

Standard Operating Procedure (SOP) 3.4.1.1

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Coliform Bacteria Analysis: Colilert 18 Hr. Method with Quanti-Trays®

Total coliform bacteria, fecal coliform bacteria, and *E. coli* are all considered indicators of water contaminated with fecal matter. Contaminated water may contain other pathogens (micro-organisms that cause illness) that are more difficult to test for. Therefore these indicator bacteria are useful in giving us a measure of contamination levels.

E. coli is a bacterial species found in the fecal matter of warm blooded animals (humans, other mammals, and birds). Total coliform bacteria are an entire group of bacteria species that are generally similar to and include the species *E. coli*. There are certain forms of coliform bacteria that do not live in fecal matter but instead live in soils. Fecal coliform bacteria are coliform bacteria that do live in fecal matter, including, but not limited to, the species *E. coli*. Most of the fecal coliform cells found in fecal matter are *E. coli*. Therefore, all *E. coli* belong to the fecal coliform group, and all fecal coliform belong to the total coliform group.

Colilert-18 is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in fresh and marine waters. It is based on IDEXX's patented Defined Substrate Technology® (DST®). When total coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert-18's nutrient indicator, MUG, the sample fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 MPN/100ml within 18 hours even with as many as 2 million other heterotrophic bacteria cells per 100ml present.

Equipment and Supplies

- 1) Disposable rubber gloves.
- 2) Sealed Whirlpack bags or sterile plastic bottles for sampling.
- 3) A clean ice chest and frozen plastic sealed "blue" ice.
- 4) A refrigerator maintained at a temperature of 4° C.
- 5) An incubator capable of maintaining a temperature of 35° C \pm 0.5° C.
- 6) An IDEXX sealer.

- 7) A tightly closed or sealed vessel of distilled water.
- 8) Antibacterial solution (e.g., Lysol spray).
- 9) Sterile 10 ml pipettes and a pipette pump.
- 10) Quanti-trays and sterile 100 ml mixing bottles supplied by IDEXX Corp.
- 11) A 6 watt, 365nm, ultra-violet (UV) light.
- 12) Sterile Colilert media, pre-packaged from IDEXX Corporation. Media will be stored at 4-25°C away from light. Media will be used prior to the manufacturer's expiration date.

Safety Considerations

- 1) When sampling in potentially contaminated waters take all precautions to prevent coming into contact with the water. These precautions include wearing rubber gloves and washing with antibacterial soap following sampling and analysis.
- 2) Never sample waters after a known sewage spill or when the waters are officially closed by a Public Health Officer.
- 3) Never sample when river, surf or beach/shore conditions are hazardous.
- 4) Never sample in stream bank areas in which there is the potential for falling into the stream. Never sample during flood stage conditions.
- 5) Never sample alone on streams or storm drains.
- 6) Never sample from bridges or roads where there is the potential for being at risk from automobile traffic.
- 7) Always wash down all surfaces used in the processing of samples with antibacterial solutions (e.g., Lysol, etc.) once the Quantitrays are in the incubator or after a spill of a sample.
- 8) All spent Quanti-trays containing live bacterial cultures (positive, yellow wells) must be autoclaved prior to disposal. Develop a partnership with a college, laboratory, public health agency or hospital that can accept and autoclave the spent Quanti-trays.

Field Sample Collection Procedure

- 1) Sterile sample bottles will be used (Whirl-Pak bags are acceptable) and will be labeled with the proper sample ID. The sampler will use aseptic technique, making certain that the bottle does not touch the benthic substrate (rock, sand, or mud on the bottom of the waterbody). Therefore, make an effort, within reason, to prevent sediment from entering the sample containers.
- 2) When samples are collected on a beach, it will be collected on an incoming wave. In all cases (beach or stream) the sampler must be downstream and away from the bottle, and the mouth of the bottle must face into the current.
- 3) After the sample is taken, the bottle will be immediately tipped to decant enough sample to ensure 1-2 inches of airspace in the sample bottle. The bottle will be tightly capped or twist closed (in the case of the Whirlpack) and promptly stored on ice (target temperature 4° C) in a clean ice chest.
- 4) Laboratory analysis should begin within 6 hours of sample collection and within two hours of receiving the sample at the lab.

