**Section 465.360 Methodology**

A laboratory shall be certified for all analytical methods listed in subsection (a) that it uses for compliance purposes. At a minimum, the laboratory shall be certified for one total coliform method and one fecal coliform/E. coli method. In addition, for laboratories that may enumerate heterotrophic bacteria (as measured by the Heterotrophic Plate Count) for compliance with the Surface Water Treatment Rule (SWTR), the laboratory shall be certified for either the Pour Plate Method or the SimPlate method for heterotrophic bacteria.

a) The following methodology with the exception of 9221D and Chromocult, as specified in the listed references, shall be followed for individual parameters:

1) Analytical Methods Approved for Compliance Monitoring under the Revised Total Coliform Rule – 40 CFR 141.852(a)(5). https://www.epa.gov/sites/default/files/2017-02/documents/rtcr\_approved\_methods.pdf

2) Analytical Methods Approved for Compliance Monitoring under the Surface Water Treatment Coliform Rule – 40 CFR 141.74(a)(1). https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P100WD7G.txt

3) Analytical Methods Approved for Compliance Monitoring under the Ground Water Rule – 40 CFR 141.402(c)(2).

https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P100WD6V.txt

4) Appendix A to Subpart C of Part 141. https://ecfr.io/Title-40/Part-141/Appendix-A#40:25.0.1.1.3.3.16.10.5

b) Water samples shall be shaken vigorously at least 25 times in a complete up and down or back and forth movement.

c) Sample volume analyzed for total coliforms in drinking water shall be 100 mL.

d) Aseptic practices shall be used for all microbiological procedures.

e) All samples shall be handled as though they are positive and have the potential to contaminate other samples if handled improperly. All spills shall be promptly disinfected.

f) Fermentation broth methods: The water level of the water bath shall be above the upper level of the medium in the culture tubes.

g) Multiple tube fermentation technique (for detecting total coliforms in drinking water and enumerating total coliforms in source water):

1) For drinking water samples: Various testing configurations can be used (Standard Methods 9221B), as long as a total sample volume of 100 mL is examined for each test.

2) For source water samples: Laboratories shall use at least three series of five tubes each with appropriate sample dilutions of source water (e.g., 0.1 mL, 0.01 mL, 0.001 mL).

h) Media

1) Lauryl tryptose broth (LTB) (also known as lauryl sulfate broth) shall be used in the presumptive test and 2% brilliant green lactose bile broth (BGLBB) in the confirmed test. Lactose broth (LB) may be used in lieu of LTB (40 CFR 141.21(O)(3)) if the laboratory conducts at least 25 parallel tests between this medium and LTB using the waters normally tested, and if this comparison demonstrates that the false positive rate and false negative rate for total coliforms, using LB, is less than 10%. This comparison shall be documented and the records retained. The final pH shall be 6.8 ± 0.2 for LTB, and 7.2 ± 0.2 for 2% BGLBB.

2) The test medium concentration shall be adjusted to compensate for the sample volume so that the resulting medium after sample addition is single strength. If a single 100-mL sample volume is used, the inverted vial shall be replaced with an acid indicator (bromcresol purple) to prevent problems associated with gas bubbles in large inverted tubes. The media shall be autoclaved at 121° C for 12 to 15 minutes.

3) Sterile media in tubes shall be examined to ensure that the inverted vials, if used, are free of air bubbles and are at least one-half to two-thirds covered after the water sample is added.

4) After the medium is inoculated, it shall be incubated at 35° ± 0.5° C for 24 ± 2 hours. If no gas or acid is detected, it shall be incubated for another 24 hours (total incubation time 48 ± 3 hours).

5) Each 24- and 48-hour tube that contains growth, acid, or gas shall be confirmed using 2% BGLBB. A completed test is not required.

6) For drinking water samples: Each total coliform positive sample shall be tested for the presence of E. coli.

i) Invalidation of total coliform-negative samples

1) For drinking water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, shall be invalidated. The laboratory shall collect, or request that the system collect, another sample from the same location as the original invalidated sample within 24 hours. Before invalidation, the laboratory may perform a confirmed E. coli test on the total coliform‑negative culture to check for coliform suppression. If the confirmed test is coliform positive or E. coli is detected, the sample shall be reported as such. An E. coli-positive result is considered a total coliform positive, E. coli‑positive sample, even if the presumptive or confirmed total coliform test is negative. If the follow-up test or tests are negative, the sample shall be invalidated because high levels of non-coliform bacteria in the presumptive tubes may have injured, killed, or suppressed the growth of any coliforms in the sample.

