Designation: ~~E1154 – 14~~ E1154 – 23

Standard Specification for Piston or Plunger Operated Volumetric Apparatus and Operator Qualification[1]

This standard is issued under the fixed designation E1154; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This specification covers requirements, operating conditions, and test ~~methods~~procedures for piston or plunger operated volumetric apparatus ~~(POVA).~~(POVA), as well as requirements for pipette operator training and qualification.

1.2 This specification ~~includes specifications applicable for~~is applicable to all types of ~~POVA or those given by the manufacturer.~~ POVA. The following precautionary caveat pertains only to the test ~~method~~procedure portion, ~~Section~~Annex A1 and ~~13~~Annex A2, of this specification: *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate ~~safety and health~~safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.3 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E288 Specification for Laboratory Glass Volumetric Flasks
E456 Terminology Relating to Quality and Statistics
E542 Practice for Gravimetric Calibration of Laboratory Volumetric Instruments
E617 Specification for Laboratory Weights and Precision Mass Standards
E898 Practice for Calibration of Non-Automatic Weighing Instruments
E969 Specification for Glass Volumetric (Transfer) Pipets

2.2 *ISO ~~Documents:~~Standard:*[3]

~~ISO 3534 Statistics—Vocabulary and Symbols~~
~~ISO 653 Long Solid-Stem Thermometers for Precision Use~~
~~ISO 655 Long Enclosed-Scale Thermometers for Precision Use~~
~~ISO 4787~~ISO 3696 ~~Laboratory Glassware—Volumetric Glassware—Methods for Testing and Use~~Water For Analytical Laboratory Use – Specification And Test Methods

[1] This specification is under the jurisdiction of ASTM Committee E41 on Laboratory Apparatus and is the direct responsibility of Subcommittee E41.06 on Laboratory Instruments and Equipment.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from ~~American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.~~International Organization for Standardization (ISO), ISO Central Secretariat, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, https://www.iso.org.

2.3 *Other ~~Documents~~Documents:*[4]

OIML R 111-1 Weights of classes E_1, E_2, F_1, F_2, M_1, M_{1-2}, M_2, M_{2-3} ~~and~~ and M_3: Part 1: Metrological and ~~technical requirements~~Technical Requirements

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *accuracy*[5]—the accuracy of ~~an instrument~~ a volumetric apparatus is the closeness of agreement between the ~~nominal~~selected volume and the mean volume, obtained by applying one of the test ~~procedure~~procedures specified in Section 13 of this ~~specification.~~Specification. It is quantified by the inaccuracy of the mean.

3.1.2 *dead volume*—the dead volume is that part of the total liquid volume, held in the operational part of the device, which is not delivered.

3.1.2.1 *Discussion*—

The dead volume should not be confused with the dead air space of an air displacement ~~instrument.~~apparatus. The dead air space is the air gap between the piston and sample liquid in air-displacement devices and is sometimes referred to as air cushion.

3.1.3 *disposable*—those parts of ~~an instrument~~ a volumetric apparatus that are intended to be used once only and then discarded. Disposable parts are generally intended for use in applications where sample carryover is intolerable.

3.1.4 *maximum error*—the maximum difference between the ~~nominal~~selected volume and any single individual volume obtained by applying one of the test ~~procedure~~procedures specified in Section 13 of this Specification.

3.1.5 *maximum expectable error*—with more than 95 % probability, the maximum expectable error (MEE) is calculated ~~as follows:~~according to Eq 1:

$$\pm(1E_T 1+2s) \quad (1)$$

$$MEE = \pm(|E_t| + 2s_r) \quad (1)$$

where:

~~E_T = inaccuracy of the mean, and~~
~~s = standard deviation from the repeatability test in Section 13.~~

MEE = maximum expectable error,
E_t = inaccuracy of the mean, and
s_r = standard deviation of repeatability, see A1.4.8 and A2.5.7.

~~3.1.6 *nominal volume(s)*—the stated volume(s) for which performance is specified.~~

3.1.6 ~~*piston or plunger*~~*piston- or plunger- operated volumetric apparatus* (POVA)—the volume of liquid to be measured with POVA is defined by one or more strokes of one or more pistons or plungers. POVA may be operated manually or mechanically (for example, electrically, pneumatically or by hydrostatic pressure).

3.1.6.1 *Discussion*—

In the following text the word ‘piston’ means ‘piston or plunger.’

3.1.7 *precision*[5]—the closeness of agreement between the individual volumes obtained by applying one of the test ~~procedure~~procedures specified in this specification. It is quantified by the ~~imprecision.~~ coefficient of variation (CV).

3.1.7.1 *Discussion*—

The ~~test procedure specified gives only~~ specified test procedures give a measure of the repeatability ~~(see ISO 3534)~~ under controlled ~~conditions.~~ conditions (see E456).

