Determination of Thermotolerant Coliforms in Water by Multiple-Tube Fermentation Technique

Parameter Coliform, Thermotolerant
Analytical Method Multiple Tube Fermentation

Introduction
This method is prescriptive. It describes the statistical estimation of total coliform density in environmental water sources such as fresh water, surface water, ground water, etc. This test can also be applied to wastewater and effluent samples.

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
Thermotolerant coliforms (those that ferment lactose to produce gas at 44.5°C), are a subset of the coliform group and were traditionally called fecal coliforms. However, they have also been documented in organically rich waters or tropical climates in the absence of recent fecal contamination. Therefore, testing for \textit{E. coli}, a specific indicator of fecal contamination, is recommended.

Current regulations may require that Thermotolerant (formerly fecal) coliforms be identified and enumerated. In the multiple-tube fermentation technique, this group of organisms is identified by their ability to ferment lactose to produce gas at 44.5°C.

### MDL(s) and EMS Analyte Code(s)

<table>
<thead>
<tr>
<th>Analyte Code(s)</th>
<th>Analyte</th>
<th>Approx. MDL (units)</th>
<th>EMS Analyte Code</th>
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</thead>
<tbody>
<tr>
<td>MTF-Quantitative</td>
<td>Coliform, Thermotolerant Confirmed</td>
<td>1 MPN/100 mL</td>
<td>0450 X015</td>
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Matrix Water

Interferences and Precautions
The precision of the fermentation test in estimating coliform density depends on the number of tubes used. The most satisfactory information will be obtained when the largest sample inoculum examined shows acid and/or gas in some or all of the tubes and the smallest sample inoculum shows no acid and/or gas in any or a majority of tubes.
Use the completed test on as a quality control measure on at least 10% of coliform-positive non-potable water samples on a seasonal basis to ensure false positive test results are not reported.

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 ± 2.5 mL (APHA 9060 B.1, 2006).

**Storage:** The sample should be kept cool (at <8°C) during transport and storage until analysis. Do not freeze samples (APHA 9060 B.1, 2006).

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 are contend in APHA 9221 E.

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 in APHA 9221 C.

**Quality Control**

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.
# Summary of QC Requirements

<table>
<thead>
<tr>
<th>QC Component</th>
<th>Minimum Frequency</th>
<th>Minimum Data Quality Objectives</th>
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</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>

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:** Two are recommended. Using both each day confirms that the test 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.

**References**

1. APHA 9221 (2006) Multiple-Tube Fermentation Technique for Members of the Coliform Group
3. APHA 9020 (2005) Quality Control

**Revision History**

- November 14, 2002: SEAM Codes replaced by EMS Codes

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