



Designation: D4994 – 24

Standard Practice for Recovery of Replicable Viruses from Municipal Wastewater Sludges¹

This standard is issued under the fixed designation D4994; 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 is used for the recovery of viruses from municipal wastewater sludges and favors the enteroviruses of human origin.

1.2 Both procedures are applicable to raw, digested, and dewatered sludges.

Procedure A—Adsorption
Procedure B—Sonication

Sections

7 to 11
12 to 16

1.3 This practice was tested on standardized sludges as described in 11.1. It is the user's responsibility to ensure the validity of this practice for untested matrices.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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.*

1.6 *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,² for work with potentially hazardous biological organisms.*

1.7 *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 Although many laboratories are presently isolating viruses from sludge, a valid comparison of data generated has not been possible because of the lack of a standard test method(s).

5. Apparatus

5.1 *Centrifuge(s)*, refrigerated, capable of attaining $10\,000 \times g$, screw-capped 100 mL centrifuge bottles that can withstand $10\,000 \times g$, and 250 mL screw-capped centrifuge bottles capable of withstanding $2500 \times g$.

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

5.3 *Filter Apparatus*, for membrane filtration,^{4,5} 0.2 μm , with 47 mm diameter filter holder and 50 mL slip-tip syringe (see 7.7 for type of filter material).

6. Purity of Reagents

6.1 *Purity of Reagents*—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,

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 Richardson, J. H., and Barkley, W. E., *Biological Safety in Microbiological and Biomedical Laboratories*, 2nd edition, U.S. Dept. of Health and Human Services, Public Health Service, Center for Disease Control, and National Institutes of Health and Human Services, 1988.

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at www.astm.org/contact. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

⁴ The sole source of supply of the apparatus, Swinnex filter (No. SX0047000), known to the committee at this time is Millipore Corp., 80 Ashby Rd., Bedford, MA 01730.

⁵ 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.

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