3.1.8 *reference temperature*—the temperature at which the ~~instrument~~apparatus is designed to deliver its ~~nominal~~selected volume(s).

[4] Available from International Organization of Legal Metrology, 11 rue Turgot, 75009 Paris, France. www.oilm.org/en/

[5] These definitions apply only in the cases where the distributions are Gaussian.

3.1.8.1 *Discussion*—
At that temperature the closest agreement between manufacturer's performance claims and test results may be expected.

3.1.9 *reference temperature range*—~~that~~the temperature range for which the tolerances for accuracy and precision are specified.

3.1.10 *reusable*—those parts of an ~~instrument~~apparatus that are meant to be used more than once. As the reusability of some parts can rarely be quantified, any institution or individual who reuses a reusable part must see to its safety and effectiveness. Reusable parts are generally intended for use in applications where sample carryover is tolerable, or can be adequately prevented.

3.1.11 *sample carryover*—~~that~~the portion of the sample that is retained in the ~~instrument~~apparatus and that may affect subsequent samples.

3.1.12 *selected volume(s)*—the volume setting(s) at which performance is tested.

3.1.13 *stated feature*—any feature claimed by the manufacturer.

3.1.14 *unit of volume*—the ~~millilitre~~milliliter or the ~~microlitre, that~~microliter, which are accepted substitutes for the cubic ~~centimetre~~centimeter or cubic ~~millimetre.~~millimeter.

3.1.14.1 *Discussion*—
~~It is recommended that volumes~~ Volumes should be specified in ~~microlitres~~microliters up to 999 µL, and in ~~millilitres~~milliliters from 1 mL.

3.1.15 *working range*—~~that~~the part (of the total range) for which manufacturer's performance specifications are given.

3.1.16 *working temperature range*—~~that~~the range of temperatures for which manufacturer's performance specifications are given.

4. Classification

4.1 *Types of POVA*—Piston or plunger operated volumetric apparatus (POVA) are classified as follows:

4.1.1 *Pipette*—A measuring ~~instrument~~apparatus for the transfer of a predetermined volume of liquid from one vessel to another. It is not connected to a reservoir.

4.1.2 *Dispenser*—A measuring ~~instrument~~apparatus for delivering predetermined volumes of liquid from a reservoir. The reservoir may be integrated with the ~~instrument~~apparatus or connected externally.

4.1.3 *Dilutor*—A measuring ~~instrument~~apparatus for taking up different liquids (for example, sample and diluent) and delivering them in combination so as to comprise a predetermined ratio, or predetermined volumes, or both. The reservoir of diluent may be integrated with the ~~instrument~~apparatus or connected externally.

4.1.4 *Displacement ~~Buret—~~Burette*—A measuring ~~instrument~~apparatus from which the volume delivered is determined by an external indicator. The volume delivered can then be read.

4.2 *Types of Displacement:*

4.2.1 Displacement with an air interface ("air displacement"). The delivered liquid is displaced by an air interface (indirect action), ~~(see~~see ~~Figs. 1 and 2~~Fig. 1~~).~~.

4.2.2 Displacement without an air interface ("positive displacement"). The delivered liquid is displaced either by ~~a liquid interface (indirect action) or by~~ actual contact with the piston (direct action), ~~(see~~or ~~Fig. 3 and~~by a ~~Fig. 4).~~ liquid interface (indirect action) see Fig. 2.

5. Performance Requirements

5.1 *Performance Tolerances:*

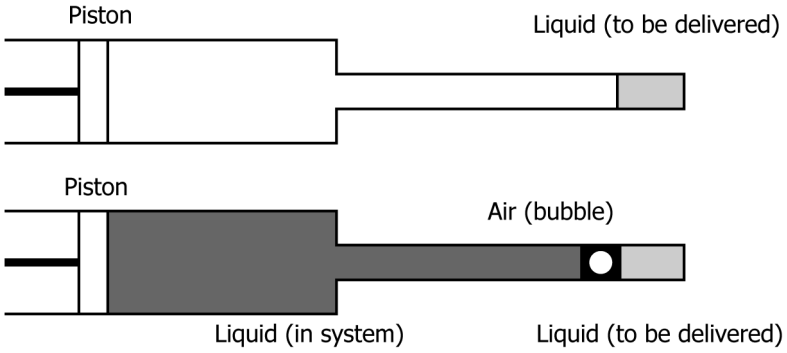

FIG. 1 Displacement With an Air Interface (Air Displacement)

