Detection of Total Coliforms, Thermotolerant (Fecal) Coliforms and *E. coli* by Colilert® in Water - Prescriptive

**Parameter** Coliforms [Total, Thermotolerant (Fecal), and *E. coli*]  
**Analytical Method** Enzyme Substrate Test

**Introduction**  
This method is prescriptive. It describes the selective isolation of total coliforms, thermotolerant (fecal) coliforms, and *E. coli* from environmental water sources such as fresh water, surface water, ground water, etc. This test can also be applied to wastewater and effluent samples.  

For seawater, Colilert-18 can be used for *E. coli* detection (but not for total coliforms or thermotolerant (fecal) coliforms).

Note that in British Columbia, drinking water testing must be performed by approved test methods as defined by the BC EWQA Program and in compliance with the BC Drinking Water Protection Act. This method does not meet the prescriptive elements required for testing and reporting drinking water samples. It is intended for the analysis of environmental test samples (including those that may potentially be used as drinking water sources), but it is not intended as a method to confirm suitability of drinking water for human consumption.

A licence must be obtained from the Public Health Agency of Canada (PHAC) to purchase the control organisms required for this test. Refer to the PHAC website.

**Method Summary**  
The Colilert and Colilert-18 reagents simultaneously detect the presence of total coliforms and *E. coli*. Two nutrient-indicators, ONPG and MUG, are metabolized by the coliform enzyme β-galactosidase and the *E. coli* enzyme β-glucuronidase, respectively. As coliform organisms grow during incubation at 35.0 ± 0.5°C, they use β-galactosidase to metabolize ONPG and change it from colourless to yellow. *E. coli* use β-glucuronidase to metabolize MUG and create fluorescence. Since most non-coliforms do not have these enzymes, they are unable to grow, and therefore they do not interfere or cause false positives. The few non-coliforms that do have these enzymes are selectively suppressed by the Colilert formulation.

Thermotolerant (fecal) coliforms are detected on a separate sample aliquot using the Colilert-18 hour reagent and an elevated incubation temperature of 44.5 ± 0.2°C, which is necessary to eliminate non-thermotolerant organisms.

The Presence/Absence tests for Total Coliforms and *E. coli* is performed directly in sample bottles. The Presence/Absence test for thermotolerant (fecal) coliforms is also performed directly in the same bottles, using a separate sample and the Colilert-18 hour reagent.

IDEXX Quanti-Tray and Quanti-Tray 2000 are semi-automated quantification methods based on the Most Probable Number (MPN) model as described in *Standard Methods for the Analysis of Water and Wastewater*. The 51-well tray is used for samples such as drinking and clean surface waters with an expected concentration range of 1 to 200 MPN / 100 mL. The 97-well tray has an auto-dilution feature that allows quantification from 1 to 2,419 MPN / 100 mL, and is suitable for clean water samples, effluents, wastewaters, or other samples where a higher count is expected.

Quantitative enzyme substrate tests for total coliforms, thermotolerant (fecal) coliforms, and *E. coli* from can also be performed in a multiple-tube format that
results in a higher detection limit than the Quanti-Tray test. In the multiple-tube test, a series of tubes are inoculated and incubated, and the resulting reaction is converted to MPN units. Refer to IDEXX for supplies and instructions.

<table>
<thead>
<tr>
<th>MDL(s) and EMS Analyte Code(s)</th>
<th>Method Version / Analyte</th>
<th>Approx. MDL</th>
<th>EMS Analyte / Method Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multi-well / Quantiti-tray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>1 MPN /100 mL</td>
<td>0451 / X388</td>
<td></td>
</tr>
<tr>
<td>Thermotolerant (Fecal) Coliforms</td>
<td>1 MPN /100mL</td>
<td>0450 / X388</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>1 MPN /100 mL</td>
<td>0147 / X388</td>
<td></td>
</tr>
<tr>
<td><strong>Presence / Absence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>Present or Absent</td>
<td>0451 / n.a.</td>
<td></td>
</tr>
<tr>
<td>Thermotolerant (Fecal) Coliforms</td>
<td>Present or Absent</td>
<td>0450 / n.a.</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>Present or Absent</td>
<td>0147 / n.a.</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple-Tube Fermentation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Coliforms</td>
<td>2 MPN / 100 mL</td>
<td>0451 / n.a.</td>
<td></td>
</tr>
<tr>
<td>Thermotolerant (Fecal) Coliforms</td>
<td>2 MPN / 100 mL</td>
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<td></td>
</tr>
</tbody>
</table>

***Refer to EMS Parameter Dictionary on the ministry website for all current EMS codes.

**Matrix**

Fresh water, Wastewater, Seawater

**Interferences and Precautions**

Non-coliform bacteria, such as *Aeromonas*, *Flavobacterium*, and *Pseudomonas* species may produce small amounts of the β-D-galactosidase enzyme within the incubation time if present in concentrations of more than $10^6$ CFU/100 mL.

