concentration) per liter only after tempering (44-46 °C) the medium. Final pH must be 6.95 ± 0.2. Pipet the medium into 9- × 50-mm petri dishes (5 mL/plate). Store plates inverted, refrigerated and in the dark for up to 2 weeks.

c. MI broth: Use same ingredients as MI agar, but omit the agar. Prepare and sterilize, and add cefsulodin after cooling by the methods described for MI agar. Alternately, the broth can be filter-sterilized. The final pH must be 7.1 ± 0.2. Place absorbent pads in 9- × 50-mm petri dishes and saturate with 2- to 3-mL MI broth containing 5 µg/mL cefsulodin. Store plates refrigerated in the dark, and discard after 96 h. Pour off excess broth just before using plates.

3. Procedure

a. Selection of sample size: See 9222 B.4*a*.

b. Sterile filtration units: See 9222 B.4*b*.

c. Filtration of sample: See 9222 B.4*c*. Incubate agar plates inverted and plates with a medium-saturated pad grid-side up at 35 ± 0.5 for up to 24 hours.

d. Counting: To count colonies on membrane filters, use a low-powered (10× to 15× magnification) binocular wide-field dissecting microscope or other optical device with a cool white fluorescent light source directed to provide optimal viewing. Count all blue colonies on each MI plate under ambient light and record as *E. coli* results. Expose each MI plate to long-wave UV light (366 nm), and count all fluorescent colonies [blue-green fluorescent *E. coli,* blue-white fluorescent coliforms other than *E. coli,* and blue-green with fluorescent edges (also *E. coli)*] to obtain the total coliform count. Record the data. If any blue, nonfluorescent colonies are found on the same plate, add their total to the total coliform count.

Calculate the final values using the following formulas:

$$E.coli/100\,\text{mL} = \frac{\text{no. of blue colonies}}{\text{volume of sample filtered (mL)}} \times 100$$

$$\text{TC}/100\text{ mL} = \frac{\text{no. of fluorescent colonies} + \text{no. of blue, nonfluoresent colonies (if any)}}{\text{volume of sample filtered (mL)}} \times 100$$

e. Coliform confirmation: For drinking water, a total coliform colony count is not specifically needed; however, confirm the presence of total coliform colonies (several typical or atypical colonies or swabbing of membrane surface colonies). For waters other than drinking water, confirm at a frequency established by the laboratory (see Section 9020 B.10). Laboratories may incorporate more stringent QC measures (e.g., confirm at least one colony from each typical or atypical colony type from a given membrane filter culture, confirm 10% of positive samples) based on need and sample type (see Section 9020 B.10). Adjust counts based on confirmation results. Confirmation tests are listed in 9222 B.4*g*.

4. Calculation of Coliform Density

See 9222 B.5.

References

1. Brenner KP, Rankin CC, Roybal YR, Stelma GN Jr, Scarpino PV, Dufour AP. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. Appl Environ Microbiol. 1993;59(11):3534–3544.
2. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI medium); EPA 821-R-02-024. Washington DC: Office of Water, U.S. Environmental Protection Agency; 2002.
3. Brenner KP, Rankin CC, Sivaganesan M, Scarpino PV. Comparison of the recoveries of *Escherichia coli* and total coliforms from drinking water by the MI agar method and the U.S. Environmental Protection Agency-approved membrane filter method. Appl Environ Microbiol. 1996;62(1):203–208.

9223

Enzyme Substrate Coliform Test

Approved by Standard Methods Committee, 2016. Joint Task Group: Jennifer Best (chair), Bennie L. Cockerel, Jr., Gil Dichter, Nancy H. Hall, William W. Northeimer, Viola Reynolds, Helena Solo-Gabriele.

9223 A. Introduction

Enzyme substrate tests use hydrolyzable chromogenic and fluorogenic substrates to simultaneously detect enzymes produced by total coliforms and *Escherichia coli.* In this method, total coliform bacteria produce the enzyme β-D-galactosidase, which cleaves the chromogenic substrate in the medium to release chromogen. Most *E. coli* strains produce the enzyme β-glucuronidase, which cleaves a fluorogenic substrate in the medium to release fluorogen. The release of chromogen indicates that coliform bacteria are present, and the release of fluorogen indicates that *E. coli* are present.