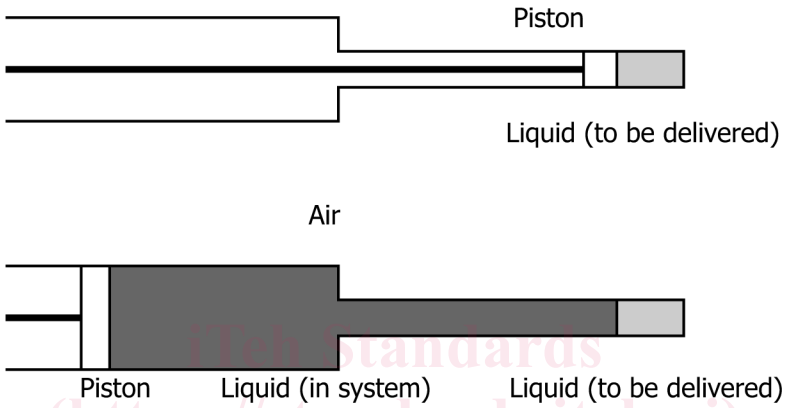

FIG. 2 Displacement Without an Air Interface (Positive Displacement)

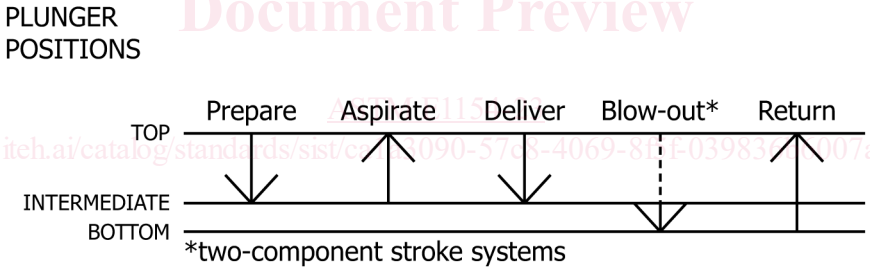

FIG. 3 ~~Pipetter~~Forward Mode of ~~Operation (Forward Mode)~~Pipette Operation

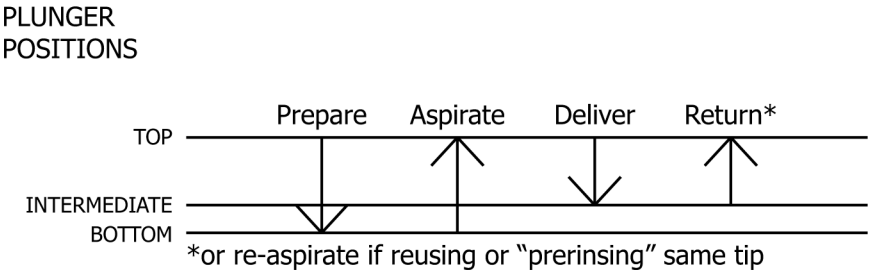

FIG. 4 ~~Pipetter~~Reverse Mode of ~~Operation (Reverse Mode)~~Pipette Operation

5.1.1 Performance tolerances specified for POVA are meant to include any thermal drift effect upon the accuracy and precision attributable to ~~hand-transmitted heat~~ heat, either hand-transmitted or from electric components, during normal use. It is, therefore, important that the ~~instrument~~apparatus being evaluated according to the referenced procedure not be preconditioned (warmed) by recent ~~handling,~~ handling or use, nor isolated from normal ~~handwarming~~warming during the test series (30 or 10 cycles).

5.1.2 Volumetric performance tolerances are not specified in this ~~specification.~~standard. The manufacturer or user shall specify the performance tolerances in terms of the ~~accuracy~~inaccuracy of the mean (~~E~~ $E_{\in c}$ in volume units, or η_c, in %) and coefficient of variation (CV_c ~~%~~), in %). Values shall be given for the minimum and maximum volumes of the working range, as well as for any intermediate volumes in the series 1, 2, 5, 10 ....

5.2 The reference temperature recommended for all POVA is ~~21.5°C, which is the mid-point of the reference temperature range, (see section~~ 20.0 °C. ~~3.1.10).~~ The use of another reference temperature must be stated by the manufacturer.

5.2.1 *Reference Temperature Range*—The reference temperature range for all POVA shall be ~~19 to 24°C,~~20 °C to 25 °C, (see section ~~3.1.9~~3.1.8 and section ~~3.1.10~~3.1.9).

5.3 *Removable Parts:*

5.3.1 The volumetric performance of POVA to be used with removable parts can depend to a large extent on the design, material, and workmanship of those parts. The test procedures described can give information only about the performance of the ~~instruments~~apparatus together with the removable parts actually used. Removable parts used during testing shall be identified in the test report to the extent possible and necessary (e.g., by manufacturer, model/type, size, batch number, etc.).

5.3.2 ~~*Single-Measurement*~~*Single-Delivery Test*—The ~~single-measurement~~single-delivery ~~ment~~ test requires either 30 or 10 randomly selected removable parts, one for each sample of the series. This test evaluates the ~~instrument's~~apparatus' performance and component of imprecision due to the variation of these parts.

