Designation: ~~D5245 – 92 (Reapproved 2012)~~ 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~~ 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 ~~the~~ 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 ~~56~~, ~~7.3.1~~5.7.3.1, and ~~Note 1~~*8.3.1* ~~*and Note 2*~~.

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.*

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.~~ 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

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[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.

Current edition approved ~~June 1, 2012~~April 1, 2019. Published ~~August 2012~~April 2019. Originally approved in 1992. Last previous edition approved in ~~2005~~2012 as D5245 – 92 ~~(2005).~~(2012). DOI: ~~10.1520/D5245-92R12.~~10.1520/D5245-19.

[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, Chapt. 2, "Cleansing Laboratory Ware and Equipment, Environmental Monitoring and Support Laboratory—Cincinnati," USEPA, Cincinnati, OH.~~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.

[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.

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

specifications are ~~available.~~ 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.

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. ~~Use them according to manufacturer's instructions. There now are a number of effective biogradable detergent products available that allow the laboratory to avoid acid cleaning of most if not all glassware.~~

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 HNO_3 slowly into 900 mL of water. ~~To avoid dangerous splatters, never pour water into concentrated acid.~~

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

5.7 *Chromic Acid ~~Solution~~—Solution:* ~~Chromic acid replacement [5] is applicable.~~
5.7.1 Sodium dichromate ($K_2Cr_2O_7$) or potassium dichromate ($Na_2Cr_2O_7$), 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 accidently 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 replacement[5] 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.

6.2 Sterilize contaminated laboratory ware and equipment before cleaning.

6.3 Transport hazardous acids only in appropriate safety carriers.

6.4 See ~~7.3~~8.3 and ~~7.4~~8.4 for details on proper cleaning with acids and ~~alkalies.~~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.

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

[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.

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 ~~7.2~~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 procedure given in ~~7.2.7~~8.2.7. This procedure is similar to that given in ~~*Footnote 7.*~~*Standard Methods for the Examination of Water and Wastewater.*[6]

8.2 *Manual Washing Procedure:*

8.2.1 Immerse vessels in detergent solution, and soak vessels overnight. Use fresh detergent solution daily. Solutions that are saved may become heavily contaminated with bacteria.

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

8.2.3 Swirl-rinse vessels ten times with cold tap water. To swirl-rinse, pour into the vessel a volume of tap water equal to about 10 % of the volume of the vessel, and swirl water around entire surface with each rinse. Swirl-rinse vessels five times with water.

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

8.2.5 *Test Tubes*—Test tubes may be washed by the procedure described in ~~7.1~~8.1, unless a washing machine is unavailable or washing machine jets are so powerful they do not allow adequate evacuation of tubes and thus interfere with washing and rinsing or by the following procedure.

8.2.5.1 Remove markings from tubes with solvent before washing.

8.2.5.2 Place test tubes open end up into covered wire basket, place basket into stainless steel or plastic vessel sufficient in size to allow complete immersion of tubes, and fill vessel with hot detergent solution.

8.2.5.3 Steam autoclave (100°C) immersed tubes for 30 min.

8.2.5.4 Empty vessel and tubes, and run cold tap water in to flush out detergent solution. Introduce tap water into bottom of vessel with a hose connected to tap. Wax pencil and other scum will wash over rim of vessel.

8.2.5.5 Fill and empty tubes in vessel ten times with cold tap water. Fill and empty tubes in vessel five times with water.

8.2.5.6 Drain and air dry tubes, or dry tubes in drying chamber.

8.2.5.7 Inspect, rewash if not clean, and use alternate cleaning method if appropriate. If glassware still does not meet requirements, discard.

8.2.6 *Pipets:*

8.2.6.1 Remove cotton plugs from pipets. If necessary, remove cotton plugs by forcing a jet of air or water through delivery tips of pipets.

8.2.6.2 Place pipets, with tips up, into pipet holder.

8.2.6.3 Place pipet holder into a pipet jar, and fill jar with hot (50 to 60°C) detergent solution. Hot tap water that exceeds 50°C is adequate for preparing detergent solution. Pipets must be completely immersed. If air bubbles are present in pipets, raise and lower pipet holder several times to remove bubbles.

8.2.6.4 Soak pipets in detergent solution for 24 h. Raise and lower pipet holder five or six times during the 24-h period to agitate detergent solution and help remove soil and debris from pipets.

8.2.6.5 Place pipet holder into automatic pipet washer, and rinse pipets through ten cycles of cold tap water.

[6] ~~*Standard Methods for the Examination of Water and Wastewater*, 17th Ed., American Public Health Association, Washington, DC, Section 9020B, 3.a, 2, 1989, pp. 9–8.~~*Standard Methods for the Examination of Water and Wastewater*, 17th ed., American Public Health Association, Washington, DC, Section 9020B, 3.a, 2, 1989, pp. 9–8.

