resulting from the use of volumetric solutions at temperatures different to the calibration temperature may be significant, although thermal expansion of the glass itself over the same range is comparatively trivial. Thermal expansion of volumetric solutions should be taken into account when working at temperatures different from the reference temperature.

- 2.8.2 In Table 3, the temperature limits at which volumetric solutions fall outside their Class A tolerances are shown for various volumes. For example, an aqueous volumetric solution occupying 100 cm³ at 20°C will have a volume of 99.9 cm³ at 13°C or 100.1 cm³ at 25°C. Thus at temperatures below 13°C, or above 25°C, the volume will exceed the Class A tolerance (100 cm³ + 0.1%).

Table 3 Suggested operating temperature limits for Class A glassware

Volume / cm ³	Temperature limits / °C
10	< 31
50	10 - 26
100	13 - 25
250	16 - 23
1000	17 - 22

2.9 Specific Notes on the Use of Volumetric Glassware

- 2.9.1 The zero mark on a burette is a defined mark. All other graduations are positioned relative to this mark, usually by establishing the full capacity mark relative to the zero mark, and then subdividing the intervening length to give intermediate graduations. Thus all graduations, except for the zero mark, are subject to uncertainty.
- 2.9.2 When using volumetric glassware, there are several recognised rules of good practice:
- wherever possible, burettes or graduated pipettes shall be chosen so that the intended delivered volume is at least 50% of the total capacity of the glassware. Similarly, measuring cylinders shall be chosen so that the measured volume is at least 50% of the total capacity of the glassware;
 - the calibration of burettes, graduated pipettes and measuring cylinders, where appropriate, should be performed at several points rather than at just a single point at the maximum capacity;
 - when using burettes, optical aids are useful to enable the scale to be read more easily.

2.10 Care of Volumetric Glassware

- 2.10.1 Care should be taken to ensure that volumetric glassware is used and maintained in a way that does not cause damage to it nor alter its calibration. Extremes of temperature, including high temperature cleaning, and oven or hot air drying, which might lead to permanent changes in the capacity, shall be avoided. Certain solvents, strong acids/alkalis or surfactants may attack or alter the wetting characteristics of the glass, which in turn may affect draining properties. Machine washing should be avoided wherever possible since this is a common source of mechanical damage. Any contamination that is not water-soluble should be removed with an appropriate solvent before the glassware is washed. Any guidance available from the supplier should be followed, this is specifically relevant to the use of volumetric plasticware. Before use, the tips of pipettes and burettes shall be examined for mechanical damage and possible obstructions.

2.10.2 Grease is sometimes used as a lubricant for burette stopcocks. As well as being an obvious source of chemical contamination, it can adversely affect the volumetric capacity. Lubricating grease, if necessary, should therefore be used carefully.

2.11 Marking and Segregation

2.11.1 Individually calibrated items shall be readily identifiable against a record of the calibration results. Any items calibrated for a specific application should be segregated from the general stock of glassware.

2.11.2 Where additional identification is required, the methods of marking shall avoid any risk of damage to the item, and the mark positioned in a non-critical area, eg above the top graduation mark. Any marking media should be resistant to solvents and other chemicals. Mechanical etching is the preferred method of marking.

2.12 Measuring Cylinders

2.12.1 These items are not intended for work demanding the highest accuracy. The original Class A and B categories are no longer officially in use.

2.12.2 For many applications involving the use of cylinders for dispensing approximate volumes of liquid reagents, no calibration is necessary. Where cylinders are used to make quantitative measurements of volumes, calibration of individual items may be necessary. For example, this may be required in the determination of the bulk density of powders, or in the measurement of the volume of liquid distillates, etc. In such cases, the level of calibration shall be appropriate to the application.

3. Related Apparatus

3.1 Scope

3.1.1 The following types of apparatus are dealt with in this section:

- (a) mechanical and electronic hand-pipettes
- (b) dispensers
- (c) diluters
- (d) piston burettes
- (e) syringes

3.1.2 Procedures for calibrating hand-pipettes are covered by British Standards that refer to these as 'piston-operated volumetric' (see Appendix A). The accuracy and precision of these items vary according to manufacturer, but will normally be quoted in the specification.

3.1.3 In all cases, the level of calibrations should be appropriate to the use of the equipment and should have traceability to national standards.

3.2 Apparatus

3.2.1 *Hand-pipettes*

These provide a convenient alternative to traditional glass pipettes. They may be fixed or variable volume. The sample volume is drawn by suction into a disposable tip, usually of polypropylene, and then fully expelled. The accuracy of the volume drawn in this manner is

dependent on the density and viscosity of the liquid, and other factors such as surface tension, speed of filling, etc. The manufacturer's operating instructions should be taken into consideration when performing calibrations.

Hand-pipettes which deliver multiple channels of the same volume simultaneously should be calibrated for the performance of each channel individually, although this can be performed calibrating all channels at the same time.

3.2.2 *Dispensers*

These are devices for repetitive dispensing of liquids, and consist of a reservoir and a dispenser head which normally has a variable volume adjustment. A pre-set amount of the reagent is drawn into the head using a plunger. Depression of the plunger then discharges the liquid through a spout into the receiving vessel. Flow is controlled by check valves.

