Enterococci Membrane Filter Technique (MF) for Fresh Water, Wastewater and Marine Water - Prescriptive

Parameter: Enterococci

Analytical Method: Membrane filtration

Introduction: This method is prescriptive. It describes the selective isolation of enterococci from environmental water sources such as fresh water, surface water, ground water, seawater, etc. This test can also be applied to wastewater and effluent 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: Enterococci are Gram-positive, catalase negative, non-spore-forming cocci. The normal habitat of enterococci is the gastrointestinal tract of animals and humans.

Non-turbid water samples are passed through a 0.45 µm membrane filter which is placed on a selective medium (mE agar) for 48 ± 4 hours incubation at 41 ± 0.5°C for growth of enterococci.

False positive test results are frequent, therefore confirm positive colonies as per APHA 9230.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Approx. MDL (units)</th>
<th>EMS Analyte Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococci MF - Quantitative Enterococci</td>
<td>1 CFU /100 mL</td>
<td>0148 X022</td>
</tr>
</tbody>
</table>

Matrix: Water

Interferences and Precautions: The MF technique has limitations, particularly when testing waters with high turbidity.

Follow all instructions in the prescriptive reference method, including safety precautions and including confirming positive colonies to prevent reporting false positive test results.

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:** Begin incubation within 24 hours of sample collection. 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 in the reference method and must be followed.

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

**TEST PROCEDURE**

Detailed, prescriptive instructions are described in APHA 9230C.

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 APHA 9230.

**QUALITY CONTROL**

Proofing of sample bottles, organisms, reagent and filtration units 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.

<table>
<thead>
<tr>
<th>Summary of QC Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>QC Component</td>
</tr>
<tr>
<td>Method Blank (MB)</td>
</tr>
<tr>
<td>Lab Duplicates (DUP)</td>
</tr>
<tr>
<td>Positive &amp; Negative Controls</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:** 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**
3. APHA 9020 (2015) Quality Control

**Revision History**
Nov 14, 2002: SEAM Codes replaced by EMS codes.