2) For source water samples: All samples that produce a turbid culture (i.e., heavy growth) in the absence of gas/acid production, in LTB or LB, shall be invalidated. The laboratory shall collect, or request that the system collect, another sample from the same location as the original invalidated sample. Before invalidation, the laboratory may perform a confirmed test on the total coliform-negative culture. If the confirmed test is total coliform positive, the most probable number shall be reported. If the test is total coliform negative, the sample shall be invalidated.

j) Enzyme (chromogenic/fluorogenic) substrate tests

1) For detecting total coliforms and E. coli in drinking water samples, a laboratory may use the MMO-MUG test (Colilert), Colisure test, E\*Colite test, Readycult Coliforms 100 Presence/Absence Test, or Modified ColitagTM test. These tests, known as enzyme substrate tests, may be available in various configurations. For enumerating total coliforms in source water, a laboratory may use the Colilert test. If a laboratory uses a fermentation method to detect total coliforms in drinking water, and the sample is total coliform positive, the laboratory may transfer the positive culture to the EC+MUG test to detect E. coli, but not to any other enzyme substrate test medium in this Section.

2) Media shall not be prepared from basic ingredients, but rather from a commercially available source.

3) Media shall be protected from light.

4) Some lots of enzyme substrate media have been known to fluoresce. Each lot of medium shall be checked before use with a 365-366 nm ultraviolet (UV) light with a 6-watt bulb. For checking Colilert, Colilert-18, Colisure, Readycult, and Modified ColitagTM media, a packet of medium shall be dissolved in sterile water in a non-fluorescing vessel. If the medium exhibits faint fluorescence, the laboratory shall use another lot that does not fluoresce.

5) If the samples plus the medium exhibit an inappropriate color change before incubation, they shall be discarded and another lot of medium used. The laboratory shall notify the medium vendor and request another water sample from the water system. Before incubation, Colilert, Colilert-18, and Modified ColitagTM shall appear colorless to a slight tinge of color, while Colisure and E\*Colite are yellow and Readycult shall appear slightly yellow.

6) Glass and plastic sample bottles and test tubes shall be tested before use with a 365-366 nm UV light source with a 6-watt bulb to ensure that they do not fluoresce. If they fluoresce, another lot of containers that do not fluoresce shall be used.

7) Air-type incubators may not bring a cold 100 mL water sample or samples to the specified incubation temperature for several hours. The problem may cause false negative results with the enzyme substrate tests and possibly other tests as well. Laboratories with air-type incubators shall observe the following instructions for chromogenic/fluorogenic substrate test:

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| **Test** | **Pre-incubation sample instructions 1,2** |
| Colilert (Presence/Absence) | Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C 1 |
| Colilert Quanti-Tray | Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C  |
| Colilert-18 (Presence/Absence) | Prewarm sample in 35° ± 0.5° C water bath for 20 minutes or 44.5° C for 7-10 minutes |
| Colilert-18 Quanti-Tray | Allow sample to equilibrate to room temperature (20-30° C) before beginning 18-hour incubation time |
| Colisure | Allow sample to equilibrate to room temperature (20-30° C) before beginning 24-hour incubation time |
| Readycult Coliforms | Specified 24-hour incubation time includes time it takes to bring sample temperature up to 35° ± 0.5° C |
| Modified ColitagTM  | If results are to be read before 22 hours, sample must be prewarmed in a 44.5° C water bath for 7-10 minutes |

1Samples shall be brought to room temperature before incubation.

2 Information based on manufacturer's instructions.

8) If a water bath is used, the water level shall be above the upper level of the medium.

9) For E. coli testing, the laboratory shall place all total coliform-positive samples under an ultraviolet lamp (365-366 nm, 6-watt) in a darkened area. If E. coli is present, the medium will emit a blue fluorescence.

10) The enzyme substrate tests shall not be used to confirm a presumptive total coliform-positive result that was obtained in fermentation broth (e.g., LTB, LB) or on a membrane filter.

11) Any sample that produces an atypical color change (e.g., greenish black or black) shall be invalidated.

12) Any reference comparator provided by the manufacturer shall be discarded by the manufacturer's expiration date.

