



Designation: E 1154 – 89 (Reapproved 1997)

Standard Specification for Piston or Plunger Operated Volumetric Apparatus¹

This standard is issued under the fixed designation E 1154; 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 for piston or plunger operated volumetric apparatus (POVA).

1.2 This specification includes specifications applicable for all types of POVA or those given by the manufacturer. The following precautionary caveat pertains only to the test method portion, Section 13, 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 practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ISO Documents:²

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 Laboratory Glassware—Volumetric Glassware—Methods for Testing and Use

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *accuracy*³—the accuracy of an instrument is the closeness of agreement between the nominal volume and the mean volume, obtained by applying the test procedure specified in Section 13 of this 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 space of an air displacement instrument.

3.1.3 *disposable*—those parts of an instrument 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 volume and any single individual volume obtained by applying the test procedure specified in Section 13 of this ISO Standard.

3.1.5 *maximum expectable error*—with more than 95 % probability, the maximum expectable error is calculated as follows:

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

where:

E_T = inaccuracy of the mean, and

s = standard deviation from the repeatability test in Section 13.

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

3.1.7 *unit of volume*—the millilitre or the microlitre, that are accepted substitutes for the cubic centimetre or cubic millimetre.

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

3.1.8 *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).

3.1.8.1 *Discussion*—In the following text the word 'piston' means 'piston or plunger.'

3.1.9 *precision*³—the closeness of agreement between the individual volumes obtained by applying the test procedure specified in this specification. It is quantified by the imprecision.

3.1.9.1 *Discussion*—The test procedure specified gives only a measure of the repeatability (see ISO 3534) under controlled conditions.

3.1.10 *reusable*—those parts of an instrument 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 portion of the sample that is retained in the instrument and that may affect subsequent samples.

¹ This specification is under the jurisdiction of ASTM Committee E-41 on Laboratory Apparatus and is the direct responsibility of Subcommittee E41.06 on Weighing Devices.

Current edition approved April 4, 1989. Published May 1989. Originally published as E 1154-87. Last previous edition E 1154-87.

² Available from American National Standards Institute, 1430 Broadway, New York, NY 10018.

³ These definitions apply only in the cases where the distributions are Gaussian.

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

3.1.13 *reference temperature*—the temperature at which the instrument is designed to deliver its nominal volume(s).

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

3.1.14 *reference temperature range*—that temperature range for which the tolerances for accuracy are specified.

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

3.1.16 *working temperature range*—that 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 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 for delivering predetermined volumes of liquid from a reservoir. The reservoir may be integrated with the instrument or connected externally.

4.1.3 *Dilutor*—A measuring instrument 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 or connected externally.

4.1.4 *Displacement Buret*—A measuring instrument 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 Figs. 1 and 2).

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 Fig. 3 and Fig. 4).

5. Performance Requirements

5.1 Performance Tolerances:

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 during normal use. It is, therefore, important that the instrument being evaluated according to the referenced procedure not be pre-conditioned (warmed) by recent handling, nor isolated from normal handwarming during the test series (30 or 10 cycles).

5.1.2 Volumetric performance tolerances are not specified in this specification. The manufacturer shall specify the performance tolerances in terms of the accuracy of the mean (\bar{E}_c %) and coefficient of variation (CV_c %). 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 3.1.14). 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, (see section 3.1.13 and section 3.1.14).

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 together with the removable parts actually used.

5.3.2 *Single-Measurement Test*—The single-measurement test requires either 30 or 10 randomly selected removable parts, one for each sample of the series. This test evaluates the instrument’s 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 performance and the component of imprecision due to the reuse of this part.

5.4 *Durability*—Any claim by a manufacturer that an instrument 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.

6. General Operating Conditions

6.1 *Relationship to Performance*—The specification of operating procedures is critical to the proper functioning of the instruments, and determines their ability to perform within specified tolerances. Changes in the operating mode can

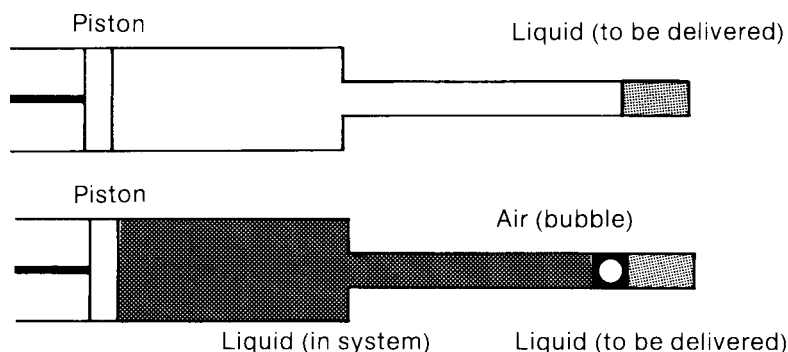


FIG. 1 Displacement With an Air Interface (Air Displacement)

