Chapter 15

E. coli protocols

Background

Escherichia coli (*E. coli*) bacteria are indicator organisms; that is they are monitored in surface waters because their presence indicates fecal contamination is present. Because it is not practical or feasible to test for all the disease-causing organisms that can be present in surface water, we use *E. coli* as an indicator because it is commonly found in human and animal wastes and is easy to quantify in the laboratory. If *E. coli* is present above certain levels, then other disease-causing organisms may be present and a potential threat to human health exists.

Over the years the choice of indicator organism used in water quality standards has changed as new studies are performed to determine which indicator correlates best with human illness. The 1992-1994 Triennial Water Quality Standards Review recommended that *E. coli* replace enterococci as the indicator for freshwater and estuarine/non-shellfish producing waters and that fecal coliform be retained as the indicator for marine/shellfish producing waters.

This protocol explains the methods for sample collection and use of the Quanti-Tray® and Quanti-Tray 2000® MPN (most probable number) Enumeration Test Procedure and Colilert® reagent, both patented by IDEXX Laboratories, Inc. The U.S. Environmental Protection Agency (EPA) approved the Colilert® procedure in 1990 and the Quanti-Tray® addition in 1996. The substrate used in the test contains two indicator compounds (ONPG and MUG) that either produce a color or fluoresce when metabolized by total coliform or *E. coli*, respectively. This method is easy to use, provides results in 24 hours, and compares favorably with other methods for quantifying *E. coli*. The IDEXX Quanti-Tray 2000® MPN Method has a maximum counting range of 2,419 E.coli per 100 ml on undiluted samples. The maximum counting range of the Quanti-Tray® MPN Method is 200 MPN/100 mL on undiluted samples. As with other bacterial enumeration methods, the counting range can be extended by serial sample dilution. The Quanti-Tray 2000® method is recommended for environmental water samples because the 200 MPN/100 mL maximum quantification of the Quanti-Tray® method is less than the state *E. coli* standard of 406 MPN/100 mL.

Colilert®-18 reagent produces results after 18, rather than 24, hours of incubation, and should be used with marine samples. Marine samples must be diluted at least ten-fold before analysis with Colilert®-18.

In brief, a water sample is mixed with the Colilert® reagent and divided up into a series of wells. After incubation at the optimal temperature the number of positive wells are recorded (the number which turn yellow and the number which fluoresce under 365 nanometer (nm) ultraviolet (UV) light). The number of positive wells depends on the bacterial concentration in the original sample. The actual bacterial concentration is read from an MPN table based on the principle that each well has a certain probability of being positive.

Equipment and Supplies

All of the equipment and supplies can be ordered directly from IDEXX Laboratories, Inc. at the phone number 1-800-321-0207.

Item	Catalog Number
Colilert® reagent packs for 100 ml samples	WP020 (20-Pack)
Colilert® reagent packs for 100 ml samples	WP200 (200-Pack)
Colilert®-18 reagent packs for 100 ml samples	WP020-18 (20-Pack)
Colilert®-18 reagent packs for 100 ml samples	WP200-18 (200-Pack)
Quanti-Tray Sealer	WQTS-110 (110 Volt) WQTS-220 (220 Volt)
Quanti-Tray/2000® Quanti-Tray®	WQT2K-100 (100 trays) WQT-100 (100 trays)
97-Well Rubber Insert	WQT RBR-2K (use with Sealer)
Colilert® Comparator with Vessel	WP104
Collection Bottles with thiosulfate 120 ml	WV120SBST-20 (20 per case) WV120SBST-20 (200 per case)
Quanti-Cult® QC Kit Quality Control Bacteria (3 sets)	WKIT-1001
UV Viewing Cabinet	WCM10
Incubator, 35°C	WI300 (110 Volt/60Hz) WI3001 (220 Volt/50Hz)
Fluorescent UV Lamp, 365nm Or other 365 nm long wave UV lamp	WL160 (110V AC cord) WEA160F (220V European cord)
UV Absorbing Safety Goggles Or UV Absorbing Safety Spectacles	WLG WLS

Calibration and Standardization

1. This equipment need not be calibrated, although the incubator temperature must be maintained within 0.5°C of 35C° during incubation. Dry incubators may need to be turned on at least 12 hours before use to ensure that the temperature is stable. The incubator temperature should be checked and recorded daily during periods of use.

2. For each batch of Colilert® reagent (check Lot Number on package), follow the quality control procedure provided with the Quanti-Cult® QC Kit. This involves inoculating three separate bottles containing 100 ml of sterile water with three different bacteria cultures and following the test procedure explained in the Methods section. The following results should be obtained:

   E. coli—yellow, fluorescent;
   Klebsiella pneumoniae—yellow, not fluorescent;
   Pseudomonas aeruginosa—clear, not fluorescent.

Methods

Refer to the instructions that accompany the reagents and equipment.