13) For the Colilert test, samples shall be incubated at 35° ± 0.5° C for 24 hours. A yellow color in the medium equal to or greater than the reference comparator indicates that the sample is total coliform positive. If the sample is yellow, but lighter than the comparator, it shall be incubated for another four hours. If the color is still lighter than the reference comparator at 28 hours, the sample shall be reported as negative. After 28 hours negative results are still considered valid, but positive results are not. A coliform-positive sample that fluoresces under an ultraviolet (UV) light indicates the presence of E. coli.Laboratories that use the Colilert-18 test shall incubate samples for 18 hours (up to 22 hours if the sample after 18 hours is yellow, but is lighter than the comparator). After 22 hours negative results are still considered valid, but positive results are not.

14) For enumerating total coliforms in source water with the Colilert, Colilert 18 test, Quanti-Tray, or Quanti-Tray 2000 may be used for each sample tested. Dilution water (if used) may be sterile deionized or sterile distilled water, but not buffered water.

15) If the Quanti-Tray or Quanti-Tray 2000 test is used, the sealer shall be checked monthly by adding a dye (e.g., bromcresol purple) to the water. If dye is observed outside the wells, maintenance shall be performed or another sealer shall be used.

16) For the Colisure test, samples shall be incubated at 35° ± 0.5° C for 24 hours. If an examination of the results at 24 hours is not convenient, then results may be examined at any time up to 48 hours. After 48 hours, negative or positive results are considered invalid. If the medium changes from a yellow color to a red/magenta color, the sample is total coliform positive. A coliform positive sample that fluoresces under a UV light indicates the presence of E. coli.

17) For the E\*Colite test, samples shall be incubated at 35° ± 0.5° C for 28 hours. If total coliforms are present, the medium changes from a yellow color to a blue or blue-green color, or a blue color in the corners of the bag. If E. coliis present, the medium will fluoresce under a UV light. If no fluorescence is observed, the sample shall be re-incubated for an additional 20 hours (for a total incubation time of 48 hours) and again checked for fluorescence. If the medium becomes red, it shall be assumed that a faulty seal has allowed the bactericide (in the third compartment of the bag) to leak into the compartment containing the medium. In this case, the sample shall be discarded and another sample shall be requested.

18) For the Readycult Coliforms 100 Presence/Absence test, the contents of a snap pack shall be added to a 100-mL water sample, followed by incubation at 35° ± 0.5° C for 24 ± 1 hours. If coliforms are present, the medium changes color from a slightly yellow color to blue-green. In addition, if E. coliis present, the medium will emit a bright light-blue fluorescence when subjected to a long wave (365-366 nm) UV light. If confirmation of E. coliis desired, Kovac's indole reagent shall be added to the broth; the immediate formation of a red ring confirms the presence of E. coli*.*

19) For the Modified ColitagTM test, samples shall be incubated at 35° ± 0.5° C for 16-48 hours. If results are to be read before 22 hours, the sample must be prewarmed in a 44.5° C water bath for 7-10 minutes. During incubation, trimethylamine-N-oxide in the Modified ColitagTM medium causes the pH of the medium to increase from 6.2 to 6.8-7.2. A yellow color in the medium indicates the presence of total coliforms. A coliform-positive sample that fluoresces under a UV light indicates the presence of E. coli.

k) Membrane filter (MF) methods

1) For source water samples (SWTR): To optimize counting, appropriate sample dilutions shall be used to yield 20 to 80 total coliform colonies or 20 to 60 fecal coliform colonies for at least one dilution or volume.

2) At least one membrane filter and filtration unit sterility check shall be conducted at the beginning and the end of each filtration series (unless using single use disposable funnels) by filtering 20 to 30 mL of dilution water through the membrane filter and testing for growth. If the control indicates contamination, all data from affected samples shall be rejected and an immediate resampling shall be requested. A filtration series ends when 30 minutes or more elapse between sample filtrations.

3) Each filtration funnel shall be rinsed after each sample filtration with two or three 20 to 30 mL portions of sterile rinse water to ensure that the entire sample is rinsed off the funnel before the filter is removed. After the filter is removed, the funnel may be rinsed again with two or three 20 to 30 mL portions of sterile rinse water or exposed to UV light with a 254-nm wavelength for at least two minutes to prevent carryover between samples, especially for surface water samples.

4) Absorbent pads shall be saturated with a liquid medium (at least 2 mL of broth) and excess medium removed by decanting the plate.

