APPENDIX D

OHIO WATER MICROBIOLOGY LABORATORY

ANALYSIS OF WATER SAMPLES USING COLILERT QUANTI-TRAY®

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. 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 Colilert reagent.
        iii. Mix to dissolve reagent. 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 35°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 20th sample.
    a. For a positive control, make up 100 mL of a 10^{-8} dilution of *E. coli* and *Serratia marcescens*; add one packet of Idexx reagent.
    b. For a negative control, make up 100 mL of a 10^{-8} dilution of *Pseudomonas aeruginosa* ATCC 10145 culture and add one packet of Idexx reagent
    c. Seal, label, and incubate these controls as stated above.
    d. Confirm the viability of the *P. aeruginosa* culture by streak plating 0.1 mL of the 10^{-4} dilution onto TSA.