Multiple-tube, multiwell, or presence-absence (single 100-mL sample) formats are available for use with these enzyme substrate tests.

1. Principle

a. Total coliform bacteria: Colilert, Colilert-18, and Colisure media use the chromogenic substrates *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and chlorophenol red-β-D-galactopyranoside (CPRG), respectively, to detect the enzyme β-D-galactosidase, which is produced by total coliform bacteria. The β-D-galactosidase enzyme hydrolyzes the chromogenic substrate that produces a color change, thereby indicating the presence of total coliforms without additional procedures.

Although noncoliform bacteria (e.g., *Aeromonas, Flavobacterium,* and *Pseudomonas* species) may produce small amounts of the enzyme β-D-galactosidase, the growth of these organisms is suppressed so they generally will not produce a false-positive result unless $>10^6$ CFU/100 mL are present.

b. Escherichia coli: The fluorogenic substrate 4-methylumbelliferyl β-D-glucuronide (MUG) is used to detect the enzyme β-D-glucuronidase, which is produced by most strains of *E. coli.* The β-D-glucuronidase enzyme hydrolyzes the fluorogenic substrate that produces bluish fluorescence when viewed under long-wavelength (365-366 nm) ultraviolet (UV) light. Together, the color change (due to β-D-galactosidase) and the fluorescence (due to β-D-glucuronidase) indicate that a sample contains *E. coli.*

Large numbers of some bacteria or strains of bacteria (e.g., some strains of *Shigella* and *Salmonella* spp.) may cause a sample to fluoresce but will not change its color because they lack β-D-galactosidase. Such samples would be considered negative for *E. coli.*

2. Applications

These enzyme substrate coliform tests are recommended for the analysis of drinking water, source water, groundwater, and wastewater samples. If laboratory personnel have not used this method before, conduct parallel testing (including seasonal variations) with the existing method to assess site-specific effectiveness and to compare results. The results of many method-performance studies are available in the literature and the rates of false positive and negative results differ among various media. Carefully select the medium and procedure that best fit the needs of the laboratory. See Section 9020 B.11 for guidance on validating new methods.

Water samples containing humic or other material may be colored. If there is a natural background color, note it. If the water is yellow enough to be misinterpreted as a weak positive after incubation, use a medium that does not turn yellow (e.g., Colisure). Some waters' high calcium-salt content can cause precipitation, but this should not affect the reaction. In samples with excessive chlorine, a blue flash may be seen while adding Colilert or Colilert-18 media. If this occurs, consider the sample invalid and discontinue testing.

Do not use the enzyme substrate test to verify presumptive coliform cultures or membrane-filter colonies, because the substrate may be overloaded by the heavy inoculum of weak β-D-galactosidase-producing noncoliforms, causing false positive results.

9223 B. Enzyme Substrate Test

1. Samples

Collect samples as directed in Section 9060 A, using sample containers specified in Section 9030 B.19. When collecting chlorinated water samples, use sodium thiosulfate as described in Section 9060 A.2. Follow the quality control (QC) guidelines for sample bottles described in Section 9020 B.5*d.* Adhere to sample holding times and conditions as described in Section 9060 B or required by regulations. Take care to ensure that samples are held at the appropriate temperature and analyzed as soon as possible after sample collection because failure to do so could compromise results. Ensure that samples meet laboratory-acceptance criteria upon receipt.

2. Quality Control

Method users must adhere to the quality assurance (QA)/QC guidelines in Section 9020, including, but not limited to, analytical QC (Section 9020 B.9), instrumentation and equipment (Sections 9020 B.4 and 9030 B), and supplies (Section 9020 B.5). Refer to Table 9020:1 for key QC procedures.

Before using each lot of new medium, verify its performance via positive and negative control organisms. To conduct culture controls, inoculate medium with 3 control bacteria: *E. coli,* a total coliform strain other than *E. coli* (e.g., *Enterobacter cloacae),* and a noncoliform (see Table 9020:6). Also analyze an uninoculated negative control. In addition, test medium and vessels (bottles, multiwell trays, tubes) to confirm sterility and lack of autofluorescence.

3. Substrate Media

Colilert, Colilert-18, and Colisure media are available commercially (IDEXX Laboratories) in premeasured packets for presence-absence testing or in disposable tubes for use in a multiple-tube format. The Quanti-Tray and Quanti-Tray/2000 (IDEXX Laboratories) are multiwell formats that may be used with the premeasured packets to quantitate the coliform bacteria present in a sample.

