Designation: D5245 – 92 (Reapproved 2012)

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 the 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 and health practices and determine the applicability of regulatory limitations prior to use.* For specific precautions, see Section 5, 7.3.1, and Note 1and Note 2.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1193 Specification for Reagent Water

3. Significance and Use

3.1 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]

4. Reagents

4.1 *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.

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

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

4.4 *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.

4.5 *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.

4.6 *Chromic Acid Solution*—Chromic acid replacement [5] is applicable.

5. Hazards

5.1 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

[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, Chapt. 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.

[4] "Reagent Chemicals, American Chemical Society Specifications, Am. Chemical Soc., 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."

[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.

cultures, reagents, and materials, and while operating sterilization and other equipment and instrumentation.

5.2 Sterilize contaminated laboratory ware and equipment before cleaning.

5.3 Transport hazardous acids only in appropriate safety carriers.

5.4 See 7.3 and 7.4 for details on proper cleaning with acids and alkalies.

6. Cleaning Rules

6.1 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.

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

6.3 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.

6.4 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.

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

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

7. Cleaning Procedures

7.1 *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.

7.1.1 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.

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

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

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

7.1.5 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. This procedure is similar to that given in Footnote 7.[6]

7.2 *Manual Washing Procedure:*

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

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

7.2.3 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.

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

7.2.5 *Test Tubes*—Test tubes may be washed by the procedure described in 7.1, 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.

7.2.5.1 Remove markings from tubes with solvent before washing.

7.2.5.2 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.

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

7.2.5.4 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.

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

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

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

7.2.6 *Pipets:*

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

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

7.2.6.3 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.

[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.

7.2.6.4 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.

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

7.2.6.6 Rinse pipets through five cycles of water.

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

7.2.6.8 Plug pipets with cotton.

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

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

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

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

7.2.7.4 Sterilize dishes in the usual manner.

7.2.7.5 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.

7.2.7.6 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.

7.2.8 *Automatic Pipetor (Brewer-Type):*

7.2.8.1 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.

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

7.2.8.3 Disassemble syringe, and remove cannula.

7.2.8.4 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.

7.2.8.5 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.

7.2.8.6 *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.

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

7.2.8.8 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.

7.3 *Cleaning With Acid:*

7.3.1 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.

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).

7.3.2 *Chromic Acid Cleaning:*

7.3.2.1 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.

7.3.3 *Acid Cleaning Procedure:*

7.3.3.1 Rinse loose debris from vessels with tap water.

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

7.3.3.3 Fill vessels to brim with nitric acid (1 + 9) or chromic acid (1 + 9). Small vessels may be immersed in a vat of acid. Do not allow acid to contact skin. When necessary wear acid-resistant gloves. Gloves must have nonskid surfaces, because acid makes vessels slippery.

7.3.3.4 Allow nitric acid (1 + 9) to remain in contact with vessel surface for 24 h. If chromic acid is used, allow acid to remain in contact about 5 min.

7.3.3.5 Neutralize nitric acid washings to pH 6 to 8 with NaOH solution (240 g/L) in ice water. Carefully pour neutralized acid down acid-resistant sewer drain, and flush with copious quantities of tap water.

7.3.3.6 Fill and empty vessel about ten times with cold tap water. Be certain that all acid is removed from outside of vessel. Swirl-rinse vessel five times with reagent water. To swirl-rinse, pour into the vessel a volume of water equal to about 10 % of the vessel volume, and swirl around entire surface with each rinse.

7.3.4 *Pipets:*

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

7.3.4.2 Place pipets, with tips up, into an acid-resistant plastic pipet holder.

7.3.4.3 Carefully place pipet holder into an acid-resistant pipet jar containing HNO_3 (1 + 9), chromic acid or replacement product.

7.3.4.4 Carefully raise and lower pipet holder several times to force air bubbles from pipets.

7.3.4.5 Soak pipets in acid for 24 h. Carefully raise and lower pipet holder five or six times during the 24-h period to agitate acid and thus help remove contaminants and debris from pipets. Carefully remove pipet holder from pipet jar and place holder and pipets in automatic pipet washer.

7.3.4.6 Take care to avoid acid splatter. Immediately rinse pipets through ten cycles of cold tap water. Do not allow acid dripping from pipets to remain in contact with metal parts of automatic pipet washer. Acid may damage metal. Rinse pipets through seven cycles of reagent water.

7.3.4.7 Remove pipets from automatic washer, and allow pipets to drain and air dry.

7.3.5 *Test for Acid Residues on Laboratory Ware:*

7.3.5.1 Select several pieces of clean glassware or plasticware for testing.

7.3.5.2 Add a few drops of 0.04 % bromthymol blue into each of the selected pieces.

7.3.5.3 Prepare 0.04 % bromthymol blue indicator by adding 16 mL of a NaOH solution (240 g/L) to 0.1 g of bromthymol blue and dilute to 250 mL with Type III reagent water.

