Designation: E672 – 87 (Reapproved 2011)

Standard Specification for Disposable Glass Micropipets[1]

This standard is issued under the fixed designation E672; 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 two different types of disposable micropipets, calibrated "to contain," used in measuring microlitre volumes of liquids.

1.2 The values stated in SI units are to be regarded as the standard.

1.3 The following precautionary caveat pertains only to paragraph 9.1.1 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:*[2]

E438 Specification for Glasses in Laboratory Apparatus

2.2 *ISO Standard:*[3]

R-1769 Color Coding for Pipets

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *accuracy*—the closeness of agreement between the nominal value and the mean volume, obtained by applying the test procedure specified in 9.4.1. It is quantified by the inaccuracy of the mean (bias).

3.1.2 *disposable micropipet*—such micropipets will only be expected to provide their specified performance during their original use or operation.

NOTE 1—The descriptions of "accuracy" and "repeatability" apply only in cases where the distributions are Gaussian.

3.1.3 *repeatability*—the closeness of agreement between the individual volumes obtained by applying the test procedure specified in 9.4.2. It is quantified by the imprecision.

4. Classification

4.1 This specification covers two different pipet designs as follows:

4.1.1 *Type I*—Disposable micropipets with calibration line and color coding (see Fig. 1 and Table 1).

4.1.2 *Type II*—Disposable micropipets void of markings (see Fig. 2 and Table 2).

NOTE 2—Type I pipets were originally specified by the Department of Defense under MIL-P-36722.

5. Materials and Manufacture

5.1 The pipets made to these specifications shall be fabricated from borosilicate glass, Type I, Class A or B or soda lime glass, Type II, in accordance with Specification E438.

6. Dimensions and Permissible Variations

6.1 *Design*—Pipets shall be of one piece construction in accordance with Table 1 and Table 2 for shape, dimensions, and permissible variations. Any cross-section of the pipet, taken in a plane perpendicular to the longitudinal axis, shall be circular.

6.2 *Capacity*—The pipet capacity shall be stated on the package label, expressed as µL (microlitre); 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 tested for capacity as specified in 9.1. The unit, microlitre, µL, may be considered as equivalent to 0.001 cm^3.

6.2.1 *Accuracy* (see 4.1)—The accuracy shall be determined as specified in 9.4 and shall be within the limits given in Table 1 and Table 2.

6.2.2 *Coefficient of Variation* (see section 4.2)—The coefficient of variation shall be determined as specified in 9.4 and shall be within the limits given in Table 1 and Table 2.

6.3 *Capacity Mark*—Pipets in 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.

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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.

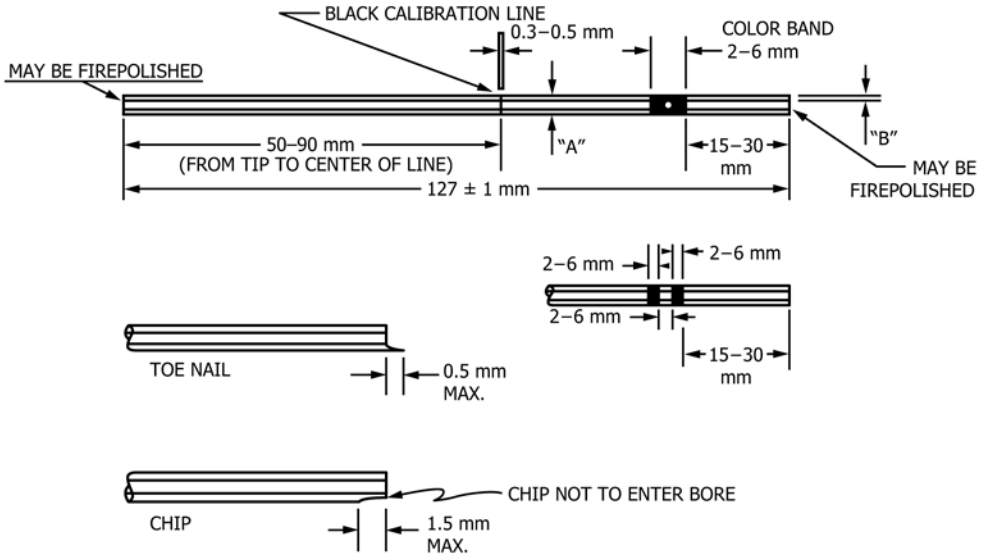

FIG. 1 Type I Pipet

TABLE 1 Dimensions for Type I Pipet

Stated Capacity, µL	Color Code	Minimum Diameter A, mm	Minimum Wall B, mm	Maximum Volumetric Deviation	
				Accuracy, %	Coefficient of Variation, %
5	white	1.0	0.35	±1.0	<1.5
10	orange	1.0	0.25	±0.5	<1.0
20	black	1.1	0.25	±0.5	<1.0
25	2 white	1.1	0.25	±0.5	<1.0
50	green	1.3	0.20	±0.5	<1.0
100	blue	1.6	0.20	±0.5	<1.0
200	red	2.2	0.20	±0.5	<1.0