5.3.3 *Replicate-Delivery Test*—The replicate delivery test uses one removable part for the 30 or 10 sample series. This test evaluates the ~~instrument's~~apparatus' performance and the component of imprecision due to the reuse of this part.

5.4 *Durability*—Any claim by a manufacturer that an ~~instrument~~apparatus is resistant to any defined conditions (for example, sterilization and chemical exposure) shall be understood in such a way that even long term or repeated exposure to those conditions (as specified by the manufacturer) will not affect the rated performance of the ~~instrument.~~apparatus.

6. General Operating Conditions

6.1 *Relationship to Performance*—The specification of operating procedures is critical to the proper functioning of the ~~instruments,~~ volumetric apparatus, and determines their ability to perform within specified tolerances. Changes in the operating mode can dramatically alter the ~~results of analyses. Most instruments~~delivered volume. Most apparatus are calibrated for certain operating modes; another manner of use may result in a change in the accuracy or precision, or both.

6.2 *Delineation*—It is the manufacturer's responsibility to delineate the modes of operation in instruction manuals and to state for which of the modes the ~~instrument~~apparatus is calibrated.

6.3 *Preparation*—The manufacturer shall provide instructions necessary for the preparation of the ~~instrument~~apparatus for use in particular operating modes (for example, mounting of removable parts, method of volume adjustment, temperature equation, isothermal requirements, testing of piston action, lubrication, priming, purging or prerinsing information, etc.).

7. Operating Conditions for ~~Pipetters~~Pipettes

7.1 Two common modes of operation are in use, the forward mode (sometimes referred to as normal mode), and the reverse mode (usable with two-component stroke mechanism systems only), ~~(see~~see Fig. 3 and Fig. 4~~).~~.

7.1.1 In general, the precision of the repetitive use of the forward mode relies upon the precise draining by air pressure (in the case of air displacement ~~pipetters)~~pipettes) or internal wiping of the ~~pipet~~pipette barrel or tip (in the case of positive displacement ~~pipetters).~~pipettes). As compared to the reverse mode, the forward mode is relatively insensitive to variations in the speed of the piston or plunger in the dispensing action. Positive displacement ~~instruments~~pipettes with relatively small delivery orifices are generally less sensitive to change in accuracy when handling liquids ~~with high wetability characteristics.~~which wet plastic tips.

7.1.2 Air displacement ~~pipetters~~pipettes with two-component stroke mechanisms are generally less sensitive than air displacement ~~pipetters~~pipettes with one-stroke mechanisms and positive displacement ~~pipetters~~pipettes to errors introduced by slight variations

of the dynamics of the liquid interface break at the end of the ~~pipet or pipet~~pipette or pipette tip during the dispensing action, due to the purging action of the air "blow-out" stroke potential.

7.1.3 The use of the reverse mode with two-component stroke mechanism ~~pipetters~~pipettes may be more advantageous when liquids that are difficult to handle in the forward mode are encountered.

7.2 *Forward Mode, General Format:*

7.2.1 *Preparation*—~~Pipetter~~Pipette and environment shall be isothermal. Volume settings and the mounting of removable or disposable ~~pipet~~pipette tips shall be accomplished according to the manufacturer's directions.

7.2.2 *Aspiration:*

7.2.2.1 Hold the ~~instrument~~pipette in a vertical position, or as prescribed by the manufacturer.

7.2.2.2 In the case of two-component stroke systems, depress the push button smoothly to the intermediate stop position.

7.2.2.3 In the case of one-component stroke systems, depress the push-button smoothly to the bottom stop position.

7.2.2.4 Immerse the ~~pipet or pipet~~pipette or pipette tip into the liquid to be pipetted to, and maintain it at the following ~~depth:~~depth (see Table 1):

TABLE 1 Immersion Depth of Pipette Tip

Volume, µL	Immersion Depth, mm
< 1	1 to 2
1 to 100	2 to 3
101 to 1000	2 to 4
1.1 to 10 mL	3 to 6

7.2.2.5 Allow the push-button to move up to the top stop position slowly and smoothly.

7.2.2.6 For air displacement ~~pipetters,~~pipettes, observe a wait of ~~1 s.~~1 second.

7.2.2.7 Withdraw the ~~pipet or pipet~~pipette or pipette tip smoothly by lifting straight up ~~either~~ from the center of the liquid surface in the ~~vessel, or up the sidewall of the~~ vessel.

NOTE 1—No further liquid contact of the ~~pipet or pipet~~pipette or pipette tip is allowed once the liquid interface is broken.