5) Membrane filters shall be handled with sterile forceps that are sterilized before each use by dipping in 95% ethyl or absolute methyl alcohol and flaming. The membrane filters shall be grasped outside the effective filtration area.

l) Media used for detecting total coliforms and E. coliin drinking water, enumerating total coliforms or fecal coliforms in source water, and detecting E. coliin ground water.

1) Using M-Endo medium agar or broth (also known as M-Endo broth MF and M-Coliform broth) or LES Endo agar (also known as M-Endo agar LES) for detecting total coliforms in drinking water or enumerating total coliforms in source water: Medium may be used in the single step or enrichment techniques. Ethanol used in the rehydration procedure shall not be denatured. Medium shall be prepared in a sterile flask and brought just to the boiling point with a boiling water bath or, if constantly attended, a hot plate with a stir bar. The medium shall not be boiled. Final pH shall be 7.2 ± 0.2 for M-Endo Agar LES and Endo medium.

2) Using m-ColiBlue24 medium for detecting total coliforms and E. coliin drinking water: Purchase this medium from a commercial vendor, it cannot be prepared from basic ingredients. Ampules of broth shall be inverted two to three times to mix contents before breaking. Then, contents shall be poured evenly over absorbent pad. Unopened refrigerated ampules may be stored in the dark until the expiration date, but shall be discarded earlier if growth is observed. The final pH of the medium shall be 7.0 ± 0.2.

3) Using MI medium (with or without agar) for detecting total coliforms and E. coli in drinking water or enumerating total coliforms in source water: Commercially made pre-sterilized bottled MI agar or broth shall not be autoclaved. Bottled agar shall be melted in a boiling water bath or by other processes recommended by the manufacturer. As soon as complete melting has occurred, the medium shall be cooled slightly and immediately poured into sterile plates. Care shall be taken to prevent overheating the agar, as excessive heat destroys the effectiveness of the antibiotic cefsulodin. If dehydrated culture medium is used, it shall be prepared and autoclaved according to the manufacturer's instructions. The agar shall be cooled, freshly prepared filter-sterilized cefsulodin shall be added, and the mixture shall be immediately poured into sterile plates. The final pH of MI agar shall be 6.95 ± 0.2; the final pH of MI broth shall be 7.05 ± 0.2. The preparation and use of MI agar and MI broth are referenced in Section 465.125(a)(4). EPA Method 1604, which can be found online at www.epa.gov/microbes, is identical.

4) m-FC broth (with or without agar) for enumerating fecal coliforms in source water shall not be autoclaved. The medium shall be brought just to the boiling point. The final pH shall be 7.4 ± 0.2.

5) When stored, prepared medium shall be refrigerated. Petri dishes containing medium shall be stored in a plastic bag or tightly closed container, and used within two weeks. Before use, refrigerated sterilized medium shall be brought to room temperature. Plates with laboratory‑prepared broth medium shall be discarded after 96 hours, poured MF agar plates discarded after two weeks, and ampules of M-Endo broth and other prepared media discarded in accordance with the manufacturer's expiration date. Broth, plates, or ampules shall be discarded earlier if growth or (for M-Endo agar) surface sheen is observed. The date and time prepared shall be recorded.

6) Incubation conditions and colony color of inoculated medium

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| **Medium**  | **Incubation**  | **Total coliforms**  | **E. coli**  |
| M-Endo medium or M-Endo agar LES  | 35° ± 0.5° C for 22-24 hrs  | Metallic (green-golden) sheen colonies (presumptive)  | N/A  |
| m-ColiBlue24  | 35° ± 0.5° C for 24 hrs  | Count all red and blue to purple colonies under normal/ambient light and record as the total coliform result.  | Count only blue to purple colonies and record as E. coli result.  |
| MI1 | 35° ± 0.5° C for 24 ± 2 hrs  | Fluorescent colonies under UV light  | Blue colonies under normal light  |
| m-FC  | 44.5° ± 0.2° C for 24 ± 2 hrs  | N/A  | Blue colonies (fecal coliforms)  |

