

**APPENDIX D2**  
**OHIO WATER MICROBIOLOGY LABORATORY**

**ANALYSIS OF WATER SAMPLES USING ENTEROLERT 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 Enterolert reagent.
    - iii. Mix to dissolve reagent by gently inverting the bottle. Do not shake vigorously; this will case 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 Enterolert reagent.
    - iii. Mix to dissolve reagent by gently inverting the bottle. Do not shake vigorously; this will case 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 41±0.5°C.
8. Count large and small positive wells that
  - a. Fluoresce under a long-wave ultraviolet light as enterococci.
  - b. Off-color fluorescence is not counted as positive results.
  - c. 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. faecium* (ATCC 35667).
  - b. For a negative control, make up 100 mL of a 10<sup>-8</sup> dilution of *S. marcescens* (ATCC 43862) and 100 mL of a 10<sup>-8</sup> dilution of *A. viridans* (ATCC 10400).
  - 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 Enterolert reagent to the appropriate dilution bottle.
- f. Seal, label, and incubate these controls as stated above.
- g. Confirm the viability of the *S. marcescens* and *A. viridans* cultures by streak plating 0.1 mL of the  $10^{-4}$  dilution onto TSA.

Method	Organism (ATCC #)	Label	Description
Enterolert	<i>Enterococcus faecium</i> (35667)	Positive Enterolert	<i>E. faecium</i> is an enterococci (gram +), so should fluoresce blue or blue-white under UV light.
Enterolert	<i>Serratia marcescens</i> (43862)	Negative G-Enterolert	<i>S. marcescens</i> a gram negative total coliform that should not exhibit fluorescence under UV light.
Enterolert	<i>Aerococcus viridans</i> (10400)	Negative G+ Enterolert	<i>A. viridans</i> is a gram positive organism that should not exhibit fluorescence under UV light.