IDEXX COLILERT® TEST METHOD FOR THE SIMULTANEOUS DETECTION OF TOTAL COLIFORMS AND *E. COLI* IN WATER

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1.0 Scope and Application

1.1 This method is intended for use in the simultaneous detection and confirmation of total coliforms and *E. coli* in water. Any positive sample for total coliforms is an indication of contamination. Any positive sample for both total coliforms and *E. coli* is an acute violation.

1.2 The minimum, non-zero number of bacterial counts detectable with this method is a function of the dilution scheme used when processing the sample.

1.3 Colilert® method can be applied to fresh waters, drinking waters and waste waters. It can be used as a Presence/Absence test or quantification with either 5, 10, or 15 tube serial dilution MPN tubes or with the Quanti-Tray™ system (see package insert) (1).

1.4 Since there can be a wide range of coliform levels in surface waters and wastewaters, dilutions can be used with this method for detecting and enumerating the actual level.

1.5 Colilert® is not for intended for use with marine waters.

2.0 Summary of Method

2.1 This method is based on Defined Substrate Technology®. This product utilizes nutrient indicators that produce color/fluorescence when metabolized by total coliforms and *E. coli.* When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/100 mL within 24 hours with as many as 2 million heterotrophic bacteria/100 mL present. This test is not intended for marine waters.

3.0 Definitions

3.1 In this method, coliform bacteria are those bacteria which produce a yellow color, and for *E. coli*, also produce a fluorescent signal under a 6-watt, 365-nm UV light after incubation at 35°C ± 0.5°C for 24 hours.

4.0 Interferences

4.1 Heterotrophic bacteria greater than 2,000,000/100 mL can yield a positive reaction for coliforms. Some water samples containing humic material may have an innate color and a control blank of the same water sample may be may be required for comparison to the inoculated sample.

5.0 Safety

5.1 The analyst/technician must know and observe the normal safety procedures required in a microbiology laboratory preparing using and disposing of samples, reagents and materials, and while operating sterilizing equipment.

5.2 Mouth-pipetting is prohibited.

6.0 Equipment and Supplies

6.1 Pipettes, sterile, T.D. bacteriological or Mohr, glass or plastic of appropriate volume.

6.2 Sterile vessels, glass or plastic (free from fluorescence), 100- to 200-mL volume.

6.3 Incubator maintained at 35°C ± 0.5°C.

6.4 6-watt, 365-nm, UV light.

7.0 Reagents

7.1 Sterile deionized or distilled water: Water conforming to specification D1193, reagent water conforming Type II, Annual Book of ASTM Standards (2). Autoclave at 121°C (15-lb pressure) for 15 minutes or sterile filter using a 0.22 micron filter into a sterile container.

7.2 Sodium thiosulfate is used to dechlorinate drinking water samples.

7.3 Store Colilert® at 4°C - 30°C away from light. The expiration date is indicated on the package (12 months from the date of manufacture).

8.0 Sample Collection, Preservation and Storage

8.1 Sampling procedures as described in detail in the USEPA microbiology methods manual, Section II, A (3) and in Standard Methods for the Examination of Water and Wastewater (4).

8.1.1 Storage Temperature and Handling Conditions: Ice or refrigerate bacteriological samples at a temperature less than 10°C during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Take care that sample vessels are not totally immersed in water during transit.

8.1.2 Holding Time Limitations: Examine samples as soon as possible after collection. For drinking water samples do not exceed 30 hours holding time from collection to analysis. For non-potable water for compliance, do not exceed 6 hours holding time and process within 2 hours.

9.0 Quality Control

9.1 Quality control should be conducted on each lot of Colilert® or more often as regulations required. Inoculate 100 mL of sterile water with Quanti-Cult™ or American Type Culture Collection (ATCC) listed below. Follow the procedure in Section 11.

Quanti-Cult™ Organism	ATCC #	Expected Result
E. coli	25922 or 11775	Yellow, fluorescent
Klebsiella pnuemoniae	31488	Yellow
Pseudomonas aeruginosa	10145 or 27853	Colorless, no fluorescence

10.0 Calibration and Standardization

10.1 Check temperatures in incubators daily to insure operation within stated limits.

10.2 Check thermometers at least annually against NIST certified thermometer or one that meets the requirements of NIST Monograph SP 250-23.

