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An American National Standard

Standard Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses[1, 2]

This standard is issued under the fixed designation D 5245; 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 practice does not purport to address all of the safety problems, 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 1 and Note 2.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water [3]

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 D 1193.

4.3 *Detergent Solution*, for machine-washing glassware and equipment. [5] 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 [6] 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 cultures, reagents, and materials, and while operating sterilization and other equipment and instrumentation.

5.2 Sterilize contaminated laboratory ware and equipment before cleaning.

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[1] This practice is under the jurisdiction of ASTM Committee D-19 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] *Annual Book of ASTM Standards*, Vol 11.01.

[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] MIR-A-KOL, available from Diversey Corp., 255 East Fifth St., Cincinnati, OH 45202, or equivalent.

[6] Chromerge, a registered trademark of Monostat Corp., 519 Eighth St., New York, NY 10018 has been found suitable for this purpose.

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.[7]

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

[7] *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.