Designation: E 733 – 80 (Reapproved 1999)ε1

Standard Specification for 44.7-µL Disposable Glass Micropipets[1]

This standard is issued under the fixed designation E 733; 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.

ε1 NOTE—Keywords were added in March 2000.

1. Scope

1.1 This specification describes two different types of disposable micropipets, calibrated "to contain," used in measuring microlitre volumes of liquids.

1.2 The following precautionary statement pertains only to the test method portion, Section 9, 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 *ASTM Standards:*
E 438 Specification for Glasses in Laboratory Apparatus[2]
E 672 Specification for Disposable Glass Micropipets[2]
2.2 *Other Standard:*
USP XIX United States Pharmacopeia

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*
3.1.1 *accuracy*—the expected distribution of mean volumes around the stated volume.
3.1.2 *coefficient of variation*—the expected distribution of individual volumes around the mean volume.
3.1.3 *disposable micropipets*—in accordance with this specification and the expected product performance expressed in this standard, those micropipets which are to be used one time only. *Any institution or individual who reuses a disposable micropipet must bear full responsibility for its safety and effectiveness.*

4. Classification

4.1 This specification covers two different pipets as follows:
Type I—Coated with heparin.
Type II—Uncoated.

5. Material

5.1 *Glass*—The pipets made to this specification shall be fabricated from borosilicate glass, Type I, Class A or B, or soda-lime glass, Type II, in accordance with Specification E 438.

5.2 *Heparin*—Heparin shall be of sodium salt isolated from the intestinal mucosa of hog origin. The heparin potency shall be 1 mg of sodium heparin compound and shall be equal to at least 100 United States Pharmacopeia (USP) units.

6. Physical Requirements

6.1 *Design*—Pipets shall be of one-piece construction for shape, dimensions, and permissible variations. Any cross section of the pipet, taken in a plane perpendicular to the longitudinal axis, shall be circular. The pipet design is similar to that expressed in Specification E 672 except for sizing, heparin coating, and specific color coding requirements.

6.2 *Capacity*—The pipet shall be calibrated "to contain" (T.C.) and shall have a capacity of 44.7 µL. This shall be known as the "stated capacity," V_1, in making subsequent calculations. The expected deviation from the stated capacity shall be expressed as accuracy *and* coefficient of variation and shall be expressed on the package label. The pipets shall be tested for capacity as specified in 9.1.

6.2.1 *Accuracy*—(see 4.1)—The accuracy from stated volume shall be within ± 0.5 % and shall be determined as specified in 9.4.

6.2.2 *Coefficient of Variation* (see section 3.1.2)—The coefficient of variation from stated volume shall not exceed 1.0 % and shall be determined as specified in 9.4.

6.3 *Capacity Mark*—Pipets (Fig. 1) shall have a capacity line that is calibrated "to contain" a volume of liquid at 20°C. The capacity line shall be 0.3 to 0.5 mm wide and shall completely encircle the pipet in a plane perpendicular to its longitudinal axis.

6.4 *Heparin Coating* (Type I, Heparinized, only)—The inner surface of Type I pipets shall be evenly coated with sodium heparin. At his option, the manufacturer may label Type I, heparinized pipets with a statement of expected units of heparin activity. An expiration date on specified units of heparin activity may be claimed by the manufacturer. The pipet

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[2] *Annual Book of ASTM Standards*, Vol 14.04.

