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SAN FRANCISCO BAYKEEPER

STANDARD OPERATING PROCEDURE

Bacteriological Analysis of Marine Waters Using Colilert-18 and Enterolert

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1. BACKGROUND AND APPLICABILITY

One of the indicators of poor water quality is the presence of pathogens, disease causing viruses, bacteria and protozoans. Often, the presence of pathogens is the result of fecal contamination which, in marine waters, can be the result of inadequately treated sewage, urban runoff, boat and marina waste, combined sewer overflows, and animal waste.

2. METHODS

Testing for directly pathogens is difficult and expensive so monitoring programs test for "indicator" species instead. Indicator species are bacteria that also occur in feces, but are usually not pathogenic. IDEXX's Colilert-18 and Enterolert methods are used to detect the presence of fecal indicator bacteria in water because they are relatively simple and inexpensive.

Both Colilert-18 and Enterolert contain a compound, or substrate, that the bacteria being tested for can metabolize and other bacteria usually can't. When the bacteria metabolize the substrate, they produce another compound that is either visible or fluoresces (can be seen under ultra-violet light). Enterolert fluoresces when enterococcus are present; Colilert-18 turns yellow when total coliforms are present and fluoresces when *E. coli* are present. This technique allows water quality monitors to detect the present of bacteria associated with feces within 18-24 hours of collecting the sample.

Colilert-18 was originally formulated for testing fresh waters, but has been used successfully to monitor marine waters with slight modifications in the sample preparations. Some studies have shown that Colilert-18 can produce false positives in marine waters when the sample is not sufficiently diluted. The Environmental Protection Agency's SOP recommends running a lab duplicate for each batch of samples when the dilution used is less than 1:100.

3. HEALTH AND SAFETY

3.1 Microbiological analyses involve the culturing of potentially pathogenic organisms, therefore care should be taken to protect oneself from contamination. Always wear sterile latex gloves when collecting samples or processes the results. *Do not perform this analysis if you are allergic to latex.* Wash hands thoroughly before and after coming in contact with samples or Quanti-Trays.

3.2 All biologically contaminated materials in the laboratory, namely the IDEXX Quanti-Trays, should be autoclaved prior to disposal. After analyses of a Quanti-Tray is complete, dispose of it, intact, in the container labeled "Used Trays."

3.3 All accidents and spills must be reported to Amy Chastain immediately.

3.4 A 6-watt ultraviolet light is used to read Quanti-Trays for *E. coli* and enterococcus results. Care should be taken not to look directly at the light, and it should be pointed away from the analyst during readings.

4. APPARATUS AND MATERIALS

4.1 Colilert-18 and Enterolert dry media in "Snap-Packs." Set out one of each for each sample to be analyzed.

4.2 Colilert Quanti-Tray 2000 MPN trays**.** Set out two trays—one for a Colilert-18 analysis and one for and Enterolert analyses—for each sample to be analyzed.

4.3 Two sterile, disposable sample bottles for each sample.

4.4 Sterile water. You will need approximately 90 mL of water for each sample to be analyzed.

4.5 70% ethanol or antibacterial disinfectant spray for sterilizing the workbench.

4.6 10 mL pipette and sterile pipette tips.

4.7 Two incubators: one at 35^{o}C and one at 42 ^{o}C.

4.8 6 watt, 365 nm UV lamp

4.9 Colilert MPN Tables and the MPN generator.

4.10 Colilert color comparator.

5. ANALYTICAL PROCEDURES

5.1 Put on sterile latex gloves.

5.2 Lay out enough labmat to cover the work surface.

5.3 Using a pipette and sterile pipette tip, place approximately 80 mL of sterile water into the sample bottle.

5.4 Add the contents of one Colilert-18 or Enterolert snap pack to the bottle. Take care not to touch the opening of the pack to the sample bottle or spill the contents. Mix the contents thoroughly until all media is dissolved.

5.5 Take the sample off ice and shake it gently but thoroughly.

5.6 Using a 10mL pipette and sterile pipette tip, transfer 10 mL of the sample from the whirl-pak into the sterile disposable sample bottle. Take care not to bring the pipette tip into contact with the whirl-pak bag or the side of the sample bottle.

5.7 Take the temperature of the remaining sample. Record this value on the Lab Data

Sheet under "receiving temperature." *Do not perform this step until you are certain that you no longer need the sample.*

5.8 Add approximately 10 mL of sterile water to the sample bottle until the meniscus is right at the graduated line on the bottle.

5.9 Place the cap on the bottle and shake gently.

5.10 Open one Quanti-Tray and pour in the contents of the bottle. Do not allow the sample bottle and the Quanti-Tray to come into contact with each other. Remove any air bubbles by tapping the wells.

5.11 Place the filled Quanti-Tray face (well) down into the orange Quanti-Tray rubber insert so that the plastic wells are protected by the rubber insert. Place the rubber insert with tray into the Sealer with the largest cutout facing out and the white backing of the Quanti-Tray facing up. Slide it through the Sealer until the motor grabs it and begins to draw it in.

5.12 Remove the sealed Quanti-Tray from the Sealer and place it in the appropriate incubator. Record the time and incubator temperature on the Lab Data Sheet.

5.13 Incubate the Colilert trays for 18-20 hours and the Enterolert trays for 24 hours.

5.14 Count the positive wells for each, and enter into the appropriate space on the Lab Data Sheet. See the table below.

Reagent	Yellow	Fluorescence	Interpretation
Colilert-18	Yes	Yes	Positive for *E. coli*
Colilert-18	Yes	No	Positive for coliform
Colilert-18	No	Yes	Negative for *E. coli*, false positive
Colilert-18	No	No	Negative for *E. coli* and coliform
Enterolert	NA	Yes	Positive for enterococcus
Enterolert	NA	No	Negative for enterococcus

6. DOCUMENTATION

6.1 Consult the Quanti-Tray MPN Table and derive a most probable number for both coliform and *E. coli.* Keep in mind that the symbol ">" means "greater than" and "<" means "less than" and must be listed as part of the number. If using a dilution, multiply the dilution ratio by the MPN number and enter that result. Samples that do not result in positive results in any wells are to be listed as <1 MPN/100mL for 1:1 dilution, <10 MPN/100mL for 1:10 dilution, <100 MPN/100mL for 1:100 dilution and not zero.

6.2 Place completed Lab Data Sheet in the basket labeled "data."

7. QUALITY CONTROL

7.1 Positive and negative controls should be run on each new lot of Colilert-18 and Enterolert.

7.2 One sterile laboratory blank should be run with each day's samples.

7.3 One field duplicate should be run with each day's samples. The field duplicate results should be within a 95% confidence interval of its pair. If the results do not meet this criterion, flag all results for the batch.

7.4 One lab duplicate should be run for each days' samples. The lab duplicate should be selected at random. The results should be within a 95% confidence interval of its pair. This lab duplicate may also be run at a higher (1:100) dilution to test for false positives. If the result is significantly lower than expected, flag all results.

REFERENCES

USEPA, *Standard Operating Procedures for Volunteer Monitoring of Surface Waters for Bacteria*, EPA SOP # 1106, Rev #3, December 2001.

USEPA, *Standing Operating Procedures for Colilert,Colilert-18 and Colisure, Coliform and E. coli Water Analysis*, EPA SOP #1103, Rev. #3, December 4, 2003.

USEPA, *Volunteer Stream Monitoring: A Methods Manual*, EPA 841-B-97-003, November, 1997.

USEPA Volunteer Estuary Monitoring: A Methods Manual, EPA 842-B-93-004, December, 1993.