



Designation: D5245 – 19

Standard Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses^{1,2}

This standard is issued under the fixed designation D5245; 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 In microbiology, clean glassware is crucial to ensure valid results. Previously used or new glassware must be thoroughly cleaned. Laboratory ware and equipment that are not chemically clean are responsible for considerable losses in personnel time and supplies in many laboratories. These losses may occur as down time when experiments clearly have been adversely affected and as invalid data that are often attributed to experimental error. Chemical contaminants that adversely affect experimental results are not always easily detected. This practice describes the procedures for producing chemically clean glassware.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For specific precautions, see Section 6, 5.7.3.1, and 8.3.1.

1.4 *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.*

¹ This practice is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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² A significant portion of this practice was taken from: Berg, G., Safferman, R. S., Dahling, D. R., Berman, D., and Hurst, C. J., *USEPA Manual of Methods for Virology*, EPA-600/4-84-013, Chapter 2, "Cleansing Laboratory Ware and Equipment, Environmental Monitoring and Support Laboratory—Cincinnati," USEPA, Cincinnati, OH.

2. Referenced Documents

2.1 *ASTM Standards*:³

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

3. Terminology

3.1 *Definitions*:

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

4. Significance and Use

4.1 This practice provides uniform guidance for cleaning the laboratory glassware, plasticware, and equipment used in routine microbiological analyses. However, tests that are extremely sensitive to toxic agents (such as virus assays) may require more stringent cleaning practices.²

5. Reagents

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.⁴ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

³ 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.

⁴ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

*A Summary of Changes section appears at the end of this standard

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean Type IV of Specification D1193.

5.3 *Bromothymol blue*, 0.4 %.

5.3.1 Bromothymol blue.

5.3.2 Sodium hydroxide solution, 240 g/L.

5.3.3 Adding 16 mL of a NaOH solution (240 g/L) to 0.1 g of bromothymol blue.

5.3.4 Dilute to 250 mL with Type III reagent water of Specification D1193.

5.4 *Detergent Solution*, for machine-washing glassware and equipment. Use according to manufacturer's instructions.

5.5 *Detergent Powder*, for hand-washing glassware and equipment.

NOTE 1—There now are effective biodegradable detergent products available that allow the laboratory to avoid acid cleaning of most if not all glassware.

5.6 *Nitric Acid* (1 + 9)—Pour 100 mL of concentrated HNO₃ slowly into 900 mL of water.

NOTE 2—To avoid dangerous splatters, always pour concentrated acid into water.

5.7 *Chromic Acid Solution*:

5.7.1 Sodium dichromate (K₂Cr₂O₇) or potassium dichromate (Na₂Cr₂O₇), 25 g.

5.7.2 Sulfuric acid, concentrated (36.8 N), 2.5 L.

5.7.3 To prepare chromic acid (1 + 9), dissolve 25 g of sodium dichromate or potassium dichromate in 2.5 L of concentrated sulfuric acid.

5.7.3.1 **Warning**—Chromic acid and nitric acid are capable of producing burns even when used in relatively dilute solutions. When working with these or with other acids, avoid inhalation of fumes. Protect eyes with safety goggles or with full-face mask. Protect clothing with acid-resistant laboratory coat or apron. If eyes are accidentally exposed to acid, immediately wash them with copious quantities of tap water for at least 15 min. Consult a physician immediately thereafter. If other parts of the body are exposed to acid, immediately remove clothing over exposed areas and flood with large volumes of tap water. Consult a physician immediately if affected area is large or if exposure has been lengthy. Subsequently, wash exposed areas of clothing with copious quantities of tap water. To avoid dangerous splatters, always add acid to water, not the reverse (see also precautions noted under Section 6).

NOTE 3—Chromic acid replacement⁵ is applicable.

6. Hazards

6.1 The analyst/technician must know and observe normal good laboratory practices and safety procedures required in a microbiology laboratory in preparing, using, and disposing of cultures, reagents, and materials, and while operating sterilization and other equipment and instrumentation.

⁵ The sole source of supply of the apparatus known to the committee at this time is Monostat Corp., 519 Eighth St., New York, NY 10018. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,¹ which you may attend.

6.2 Sterilize contaminated laboratory ware and equipment before cleaning.

6.3 Transport hazardous acids only in appropriate safety carriers.

6.4 See 8.3 and 8.4 for details on proper cleaning with acids and alkalis.

7. Cleaning Rules

7.1 Once detergent solution or acid used to clean a vessel has been rinsed away, do not touch lip or inside of vessel with hands. Detergent or acid on hands or gloves and even oil from clean skin are sources of contamination.

7.2 Do not allow soiled laboratory ware and equipment to dry. Soak glassware if cleaning is delayed.

7.3 Use only cold water for tap water rinsing. Hot water may contain grease or oil removed from plumbing. Use only cold water to wash laboratory ware heavily contaminated with proteinaceous material. Hot water may coagulate such material.

7.4 Inspect washed laboratory ware and equipment for cleanliness. Reclean by appropriate procedures. Check laboratory ware and equipment for cracks, chips, or other damage and replace.

7.5 Use nontoxic stainless steel, glass, nonbreakable plastic, or other nontoxic materials for plumbing that carries water. Do not use copper plumbing.

7.6 Use disposable glass and plasticware for pathogenic work and test conditions that severely soil or etch glassware.

8. Cleaning Procedures

8.1 *Machine Washing*—Equip washing machine with capability for delivering four water rinses. The water jets in some washing machines are not strong enough to reach all walls in tall vessels. This results in poor washing and rinsing. The water jets in other washing machines are too strong for test tubes and similar vessels and for many other narrow-necked vessels. Jets that are too powerful hold detergent and rinse water in place and do not allow them to drain properly. If washing machine is unable to wash or rinse adequately, use procedure described in 8.2.

8.1.1 Immerse washable vessels in detergent solution, and soak them overnight. If vessels are too large to immerse, fill them to brim with detergent solution, and soak them overnight.

8.1.2 Brush-wash vessels with hot (50 to 60°C) detergent solution. Hot tap water that exceeds 50°C is adequate for preparing detergent solution.

8.1.3 Machine-wash vessels. Follow manufacturer's instructions carefully. Add four water rinses if not included in manufacturer's instructions.

8.1.4 Drain and air dry vessels, or dry vessels in drying chamber.

8.1.5 Detergents used in washing may contain inhibitory substances. As necessary, test for the presence of inhibitory residues (for example, a new supply of detergent). Check clean laboratory ware and equipment for residues in accordance with