



Standard Test Method for Coliphages in Water¹

This standard is issued under the fixed designation D 4201; 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 approval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of coliphages infective for *E. coli* C in water. The test method is simple, inexpensive, and yields rapid water quality data. Its sensitivity is limited to 5 coliphages per 100 mL of water sample. This test method is applicable to natural fresh water samples.

1.2 *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.*

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water²

D 1193 Specification for Reagent Water²

D 3370 Practices for Sampling Water from Closed Conduits²

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *bacterial lawn*—confluent growth of bacteria.

3.2.2 *coliphage*—as used in this procedure, bacterial virus capable of replication using a specific strain of coliform bacteria (*E. coli* C) as a host.

4. Summary of Test Method

4.1 A measured water sample is added to a tube of melted modified nutrient agar. An *E. coli* C host culture is added to the tube, and the contents of the tube are mixed and poured into a petri dish. The dish is incubated at 35°C. The coliphages present in the water sample lyse the bacteria and form plaques; the total number of plaques represents the number of coliphages in the volume of water sample tested.

¹ This method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

Current edition approved May 10, 1996. Published July 1996. Originally published as D 4201 – 82. Last previous edition D 4201 – 82 (1989).

² *Annual Book of ASTM Standards*, Vol 11.01.

5. Significance and Use

5.1 Coliphage organisms may serve as indicators of fecal pollution. The presence of coliphages in water in the absence of a disinfectant indicates the probable presence of fecal contamination, but the absolute relationship between the number of coliforms and coliphages in natural waters has not been demonstrated conclusively.^{3, 4, 5}

5.2 The detection of coliphages in a water sample depends upon the use of a sensitive-host strain in the coliphage assay. Coliphages may be detected in 4 to 6 h to provide important same-day information on the sanitary quality of a water. The lower detection limit is 5 coliphages per 100 mL of fresh water sample.

6. Interferences

6.1 High salt concentrations, such as those found in saline or brackish water, interfere in this test method by inhibiting plaque formation.

6.2 Analysis for coliphages can be performed on disinfected waters. However, the quantitative relationship between coliphages and coliform bacteria is different from that observed in natural fresh waters. This difference is due to variations in the survival rates of coliphages and coliform bacteria exposed to disinfectants. For example, coliphages may have been shown to be more resistant to chlorine disinfection than fecal or total coliforms.⁶

7. Apparatus

7.1 *Water Bath*, 44.5 ± 0.2°C.

7.2 *Incubator*, 35 ± 0.5°C.

7.3 *Balance*.

7.4 *Petri Dishes*, sterile, 100 by 15-mm.

7.5 *Pipets*, plugged, sterile, 1-mL and 5-mL.

³ Kenard, R.P., and Valentine, R.S., "Rapid Determination of the Presence of Enteric Bacteria in Water," *Applied Microbiology*, Vol 27, 1974, p. 484.

⁴ Scarpino, P.V., "Bacteriophage Indicators," Berg, G., editor, *Indicators of Viruses in Water and Food*, Ann Arbor Science, Ann Arbor, Mich., 1978, p. 201.

⁵ Kott, Y., Ari, B., and Buras, N., "The Fate of Viruses in a Marine Environment," *Proceedings 4th International Conference on Water Pollution Research*, Jenkins, S.H., editor, Pergamon Press, Oxford, 1969, p. 823.

⁶ Kott, Y., Roze, N., Sperber, S., and Betzer, N., "Bacteriophages as Viral Pollution Indicators," *Water Research*, Vol 8, p. 165.