Detection of Total Coliforms and *E. coli* in Water by Membrane Filtration and Chromocult® Coliform Agar (CCA) - Prescriptive

**Parameter(s)**
- Coliform, total
  - *E. coli*

**Analytical Method**
- Membrane Filtration

**Introduction**
This method is prescriptive. It describes the selective isolation of total coliforms and *E. coli* 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**
Chromocult® Coliform Agar is a selective and differential chromogenic culture medium for the microbiological analysis of water samples. Within 24 hours incubation at 35°C± 0.5°C, this medium enables the simultaneous detection, differentiation and enumeration of *E. coli* and coliform bacteria.

Counting of coliform bacteria is based on the ability of β-D-galactosidase, an enzyme which is characteristic of coliform bacteria, to cleave the substrate Salmon-GAL. The reaction results in salmon red colored coliform bacteria colonies.

Counting of *E. coli* is based on the cleavage of both the substrates X-glucuronide by β-D-glucuronidase and Salmon-GAL by β-D-galactosidase, an enzyme combination, which is characteristic of *E. coli*. In the presence of *E. coli* both substrates are cleaved, resulting in colonies that take on a dark blue to violet color as opposed to the salmon red of other coliform bacteria colonies. Non-coliform bacteria appear as colorless.

Occasionally on mEndo medium, typical sheen colonies may be produced by non-coliform organisms. Verification of typical and atypical colonies is required.

**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>
</tr>
</thead>
<tbody>
<tr>
<td>MF-Quantitative</td>
<td>Total Coliforms</td>
<td>1 CFU /100 mL</td>
<td></td>
</tr>
<tr>
<td>MF-Quantitative</td>
<td><em>E. coli</em></td>
<td>1 CFU /100 mL</td>
<td></td>
</tr>
<tr>
<td>MF-Qualitative</td>
<td>Total Coliforms</td>
<td>Present or Absent</td>
<td></td>
</tr>
<tr>
<td>MF-Qualitative</td>
<td><em>E. coli</em></td>
<td>Present or Absent</td>
<td></td>
</tr>
</tbody>
</table>

**Matrix**
- Water
Interferences and Precautions

The MF technique is useful in monitoring clean, non-turbid samples. However, the MF technique has limitations when testing waters with high turbidity or large numbers of noncoliform (background) bacteria. If heterotrophic bacteria interference is exhibited, for example, sample results may need to be invalidated and new samples collected.

The type and quality of membrane filter affects the size, coloration and number of colonies significantly.

Sample Handling and Preservation

The sample is collected in the field and submitted unfiltered and unpreserved in a sterilized water bacteriology 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.

**TEST PROCEDURE**

Purchase the media from a commercial vendor; it cannot be prepared from basic ingredients.

The prescriptive procedure is described in APHA 9222J.

Follow sample size selection and filtering procedures in APHA 9222B.

Refer to APHA 9020 for guidance on quality control testing practices for the evaluation and maintenance of equipment, media and organisms.

**Data Analysis**

Refer to reading instructions in APHA 9222J.

**Quality Control**

Proofing of sample bottles, organisms, and agar 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>
</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)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 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 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**
4. EMDMillipore technical data sheet, Chromocult Coliform Agar.

**Revision History**
Nov 22, 2018        First Edition