Store media according to directions and use before expiration date. Avoid prolonged exposure of media to direct sunlight. Discard media that have changed color, appearance, or texture. Media are hygroscopic and will clump and darken if exposed to moisture.

4. Procedure

Begin analysis by mixing the sample properly to promote the even distribution of bacteria. For proper mixing to occur, samples must have 1 inch or more of headspace and be shaken vigorously for 7 s (back and forth 1 ft approximately 25 times).

Failure to properly mix sample and distribute the bacteria homogenously throughout the sample can lead to erroneous results. For instance, most probable number (MPN) results are based on a Poisson (random) distribution of cells in the sample; failure to properly mix sample before analysis will result in an MPN value that underestimates actual bacterial density. Removing a portion of sample without proper mixing—such as when performing presence-absence analyses with a single bottle (one bottle used to both collect and analyze sample)—may result in false negative results if the target organisms were clumped together and removed from the bottle without being homogenized.

If the bottle lacks enough headspace for adequate mixing, pour sample into a larger sterile vessel so it can be mixed properly. Measure the desired sample volume and proceed with analysis.

For each medium or format used, place tests in the incubator within 30 min after medium is added to sample. No matter which format is used, all media must be incubated at 35 ± 0.5 °C. Colilert medium must be incubated for ≥24 h, Colilert-18 medium must be incubated for ≥18 h, and Colisure medium must be incubated for ≥24 h.

The coliform tests described here have been developed to obtain optimal bacterial growth at the indicated incubation temperatures. Failure to maintain this temperature throughout incubation could result in false negative results, especially with the shorter incubation times for Colilert-18. To ensure that samples are at proper temperature for the entire incubation period, laboratory personnel should prewarm samples after adding the medium but before placing them in the incubator.

To prewarm a test sample, place it in a 35 ± 0.5 °C water bath for 20 min or in a 44.5 ± 0.2 °C water bath for 7 to 10 min to bring it to incubation temperature. The laboratory may need to conduct load studies to determine how long samples need to be incubated for effective prewarming (depends on number of samples being incubated). Prewarming is unnecessary if the Quanti-Tray format is used.

a. Presence-absence procedure (P/A): Aseptically add contents of the packet containing premeasured medium to a 100-mL sample in a sterile, transparent, nonfluorescent borosilicate glass or equivalent bottle or container. Aseptically cap and shake vigorously to dissolve the medium. Some medium may remain undissolved, but this will not affect test performance.

b. Multiple-tube procedure:

1) Multiple-tube procedure using a 5- or 10-tube MPN test—A 5-tube series (20 mL sample per tube) or 10-tube series (10 mL sample per tube) can be used when bacteria levels are anticipated to be fairly low or a fixed 100-mL sample volume must be analyzed (e.g., for regulatory compliance).

Add a premeasured packet of medium to a well-mixed 100-mL water sample in a container and shake vigorously to dissolve medium. Arrange tubes in rows of 5 or 10 in a test tube rack, and label each set of tubes. Aseptically dispense a 20 mL sample into each of 5 sterile tubes or 10 mL into each of 10 sterile tubes, cap tightly, and mix vigorously to dissolve the medium. If using 10 tubes already containing premeasured medium (available from manufacturer), aseptically dispense a 10 mL sample into each tube.

Some medium particles may remain undissolved; this will not affect test performance.

After incubation, refer to Tables 9221:2 and 3 to determine the MPN of total coliforms and *E. coli* present.

2) Multiple-tube procedure using 15-tube MPN test—A 15-tube test typically involves 3 serial dilutions of a sample, with each dilution inoculated into 5 tubes. Typically, 5 tubes contain undiluted sample, 5 contain a 1:10 dilution, and 5 contain a 1:100 dilution.

Use this technique when a water sample may contain higher bacteria levels and there is no requirement to analyze a fixed volume (e.g., when analyzing nonpotable waters). The number of tubes and sample volumes selected depend on the quality and characteristics of the water to be examined. To preclude any unwanted interaction with the medium, use only sterile, nonbuffered, oxidant-free water (e.g., deionized or distilled water) to prepare dilutions.

