Designation: D 5244 – 92 (Reapproved 2004)

An American National Standard

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 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.* 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 *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.

3.2.3 *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.

3.2.4 *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.

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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.

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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] 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.