8.2.6.6 Rinse pipets through five cycles of water.

8.2.6.7 Remove pipets from automatic pipet washer, and allow them to drain and air dry.

8.2.6.8 Plug pipets with cotton.

8.2.7 *Test Procedure for Suitability of Detergent Used in Washing:*

8.2.7.1 Wash and rinse six petri dishes in the usual manner. These are Group A.

8.2.7.2 After normal washing, rinse a second group of six petri dishes twelve times with successive portions of water. These are Group B.

8.2.7.3 Wash six petri dishes with the detergent wash water using detergent concentrations normally employed, and dry without rinsing. These are Group C.

8.2.7.4 Sterilize dishes in the usual manner.

8.2.7.5 Add the proper dilution (usually two different dilutions are used) of a water sample yielding 30 to 300 colonies to triplicate petri dishes from each group (A, B, and C). Proceed according to the heterotrophic plate count method.

8.2.7.6 Differences in bacterial counts of less than 15 % among all groups indicate the detergent has no toxicity or inhibitory effect. Differences in bacterial counts of 15 % or more between Groups A and B demonstrate that inhibitory residues are left on glassware after the normal washing procedure used. Disagreement in averages of less than 15 % between Groups A and B, and greater than 15 % between Groups A and C indicates that detergent used has inhibitory properties that are eliminated during routine washing.

8.2.8 *Automatic Pipetor (Brewer-Type):*

8.2.8.1 Immediately after pipettor has been used, fill reservoir with tap water and carefully pump sufficient water through the system to remove cellular debris and other materials that might adhere to apparatus. Determine whether syringe delivers properly without cannula connected.

8.2.8.2 Remove tubing from reservoir, and remove syringe from pipettor; autoclave valve, tubing, reservoir, and syringe at 121°C for 60 min.

8.2.8.3 Disassemble syringe, and remove cannula.

8.2.8.4 Cleanse syringe. Rinse plunger and barrel of syringe with copious quantities of cold tap water. Soak tubing overnight in water. Allow tubing to drain and air dry.

8.2.8.5 Fill reservoir with hot (50 to 60°C) detergent solution, and soak reservoir overnight. Hot tap water that exceeds 50°C is adequate for preparing detergent solution. Brush-wash reservoir with hot (50 to 60°C) detergent solution. If reservoir does not come clean, rinse it with tap water, and soak it overnight in HNO_3 (1 + 9) or in chromic acid (1 + 9). Then rinse reservoir ten times with cold tap water, swirl-rinse five times with water, and allow to drain and air dry.

8.2.8.6 *Valve*—If syringe has been delivering properly with the cannula removed, no further attention to valve is needed. If syringe has not been delivering properly with the cannula removed, remove valve from apparatus. Soak valve overnight in (1 + 9) HNO_3 or in chromic acid (1 + 9). Rinse copiously with cold tap water and reagent water. Allow to drain and air dry and return to apparatus.

8.2.8.7 Connect cannula to a clean syringe and force through 50 mL of water.

8.2.8.8 Rinse tubing copiously with cold tap water. If tubing does not come clean, place it in hot (50 to 60°C) detergent solution, remove air bubbles, and allow tubing to soak for ~~24 h.~~24 h.

8.3 *Cleaning With Acid:*

8.3.1 Use acid cleaning only when there is no alternative. Consider disposable glassware as a possible alternative. Chromic acid or HNO_3 (1 + 9) may be used to clean glassware. Ten percent HNO_3 requires longer contact (24 h) with tubes than chromic acid requires, but residual HNO_3 is not as likely to be toxic to microorganisms. (**Warning—**Do not expose metals or other materials to acids unless certain that those substances are acid-resistant. Chromic acid cleaning solutions[5] and other acids may react violently with organics or other oxidizable substances. Take care to avoid such reactions.)

~~NOTE 1—**Warning:** Do not expose metals or other materials to acids unless certain that those substances are acid-resistant. Chromic acid cleaning solutions[5] and other acids may react violently with organics or other oxidizable substances. Take care to avoid such reactions.~~

~~NOTE 2—**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 accidently 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 5).~~

8.3.2 *Chromic Acid Cleaning:*

8.3.2.1 Chromic acid should be used only when stubborn contaminants are not effectively removed by other cleaning reagents. Replacement products for chromic acid are offered by several manufacturers.

~~7.3.2.2 To prepare chromic acid (1 + 9), dissolve 25 g of sodium dichromate ($Na_2Cr_2O_7$) or potassium dichromate ($K_2Cr_2O_7$) in 2.5 L of concentrated sulfuric acid. Follow instructions in Note 2.~~

8.3.3 *Acid Cleaning Procedure:*

8.3.3.1 Rinse loose debris from vessels with tap water.

8.3.3.2 Wash glassware with detergent solution and rinse well with tap water.