Designation: D 5244 – 92 (Reapproved 1998)

Standard Practice for Recovery of Enteroviruses from Waters [1]

This standard is issued under the fixed designation D 5244; 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 practice covers a uniform procedure for the concentration of viruses from collected samples.

1.2 This practice describes a virus adsorption-elution cartridge filter procedure for recovering viruses from drinking water. Volumes of 400 L or more are processed for samples of drinking water quality.

1.3 The principles of this practice are also applicable to sewages, effluents, and surface waters without technical modifications.

1.4 Although specifically designed for recovery of human enteroviruses, this practice also may be applied to some other human enteric viruses, that have to be determined by specific testing.

1.5 The consistency of this practice was determined from method evaluation studies with poliovirus-seeded drinking water samples.

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

1.7 *This standard 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.* Only adequately trained personnel should be allowed to perform these procedures and should use safety precautions recommended by the U.S. Public Health Service Center for Disease Control for work with potentially hazardous biological organisms. [2]

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water [3]

D 1193 Specification for Reagent Water [3]

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this practice, refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *cell monolayer*—a single layer of cells grown on a glass or plastic surface to which they are securely attached.

3.2.2 *enterovirus*—a genus of the family *Picornaviridae*. Members of this genus are 22 to 30 nm in diameter, contain a positive single-stranded RNA, are stable under acid conditions and are resistant to ether. Included in this genus are poliovirus, coxsackievirus, and echovirus.

3.2.3 *enteric virus*—a general term denoting a virus that normally enters by the oral route, is capable of multiplying in cells of the alimentary canal and is found in stool specimens. In addition to the enterovirus, included under this general term are such agents as adenovirus, rotavirus, Norwalk virus, astrovirus, and calicivirus.

3.2.4 *plaque*—an area of clearing caused by the cytopathic effects of virus on a susceptible cell monolayer.

4. Summary of Practice

4.1 A commercially available negatively charged cartridge-type filter is used to recover low levels of virus from water. The viruses adsorbed to this filter matrix are released by passage of beef extract-glycine reagent (pH 9.0) through the filter. The eluted viruses are further concentrated by organic flocculation. This consists of lowering the pH of the beef extract to 3.5, separating the resulting floc, and solubilizing the floc in a relatively small volume of phosphate solution to release the bound viruses.

5. Significance and Use

5.1 Enteric viruses of public health significance are present in the aquatic environment.

5.2 Enteric viruses have been detected in treated water supplies.

5.3 Enteric viruses are responsible for a wide range of illnesses, ranging from hepatitis to gastroenteritis.

5.4 This practice is applicable to the recovery of many plaque-forming enteric viruses from waters when used in conjunction with cell culture assay systems.

5.5 The principles of this practice are applicable without technical modifications for monitoring for viruses based on the use of gene probe technology.

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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] *Biological Safety in Microbiological and Biomedical Laboratories*, Richardson, J. H., and Barkley, W. E., Eds., U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and National Institutes of Health, HHS Publication No. (NIH) 88-8395, 2nd Ed, May 1988.

[3] *Annual Book of ASTM Standards*, Vol 11.01.

6. Apparatus

6.1 *Holder*, for 10-in. (25.4 cm) cartridge filter.

6.2 *Filterite* [4] *Cartridge Filter*, negatively charged pleated fiberglass, 10-in. (25.4 cm), 0.45-µm pore size.

6.3 *pH Meter*, measuring to an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

6.4 *Magnetic Stirrer*, with stir bars.

6.5 *Positive Pressure Source*, equipped with a pressure gage. Deliver to filter holder no more pressure than recommended by the manufacturer (5.3 kg/cm ^{2}). Do not exceed a pressure of 0.4 kg/cm ^{2} when eluting viruses so that buffered 3 % beef extract solution passes through cartridge filter slowly thereby maximizing elution contact period.

6.6 *Dispensing Pressure Vessel*.

6.7 *Refrigerated Centrifuge*, capable of attaining 2500 × *g*.

7. Reagents and Materials

7.1 *Purity of Reagent*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the committee on Analytical Reagents of the American Chemical Society. [5] 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.

7.2 *Purity of Water*— Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type II of Specification D 1193.

7.3 *Aluminum Chloride Solution*—Dissolve 100 g of $AlCl_3 \cdot 6H_2O$ in water and dilute solution to 1 L.

7.4 *Beef Extract Powder*—Capable of floccing at pH 3.5.