3.2.3 *Diluters*

These devices take up a measured amount of reagent and then discharge it together with a measured amount of diluent, taken from a reservoir. The principle of operation is very similar to that for mechanical hand-pipettes and dispensers.

3.2.4 *Piston burettes*

These are used in auto-titration systems, and operate on very similar principles to diluters and dispensers. The burette barrel consists of a precision cylinder which is filled by titrant using a moving piston. The same piston discharges the titrant until an end point to the titration is detected. The titre reading is automatically calculated from the piston travel multiplied by the cross-sectional area of the burette.

3.2.5 *Syringes*

Syringes used in laboratories fall into two broad categories: high precision metal and glass instruments, often designed for a specific application, and disposable, general purpose, low precision, plastic items. High precision syringes often have a claimed volumetric accuracy and precision of better than 1%. Plastic syringes normally have relatively low volumetric accuracy, typically $\pm 5\%$.

3.3 **Operation**

3.3.1 The performance of these instruments, in particular syringes, may be user or application dependent. For this reason, it is important that suitable operating procedures are documented and followed, and that operators are specifically trained in the correct use of the instruments. Users should be cautious in relying on manufacturers' performance figures. It is more appropriate for the laboratory to determine its own data, taking different users into account.

3.4 **Calibration Intervals**

3.4.1 The time taken for the calibration of a particular instrument to become invalid should be monitored and the re-calibration interval set accordingly. For instruments with a variable volume setting, it may be advisable that the volume is always set from the same direction and the accuracy of the set volume checked every time the setting is altered. The tolerance specified by the manufacturer should give a guide to the achievable performance.

3.5 **Calibration Requirements**

3.5.1 These instruments should be checked for the accuracy of the delivered volume against the set volume (at several different settings in the case of variable volume instruments covering at least

the range of volumes used), and the precision of repeat deliveries should be measured (at several different settings in the case of variable volume instruments). In the case of variable volume instruments, the accuracy of the selected volume setting may be dependent on whether the adjustment is made from 'up-volume' or 'down-volume'.

- 3.5.2 For syringes used for chromatographic sample injection, the absolute accuracy of the volume delivered is relatively unimportant, provided that the injected volumes of sample standard are the same, since the syringe is used in a comparative way, i.e. one injection will be compared with another. Precision is important, but it is normally sufficient to determine the overall precision of the syringe and the application for which it is being used. This can be achieved from repeat injections and the determination of the variation of the results obtained. Clearly, for optimum results, the user needs to develop a reliable injection technique. An alternative approach is to use an internal standard which can be used to calculate the relative volumes of each injection.

3.6 Care of Apparatus

- 3.6.1 Although these instruments are generally made of inert materials, care should be taken when handling corrosive solvents, or in microbiological or clinical applications. Volatile solvents may damage seals. In all cases, care should be taken that the liquids being handled only come into contact with the disposable tip, and not the main body of the instrument.
- 3.6.2 For some applications, it may be necessary to sterilise syringes by autoclave. This may result in irreversible changes in the volume of the syringe. If used in an application where its volumetric accuracy is critical, it should be re calibrated before re use. A syringe should be dismantled into its component parts before being autoclaved.
- 3.6.3 The manufacturer's operating manual generally gives clear advice on instrument care.

3.7 Marking

- 3.7.1 The instruments shall be clearly and indelibly marked so that they can be unambiguously related to their calibration data.

3.8 Calibration Procedures

- 3.8.1 Hand-pipettes should be calibrated according to the relevant British Standard method (see Appendix A). Dispensers, diluters and syringes (down to 0.01 cm³ capacity) may be calibrated using a suitable method, based on these standards methods. It may also be desirable to make occasional checks for gross errors, e.g. a 2 cm³ pipette might be checked by discharging 5 successive aliquots into a 10 cm³ volumetric flask.
- 3.8.2 For calibration of syringes of capacity less than 0.01 cm³, gravimetric errors become significant, and such methods may be impractical unless very high specification microbalances are available.
- 3.8.3 For some syringes, the volumetric capacity is so small that problems may occur because of factors such as surface tension, and difficulty in eliminating carry-over. Similar considerations apply to any other very small or micro volume apparatus such as micro-pipettes. These and automatic pipettes especially require specialist knowledge and procedures over which great care needs to be taken.

Appendix A: Standards Relating to Volumetric Apparatus

Note: where ISO standards are listed, equivalent BS EN ISO standards are available

ISO 4788:2005	Laboratory glassware. Graduated measuring cylinders
ISO 835:2007	Laboratory glassware. Graduated pipettes
ISO 4787:2010	Laboratory glassware – Volumetric instruments – Methods for testing capacity and for use
BS 1132:1987	Specification for automatic pipettes.
ISO 648:2008	Laboratory glassware. Single-volume pipettes
ISO 1042:1998	Laboratory Glassware – One-mark volumetric flasks
ISO 3696:1987	Water for analytical laboratory use - Specification and test methods
BS 3996:1978	Specification for colour coding for one-mark and graduated pipettes (including requirements for the service performance of the colour coding enamels)
ISO 8655-1:2002	Piston-operated volumetric apparatus. Terminology, general requirements and user recommendations
ISO 8655-2:2002	Piston-operated volumetric apparatus. Piston pipettes
ISO 8655-6:2002	Piston-operated volumetric apparatus. Gravimetric methods for the determination of measurement error