When working with diluted samples, best laboratory practice is to ensure that all tubes are in place and labeled before analysis begins. Additionally, use clean, sterile pipets to pipet each dilution because bacterial carryover from soiled pipets render test results inaccurate.

a) Using disposable tubes containing premeasured medium (available from manufacturer)

i) Prepare sample for the undiluted series—Aseptically pipet 10 mL of well-mixed sample into each of 5 tubes containing predispensed medium. Cap tubes and mix vigorously to dissolve medium.

ii) Prepare 1:10 dilution—Aseptically pipet 10 mL of well-mixed sample into a sterile vessel containing 90 mL of sterile, nonbuffered, oxidant-free water (e.g., deionized or distilled water). Mix well. Aseptically pipet 10 mL of this dilution into each of 5 tubes containing pre-dispensed medium. Cap tubes and mix vigorously to dissolve medium.

iii) Prepare 1:100 dilution—Aseptically pipet 10 mL of well-mixed sample from the 1:10 dilution into a sterile vessel containing 90 mL of sterile, nonbuffered, oxidant-free water (e.g., deionized or distilled water). Mix well. Aseptically pipet 10 mL of this dilution into each of 5 tubes containing predispensed medium. Cap tubes and mix vigorously to dissolve medium.

b) Using packets of premeasured medium

i) Prepare sample for the undiluted series—Add 1 packet of premeasured medium to a sterile vessel containing 100 mL of well-mixed sample, and mix vigorously to dissolve medium. Aseptically pipet 10 mL of sample/medium mixture into each of 5 sterile, nonfluorescing tubes.

ii) Prepare 1:10 and 1:100 dilutions—Add 1 packet of premeasured medium to 100 mL sterile, nonbuffered, oxidant-free water (e.g., deionized or distilled water) in a sterile container, and mix vigorously to dissolve medium. Aseptically pipet 9 mL of prepared medium into 10 sterile, nonfluorescing tubes. This preparation of enzyme substrate medium must be completed in 1 h or less of adding sample to prepared medium.

iii) Inoculate tubes for 1:10 dilution—Aseptically pipet 1 mL of well-mixed sample into each of 5 tubes containing 9 mL of prepared medium. Cap and mix well.

iv) Inoculate tubes for 1:100 dilution—Pipet 10 mL of well-mixed sample into a vessel containing 90 mL sterile, nonbuffered, oxidant-free water (e.g., deionized or distilled water). Close and mix well to dissolve medium. Aseptically pipet 1.0 mL of this diluted sample into 5 tubes containing 9 mL of prepared medium. Cap and mix well.

For any additional dilutions needed, continue with the dilution process as described above.

After incubation, use Table 9221:4 to determine the MPN for both total coliforms and *E. coli*. If further dilutions were performed, the MPN value must be multiplied by the dilution factor to obtain the proper quantitative results.

c. Multiwell procedure: This procedure is performed with sterilized disposable multiwell trays [either the Quanti-Tray (51 well) or Quanti-Tray/2000]. Aseptically add premeasured medium from packet to a 100-mL water sample in a container and shake vigorously to dissolve medium. To open Quanti-Tray, use one hand to hold unit upright (with the well side facing the palm) and squeeze the upper part of the tray so it bends toward the palm. Gently pull foil tab to separate foil from tray, being careful not to touch the inside of either foil or tray. Add reagent-water sample mixture directly into tray, avoiding contact with foil tab. Gently tap the small wells (Quanti-Tray/2000) 2 to 3 times to release any air bubbles that may be trapped. Allow foam to settle, although some foam is acceptable. Place tray into the appropriate rubber insert with the well (plastic) side facing down, and feed it into the Quanti-Tray sealer. The sealer disperses the sample into the wells and seals the package.

5. Interpretation

a. Total coliform bacteria: The bacterial enzyme β-D-galactosidase hydrolyzes ONPG (Colilert and Colilert-18) to yield a yellow color and hydrolyzes CPRG (Colisure) to yield a red or magenta color. After the minimum incubation period, examine for the appropriate color change (Table 9223:1). If the color response is not uniform throughout sample, mix by inversion before reading.

Use an unexpired color comparator (available from manufacturer) to ensure that Colilert and Colilert-18 test results are read accurately. The comparator used must have the same volume in the same type of container as the sample.

