

Topic	Testing for Fecal Coliforms in Wastewater Using Colilert*-18
Title	<i>IDEXX Colilert*-18 and Quanti-Tray* Test Method for the Detection of Fecal Coliforms in Wastewater</i>
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Highlights

- The Colilert-18 method can be used to test for fecal coliforms in wastewaters when used with the Quanti-Tray system.
- In this method, fecal coliform bacteria are defined as bacteria that produce a yellow color after 18-22 h incubation at 44.5° +/- 0.2° C. Positive results are visualized only by color change, not fluorescence.
- Quality control should be conducted on each lot of Colilert-18, or more often as regulations require. Quality control procedures are identified in the product package insert (06-02027-19).
- The U.S. EPA has determined that Colilert-18 is a suitable method for the detection of fecal coliforms in wastewater when incubated at 44.5° C. See article 15A.

**IDEXX COLILERT[®]-18 TEST and QUANTI-TRAY[®]
METHOD FOR THE DETECTION OF FECAL
COLIFORMS IN WASTEWATER**

IDEXX Colilert[®]-18 and Quanti-Tray[®] Test Method For The Detection Of Fecal Coliforms In Wastewater

1.0 Scope and Application

- 1.1 This method is intended for use in the detection and confirmation of fecal coliforms in wastewater.
- 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 The Colilert-18 method can be applied to test for fecal coliforms in wastewaters with the Quanti-Tray[®] system (see package insert) (20.1).
- 1.4 Since there can be a wide range of fecal coliform levels in wastewaters, dilutions can be used with this method for detecting and enumerating the actual level.

2.0 Summary of Method

- 2.1 The method is based on Defined Substrate Technology[®]. The product utilizes a nutrient indicator (ONPG) that produces a yellow color when metabolized by fecal coliforms at 44.5°C. When the reagent is added to the sample and incubated, it can detect these bacteria at 1 CFU/ml at 18 hours and up to 22 hours.

3.0 Definitions

- 3.1 In this method, fecal coliform bacteria are those bacteria which produce a yellow color after incubation at 44.5 ±0.2°C at 18 hours and up to 22 hours.
- 3.2 Fecal coliform detection is based on the presence of the enzyme β-D-galactosidase which is known to be present in fecal coliform bacteria.
- 3.3 For the detection of β-D-galactosidase, Colilert-18 utilizes the chromogenic nutrient indicator ortho-nitrophenyl-β-D-galactopyranoside (ONPG) which produces a distinct yellow color when hydrolyzed by β-D-galactosidase.

4.0 Interferences

- 4.1 Some samples containing humic material may have an innate color and a control blank of the same water sample may be required for comparison to the inoculated sample.

5.0 Safety

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

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 containing sodium thiosulfate to neutralize up to 15 mg/L of chlorine. Vessels should be at least 120 mL to hold 100 mL of sample.
- 6.3 Quanti-Tray (51 well) or Quanti-Tray/2000
- 6.4 Quanti-Tray Sealer

6.5 Incubator maintained at 44.5 ±0.2°C. (Dry incubator or water bath that can maintain the required temperature tolerance).

7.0 Reagents

7.1 Sterile deionized water or distilled water. Water conforming to specification D1193, “Reagent water conforming to 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. (20.2)

7.2 Colilert-18, stored at 2 -25°C away from light. The expiration date is indicated on the package (15 months from the date of manufacture).

7.3 Quanti-Tray or Quanti-Tray/2000 Comparator

7.4 Antifoam reagent

7.5 Sodium thiosulfate reagent (Standard Methods for the Examination of Water and Wastewater, [20.3 & 20.4]) or sterile vessels containing sodium thiosulfate to neutralize up to 15mg/L chlorine

8.0 Sample Collection, Preservation and Storage

8.1 Sampling procedures as described in detail in Standard Methods for the Examination of Water and Wastewater (20.3 & 20.4).

8.1.1 Storage Temperature and Handling Conditions: Ice or refrigerate bacteriological samples at a temperature less than 10°C (2-10°C) during transit to the laboratory. Use insulated containers to assure proper maintenance of storage temperature. Ensure the sample vessels are not totally immersed in water during transit.

8.1.2 Holding Time Limitations: Examine samples as soon as possible after collection. The required hold time of samples is 6 hours from collection to initiation of testing (20.7).

9.0 Quality Control

9.1 Quality control should be conducted on each lot of Colilert-18 or more often as regulations requires or accept the IDEXX Certificate of Quality (obtained from the website; www.idexx.com/water) . Inoculate 100 ml of sterile water with Quanti-Cult™ or American Type Culture Collection (ATCC) listed below or equivalent. Follow the procedure in section 11.0

Bacteria spp.		Expected Result
E.coli	ATCC 11775 or Quanti-Cult™ Organism (from IDEXX)	Yellow
Pseudomonas aeruginosa	ATCC 10145 or 27853 or Quanti-Cult™ Organism (from IDEXX)	Colorless

10.0 Calibration and Standardization

10.1 Check the temperature in the incubators daily as a minimum to insure it is within the stated limits. It is preferable to check the temperature in the morning and in the late afternoon. Record the date, temperature, time of reading and initial.

