APPENDIX D1
OHIO WATER MICROBIOLOGY LABORATORY
COLLECTION AND ANALYSIS OF SEDIMENT SAMPLES USING COLILERT QUANTITRAY

SAMPLE COLLECTION

Wading or diving sites

Due to the heterogeneity of *E. coli* in sediments, three sterile, polypropylene Nalgene 125-mL or 250-mL jars are used for sample collection:

1. Secure the lid on a sterile jar before diving or reaching to the bottom.
2. Upon reaching the bottom, open the jar and scoop the bed sediments into jar.
3. Close jar before surfacing.
4. Repeat for the remaining two jars.
5. Immediately place on ice in a cooler until processing.

Ponar sediment-sampler sites

When using a Ponar sediment sampler, three grab samples are collected and composited into one sterile, Nalgene 250-mL jar in the field:

1. Sterilize the sampler prior to use.
    a. Always wear gloves when sterilizing the Ponar.
    b. Wash the sampler in soap and rinse with tap water and then deionized or distilled water.
    c. Soak the sampler in 0.005% bleach solution for 10 to 20 minutes.
        i. Solution is prepared by adding 1 mL or household bleach to 999 mL of deionized or distilled water.
    d. Soak in sterile 0.005% sodium thiosulfate solution for 5 minutes.
        i. 10% stock solution is prepared by dissolving 100g of sodium thiosulfate in 1 L of deionized or distilled water. Autoclave for 15 minutes. Store at room temperature.
        ii. 0.005% working solution is prepared by adding 0.5 mL of the 10% stock to 999.5 mL of sterile deionized or distilled water.
2. Lower the sampler through the water column and collect the sediment sample per the manufacturer's instructions. After bringing the sampler to the surface, drain off the excess water. Deposit the sediment into a clean and sterile washtub.
    a. Washtub is sterilized by following the same procedure described above for sterilizing the Ponar followed by a rinse in sterile deionized or distilled water. Once sterilized, washtubs can be stored individually in new, clean garbage bags until ready for use.
3. Repeat above step two more times to collect a total of three grab-samples.
4. Use a sterile spatula to mix the three samples thoroughly and then deposit into a sterile, 250-mL jar. The jar should be ½ to ¾ full.
5. Immediately place on ice in a cooler until processing.
6. Sterilize sampler before using at another site.

PREPARATION OF SEDIMENT SAMPLES FOR ANALYSIS

1. Using a sterile spatula, remove 50 g of sediment from each of three replicate sample jars. Composite the sediments into sterile, 1-L jars and mix thoroughly. (If sample was composited in the field, skip this step.)
2. Place 20 g of the sediment composite into a labeled bottle containing 200 mL of phosphate buffered dilution water (PB) (U.S. Environmental Protection Agency, 2002). Immediately prepare a sample for dry-weight analysis (see below). NOTE: If preparing a replicate sample, Place 30 g of the sediment composite into a bottle containing 300 mL of PB.
3. Label the lid of this bottle (sample and buffer) with the time the bottle should be removed from the shaker (time on shaker: 45 minutes).
4. Place the bottle on the wrist-action shaker.
5. After 45 minutes, remove the bottle from the shaker and let it stand for 30 seconds undisturbed. Pour off the supernatant into a new, labeled sterile bottle.
6. Discard the sediment and analyze the supernatant by the Colilert Quantitray-2000 method for *E. coli*. (Idexx Laboratories, Westbrook, Maine).

DRY WEIGHT OF SEDIMENT SAMPLES

All sediment samples will be analyzed for dry weight.

1. After processing a sample for *E. coli*, weigh a clean, dry heat-tolerant glass or metal dish and record as "empty dish weight."
2. Add about 25 grams of composited sediment and record the dish weight with sediment.
3. Place in a 105^{o}C oven for 24 hours, or until a constant weight is obtained. If an oven is not available, dry in a desiccator until a constant weight is obtained.
4. Record the dish weight after drying and compute dry weight.
    a. Use the following equation to calculate dry weight:

$$\frac{\text{Weight after drying - empty dish weight}}{\text{Weight before drying - empty dish weight}}$$

COLILERT QUANTITRAY-2000 METHOD FOR SEDIMENT SAMPLES

1. Prepare lab forms. Fill out appropriate lab forms for each sample and check off the dilutions to prepare, if any.
2. Label Quantitray. Label the bottom of tray with sample information. Label the tray with site name, date, and dilution (if any).
3. 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 supernatant with one packet of Colilert reagent in a sterile bottle.
        iii. Mix to dissolve reagent.
    b. For dilution:

i. If you suspect the water to have greater than 2,400 MPN/100 mL, make an appropriate dilution (for example, 1/10, 1/100, etc.).
ii. For a 1/10 dilution
    1. Add 10 mL of the supernatant to 90 mL of sterile water and combine with Colilert reagent.
    2. Mix to dissolve reagent.

4. Pour the reagent/sample mixture into the Quantitray. Tap the small wells to release any air and allow foam to settle.
5. Run the tray through the Quantitray sealer.
6. Incubate for 24 hours at 35°C.
7. 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 (if required for the project).
8. 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. NOTE: Confidence limits for all MPN results have been issued by Idexx Laboratories and can be obtained at http://www.idexx.com/water/refs/qt2k95.pdf.
9. Concentrations of *E. coli* in sediment are reported as MPN per gram of dry weight sediment (MPN/g_{dw}). Calculate col/g_{dw} as described below.
    a. A sediment dilution factor was calculated based on beach sediments from Lake Erie (Francy and Darner, 1998) as follows: Because 20 g of dry sediment displaces approximately 10 mL of buffer, the total volume of the sediment/buffer mixture is 210 mL. The dilution factor for the sediment sample is therefore, 10.5 mL/g (210 mL/ 20g).
    b. Use the following equation to obtain your results:

$$\text{MPN/g}_{dw} = \frac{\text{MPN}}{100\ \text{mL}} \times \frac{10.5\ \text{mL}}{\text{g}} \times \frac{1}{\text{dry weight}}$$

REFERENCES CITED:

Francy, D. S. and Darner, R.A., 1998, Factors affecting *Escherichia col* concentrations at Lake Erie public bathing beaches: U.S. Geological Survey Water-Resources Investigations Report 98–4241, p. 9.

U.S. Environmental Protection Agency, 2002, Method 1603–*Escherichia coli* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar: Washington, D.C., EPA 821-R-02-023, p. 3-4.