Background colour in a water sample may interfere with interpretation of the colour produced after incubation. This interference is eliminated by comparing the colour produced by the sample to the colour of a control blank of the same sample, to which no reagent was added. Samples with a very high background colour must be diluted prior to analysis.

Excessive chlorine may interfere with this test. If a blue flash is seen when adding the Colilert reagent, the sample is considered to be invalid and the test must not be completed.

Colilert reagent is not intended to be used for samples altered by pre-enrichment or concentration, and therefore this test cannot be used as a confirmation step for cultures isolated by other tests.

Use on effluents that have been treated with brighteners or surfactants should be confirmed by running successive dilutions, as these products can produce fluorescence which can interfere with *E. coli* analysis.

**Sample Handling and Preservation**

The sample is collected in the field and submitted unfiltered in a sterilized bacteriology water bottle containing sufficient sodium thiosulfate to neutralize up to 15 mg/L residual chlorine, or a minimum of 10 mg/container. Sodium Thiosulfate is effective in neutralizing the bactericidal effect of chlorine, neutralizing residual halogens, and preventing continuation of bactericidal action during sample transit.

**Stability**

**Holding Time:** Incubation must begin within 30 hours of sample collection for results to be valid. Minimum volume required for analysis is 100 mL (APHA 9060A 2013).

**Storage:** The sample should be kept cool (at <10°C) during transport and storage until analysis. Do not freeze samples (APHA 9060B, 2013).
**Procedure**

**PRECAUTIONS**

Work aseptically to prevent contamination of lab personnel and the lab area, and to prevent cross-contamination between samples. Refer to the *Government of Canada Canadian Biosafety Standard* for more information.

Incubation temperatures and times are important to prevent false positive and false negative reactions. The incubation details are provided by the manufacturer and must be followed.

Where subsampling occurs, be sure to homogenize the sample well prior to subsampling.

If dilutions are needed, do not dilute the sample in buffered water. The reagents are already buffered, and excessive buffer compounds can adversely affect the growth of the target organisms.

**TEST PROCEDURE**

Detailed, prescriptive instructions for the quantitative, presence/absence, and multiple-tube tests accompany the supplies purchased from the Colilert® test method supplier (IDEXX), and are also available on their website.

Seawater samples must be diluted at least tenfold prior to testing.

Note that use and handling instructions for control organisms, and quality control practice guidelines are not described in the manufacturer's instructions. Refer to APHA 9020 for guidance on these topics.

**DATA ANALYSIS**

Refer to the MPN table provided by the manufacturer for the specific test performed.

**Quality Control**

<table>
<thead>
<tr>
<th>QC Component</th>
<th>Minimum Frequency</th>
<th>Minimum Data Quality Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Blank (MB)</td>
<td>One per batch (max 20 samples)</td>
<td>Less than reported DL or Absent for P/A tests</td>
</tr>
<tr>
<td>Lab Duplicates (DUP)</td>
<td>1 per batch (max 20 samples)</td>
<td>± 65% RPD</td>
</tr>
<tr>
<td>Positive &amp; Negative Controls</td>
<td>One each per day per incubator</td>
<td>Expected reaction to confirm proper operation of incubator and performance of the test.</td>
</tr>
</tbody>
</table>

*B.C. EWQA Program QC requirements for drinking water testing are more stringent, requiring duplicate samples at a frequency of 1 in 10 samples.*

If DQOs are not met, repeat testing or report qualified test results. If Analyst precision criteria is not met additional training may be needed.

**Method Blank:** The method blank is 100 mL sterile water poured into a 120 mL sample bottle, (containing sodium thiosulfate if used with test samples).

**Laboratory Duplicates:** Sample duplicates are prepared when sufficient sample is received to subsample for laboratory duplicates. Homogenize the sample well prior to subsampling into individual 120 mL sample bottles.

**Positive / Negative Controls:** Three are recommended: *E. coli*, a total coliform other than *E. coli* and a non-coliform. Using all three each day confirms that the reagent is performing as expected for all target and non-target organisms and that the incubator is operating as expected (gets to the right temperature at the right
rate). Refer to APHA 9020 for more information.

Proofing of sample bottles, organisms, reagent and trays by lot is recommended to demonstrate sterility and performance prior to use. Refer to APHA 9020 for more information on recommended Quality Control practices for this test.

References

4. IDEXX Instructions. Prescriptive instructions available on the IDEXX website.

Revision History

Nov 14, 2002  SEAM codes replaced by EMS codes
Nov 14, 2004  Formatting changes. Addition of Revision History section.
Nov 15, 2018  Revised format, updated references: APHA 9223 revised 2016. APHA 9060 Samples revised 2013, APHA 9020 revised 2015. Confirmed that test is prescriptive with reference to detailed procedures on IDEXX website. Added Thermotolerant (Fecal) Coliform testing to method. Updated QC section to include Method Blanks and Duplicate Samples. Changed sample storage temperature to <10C as per APHA 9060 (2013).