1) Colilert—If the sample color is as yellow as or darker yellow than the comparator, then it is positive for total coliforms. If not, then the sample is negative for total coliforms.

However, if the chromogenic response is ambiguous (color cannot be discerned) after 24 h, incubate the sample for up to 4 h longer to allow the test color to intensify. If the color does become as yellow as or darker yellow than that of the comparator within this period, then the sample is positive for total coliforms. If not, then the sample is negative for total coliforms.

Colilert can be incubated for 28 h or less. After 28 h, negative test results are still considered valid, but positive results are not.

2) Colilert-18—If the sample color is as yellow as or darker yellow than the comparator, then it is positive for total coliforms. If not, then it is negative for total coliforms.

However, if the chromogenic response is ambiguous (color cannot be discerned) after 18 h, incubate the sample for up to 4 h longer to allow the test color to intensify. If the color does become as yellow as or darker yellow than that of the comparator within this period, then the sample is positive for total coliforms. If not, then the sample is negative for total coliforms.

Colilert-18 can be incubated for 22 h or less. After 22 h, negative test results are still considered valid, but positive results are not.

3) Colisure—If the sample has a red or magenta color, it is positive for total coliforms. If the chromogenic response is questionable after 24 h (color may be orange or pink), incubate the sample for up to 24 h longer to allow test color to intensify. If color becomes red or magenta within this period, then the sample is positive for total coliforms.

Table 9223:1. Color Changes for Various Media

Substrate	Total Coliform Positive	*E. coli* Positive	Negative Result
Colilert and Colilert-18	Yellow	Blue fluorescence	Colorless or color lighter than the comparator with no fluorescence
Colisure	Red or magenta	Blue fluorescence	Yellow, pink, or orange with no fluorescence

Colisure tests turn yellow after medium is added; if color does not change to red or magenta after incubation, then the sample is negative for total coliforms.

Colisure can be incubated for ≤48 h. After 48 h, results are not valid.

Sometimes a sample's high calcium-salt content can cause precipitation, but this will not affect the reaction. However, if the test medium turns an inappropriate color (e.g., green or black) that interferes with test-result reading, another method must be used.

b. Escherichia coli: The fluorogenic substrate MUG is hydrolyzed by the bacterial enzyme β-D-glucuronidase to yield a bluish fluorescence when viewed under long-wavelength (365-366 nm) UV light. The color change (indicating β-D-galactosidase is active) and fluorescence (indicating β-D-glucuronidase is active) together show that *E. coli* is present.

After the minimum incubation period, examine positive total coliform tests for a bluish fluorescence; use a long-wavelength (365-366 nm) UV lamp with a 6-W bulb and hold it within 5 in. of sample in a dark environment. Use a color comparator (available from the manufacturer) before its expiration date to ensure that test results are read accurately. The comparator used must have the same volume in the same type of container as the sample.

1) Colilert—If the sample has a bluish fluorescence equal to or greater than that of a total-coliform-positive comparator, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned) after 24 h, the sample may be incubated for up to 4 h longer to allow the fluorescence to intensify. If sample fluorescence does intensify to equal to or greater than that of the comparator within this period, then the sample is positive for *E. coli*.

If sample fluorescence remains less than that of the comparator after 28 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

2) Colilert-18—If the sample has a bluish fluorescence equal to or greater than that of a total-coliform-positive comparator, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned), the sample may be incubated for up to 4 h longer to allow the fluorescence to intensify. If sample fluorescence does intensify to equal to or greater than that of the comparator within this period, then the sample is positive for *E. coli*.

If sample fluorescence remains less than that of the comparator after 22 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

3) Colisure—If a total-coliform-positive sample fluoresces, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned), the sample should be incubated for up to

24 h longer to allow the fluorescence to intensify. If the sample clearly fluoresces within this period, then it is positive for *E. coli*.

If sample does not fluoresce after 48 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

6. Reporting

For the presence-absence procedure, report results as total coliforms and *E. coli* present or absent in a 100-mL sample.

For the multiple-tube procedure, calculate the MPN value for total coliforms and *E. coli* from the number of positive tubes, as described in Section 9221C.

For the multiwell procedure, determine the MPN from the appropriate MPN tables obtained from the tray manufacturer.

Bibliography

Edberg SC, Allen MJ, Smith DB; The National Collaborative Study. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube fermentation method. Appl Environ Microbiol. 1988;54(6):1595–1601.