7.3.5.4 Observe color reaction. Color reaction should be blue-green in the neutral pH range. A yellow color reaction identifies the presence of an acid residue. The presence of an alkaline residue is distinguished by a blue color reaction.

7.4 *Cleansing with Alkalies*—Alkalies such as sodium metasilicate, trisodium phosphate, sodium carbonate, and soft soaps can be used to cleanse laboratory ware and equipment. Alkalies, however, tend to etch the glassware much more than acids.

8. Waste Disposal Guidelines

8.1 Waste disposal measures must be taken to protect workers, the public, and the environment. Emphasis should be placed on recycling both nonhazardous and decontaminated hazardous wastes. Where possible, reduce waste at the generating source by reuse of materials.

8.1.1 *Disposal of Infectious Supplies:*

8.1.1.1 Place solid contaminated waste materials in plastic bag certified for infectious waste use by the manufacturer. Waste materials include clothing, towels, and plastic and unbroken glass laboratory ware. Plastic bags must be autoclavable, leakage and tear resistant, and imprinted with biohazard symbol.

8.1.1.2 Place contaminated sharp waste materials in rigid container certified for sharp material use by manufacturer. Sharp waste material include hypodermic needles, broken glass, and other laboratory ware with cutting or piercing edges. Sharp waste containers must be autoclavable, rigid, puncture and leakage resistant, and marked with a biohazard symbol.

8.1.1.3 Sterilize filled plastic bags or sharp waste containers. Use care while handling bags so as not to puncture them. Containers should be closed tightly to prevent contamination of workers while handling them.

8.1.1.4 If bags or containers must be moved from laboratory area for decontamination, they must be securely sealed to prevent leakage and transported in rigid, sturdy outer containers that are taped closed. The outer containers must be properly labelled with the biohazard symbol and the location and name of the person disposing of the infectious waste.

8.1.1.5 After decontamination, label plastic bags or containers “nonhazardous” and dispose of in appropriate waste receptacle.

8.2 *Disposal of Agar Media:*

8.2.1 Place used culture media in autoclavable, leakage- and tear-resistant plastic bags imprinted with biohazard symbol. Place plastic bags in a durable leakproof receptacle. Containers to be recycled may be placed in a metal pan. Autoclave or otherwise sterilize bags and containers. Flush spent agar from containers into sewer system with hot tap water.

8.2.2 If bags are moved from laboratory area for decontamination, seal to prevent leakage and transport in rigid, sturdy outer containers that are taped closed. The outer containers must be properly labelled with the biohazard symbol and the location and name of the person disposing of the infectious waste.

8.2.3 After decontamination, label plastic bags “nonhazardous.”

8.2.4 Dispose of waste in appropriate trash receptacle.

8.3 *Disposal of Liquid Media*—Autoclave or otherwise sterilize the liquid media. Flush decontaminated spent liquid media into sewer system with large amounts of tap water.

8.4 *Disposal of Nonhazardous Waste:*

8.4.1 *Glassware:*

8.4.1.1 Place broken or disposable glassware in plastic-lined puncture-resistant container.

8.4.1.2 Minimize waste production by recycling waste glassware whenever possible; otherwise dispose of waste in appropriate trash receptacle.

8.4.2 *Plasticware:*

8.4.2.1 Place disposable plastic laboratory ware in plastic-lined trash can.

8.4.2.2 Minimize waste production by recycling disposable plastic laboratory ware whenever possible; otherwise dispose of waste in appropriate trash receptacle.

8.5 *Disposal of Chemical Wastes:*

8.5.1 *Hazardous Chemicals:*

8.5.1.1 Collect chemical in a suitable container. Container must be sturdy, nonleaking, and sealable.

8.5.1.2 Label container with the name of the chemical waste, its hazardous properties (for example, corrosive, caustic, toxic, flammable, irritant), quantity, date of disposal, and location and name of person disposing of the waste.

8.5.1.3 Transfer chemical to hazardous waste storage facility for ultimate disposition by safety- or waste-control officer.

8.6 *Acids:*

8.6.1 Potassium and sodium dichromate are strong oxidizing agents and must be handled cautiously. Spent chromic acid, being a hazardous waste, is not disposed of by conventional means. Contact safety- or waste-control officer for waste management guidelines for its safe disposal.

8.6.2 Sulfuric and nitric acid must be neutralized before disposal into sewage system. Neutralize to a pH of 6 to 8, by adding acid to a large volume a sodium hydroxide solution in ice water. For concentrated acid use NaOH solution (240 g/L). Flush the neutralized solution down the drain with a copious flow of tap water.

9. Keywords

9.1 acid cleaning; alkali-cleaning; cleaning glassware; cleaning plasticware; glasswasher; laboratory waste disposal