Prevention of Contamination or Interferences in the Laboratory

- 1) Laboratory Sterile Technique: When performing bacterial analysis all laboratory personnel will wash hands prior to beginning tests and will wash hands thereafter whenever their hands become soiled with samples, etc. All counters must be cleaned with a bactericide prior to performing tests. Sample bottles or Whirlpacks must be inverted (to mix) prior to opening in the lab. After opening the samples, subsamples for dilutions must be performed as soon as possible to minimize the potential for contamination. Only sterile pipettes must be used for subsampling.
- 2) Sterile Dilution Water: Water used to prepare culture media and reagents will be sterile distilled water stored out of direct sunlight to prevent growth of algae. All marine water samples must be diluted by at least 1:10 with distilled sterile water.
- 3) In samples with excessive chlorine, a blue flash may be seen when adding Colilert-18. If this is seen, consider sample invalid and discontinue testing.
- 4) False Positives: These are wells in which a different bacteria species (other than coliform bacteria) has grown and caused a color change. Even when all of the above precautions are followed there may still be false positive wells in the incubated quanti-trays. Do not record false positives as positive wells. However, you should make a note in your lab book or lab data sheet regarding the presence of the false positives for future reference.

Lab Procedures

- 1) Different types of water samples require different types of preparation as follows:
 - a) For sterile (blank) water or relatively clean fresh water pour 100 ml of sterile water or sample directly into the sterile 100 ml mixing bottle (by filling to the 100 ml line) and add one package of the reagent. Cap and shake until dissolved.
 - b) For fresh water that is suspected to contain contamination, pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, after the foam subsides, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.
 - c) For all marine or estuarine water samples (salinity greater than 5 ppt), pour 50 ml of sterile distilled water into the mixing bottle and add one package of the reagent. Cap and shake until dissolved. Then, using a sterile pipette add 10 ml of sample and top off with 40 ml (to the 100 ml line). Cap and shake again. This is a 1:10 dilution.
- 2) Make sure there is little or no foam left in the headspace of the mixing bottle prior to moving on to the next step.
- 3) Pour sample/reagent mixture from the mixing bottle into a quanti-tray and seal in the IDEXX Sealer.
- 4) Place the sealed tray in a $35\pm 0.5^{\circ}\text{C}$ incubator for a minimum of 18 hours and a maximum of 22 hours (includes warming time). This is the incubation period.

Quality Control Elements

- 1) Duplicate Analyses: A minimum of 5 % of the samples on a given day should be subsampled and run in duplicate. At least one duplicate should be run on every day in which the analyses are run. An attempt will be made to select samples that yield positive results (i.e., suspected of contamination) for the duplicate analyses.
- 2) Negative Blanks: One blank (sterile) water sample will be analyzed per batch of samples processed.
- 3) External Reference Samples: A positive control is a sample prepared in the lab to contain a known approximate concentration of coliform bacteria. An external reference sample is a positive control prepared and provided by a professional laboratory. The external reference sample is split. You should

analyze the split external reference and compare your results to the professional lab. At least two external reference samples must be run per year.

Determining the Most Probable Number of Coliform Cells Per 100ml of Sample

- 1) Following the incubation period, observe and count the number of positive wells. For *E. coli* look for fluorescence with a 6 watt, 365nm, UV light within 5 inches of the sample. Face light away from your eyes and towards the sample.
- 2) For total coliform and *E. coli* use the following **Result Interpretation Table**:

Appearance	Result
Colorless or slight tinge	negative for total coliforms and <i>E. coli</i>
Yellow equal to or greater than the comparator (supplied by IDEXX)	positive for total coliforms
Yellow equal to or greater than the comparator <u>and</u> fluorescence	positive for <i>E. coli</i>

Note: Fluorescent wells that are not yellow (i.e., wells which are not positive for total coliforms) cannot be considered positive for *E.coli*. **In other words, these are false positives for *E. coli*.**

- 3) Refer to the MPN table (provided by IDEXX) specific to the type of quanti-tray used (51 well or 97 well type of quanti-tray) to obtain a Most Probable Number per 100 ml of sample.
- 4) If a dilution was performed, after obtaining the initial MPN result from the table, multiply that result by the dilution level to obtain the final result (e.g., if a 1:10 dilution was employed, multiply the result from the MPN table by 10 to get the final result in MPN/100 ml).
- 5) Samples are negative if at any time after the incubation period is complete there is no yellow or yellow/fluorescence. Yellow or yellow/fluorescence observed before 18 hours is a valid positive. However, after 22 hours from inoculation, heterotrophic bacteria may overwhelm Colilert-18's inhibition system. Therefore, yellow or yellow/fluorescence first observed after 22 hours from inoculation is not a valid positive.

Technical Assistance

For IDEXX technical assistance call 1-800-321-0207 or 207-856-0496.

References:

- 1) IDEXX Colilert (18 hour) Manual

- 2) Enzyme Substrate Coliform Test Method 9223, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington D.C., 1998
- 3) Quality Assurance Plan for the Southern California Bight 1998 Project, Microbiology Committee, Southern California Coastal Water Research Project