Edberg SC, Edberg MM. A defined substrate technology for the enumeration of microbial indicators of environmental pollution. Yale J Biol Med. 1988;61(5):389–399.

Covert TC, Shadix LC, Rice EW, Haines JR, Freyberg RW. Evaluation of the Autoanalysis Colilert test for detection and enumeration of total coliforms. Appl Environ Microbiol. 1989;55:2443.

Edberg SC, Allen MJ, Smith DB; The National Collaborative Study. National field evaluation of a defined substrate method for the simultaneous detection of total coliforms and *Escherichia coli* from drinking water: comparison with presence-absence techniques. Appl Environ Microbiol. 1989;55(4):1003–1008.

Edberg SC, Smith DB. Absence of association between total heterotrophic and total coliform bacteria from a public water supply. Appl Environ Microbiol. 1989;55:380.

U.S. Environmental Protection Agency. 1989. National Primary Drinking Water Regulations: Analytical techniques; Coliform Bacteria; Final Rule. 40 CFR Part 141; Fed. Reg. 54:29998.

Edberg SC, Allen MJ, Smith DB, Kriz NJ. Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. Appl Environ Microbiol. 1990;56(2):366–369.

Rice EW, Allen MJ, Edberg SC. Efficacy of β-glucuronidase assay for identification of *Escherichia coli* by the defined-substrate technology. Appl Environ Microbiol. 1990;56(5):1203–1205.

Edberg SC, Allen MJ, Smith DB. Defined substrate technology method for rapid and simultaneous enumeration of total coliforms and *Escherichia coli* from water: Collaborative study. J Assoc Offic Anal Chem. 1991;74:526.

Edberg SC, Ludwig F, Smith DB. The Colilert® system for total coliforms and *Escherichia coli*. Denver (CO): American Water Works Association Research Foundation; 1991.

Rice EW, Allen MJ, Brenner DJ, Edberg SC. Assay for β-glucuronidase in species of the genus *Escherichia* and its application for drinking- water analysis. Appl Environ Microbiol. 1991;57(2):592–593.

Shadix LC, Rice EW. Evaluation of β-glucuronidase assay for the detection of *Escherichia coli* from environmental waters. Can J Microbiol. 1991;37(12):908–911.

Covert TC, Rice EW, Johnson SA, Berman D, Johnson CH, Mason PJ. Comparing defined-substrate coliform tests for the detection of *Escherichia coli* in water. J Amer Water Works Assoc. 1992;84(5):98–104.

McCarty SC, Standridge JH, Stasiak MC. Evaluating a commercially available defined-substrate test for recovery of *E. coli*. J Amer Water Works Assoc. 1992;84(5):91–97.

U.S. Environmental Protection Agency. 1992. National Primary Drinking Water Regulations: Analytical techniques; Coliform Bacteria; Final Rule. 40 CFR Part 141; Fed. Reg. 57:24744.

Clark JA, Shaarawi AH. Evaluation of commercial presence-absence test kits for detection of total coliforms, *Escherichia coli*, and other indicator bacteria. Appl Environ Microbiol. 1993;59:380.

U.S. Environmental Protection Agency. National Primary and Secondary Drinking Water Regulation: Analytical methods for regulated drinking water contaminants; Final Rule. 40 CFR Parts 141 & 143; Fed. Reg. 1994;59:62456.

McFeters GA, Broadway SC, Pyle BH, Pickett M, Egozy Y. Comparative performance of Colisure™ and accepted methods in the detection of chlorine-injured total coliforms and *E. coli*. Water Sci Technol. 1995;31(5-6):259–261.

9224 DETECTION OF COLIPHAGES

Approved by Standard Methods Committee, 2022. Joint Task Group: Stephanie D. Friedman and Yildiz Chambers (co-chairs), Jeremy M. Olstadt., Robert S. Salter.

9224 A. INTRODUCTION

1. General Discussion

Bacteriophages, viruses that infect bacteria, were first noted independently by Frederick William Twort[1] and Felix Hubert d'Hérelle[2] between 1915 and 1917. Coliphages are a heterogeneous group of bacteriophages that infect and replicate in Gram-negative bacteria including coliforms and *Escherichia coli*. Coliphages are part of the gut microbiome and are excreted in the feces of humans