



Standard Test Method for Low Levels of Coliphages in Water¹

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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 an indication of water quality within 6.5 h. This coliphage method can determine coliphages in water down to 1 coliphage per volume of water sampled.

1.2 The test method is applicable to natural fresh water samples and to settled, filtered or finished water samples.

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

1.4 *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:*²

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D3370 Practices for Sampling Water from Closed Conduits

D4201 Test Method for Coliphages in Water (Withdrawn 2005)³

3. Terminology

3.1 For definitions of terms used in this test method, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *bacterial lawn*, *n*—confluent growth of bacteria cultured on an agar plate.

3.2.2 *coliphage*, *n*—bacterial virus capable of plaquing on the wide-range *E. coli* host strain used in this assay.

3.2.3 *plaque*, *n*—the circular zone of clearing (lysis) of the visible growth of bacteria on a one or two layer agar plate, caused by the action of one or more bacteriophage.

3.2.4 *plaque forming unit (PFU)*, *n*—the term used to report the number of plaques formed on an agar culture plate previously seeded with a microorganism susceptible to a bacteriophage. Although theoretically, each plaque develops from the action of a single bacteriophage, microbiologists use the term, PFU, to acknowledge that a plaque may have been formed from the action of two or more bacteriophage in close proximity, which is indistinguishable from that formed by a single phage.

4. Summary of Test Method

4.1 A measured water sample is adjusted to pH 6.0 with HCl or NaOH and filtered through a positively-charged filter. The coliphages trapped in the filter are eluted with Trypticase Soy Broth (TSB) at pH 8.5. The total eluate is divided between four Tubes of melted modified nutrient agar (MNA) and *E. coli* C host culture is added to each tube. The contents of each-tube are mixed and poured into a petri plate. The plates are incubated at 35°C for 6 h. The coliphages present infect the host bacteria and form plaques. The total number of plaques on the four plates represents the number of coliphages in the volume of water sample filtered.

5. Significance and Use

5.1 Coliphage organisms may serve as indicators of fecal contamination. The presence of coliphages in water in the absence of a disinfectant indicates the probable presence of fecal contamination. The absolute relationship between the number of coliforms and coliphages in natural waters has not been conclusively demonstrated. Coliphages are generally more resistant than coliforms to chlorination and may have some advantage over coliforms as an indicator of treatment efficiency in disinfected waters. 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 by this concentration procedure in 6.5 h to provide important same-day information on the sanitary quality of water. The lower detection limit of this concentration procedure is 1 coliphage per volume of water sample tested.

¹ This test method 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 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.

³ The last approved version of this historical standard is referenced on www.astm.org.