

The following methods are recommended by the Environmental Laboratory Accreditation Program (ELAP) and the Microbiological Disease Laboratory (MDL) for the analysis of recreational marine water for compliance with Health and Safety Code §115880 [Assembly Bill 411 (AB 411), Statutes of 1997, Chapter 765].

Recommended Methods for the Analysis of Recreational Marine Water for AB 411

TOTAL COLIFORM BACTERIA

- Total Coliform by Multiple Tube Fermentation (MTF) — SM 9221 B ^(1,2)
- Total Coliform by Membrane Filtration (MF) Using m-Endo — SM 9222 B ^(1,2)

See comments below on Colilert™ 18 Medium (IDEXX) (Quanti-Tray™) for total coliforms

FECAL COLIFORM BACTERIA

- Fecal Coliform* by Multiple Tube Fermentation (MTF) Using EC Medium — SM 9221 C, E ^(1,2)
- Fecal Coliform* by Membrane Filtration Using m-FC ^(7,8) — SM 9222 D ^(1,2)

* With the written approval of the local health officer and with data showing comparative numbers for fecal coliforms and E. coli, a laboratory may instead test for E. coli, a subset of fecal coliforms (US EPA's definition), using Colilert™ 18 Medium (IDEXX). Guidance on comparative testing is available in [DHS Salt Water Beaches Guidance](#). Comparative testing must be performed only by laboratories certified by ELAP for the methods being compared. Laboratories must retain the results of the parallel testing in their files, consistent with their record retention procedures, and must make these data available for review upon request by the State.

ENTEROCOCCUS BACTERIA

- Enterococci by Membrane Filtration (MF) Using mE ⁽⁹⁾ or mEI ⁽¹¹⁾ — SM 9230 C ^(1,2); EPA Method 1600 ⁽¹⁰⁾
- Enterococci by Enterolert™ ^(3,4,5) — IDEXX Co.

JUSTIFICATION:

- The US EPA in its publication Ambient Water Quality Criteria for Bacteria –1986 ⁽¹³⁾ has recommended the use of E. coli or enterococci for testing fresh waters and enterococci only for marine waters.
- AB 411 and its implementing regulations require that marine water be tested for total coliforms, fecal coliforms and enterococci.
- Testing for three groups of indicator organisms with traditional methods for water testing requires much staff time, media and equipment space. Streamlined test methods with fast turnaround times and acceptable data are desired.

- Because of these concerns, ELAP and MDL reviewed the published literature to see if we could justify the use of two rapid methods using chromogenic/fluorogenic substrates, one that identifies both total coliforms and *E. coli* ^(5,10) and the other, enterococci. ^(3,4,5)
- A number of laboratories have indicated a desire to test for *E. coli* in place of fecal coliforms. In US EPA's Action Plan for Beaches and Recreational Waters ⁽¹⁴⁾, *E. coli* is defined as "a subset of the fecal coliform group that is part of the normal intestinal flora in humans and animals and is, therefore, a direct indicator of fecal contamination of the water." *E. coli* is considered to be a more specific indicator of fecal contamination. ^(6,13)
- Colilert™ 18 Medium (IDEXX) (Quanti-Tray™) is being used by these laboratories to report *E. coli* in place of fecal coliforms.
- Each method has its advantages and its shortcomings. Since California is examining marine water for three groups of indicator organisms, it is felt that, based on EPA's definition of *E. coli*, public health will not be compromised if ELAP is flexible on the substitution of *E. coli* for fecal coliforms.
- Colilert 18 is not recommended for the enumeration of total coliforms from marine water. Published studies suggest there are substantial false positives, yielding higher total coliform counts from marine water. ^(5,12) However, it is recognized that this method is easy to use, gives rapid and sensitive results, and has greater precision, when used for quantitative information, than the multiple tube fermentation test.
- If Colilert 18 is to be used for AB 411 monitoring for total coliforms it must be acknowledged that this method may result in an overestimation of true total coliform numbers, which errors in favor of protecting public health. Total coliform results that repeatedly exceed the AB 411 standards should be verified with a more conservative method. Such tests must be performed only by laboratories certified by ELAP for the method.
- Enterolert™ medium in Quanti-Trays™ (IDEXX) is a 24-hour method for enterococci. Published literature supporting the use of this medium is available ^(3,4,5). The medium is approved for use in some states for the testing of marine recreational water.

Review of Available Methods:

Total Coliforms

Multiple Tube Fermentation (MTF)

- Pros: Much historical data. Substantial scientific support. Allows testing of all kinds of waters, including colored and turbid.
- Cons: Requires up to 4 days for completion. Requires increase in tubes, media, incubator space, labor and time. Imprecision of MPN enumeration. 95% confidence limits are broad.

Membrane Filtration (MF)

- Pros: Substantial scientific support. Much historical data. A direct count of organisms. Greater precision and accuracy.
- Cons: Can require up to 3 days for completion. Verification of colonies required. May not pick up viable but stressed organisms. Not all waters can be filtered. Technically more complex than other methods. Labor intensive.

Colilert™ 18 Quanti-Tray

- Pros: Easy to use. More sensitive. Results in 24 hrs. Greater precision in quantitation compared with MTF (5 tube mpn) Less staff time, media and incubator space required.
- Cons: Substantial false positives with marine water yielding higher counts than MTF method.⁽¹²⁾

Fecal Coliforms or E. coli

Multiple Tube Fermentation (MTF) for fecal coliforms

- Pros: Much historical data. Substantial scientific support. Allows testing of all kinds of waters, including colored and turbid.
- Cons: Requires up to 4 days for completion. Requires increase in tubes, media, incubator space, labor and time. Not all thermal tolerant fecal coliforms are E. coli.^(1,2) Imprecision of MPN enumeration. 95 % confidence limits are broad.

Membrane Filtration (MF) for fecal coliforms

- Pros: Much historical data. Substantial scientific support. Provides direct count of organisms.
- Cons: Can require up to 3 days for completion. Verification of colonies required. Not all waters can be filtered. Technically more complex than other methods. Not all thermal tolerant fecal coliforms are E.coli.

Colilert™ 18 Quanti-Tray™ for E. coli

- Pros: Easy to use. Results in 24 hrs. Greater precision in quantitation compared with MTF. Sensitive. E. coli is a (usually major) subset of fecal coliforms.
- Cons: Not listed as one of the organisms required by AB 411. Not all E. coli are mug positive – false negatives; not all fluorescent organisms are E. coli – false positives.⁽¹²⁾

June Kani, ELAP, and Dan Mills, Ph.D., MDL
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