1 If any blue, non-fluorescent colonies are found on the same plate, add their total to the Total Coliform count.

m) Invalidation of a total coliform-negative drinking water sample: All samples resulting in confluent or TNTC (too numerous to count) growth shall be invalidated unless total coliforms are detected. If no total coliforms are detected, the sample shall be recorded as "confluent growth" or "TNTC" and an additional sample shall be requested from the same sampling site. Confluent growth is defined as a continuous bacterial growth covering the entire membrane filter without evidence of total coliform type colonies. TNTC is defined as greater than 200 colonies on the membrane filter in the absence of detectable coliforms. Laboratories shall not invalidate samples when the membrane filter contains at least one coliform type colony (i.e., sheen colony for M-Endo medium, red or blue colony for m-ColiBlue24 agar, fluorescent or blue colony for MI agar. Before invalidation, the laboratory shall perform a verification test on the total coliform negative culture, i.e., on confluent or TNTC growth, and an E. colitest. If the verification test is total coliform positive, the sample shall be reported as total coliform positive. If the test is total coliform negative, the sample shall be invalidated. An E. colipositiveresult is considered a total coliform-positive, E. coli positive sample, even if the sample tests negative for total coliform.

n) Invalidation of source water samples (SWTR): Laboratories shall invalidate any sample that results in confluent growth or TNTC, even when total coliform or fecal coliform colonies are present.

o) For drinking water samples (to verify colonies on Endo-type medium): The entire surface of the membrane filter shall be wiped with a sterile cotton swab and the verification media (LTB, then BGLBB) shall be inoculated. Alternatively, at least five typical sheen colonies and five nontypical colonies shall be verified using either single strength lactose broth (LB) or lauryl tryptose broth (LTB) and then single strength 2% brilliant green lactose bile broth (BGLBB). In addition, sheen colonies may be verified rapidly using a cytochrome oxidase and b-galactosidase procedure. Individual colonies can be transferred with a sterile needle or loop, or applicator stick. If no sheen colonies are observed, up to five red questionable sheen colonies and up to five red non-sheen colonies representing different morphological types shall be verified.

p) For drinking water samples: Total coliform-positive colonies shall be tested for E. coli. The membrane filter tests approved by USEPA do not require additional media for such a test, except for those using Endo-type medium (M-Endo medium or M-Endo agar LES). USEPA has approved several options for testing a total coliform-positive colony on Endo-type medium for E. coli. When coliforms or EC Medium-MUG is used, the colonies shall be transferred by employing one of the options specified by the Total Coliform Rule at 40 CFR 141.21(f)(5) (see Appendix G of the USEPA Manual for the Certification of Laboratories Analyzing Drinking Water). For the swab technique, a single swab can be used to inoculate a presumptive total coliform-positive culture into three different media, EC-MUG Medium, LTB, and BGLBB, in that order. If Nutrient Agar-MUG is used, the Nutrient Agar-MUG section shall be followed*.*

q) For source water samples: Initial total coliform counts shall be adjusted based upon verified data, as in Standard Methods,Section 9222B(5).

r) Nutrient Agar-MUG Test (for detection of E. coliin drinking water or ground water)

1) Medium shall be autoclaved at 121° ± 1° C for 15 minutes. MUG may be added to Nutrient Agar before autoclaving. Nutrient Agar-MUG is also available commercially. The final MUG concentration shall be 100 µg/mL. The final pH shall be 6.8 ± 0.2.

2) Positive and negative controls shall be tested as stated in Section 465.400(p). Control cultures shall be filtered or spot-inoculated onto a membrane filter on M-Endo agar LES or M-Endo broth or agar, and shall be incubated at 35° ± 0.5° C for 22 to 24 hours. The filter shall then be transferred to Nutrient Agar-MUG and incubated at 35° ± 0.5° C for another four hours. The results shall be read and recorded.

3) The membrane filter containing a coliform colony or colonies shall be transferred from the total coliform medium to the surface of Nutrient Agar-MUG medium. Each sheen colony shall be marked with a permanent marker on the lid. Also, the lid and the base shall be marked with a line to realign the lid if it is removed. A portion of the colony may be transferred with a needle to the total coliform verification test before transfer to Nutrient Agar-MUG or after the 4-hour incubation time. Another method is to swab the entire membrane filter surface with a sterile cotton swab after the 4-hour incubation time on Nutrient Agar-MUG medium, and transfer to a total coliform verification test.

4) The fluorescence shall be checked using an ultraviolet lamp (365-366 nm) with a 6-watt bulb in a darkened area. Any amount of fluorescence in a halo around a sheen colony shall be considered positive for E. coli.

s) Heterotrophic Plate Count (for enumerating heterotrophic bacteria in drinking water)

1) The Pour Plate Method (Standard Methods 9215B) or the SimPlate (Standard Methods 9215E) Method shall be used for determining compliance with 40 CFR 141.74(a)(l) and shall also be used for testing reagent grade water.