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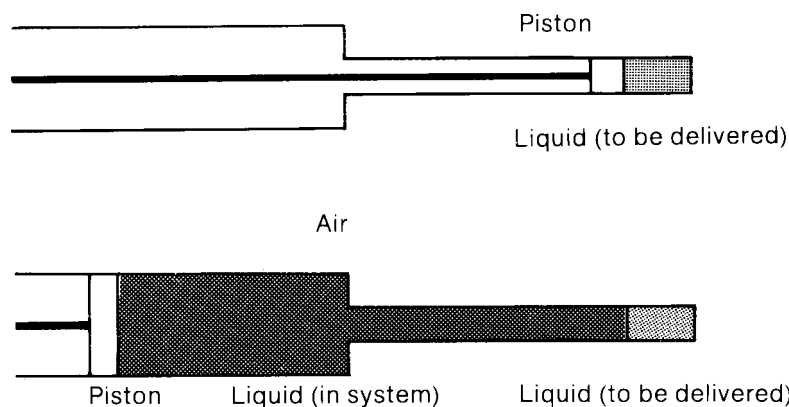
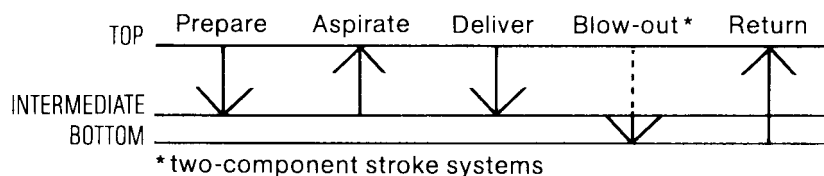


FIG. 2 Displacement Without an Air Interface (Positive Displacement)

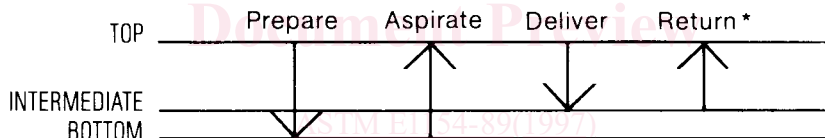
PLUNGER POSITIONS



* two-component stroke systems

FIG. 3 Pipetter Mode of Operation (Forward Mode)

PLUNGER POSITIONS



* or re-aspirate if reusing or "prerinsing" same tip

FIG. 4 Pipetter Mode of Operation (Reverse Mode)

dramatically alter the results of analyses. Most instruments 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 is calibrated.

6.3 *Preparation*—The manufacturer shall provide instructions necessary for the preparation of the instrument 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 Pipettors

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 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 pipettors) or internal wiping of

the pipet barrel or tip (in the case of displacement pipettors). 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 with relatively small delivery orifices are generally less sensitive to change in accuracy when handling liquids with high wettability characteristics.

7.1.2 Air displacement pipettors with two-component stroke mechanisms are generally less sensitive than air displacement pipettors with one-stroke mechanisms positive displacement pipettors to errors introduced by slight variations of the dynamics of the liquid interface break at the end of the pipet or pipet 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 pipettors 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 and environment shall be isothermal. Volume settings and the mounting of removable or

disposable pipet tips shall be accomplished according to the manufacturer's directions.

7.2.2 *Aspiration:*

7.2.2.1 Hold the instrument 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 tip into the liquid to be pipetted to, and maintain it at the following depth:

Volume, μL	Immersion Depth, mm
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, observe a wait of 1 s.

7.2.2.7 Withdraw the pipet or pipet 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 tip is allowed once the liquid interface is broken.

7.2.2.8 Wipe the pipet or pipet tip *only* if there are extraneous droplets. Contact with the orifice of the pipet or pipet 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 tip at an angle (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, depress the push-button to the bottom stop position as the pipet or pipet 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 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 tip immersion.

7.3.3 *Delivery:*

7.3.3.1 Place the pipet or pipet tip at an angle (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 wait, remove the pipet or pipet tip from the sidewall, in accordance with 7.2.3.

7.3.3.4 In the case of the pipet 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 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 contacting part(s) with a thin film of the same liquid to be pipetted. 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 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 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 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 tip.

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

8. Operating Conditions

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 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 Fig. 6).

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: