

APPENDIX D
OHIO DISTRICT MICROBIOLOGY LABORATORY
ANALYSIS OF *E. COLI* AND TOTAL COLIFORMS USING COLILERT QUANTITRAY

- Warm up the sealer (this takes about 15 minutes) and set the incubator to 35°C.
- Shake the sample well and measure 100 mL of sample using a sterile graduated cylinder. Pour the 100 mL-sample into a sterile plastic or glass bottle.
- Add one packet of pre-measured commercial reagent (Idexx).
- Let dissolve for several minutes, especially if sample is cold. Shake well.
- Pour mixture into an Idexx Quantitray 2000 or Quantitray 200. Be careful not to touch the lip of the sterile vessel or the inside of the Quantitray with your hands.
- Tap the wells to encourage air bubbles to rise out of the tray. Place the Quantitray WELL-SIDE DOWN onto the appropriate orange rubber pad in the sealer tray.
- Hit the red button and seal the sample.
- Label the back of the tray with sample ID, date, and your initials.
- Incubate for 24-28 hours at 35°C. For Colilert-18, incubate for 18-22 hours.
- Yellow is positive for total coliforms, and whitish-blue fluorescence is positive for *E. coli*. A comparator (Idexx) is used to set threshold levels for positive results. Dim yellow color and dim or off-color fluorescence are not counted as positive results.
- On the Quantitray 2000, the large overflow well is counted as a large well. When some wells do not fill, assume positive or negative result based on the rest of the tray.
 - If one well doesn't fill, and 80% of the wells were positive, score the empty well as a positive result.
- Calculate the results (in MPN/100 mL) using the MPN table provided by Idexx for both *E. coli* and total coliforms. Record positive-well-count data and results on the Results Worksheet.
- Positive and negative controls must be performed once every 20th sample.
 - For a positive control, make up 100 mL of a 10⁻⁸ dilution of *E. coli* and add one packet of Idexx reagent.
 - 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
 - Seal, label, and incubate these controls as stated above.
 - Confirm the viability of the *P. aeruginosa* culture by streak plating 0.1 mL of the 10⁻⁴ dilution onto TSA.