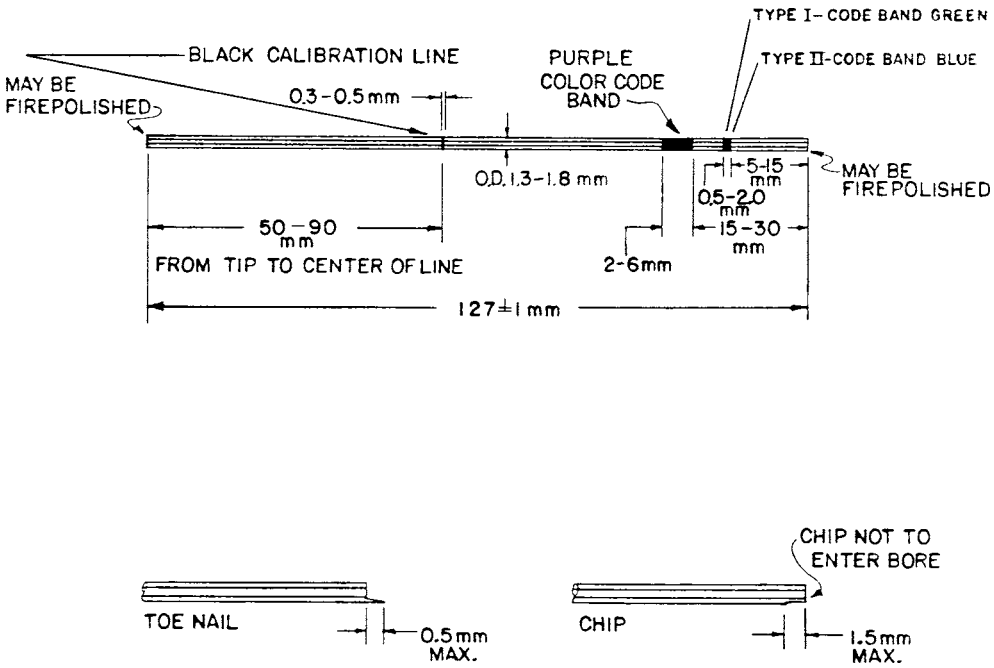

FIG. 1 44.7-µL Disposable Glass Micropipets: Type I—Coated with Heparin, Type II—Uncoated

shall contain a minimum of 5 and a maximum of 10 units of heparin that shall be present when tested as specified in 9.5. The manufacturer may heparin coat the pipet only to the calibration line. In this instance, a minimum of 2 and a maximum of 5 units of heparin shall be present when tested as specified in 9.5.

6.5 *Lot or Control Number*—A lot or control number shall be indicated on the pipet container package. This lot, or control number, shall be traceable to the origin of the heparin lot purchase or mix, or both.

6.6 *Identification Markings*:

6.6.1 *Type I, Heparinized*—The pipet shall be identified for capacity with a purple color code marking on each pipet that is 2 to 6 mm wide. An additional green color band that is 1 to 2 mm wide shall be located above the capacity color code marking to identify the existence of heparin content in the pipet. The location of these color bands shall be as specified in Fig. 1.

6.6.2 *Type II, Uncoated*—The pipet shall be identified for capacity with a purple color code marking on each pipet that is 2 to 6 mm wide. An additional green color band that is 1 to 2 mm wide shall be located above the capacity color code marking to identify the existence of heparin content in the pipet. The location of these color bands shall be as specified in Fig. 1

7. Workmanship

7.1 The pipets shall be free of defects that will detract from their appearance or may impair their serviceability. The pipets shall be free of significant foreign matter, loose or embedded lint or chips that affect the bore, or stains when viewed under normal room lighting. Type I, heparinized pipets, may appear cloudy. This is a phenomena prevalent with drying of sodium heparin within a capillary tube and will not affect the functional use of the pipet.

7.2 The calibration line and color codes on Type I and Type II pipets shall be applied to the glass pipet at locations specified in Fig. 1. The calibration line shall be sufficiently deposited on the glass to enable the setting of a meniscus, and the color band shall be sufficiently deposited on the glass to identify the pipet as to its stated volume.

8. Reading and Setting the Meniscus

8.1 *Reading a Water Meniscus*—For all pipets, the reading is made on the lowest point of the meniscus. In order that the lowest point may be observed, it is necessary to place a shade of some dark material immediately below and behind the meniscus, which renders the profile of the meniscus dark and clearly visible against a light background.

8.1.1 *Setting a Water Meniscus*—Setting of the meniscus shall be performed by one of the following methods. Wherever practical, the meniscus should descend to the position of setting.

8.1.1.1 *Method A*—The position of the lowest point of the meniscus with reference to the graduation line is horizontally tangent to the plane of the *upper edge of the graduation line.* The position of the meniscus is obtained by having the eye in the same plane of the upper edge of the graduation line.

8.1.1.2 *Method B*—The position of the lowest point of the meniscus with reference to the graduation line is such that it is in the plane of the *middle of the graduation line.* This position of the meniscus is obtained by making the setting in the center of the ellipse formed by the graduation line on the front and the back of the tube as observed by having the eye slightly below the plane of the graduation line. The setting is accurate if, as the eye is raised and the ellipse narrows, the lowest point of the meniscus remains midway between the front and rear portions of the graduation line. By this method, it is possible to observe the approach of the meniscus from either above or below the line to its proper setting.