7.2.2.8 Wipe the ~~pipet or pipet~~pipette or pipette tip *only* if there are extraneous droplets. Contact with the orifice of the ~~pipet or pipet~~pipette or pipette tip, especially with absorbent material, must be avoided, as large components of random or systematic error may be introduced.

7.2.3 *Delivery*—Place the ~~pipet or pipet~~pipette or pipette tip at an angle ~~(10~~(10° to 45°, or as prescribed by the manufacturer) against the inside wall of the receiving vessel.

7.2.3.1 For two-component stroke systems, depress the push-button smoothly to the intermediate stop position. After a wait of 1 ~~s,~~second, depress the push-button to the bottom stop position as the ~~pipet or pipet~~pipette or pipette tip end is removed from the sidewall by either a sliding action up the wall or a movement away from the wall ("touching off").

7.2.3.2 For one-component stroke systems, depress the push-button smoothly to the bottom stop position as the ~~pipet or pipet~~pipette or pipette tip end is removed from the sidewall by either a sliding action up the wall, or a movement away from the wall.

7.2.3.3 Allow the push-button to move up to the top stop position.

7.3 *Reverse Mode, General Format:*

7.3.1 *Preparation*—Prepare in accordance with 7.2.1, forward mode.

7.3.2 *Aspiration*—Aspirate in accordance with 7.2.2, except that the push-button is depressed to the *bottom* stop position prior to ~~pipet~~pipette tip immersion.

7.3.3 *Delivery:*

7.3.3.1 Place the ~~pipet or pipet~~pipette or pipette tip at an angle ~~(10~~(10° to 45°, or as prescribed by the manufacturer) against the inside wall of the receiving vessel.

7.3.3.2 Depress the push-button smoothly to the intermediate stop position.

7.3.3.3 After a ~~1-s~~ 1 second wait, remove the ~~pipet or pipet~~pipette or pipette tip from the sidewall, in accordance with 7.2.3.

7.3.3.4 In the case of the ~~pipet~~pipette tip being reused, allow the push-button to remain in the intermediate stop position for subsequent immersion for the next pipetting cycle. In the case of the ~~pipet~~pipette tip to be changed, allow the push-button to return to the top stop position.

NOTE 2—Top and bottom stop positions, as described in the procedures above, are not meant to include auxiliary stroke positions (for example, for tip ejection).

7.4 *Prerinsing (Forward Mode):*

7.4.1 Prerinsing is the action of precoating the inside of the liquid contracting part(s) with a thin film of the same liquid to be ~~pipetted.~~ pipetted, and for increasing the humidity in the air cushion (air displacement pipettes only). It is accomplished by duplicating the exact motion of a forward mode pipetting cycle, except that the liquid is dispensed back into the original vessel, or preferably discarded.

7.4.2 Prerinsing in the forward mode is advantageous when reusing (the same liquid and volume setting only) the ~~pipet or pipet~~pipette or pipette tip for subsequent immediate pipettings. Eliminating the dispensed amount from the first wetting from the sample group formed by subsequent wettings and thus the removal of its value from the calculation of a precision statistic for the group, will result in a more precise distribution.

7.4.3 Prerinsing may also be practiced when a removable ~~pipet~~pipette tip is to be used only once (for example, when pipetting different liquids), but the increase in time required to accommodate prerinsing each tip reserves this practice for pipetting different liquids which may be especially difficult to handle (for example, different patient sera). The need for prerinsing is also related to the surface properties of the ~~pipet~~pipette tip as well as due to the physical characteristics of the liquid(s).

7.5 *Positioning the Residual Volume (Reverse Mode)*—Positioning the residual volume for the reverse mode is the functional equivalent of prerinsing for the forward mode. It is accomplished by duplicating the exact motion of a reverse mode pipetting cycle, except that the liquid is dispensed back into the original vessel, or preferably discarded, and the push-button kept at the intermediate stop position instead of being allowed to return to the top stop position, when reusing the ~~pipet~~pipette tip.

7.6 *Disposable ~~Pipet~~Pipette Tips*—Discarded ~~pipet~~pipette tips contain liquid residues, particularly when used in the reverse mode. Suitable precautions should be taken with their disposal.

8. Operating Conditions for Dispensers

8.1 *Dispensers with Valves(s)*—The aspiration tube must be immersed in the reservoir for operation. When the system is filled (free of air bubbles, according to manufacturer's instructions), the movement of the piston in one direction aspirates liquid. While moving in the opposite direction, the adjusted volume of liquid is dispensed, ~~(see~~see Fig. 5~~)~~.

8.2 *Dispensers Without Valve*—When the system is filled (free of air bubbles, according to manufacturer's instructions), the movement of the piston in one direction aspirates liquid. While moving in the opposite directions, the adjusted volume of liquid is dispensed, ~~(see~~see Fig. 6~~)~~.

