Total Coliforms in Water by Multiple-Tube Fermentation - Prescriptive

Parameter
Coliforms, Total

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, seawater, etc. This test can also be applied to wastewater and effluent samples.

Drinking water testing in BC must be performed by test methods approved by the BC Enhanced Water Quality Assurance (EWQA) Program, in compliance with the BC Drinking Water Protection Act. This method does not meet all EWQA requirements for drinking water testing, and does not define regulatory requirements for the analysis of drinking water samples originating in BC.

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 coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method used for detection, lactose fermentation, rather than on the tenets of systematic bacteriology. Accordingly, when the fermentation technique is used, this group is defined as all facultative, anaerobic, gram-negative, non-spore forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours incubation at 35°C.

MDL(s) and EMS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approx. MDL</th>
<th>EMS Analyte / Method Codes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>1 MPN /100 mL</td>
<td>0451 / X015</td>
</tr>
<tr>
<td>Total Coliforms, Confirmed</td>
<td>1 MPN /100 mL</td>
<td>0451 / 2495</td>
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</tbody>
</table>

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

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.

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.

Sample Handling and Preservation
The sample is collected in the field and submitted unfiltered in a sterilized bacteriology bottle containing sufficient sodium thiosulfate to neutralize up to 15 mg/L residual chlorine, or a minimum of 10 mg anhydrous / 120 mL container or 15 mg of the pentahydrate form. Sodium Thiosulfate is effective in neutralizing the bactercidal effect of chlorine, neutralizing residual halogens, and preventing continuation of bactercidal action during sample transit.

Holding Time: Incubation must begin within 30 hours of sample collection for results to be valid (APHA 9060B, 2013). Minimum volume required for analysis is 100 mL (APHA 9221A, 2014).
Storage: The sample should be kept cool (at <10°C) during transport and storage until analysis. Do not freeze samples (APHA 9060A, 2013).

Procedure

Refer to detailed instructions provided within the APHA reference method for guidance on the execution of this test:

APHA 9221 Multiple-Tube Fermentation Technique for Members of the Coliform Group.

The APHA guidance for this test is prescriptive and must be followed without modification.

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.

Use the completed (confirmed) 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.

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

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</td>
</tr>
<tr>
<td>Lab Duplicates (DUP)</td>
<td>1 per batch (max 20 samples)¹</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>

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.

If DQOs are not met, repeat testing or report qualified test results.

Method Blank: The method blank is 100 mL sterile water that proceeds through the same sample handling processes as test samples, (including sodium thiosulfate if used with test samples; recommend preparing Method Blank in a sample bottle).

Laboratory Duplicates: Sample duplicates are prepared when sufficient sample is received to subsample for laboratory duplicates. Homogenize the sample well prior to subsampling. Process both aliquots through the same sample handling processes as test samples.

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 (reaches correct temperature at correct rate). Refer to APHA 9020 for more information.

Proofing of sample bottles, organisms, reagents, and supplies 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


Revision History

Nov 14, 2002  SEAM codes replaced by EMS codes.
Dec 20, 2019  Updated to BC Lab Manual Prescriptive Method format. APHA 9221 was revised in 2013. APHA 9060 was revised in 2013. APHA 9020 was revised in 2015. Prescriptive nature of test is confirmed. QC section updated to include Method Blanks and Duplicate Samples. Changed sample storage temperature to <10°C as per APHA 9060 (2013).