7.5 *Sodium Phosphate Solution* (0.15 *M)*—Dissolve 40.21 g of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 7H_2O$) in water and dilute solution to 1 L.

7.6 *Glycine*.

7.7 *Hydrochloric Acid* (1 + 9)—Add one volume of concentrated HCl (sp gr 1.19) to nine volumes of water.

7.8 *Sodium Hydroxide Solution* (40 g/L)—Dissolve 40 g of NaOH in water and dilute solution to 1 L.

7.9 *Sodium Thiosulfate Solution*—Dissolve 100 g of $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute solution to 1 L.

7.10 *Buffered 3 % Beef Extract Reagent*:

7.10.1 Dissolve 60 g of beef extract powder and 7.5 g of glycine in 2 L of water.

7.10.2 Autoclave beef extract solution at 121°C for 20 min.

7.10.3 Adjust to pH 9 with NaOH solution (40 g/L).

8. Procedure

8.1 *Conditioning of Sample*:

8.1.1 Dechlorinate water, if necessary, with 0.8 mL of sodium thiosulfate solution for each litre of test sample to be collected.

8.1.2 Acidify test sample to pH 3.5 with HCl (1 + 9). Rapidly mix acid into sample to prevent pH levels from becoming sufficiently low in parts to inactivate viruses.

8.1.3 Condition each litre of acidified test sample with 1.2 mL of aluminum chloride solution. Final concentration of $AlCl_3$ is 0.0005 M.

8.2 *Adsorption of Viruses*:

8.2.1 Force the pH adjusted test sample from 8.1.3 through a 0.45-µm pleated fiberglass Filterite [4] cartridge filter.

8.2.2 Drain remaining water from cartridge filter holder.

8.3 *Elution of Viruses*:

8.3.1 Force 1600 mL of buffered 3 % beef extract reagent through cartridge filter and collect in a beaker.

8.3.2 Drain remaining buffered beef extract reagent from cartridge filter holder and add to beaker.

8.4 *Concentration of Viruses*:

8.4.1 Mix recovered beef extract on magnetic stirrer. Mix at a speed sufficient to develop vortex, but not so fast as to create excessive foaming.

8.4.2 While mixing, add HCl (1 + 9) until pH reaches 3.5.

8.4.3 Mix forming floc for 30 min.

8.4.4 Centrifuge flocced beef extract suspension in refrigerated centrifuge (4°C) for 15 min at 2500 × *g*.

8.4.5 Pour supernate into graduated cylinder and record volume.

8.4.6 Discard supernate.

8.4.7 Add 0.5 mL of sodium phosphate solution to floc for each 10 mL of supernate recorded.

8.4.8 Mix floc on magnetic stirrer until floc has dissolved completely.

8.4.9 Refrigerate concentrate immediately at 4°C if assay will be undertaken within 8 h, otherwise store concentrate immediately at − 70°C.

8.5 *Viral Assay*:

8.5.1 Accomplish virus quantitation by plaque assay procedure described in the *USEPA Manual of Methods for Virology.* [6] This plaque assay procedure has not been round-robin tested at this time by ASTM.

8.5.2 For data on the quantitation of viruses using this practice, see publication by Melnick et al. [7]

9. Keywords

9.1 adsorption; cartridge filter; concentration; elution; enteric viruses; enteroviruses; human pathogens; water monitoring; water quality

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[4] The cartridge Filterite filter (product no. DFN 0.45-10AN, available from Filterite Division of Memtec America Corp., 2033 Greenspring Drive, Timonium, MD 21093), or equivalent, has been found suitable for this purpose.

[5] "Reagent Chemicals, American Chemical Society Specifications," Am. Chem. 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."

[6] Berg, G., Safferman, R. S., Dahling, D. R., Berman, D., and Hurst, C. J., *USEPA Manual of Methods for Virology*, Publication no. EPA-600/4-84-013 (Chapters 9 and 10, revised), U.S. Environmental Protection Agency, Cincinnati, OH 45268, 1984.

[7] Melnick, J. L., Safferman, R., Rao, V. C., Goyal, S., Berg, G., Dahling, D. R., Wright, B. A., Akin, E., Stetler, R., Sorber, C., Moore, B., Sobsey, M. D., Moore, R., Lewis, A. L., and Wellings, F. M., Round Robin Investigation of Methods for the Recovery of Poliovirus from Drinking Water, *Applied and Environmental Microbiology* 47:144–150, 1984.