2) Media

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| **Method** | **Medium** | **Final pH** |
| Pour Plate  | Plate count agar, also known as tryptone glucose yeast agar | 7.0 ± 0.2  |
| SimPlate  | Multiple enzyme substrate  | 7.0 ± 0.3  |

3) (For Pour Plate Method) Melted agar shall be tempered at 44°-46° C in a water bath before pouring. Agar temperature control accompanies media from tempering through use. Melted agar shall be held no longer than three hours. Sterile agar medium shall not be melted more than once. The center of media in containers shall be no greater than 2.5 cm from some surface.

4) Refrigerated medium may be stored in bottles or in screw-capped tubes for up to three months, or in petri dishes for up to two weeks.

5) For most potable water samples, countable plates can be obtained by plating 1.0 mL and/or 0.1 mL volumes of the undiluted sample (dilutions may not be necessary for SimPlate, which has a counting range up to 738/mL). At least duplicate plates per dilution shall be used. Duplicate dilutions are not required for SimPlate.

6) (For Pour Plate Method) The sample shall be aseptically pipetted onto the bottom of a sterile petri dish. Then at least 10-12 mL of tempered melted (44°-46° C) agar shall be added to each petri dish. The sample and melted agar shall be mixed carefully to avoid spillage. After agar plates have solidified on a level surface, the plates shall be inverted and incubated at 35° ± 0.5° C for 48 ± 3 hours. Plates shall be stacked no more than four high and shall be arranged in the incubator to allow proper air circulation and to maintain uniform incubation temperature. Excessive humidity in the incubator shall be avoided to reduce the possibility of spreader formation on the agar medium. Excessive drying of the agar medium shall also be avoided; agar medium in plates shall not lose more than 15% by weight during 48 ± 3 hours of incubation. Agar weight loss shall be determined quarterly.

7) (For SimPlate Method) Unit Dose (for a single sample): A 10.0 mL volume of test sample shall be added to a test tube containing dehydrated SimPlate medium. Then the dissolved medium shall be poured onto the center of a plate containing 84 small wells (provided by the manufacturer, IDEXX Laboratories, Inc.). Alternatively, 9.0 mL of sterile diluent (D.I. water, distilled water, or buffered water (Standard Methods, 9050C, 1 a)) can be added to the tube, followed by a 1.0 mL sample. Then the procedure indicated for the 10.0 mL sample shall be followed. The mixture shall be distributed evenly to the 84 wells on the plate, and the excess liquid shall be drained into an absorbent pad on the plate. The plate shall then be inverted (the fluid in each well is held in place by surface tension), and incubated for 48 ± 3hours at 35° ± 0.5° C.Bacterial density is determined by counting the number of wells that fluoresce under a 365‑366 nm UV light, and converting this value to a Most Probable Number using the Unit Dose MPN table provided by the manufacturer. If a 10.0 mL sample is used, the Unit Dose MPN/mL shall be read directly. If a 1.0 mL sample is used, then the MPN/mL value shall be corrected by multiplying it by 10.

8) (For SimPlate Method) Multiple Dose (for 10 samples of 1.0 mL each): A 100-mL sterile diluent shall be added to the dehydrated SimPlate medium to reconstitute and shaken to dissolve. Then a 1.0 mL test sample shall be pipetted to the center of a plate containing 84 small wells, followed by 9.0 mL of the reconstituted medium. The plate shall be gently swirled to mix the sample and medium, and the mixture shall be distributed evenly to the 84 wells on the plate. Then the procedure indicated in subsection (s)(7) shall be followed, except that the Multi-Dose table supplied by the manufacturer shall be used to determine the MPN/mL. If a dilution is made during sample preparation, then the MPN/mL value shall be multiplied by the dilution factor.

9) (For Pour Plate Methods) Colonies shall be counted manually using a dark-field colony counter. In determining sample count, laboratories shall count only plates having 30 to 300 colonies. For plates inoculated with 1.0 mL of undiluted sample, counts less than 30 are acceptable. Fully automatic colony counters are not suitable because of the size and small number of colonies observed when potable water is analyzed for heterotrophic bacteria.

10) Each batch or flask of agar shall be checked for sterility by pouring a final control plate. Data shall be rejected if control is contaminated.

(Source: Amended at 46 Ill. Reg. 19150, effective November 17, 2022)