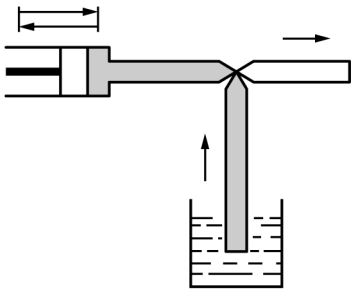

FIG. 5 Dispenser With Valve

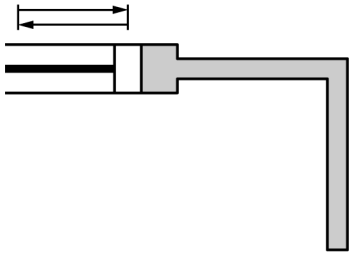

FIG. 6 Dispenser Without Valve

9. Operating Conditions for Dilutors

9.1 During operation the entire system, except the end of the probe tube, is filled with diluent. Any movement of the piston (V) in the direction (A) aspirates diluent. The diluent is aspirated as follows:

9.1.1 In the case of dilutors with valve(s), through the aspiration tube, ~~(see~~see Fig. 7~~)~~., and

9.1.2 In the case of dilutors without valve, through the probe tube, ~~(see~~see Fig. 8~~)~~..

9.2 Any movement of the piston (*P*) in the direction (*A*) aspirates sample liquid through the probe tube.

9.3 A movement of the pistons (*V*) and (*P*) in the direction (*B*) expels diluent and sample liquids in the adjusted ratio. Fig. 7 and Fig. 8 show dilutors with two separate pistons. Dilutors may also operate with one piston or with telescopic pistons. For the functioning of a dilutor it is irrelevant whether the pistons operate in the same direction, and simultaneously, or in opposite directions at different times.

10. Operating Conditions for Displacement ~~Burets~~Burettes

10.1 ~~*Burets*~~*Burettes with Valves(s)*—The aspiration tube must be immersed in the reservoir for operation. When the system is filled (free of air bubbles, according to manufacturer's instructions), the movement of the piston in one direction aspirates liquid. The movement of the piston in the opposite direction expels liquid, after which a reading can be taken, ~~(see~~see Fig. 9~~)~~..

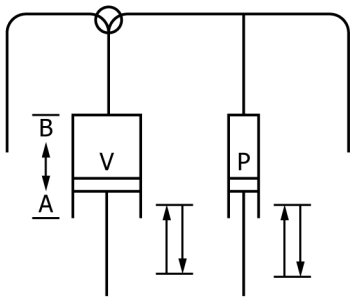

FIG. 7 Dilutor With Valve

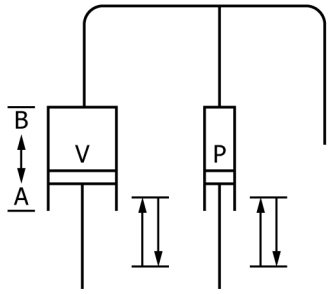

FIG. 8 Dilutor Without Valve

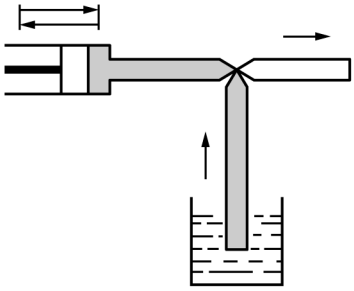

FIG. 9 Burette With Valve

10.2 ~~*Burets*~~*Burettes Without Valve*—When the system is filled (free of air bubbles, according to manufacturer's instructions), the movement of the piston in one direction ~~aspiratesliquid.~~ aspirates liquid. The movement of the piston in the opposite direction expels liquid, after which a reading can be taken, ~~(see~~see Fig. 10~~).~~.

11. Number of Tests and Retests

11.1 *Functional Test*—A functional test (for example, tests for leakage, broken parts, existence of air bubbles, contamination) shall be performed daily.

11.2 *Volumetric Tests:*

11.2.1 An appropriate single or replicate measurement test should also be performed following a change in the source of any removable parts of the delivery system (for example, as indicated by control or lot numbers of ~~pipet~~pipette tips, or change in dispensing cannulae).

11.2.2 A quick check four sample test measuring accuracy and roughly estimating precision should be performed at least monthly, or more frequently as indicated by the physical condition or extent of use of the apparatus.

11.2.3 A ten sample test measuring both accuracy and precision should be performed on all delivery systems upon introduction to service, following routine and other maintenance, and as otherwise necessary to provide a comprehensive evaluation on at least a quarterly basis.