- 10.2** Check thermometers at least annually against NIST certified thermometer or one that meets the requirements of NIST Monograph SP 250-23 (20.3).
- 11.0 Quantification Procedure [follow package insert for quantification] (20.5)**
- 11.1** Colilert-18 and the IDEXX Quanti-Tray System with either the 51 Well Quanti-Tray or the Quanti-Tray/2000.
- 11.2** Carefully separate one blister pack from the strip taking care not to accidentally open the adjacent pack.
- 11.3** Ensure the powder is at the bottom of the blister pack.
- 11.4** Hold the blister pack face down (paper side up) at the top and towards the back and snap back the scoreline forming a “v” with the opening facing the open vessel.
- 11.5** Allow the powder to fall into the vessel containing the room temperature sample. Mix the sample well to dissolve the reagent.
- 11.6** If a dilution is necessary, use sterile deionized or distilled water, not buffered water for making the dilution prior to adding Colilert-18. Colilert-18 should be added to the diluted sample.
- 11.7** The use of IDEXX Antifoam reagent may be necessary to reduce foaming and eliminate excess bubbles in the wells of the Quanti-Tray. Alternatively, let the sample sit for a few minutes to allow the foam to dissipate.
- 11.8** Remove the sterile trays from the plastic bag by opening the bag at the green line (follow the package insert). Reseal the bag with tape or a clip. Pour the sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 by squeezing the top (see package insert) and seal with the IDEXX Quanti-Tray Sealer.
- 12.0 Incubation:**
- 12.1** Place the sealed tray in a $44.5 \pm 0.2^\circ\text{C}$ incubator for 18 hours and up to 22 hours (prewarming to 35°C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring or a bungee cord or metal rack. (do not use a rock or a bottle containing water to hold down the trays).
- 13.0 Results Interpretation:**
- 13.1** Read results according to the Result Interpretation Table. Count the number of positive wells and refer to the MPN Table provided with the Quanti-Tray to obtain a Most Probable Number.
- 13.2** Less yellow than the comparator when incubated at $44.5 \pm 0.2^\circ\text{C}$ is negative for fecal coliforms.
- 13.3** Yellow equal to or greater than the comparator when incubated at $44.5 \pm 0.2^\circ\text{C}$ is positive for fecal coliforms
- 13.4** If a dilution is required, use sterile deionized or distilled water, not buffered water for making the dilutions. Always add Colilert-18 to the final 100ml diluted sample only.
- 13.5** Colilert-18 results are definitive at 18–22 hours. In addition, positives for fecal coliforms observed before 18 hours and negatives observed after 22 hours are also valid.
- 14.0 Data Analysis and Calculations**
- 14.1** Follow the same interpretation directions from section 13.0; Count the number of positive wells. Refer to the Quanti-Tray MPN table provided by IDEXX to

determine the most Probable Number (MPN) for total coliforms (yellow wells) in the sample. Correct the MPN value for any dilution made. The color of positive wells may vary. Use the appropriate Quanti-Tray MPN comparator following the instructions as indicated.

15.0 Method Performance-40CFR136.3 ATP evaluation (20.6)

- 15.1** Colilert-18 found to be equally sensitive to m-FC
- 15.2** Variances across the ten sites were similar using the Bartlett's test with Colilert-18 having a p-value (>0.05) of 0.308
- 15.3** False positives = 0%; False negatives = 7%
- 15.4** The EPA determined that Colilert-18 is a suitable method for the detection of fecal coliforms in wastewater when incubated at 44.5°C.

16.0 Reporting Results

- 16.1** Report results as MPN/100mL for fecal coliforms

17.0 Verification Procedure

- 17.1** Not applicable

18.0 Pollution Prevention

- 18.1** The solutions and reagents used in this method pose no threat to the environment when recycled and managed properly.
- 18.2** Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

19.0 Waste Management

- 19.1** 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 regulations. Compliance with all sewage discharge permits and regulations is also required.
- 19.2** Samples, reference materials and equipment known or suspected to have viable bacteria attached or contained must be sterilized prior to disposal.

20.0 References

- 20.1** Colilert-18 Package Insert from IDEXX.
- 20.2** Annual Book of ASTM Standards, Vol. 11.01, American Society for Testing Materials, Philadelphia, PA 19103, (2005)
- 20.3** 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. (December 1978)
- 20.4** 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.
- 20.5** Quanti-Tray Package Insert from IDEXX.
- 20.6** ATP summary report and letter from EPA
- 20.7** Federal Register/Vol72, NO 47, Monday March 12, 2007 Page 11236, Table Holding Time