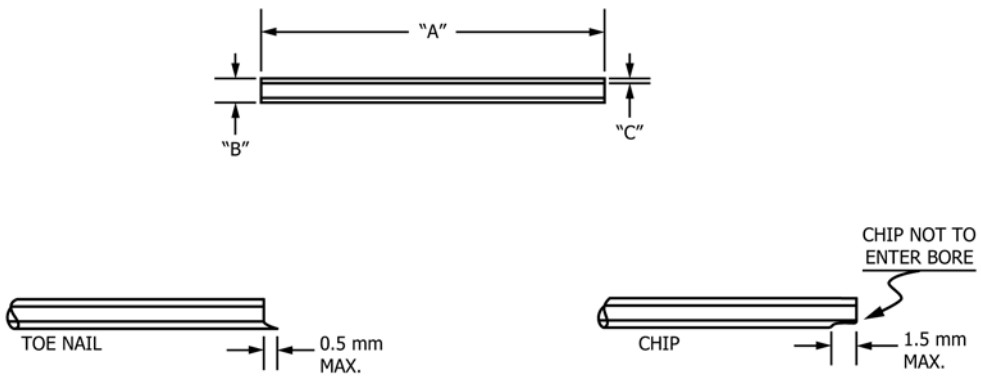

FIG. 2 Type II Pipet

6.4 *Identification Markings:*

6.4.1 *Type I*—The pipets in Fig. 1 shall be identified for capacity by a color code marking on each pipet consisting of one or two color bands (see Table 1). For a code consisting of one band, the band shall be from 2 to 6 mm wide; for a code consisting of two bands, each band shall be 2 to 6 mm wide and separated with a space of 2 to 6 mm. The color code band, or bands, shall completely encircle the pipet in a plane perpendicular to its longitudinal axis. The location of color band, or bands, shall be as specified in Fig. 1 with the selection of color, to designate capacity, according to ISO Recommendation R-1769.

6.4.2 *Type II*—Pipets in Fig. 2 are devoid of any markings and shall be identified for capacity on the package label.

7. Workmanship, Finish, and Appearance

7.1 The pipets in Fig. 1 and Fig. 2 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.

7.2 The calibration line and color code on Type I pipets shall be applied to the glass pipet at locations specified in Fig. 1. The

TABLE 2 Dimensions for Type II Pipet

Stated Capacity, µL	Minimum Length A, mm	Minimum Diameter B, mm	Minimum Wall C, mm	Maximum Volumetric Deviation	
				Accuracy, %	Coefficient of Variation, %
1	20	0.5	0.20	±1.5	<2.0
2	20	0.5	0.20	±1.2	<1.5
3	20	0.6	0.20	±1.2	<1.5
4	20	0.6	0.20	±1.2	<1.5
5	20	0.6	0.20	±1.2	<1.5
10	20	0.6	0.10	±1.0	<1.2
20	20	0.6	0.10	±1.0	<1.2
25	30	0.6	0.10	±1.0	<1.2
50	30	1.0	0.10	±1.0	<1.2
100	50	1.3	0.10	±1.0	<1.2

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 Liquid Meniscus* (Type I only)—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 Liquid 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.

8.2 *Reading a Mercury Meniscus* (Type I only)—For all pipets, the reading is made at the highest point of the meniscus. In order that the highest point may be observed, it is necessary to place a shade of some light material immediately above and behind the meniscus, which renders the profile of the meniscus dark and clearly visible against a light background.

8.2.1 *Setting a Mercury 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.

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

8.2.1.2 *Method B*—The position of the highest 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 above the plane of the graduation line. The setting is accurate if, as the eye is lowered and the ellipse narrows, the highest 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.

NOTE 3—The difference between meniscus positions resulting from the alternative methods of adjustment is the volume equivalent of one half the thickness of the graduation line. When working to the highest attainable accuracy, the difference between the two methods of adjustment is unlikely to exceed 0.4 % volumetric error from stated capacity and a correction can be calculated where necessary.

9. Testing

9.1 *Capacity (Single Pipet):*

9.1.1 *Type I (Using Mercury)*—Allow a dry pipet and a container of triple distilled mercury to stand at room temperature of 20 to 25°C for 2 h. Fill the pipet with mercury and adjust to the calibration line in accordance with 8.2 and 8.2.1. Discharge the mercury in the pipet into a clean tared dish, and reweigh the dish, together with the mercury content. Record the room temperature. From the recorded weight of the mercury discharged into the dish and the recorded temperature, calculate the volume of mercury (representing the observed capacity of the pipet) in accordance with 9.2 and Table X1.1.