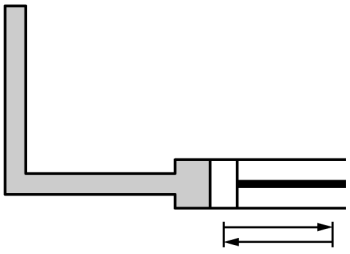

FIG. 10 Burette Without Valve

12. Sample Size

12.1 For purposes of ~~specifying or testing the volumetric performances of a single instrument~~ establishing volumetric performance specifications of a POVA by the manufacturer, supplier, or testing agent, the procedures specified in Section ~~13~~13 shall be repeated at least 30 times.

12.2 For ~~control purposes~~ calibration and verification of accuracy and precision, ten replicate measurements may be sufficient.

12.3 For quick checks of accuracy, four replicate measurements are sufficient.

13. Test Procedures

13.1 *Scope*—These test procedures cover the testing of POVA under prescribed conditions.

13.2 *Significance and Use*—These test procedures are intended to provide uniform reference procedures that can be used by anyone to assess the errors of POVA. These test procedures are recommended for use in establishing performance claims, in quality control procedures, as well as in quick checks throughout the working life of a POVA.

13.3 *Summary of the Gravimetric Procedure*—The gravimetric test procedure is based upon the determination of the weighing result of water samples delivered by the POVA. The values are corrected for evaporation, then true mass and volume are calculated simultaneously, based upon the knowledge of the density of water at specific temperatures and corrections for air buoyancy (see E542). The gravimetric test procedure is described in Annex A1.

13.4 *Summary of the Photometric Procedure*—The dual-dye ratiometric photometric test procedure is based on the Beer-Lambert Law, which correlates the concentration of a chromophore in solution with its absorbance. The unknown volume of a test solution (of known Ponceau S concentration) is added to a known amount of copper(II) chloride solution of known concentration. Ratiometric application of the Beer-Lambert Law allows the calculation of the delivered volume of test solution. The photometric test procedure is described in Annex A2.

14. ~~Gravimetric Test Method~~ Dispense Procedures:

14.1 ~~*Scope—General*—These test methods cover the testing of POVA under prescribed conditions.~~Ensure that all equipment and materials, including a sufficient number of removable parts, are properly selected and conditioned, the desired volume is set (if applicable) and the electronic balance (if used) or spectrophotometer (if used) has had the warm-up time specified by the manufacturer.

~~13.2 *Summary of Method*—The general procedure is based upon the determination of the weighing result of water samples delivered by the instrument. The values are corrected for evaporation, then true mass and volume are calculated simultaneously, based upon the knowledge of the density of water at specific temperatures and corrections for air buoyancy (see ISO 4787).~~

~~13.3 *Significance and Use*—These test methods are intended to provide uniform reference procedures that can be used by anyone to assess the errors of instruments. These test methods are recommended for use in the establishing performance claims, in quality control procedures during manufacture, as well as in control checks throughout the working life of an instrument.~~

~~13.4 *Apparatus:*~~

~~13.4.1 The resolution requirement of the weighing equipment shall be to one tenth of one percent of the water sample weight. The imprecision requirement of the weighing equipment is determined as the standard deviation of at least ten repeated weighings of a metal weight of a mass similar to the mass of the water sample. The minimum requirements for the balance are as shown in Table 1. Balances shall be calibrated and maintained at least annually, and re-calibrated after being moved. Balance calibration shall be checked at least daily. (See Test Method E898 for balance calibration and OIML R 111-1 or Specification E617 for weight requirements.)~~

~~13.4.2 *Weighing Vessel,* shall be such that the instrument can be operated according to the manufacturer's instructions. The total volume of the weighing vessel shall be as small as practicable and preferably smaller than 50 times the volume to be tested. In~~

the case of test volumes smaller than 100 µL, the weighing vessel shall be covered with a cap to avoid excessive errors due to the evaporation of water during weighing, unless conditions such as high ambient relative humidity make this unnecessary. The cap must not come into contact with the liquid.

13.4.2.1 The vessel and cover shall be made of nonporous material.

13.4.2.2 The opening shall be as small as possible. The top edge angle shall be such as not to affect the normal operation of the instrument under test.

13.4.3 *Thermometer,* used for measuring the ambient and water temperature shall show a maximum permissible error of ± 0.1°C, for example, thermometer STL/0.1/−5/ + 25 in accordance with ISO 653, or thermometer EL/0.1/−5/ + 25 in accordance with ISO 655.

13.5 *Materials and Environment:*

13.5.1 Water shall be distilled and reasonably free of dissolved air.

13.5.2 *Ambient Test Conditions*—The instruments shall be tested under referenced ambient conditions. The ambient conditions for the tests shall be as follows:

13.5.2.1 The temperatures of the test environment, including the analytical equipment, material, test water, instrument to be evaluated (including removable parts) should be identical, and as stable as possible (± 0.5°C) at least 2 h prior to and throughout the evaluation period.