11.0 Procedure

11.1 Presence/Absence

11.1.1 Carefully separate one Snap Pack from the strip taking care not to accidentally open adjacent pack.

11.1.2 Tap the Snap Pack to ensure that all of the Colilert® powder is in the bottom of the pack.

11.1.3 Open one pack by snapping back the top at the scoreline.

11.1.4 Add the reagent to the 100-mL water sample contained in a sterile, non-fluorescent vessel.

11.1.5 Aseptically cap and seal the vessel.

11.1.6 Shake until dissolved.

11.1.7 Incubate for 24 hours at 35°C ± 0.5°C

11.1.8 Read the results at 24 hours. Compare each result against the comparator dispensed into an identical vessel.

11.1.9 If no yellow color is observed, the test is negative.

11.1.10 If the sample has a yellow color equal to or greater than the comparator, the presence of total coliforms is confirmed. If color is not uniform, mix by inversion, then recheck.

11.1.11 If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (but no more than 28 hours total). If the sample is total coliform positive, the color will intensify. If it does not intensify, the sample is negative.

11.1.12 If yellow is observed, check vessel for fluorescent by placing a 6-watt, 365-nm UV light within five inches of the sample in a dark environment. Be sure the light is facing away from your eyes and towards the vessel. If the fluorescence is greater or equal to the fluorescence of the comparator, the presence of *E. coli* is confirmed.

11.2 Quantification

11.2.1 Colilert® can be used for multiple tube MPN analysis (e.g., 5-tube, 10-tube, or 15-tube serial dilutions). Consult Standard Methods for appropriate MPN tables. For accuracy and counting range, use the IDEXX Quanti-Tray™ or Quanti-Tray 2000™ and follow the above Presence/Absence Sections 11.1.1 through 11.1.5.

11.2.2 If a dilution is required, use sterile water, not buffered water for making dilutions. Colilert® is already buffered. Always add Colilert® to the proper volume of diluted sample after making dilutions.

11.2.3 Follow the package insert for Quanti-Tray™ (5). Remove a sterile tray from the plastic bag and squeeze the tray at the top to open. Pour the sample reagent mixture from step 11.1.5 above directly into the tray avoiding contact with the foil tab, and then seal the tray with the Quanti-Tray™ sealer.

11.2.4 Incubate at 24 hours at 35°C ± 0.5°C.

12.0 Data Analysis and Calculations

12.1 Quanti-Tray™

12.1.1 Follow the same interpretation directions from Section 11.1.8 above to count the number of positive wells. Refer to the MPN table provided with the Quanti-Tray™ to determine the Most Probable Number (MPN) of total coliforms (yellow wells) and *E. coli* (yellow/fluorescent wells) in the sample. The color and fluorescent intensity of positive wells may vary.

12.1.2 Record the results as the Most Probable Number/100 mL. If any dilutions were made, multiply the MPN/mL by the dilution factor to obtain the final MPN/100 value.

13.0 Method Performance

13.1 Colilert® found to be equally sensitive to LTB, EC+MUG (6).

13.2 Correlation of 0.905 found between Colilert® and m-Tec for *E. coli* (6).

14.0 Reporting Results

14.1 Report results as Presence or Absence for total coliforms and for *E. coli*. For quantification, report results as MPN/100 mL for total coliforms and *E. coli*.

15.0 Verification Procedure

15.1 Not applicable.

16.0 Pollution Prevention

16.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

16.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

17.0 Waste Management

17.1 It is the laboratory's responsibility to comply with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous identification rules and land disposal restrictions. Compliance with all sewage discharge permits and regulations is also required.

17.2 Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

18.0 References

18.1 Colilert® Package Insert from IDEXX.

18.2 Annual Book of ASTM Standards, Vol. 11.01, American Society for Testing Materials, Philadelphia, PA 19103.

18.3 Bordner, R., J.A. Winter and P.V. Scarpino (eds.). Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA-600/8-78-017. Office of Research and Development, USEPA.

18.4 Clesceri, L.S., A.E. Greenberg, A.D. Eaton (eds.). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition, American Public Health Association, Washington, DC.

18.5 Quanti-Tray™ Package Insert from IDEXX.

18.6 *Federal Register* / Vol. 66, No. 169 / Thursday, August 30, 2001, page 45818.