

**APPENDIX D**  
**OHIO WATER MICROBIOLOGY LABORATORY**  
**ANALYSIS OF WATER SAMPLES USING COLILERT QUANTI-TRAY®**

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1. Warm up the sealer; this takes about 10 minutes.
2. Prepare lab forms. Fill out appropriate lab forms for each sample and check off the dilutions to prepare, if any.
3. Label the bottom of the incubation tray with sample information. Label the tray with site name, date, and dilution (if any).
4. Prepare sample/reagent mixture.
  - a. For undiluted sample:
    - i. The system will enumerate between 1 and 2,400 MPN/100 mL for the undiluted sample.
    - ii. Combine 100 mL of sample with one packet of Colilert reagent.
    - iii. Mix to dissolve reagent by gently inverting the bottle. Do not shake vigorously; this will cause foam.
  - b. For dilution:
    - i. If you suspect the water to have greater than 2,400 MPN/100 mL, make a 1:10 dilution
    - ii. Add 10 mL of the sample to 90 mL of sterile deionized water and combine with Colilert reagent.
    - iii. Mix to dissolve reagent by gently inverting the bottle. Do not shake vigorously; this will cause foam.
5. Pour the reagent/sample mixture into the incubation tray. Tap the small wells to release any air and allow foam to settle.
6. Run the tray through the Quanti-Tray sealer.
7. Incubate for 24–28 hours at 35±0.5°C.
8. Count large and small positive wells that
  - a. Fluoresce under a long-wave ultraviolet light as *E. coli*
  - b. Appear yellow under ambient light as total coliforms
  - c. Dim yellow color and dim or off-color fluorescence are not counted as positive results.
  - d. The large overflow well at the top of the tray is counted as a large well.
9. Refer to the MPN table to obtain results. For a 1:10 dilution, multiply the result in the table by 10 to get MPN/100 mL.
10. Positive and negative controls must be performed once every 20<sup>th</sup> sample.
  - a. For a positive control, make up 100 mL of a 10<sup>-8</sup> dilution of *E. coli* (ATCC 25922) and 100 mL of a 10<sup>-8</sup> dilution of *Serratia marcescens* (ATCC 31488).
  - b. For a negative control, make up 100 mL of a 10<sup>-8</sup> dilution of *P. aeruginosa* (ATCC 10145).
  - c. Prepare 3 sterile, test tubes with 5mL of sterile DI (use a pipet to measure this amount accurately). Appropriately label each tube (labels are listed in the table below).
  - d. Using a small, sterile loop (1µL), collect a loop of appropriate organism from generation 2 freezer stock and place in the appropriate tube. Be sure to twirl the

loop in the buffer. Repeat for remaining organisms. Vortex both tubes for at least 10 seconds immediately prior to further use

- e. Label dilution bottles. There should be 2 bottles for each control type. Add Colilert reagent to the appropriate dilution bottle.
- f. Seal, label, and incubate these controls as stated above.
- g. Confirm the viability of the *P. aeruginosa* culture by streak plating 0.1 mL of the  $10^{-4}$  dilution onto TSA.

Method	Organism (ATCC #)	Label	Description
Colilert	<i>Escherichia coli</i> (25922)	Positive EC Colilert	<i>E. coli</i> is a total coliform, so should appear yellow under ambient light. Colilert media also differentiates <i>E. coli</i> from other total coliforms, so <i>E. coli</i> wells will also fluoresce blue or blue-white under UV light.
Colilert	<i>Klebsiella pneumoniae</i> (31488)	Positive TC Colilert	<i>K. pneumoniae</i> is a total coliform, so should appear yellow under ambient light.
Colilert	<i>Pseudomonas aeruginosa</i> (10145 or 27853)	Negative Colilert	<i>P. aeruginosa</i> should be inhibited by the Colilert media, but may show some greenish fluorescence under UV light.