13.5.2.2 The relative humidity should be maintained at 45 to 75 %, in order to reduce the evaporation rate and control the buildup of electrostatic potentials. In the immediate weighing area the relative humidity may be increased, but care should then be taken against condensation of water.

13.5.2.3 The balance area shall be reasonably free of vibration and air currents.

13.5.2.4 The ambient air shall be reasonably clean.

13.5.2.5 The lighting shall be of necessary intensity, and glare-free. Diffused light is preferred (direct sunlight must be avoided).

13.5.2.6 The working surface directly in front of the balance should be a dark color and glare-free.

13.5.2.7 The average barometric pressure in the test laboratory shall be known to ± 25 m bar.

14.2 *Procedures:* *Pipettes—*

13.6.1 *General*—Ensure that all equipment and materials including a sufficient number of removable parts are properly selected and conditioned, the desired volume is set (if applicable) and the electronic balance (if used) has had the warm-up time specified by the manufacturer. Select the following test conditions: pipetting operating mode, option regarding prerinsing or not, whether to reuse or dispose of pipette tips, and a cycle time for the procedure.

NOTE 3—The cycle time shall be consistent throughout a series of measurements.

14.2.1 *Pipetters*—Select the following test conditions: pipetting operating mode, option regarding prerinsing or not, whether to reuse or dispose of pipet tips, and a cycle time for procedure. Mount removable pipette tip.

NOTE 3—The cycle time shall be consistent throughout a series of measurements.

13.6.2.1 Mount removable pipet tip.

13.6.2.2 Measure the temperature of the water to ≤0.1°C and record it.

13.6.2.3 Place a small amount of water in the weighing vessel (between 2 and 30 sample amounts, or a minimum of 0.5 mL).

~~13.6.2.4 Place the cap on the weighing vessel, if necessary, and the weighing vessel on the balance pan.~~

~~13.6.2.5 While the balance is equilibrating the pipet tip may be prerinsed, and the sample aspirated, according to the operating mode selected.~~

~~13.6.2.6 Tare the weighing vessel and record the value, if necessary.~~

~~13.6.2.7 Note the time.~~

~~13.6.2.8 Deliver the sample according to the operating mode selected and replace the cap, if used.~~

~~13.6.2.9 Weigh the weighing vessel and record the time and weighing result.~~

~~13.6.2.10 If a series of measurements shall be carried out: repeat 13.6.2.5 through 13.6.2.9 until the desired number of measurements is achieved.~~

~~13.6.2.11 Perform a control blank for estimation of evaporation by repeating 13.6.2.6 through 13.6.2.9 exactly as in a normal sample weighing but without actually delivering any liquid to the weighing vessel.~~

~~NOTE 4—It is suggested that this evaporation control check be performed at the beginning and end of each series of measurements, and between each group of 10 samples in larger series.~~

~~13.6.2.12 Measure the temperature of the water and record a second time.~~

~~13.6.2.13 The procedure in 13.6.2.5 through 13.6.2.9 should be performed as quickly as practicable but without compromise to the integrity of the liquid delivery, precision of the technique of the operator, or time intervals.~~

14.2.2 Measure the temperature of the test liquid to ≤0.1 °C and record it.

14.2.3 Follow the respective test procedure in Annex A1 or Annex A2 for preparing the balance and weighing vessel, or spectrophotometer and cuvettes, and for the measurement of delivered test liquid volumes.

14.2.4 Prerinse the pipette tip, if desired.

14.2.5 ~~*Dispensers:*~~ Aspirate the test liquid and deliver the sample according to the operating mode selected against the side wall of the weighing vessel or cuvette.

~~13.6.3.1 Measure the temperature of the water to ≤0.1°C and record.~~

~~13.6.3.2 Connect or fill the reservoir and prime the dispenser according to the manufacturer's instructions before equilibrating it for normal use.~~

~~13.6.3.3 Place a small amount of water in the weighing vessel (between 2 and 30 sample amounts, or a minimum of 0.5 mL).~~

~~13.6.3.4 Place the cap on the weighing vessel, if necessary, and the weighing vessel on the balance pan. Equilibrate the dispenser to normal operation by actuating at least one complete cycle and discarding the first dispensing.~~

~~13.6.3.5 Tare the weighing vessel and record the value, if necessary.~~

~~13.6.3.6 Note the time.~~

~~13.6.3.7 Actuate a complete dispensing cycle to deliver the sample into the weighing vessel and replace the cap, if used.~~

~~13.6.3.8 Weigh the weighing vessel and record the time and weighing result.~~

~~13.6.3.9 If a series of measurements shall be carried out, the procedure in 13.6.3.5 – 13.6.3.8 are to be repeated until the desired number of measurements is achieved.~~