9.1.2 *Type I (Using Water)*—Allow a dry pipet and a container of distilled water to stand at room temperature of 20 to 25°C for 2 h. Weigh the dry pipet and record the weight. Fill the same pipet with water and adjust to the calibration line in accordance with 8.1 and 8.1.1. Then reweigh the pipet with water content and record the weight. Record the room temperature. Subtract the recorded weight of the dry pipet from the recorded weight of the pipet filled with distilled water representing the apparent mass of the contained water. Calculate the volume, *V*, in accordance with 9.2 and Appendix X2.

9.1.3 *Type II*—Allow a dry pipet and a container of distilled water to stand at room temperature 20 to 25°C for 2 h. Weigh

the dry pipet and record the weight. Then fill the same pipet with distilled water, by capillary attraction with specific care to remove all water from the exterior of the pipet with a dry cloth or gauze. Then reweigh the pipet with water content, and record the weight. Record the room temperature. Subtract the recorded weight of the dry pipet from the recorded weight of the pipet filled with distilled water representing the apparent mass of the contained water. Calculate the volume, *V*, in accordance with 9.2 and Table X1.2.

NOTE 4—To accurately perform the test methods outlined in 9.1.1, 9.1.2, and 9.1.3, the reliability of the weighing instrument used should be confirmed against a known standard and the weighing instrument should possess a minimum sensitivity that does not exceed the following:

Stated Capacity	Minimum Sensitivity
1 to 5 µL	0.001 mg
10 to 200 µL	0.01 mg

9.2 *Calculations*—Calculate the volume, *V*, of a micropipet from the weighings, in air, using the following equation:

$$V = W \times Z \tag{1}$$

where:

W = apparent mass of liquid (mercury/water), weighed in air, and
Z = apparent specific volume, (mercury/water).

Values of *Z* for mercury and water are given in Appendixes X1 and X2, respectively.

9.3 *Capacity Deviation (Single Pipet)*—In accordance with the methods outlined in 9.1.1, 9.1.2, and 9.1.3, using either mercury or water, the capacity deviation is the difference between the stated capacity and the observed capacity of the pipet as follows:

$$\text{Capacity Deviation, \%} = \frac{(V_c - V_1) \times 100}{V_1} \tag{2}$$

$$V_c = \frac{V_t}{1 + a(t - 20°C)}$$

where:

V_t = observed volumetric capacity at t°C, µL,
V_c = corrected volumetric capacity at 20°C,
a = coefficient of cubical expansion of pipet glass; 0.000010/°C for Type I, Class A borosilicate; 0.000015/°C for Type I, Class B (noncorrosive borosilicate); and 0.000025/°C for Type II, (sodalime).
V_1 = stated capacity of pipet, and
t = temperature, °C.

9.4 *Capacity Deviation (Number of Pipets)*—Test a minimum of 30 Type I or Type II pipets, or both, taken at random from a completed manufactured production lot, in accordance with 9.1.1, 9.1.2, or 9.1.3. Calculate the volumetric deviation for the 30 pipets as follows:

9.4.1 *Accuracy:*

$$\text{Accuracy, \%} = \frac{100\,(\bar{x} - V_1)}{V_1} \tag{3}$$

where:

$\bar{x}$ = mean of sample measurements, and
V_1 = stated capacity of pipet.

9.4.2 *Coefficient of Variation:*

$$\text{Coefficient of Variation, \%} = \frac{100s}{\bar{x}} \tag{4}$$

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

where:

x = individual sample measurement,
$\bar{x}$ = mean of sample measurements, and
n = number of pipets measured.

10. Keywords

10.1 disposable; glass; micropipets

APPENDIX

(Nonmandatory Information)

X1. Density and Z Factor Tables

TABLE X1.1 Density and *Z* Factor for Mercury

Temperature, °C	Density, g/cm³	*Z*, cm³/g
20	13.546	0.07382
21	13.544	0.07383
22	13.541	0.07385
23	13.539	0.07386
24	13.536	0.07387
25	13.534	0.07389
26	13.531	0.07390
27	13.529	0.07391
28	13.527	0.07392
29	13.524	0.07394
30	13.522	0.07395

TABLE X1.2 Density and *Z* Factor for Water

Temperature, °C	Density, g/cm^3	*Z*, cm^3/g
20	0.99820	1.0029
21	0.99799	1.0031
22	0.99777	1.0033
23	0.99754	1.0035
24	0.99729	1.0037
25	0.99704	1.0040
26	0.99678	1.0042
27	0.99651	1.0045
28	0.99623	1.0047
29	0.99594	1.0051
30	0.99564	1.0054