1. Remove the lid from a 120 ml clean, sterile bottle without touching the bottle neck or cap threads. The bottle should have a 100 ml fill line like the IDEXX Collection Bottles listed in the equipment and supply list, and adequate volume to allow for vigorous mixing of the sample. For chlorinated water, use sample containers containing sodium thiosulfate so that chlorine will be neutralized.

2. After collecting sample, pour out excess sample so that the final volume is approximately 100 ml. Tightly cap the bottle and shake to dissolve the sodium thiosulfate, if present. If the sample was collected in a Whirl-Pak bag or a larger sterile container, transfer 100 ml into a clean, sterile bottle. Sample transfer should be done in the laboratory with a pipette for sterile transfer.

3. If the sample *E. coli* concentration is likely to exceed an MPN of 2,419 per 100 mL (200 MPN/100 mL for Quanti-Tray®) **or if the sample is saline**, the sample should be diluted with sterile distilled water. Use an aseptic pipette to transfer a portion of sample into a prepared sterile dilution water blank. For example, a ten-fold dilution is accomplished by transferring with a pipette 10 mL of sample into 90 mL of water. The diluted sample is then capped, shaken vigorously, and treated like a regular sample.

4. Carefully separate one Snap Pack of Colilert® or Colilert®-18 (for saline water) reagent from the strip. Tap it so that all of the powder is on the bottom of the pack.

5. Open the Snap Pack by snapping back the top at the line. Do not touch the opening.

6. Add the reagent to the 100 mL water sample (Figure 1).

Figure 1

7. Cap the sample jar tightly without touching the bottle neck or cap threads.

8. Shake the sample vigorously until the reagent powder is dissolved.

9. Allow sample to sit undisturbed for a few minutes to reduce foaming.

10. Open the Quanti-Tray or Quanti-Tray/2000® and hold it in one hand in a U-shape as you pour the entire sample into it, touching only the foil tab (Figure 2). Tap the small wells two to three times to eliminate air bubbles.

Figure 2

11. Follow the manufacturer instructions to send the sample tray in the insert through the sealing machine.

Figure 3

12. Incubate the tray filled with sample for 24 hours (with Colilert®) or 18 hours (with Colilert®-18) at 35+/-0.5°C. Do not overload incubators or water baths with sample trays because samples will not achieve proper incubation temperature. The IDEXX incubator holds a maximum of 12 trays, six on the bottom and six on the shelf.

13. Prepare the comparator sample by aseptically transferring the comparator from the glass bottle to a sterile Quanti-Tray® or Quanti-Tray/2000® and sending it through the sealing machine. Record the lot number and expiration date of the comparator on the tray. Store the comparator sample in the dark between 4 and 30°C when not in use.

14. After 18 (for Colilert®-18) or 24 (for Colilert®) hours of incubation, read and record the results of the test.

- If the wells in the Quanti-Tray® or Quanti-Tray/2000® do not have a yellow color, the test is negative.
- If the wells are yellow but a lighter yellow than the comparator, the tray may be incubated an additional four hours (no longer than 22 or 28 hours total, respectively, for Colilert®-18 and Colilert®) and reexamined. If they are still lighter yellow than the comparator after an additional four hours of incubation, the test is negative.
- Wells that have turned as yellow as the comparator indicate the presence of total coliform bacteria (Figure 4).

Figure 4

15. If the wells are at least as yellow as the comparator, check each well for fluorescence (Figure 5) by placing a 6 watt 365 nm UV light within five inches of the sample in a dark place. For convenience and safety, use the IDEXX viewing cabinet. If a cabinet is not available, use UV protective eyewear.

Figure 5

16. If using the Quanti-Tray/2000® read and record the number of small wells that fluoresce and separately record the number of large wells that fluoresce, including the large well at the top of the tray.

17. If using the Quanti-Tray® read and record the number of wells that fluoresce, including the large well at the top of the tray.

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LABORATORY CHEMICAL SAFETY

Ultraviolet (UV) light damages the human eye. Wear UV eye protection if viewing the sample with the light outside of a dark, enclosed box.

If comparator comes in contact with eyes or skin, flush thoroughly with water.

QUANTI-CULT contains live microorganisms and should be used only by individuals with bacteriological training. Properly disinfect any spills and sterilize all used containers according to appropriate regulations before disposal.

Calculations and Data Reporting

Refer to the MPN table provided with the Quanti-Tray® or Quanti-Tray/2000® to obtain the Most Probable Number (MPN) of *E. coli* in the sample.

If the sample was diluted, multiply the result by the appropriate dilution factor.

If all the wells in the tray are positive, the results must be reported as >2,419 MPN/100 mL (Quanti-Tray/2000®) or >200 MPN/100 mL (Quanti-Tray®). Remaining sample, if it exists and has been stored at 4°C, may be diluted, prepared, and placed in the incubator within 30 hours of collection. If incubation begins more than 30 hours after sample collection, any results must be reported as estimates.

References

American Public Health Association, American Water Works Association, and Water Environment Federation. 1999.

Standard Methods for the Examination of Water and Wastewater (20th Edition). Section 9223. American Public Health Association, Washington, DC.