

METHOD STATEMENT



Determinand:

Enterococci

Matrix:

Sample Type: Effluents and Recreational Waters

Principle of Method:

A known volume of water is filtered through a membrane filter with 0.45µm pores upon which bacteria are entrapped. The filter is then placed on a selective growth medium, Slanetz and Bartley Agar (S&B) and incubated at 37°C ± 1 for 40-48 hours or 30°C ± 1 for 4 hours followed by 44°C ± 0.5°C for 44 hours after which characteristic colonies are counted and picked off for confirmation. Confirmation is carried out on presumptive colonies using Kanamycin Aesculin Azide Agar. The confirmation is based on the ability of isolated colonies to hydrolyse aesculin.

Sampling and Sample Preparation:

Once taken, microbiological samples should be transferred immediately to dark storage conditions and kept at a temperature between 2 - 8°C for transport to the laboratory. If samples are not analysed immediately on receipt in the laboratory, they should be kept at a temperature between 2 - 8°C, in dark conditions until analysis commences.

Samples should be analysed as soon as practicable on the day of collection. In exceptional circumstances, if there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

Where an exceedance has occurred, the customer should be informed or a statement reflecting this should be included with the report (except where the customer has been already made aware that this is occurring on a regular basis and requests this not to be applied).

Interferences

Samples with high turbidity's may not be suitable for this method. The particulates may block the filter and limit the volume that can be examined. Accumulated deposits on the membrane may mask or inhibit the growth of the target organisms. High numbers of competing organisms may also mask or inhibit the growth of the target organisms.

Accumulated deposits on the filter membrane may also inhibit the growth of indicator organisms. For samples containing a lot of debris, the debris should be allowed to settle before filtration. More than one sample volume/dilution should also be filtered to enable easy reading of the final plates.

Performance of Method:

Range of Application:

Presumptive and confirmed Enterococci are expressed in colony forming units (cfu) per 100ml of sample.

Limit of Detection:

The limit of detection is not calculated for this method

Uncertainty of measurement:

The uncertainty of measurement is not calculated for this method

References:

Standing Committee of Analysts, The Microbiology of Recreational and Environmental Waters (2015) – Part 4 - Methods for the isolation and enumeration of enterococci.

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Standing Committee of Analysts, The Microbiology of Drinking Water (2012) - Part 5 -Methods for the Isolation and enumeration of enterococci

GELDREIH, EC and KENNER, BA (1969) Concepts of Faecal Streptococci in Stream Pollution. Journal of Water Pollution Control Federation, 41, R336-R353.

ROSSER, PAE and SARTORY, DP (1982) A Note on the Effect of Chlorination of Sewage Effluents on Faecal Coliform to Faecal Streptococci ratios in the Differentiation of Faecal Pollution Sources. Water SA, 8, 66-68.

SLANETZ, LW and BARTLEY, CH (1957) Numbers of Enterococci in Water, Sewage and Faeces determined by the Membrane Filter Technique with an improved medium. Journal of Bacteriology, 74, 591-595.

Collins and Lyne's Microbiological Methods. Sixth Edition 1989. Page 133, Membrane Filter Counts.

Environment Agency - The Microbiology of Drinking Water (2002) Part 2 - Practices and Procedures for Sampling.

A Performance Evaluation of the Faecal Streptococci Membrane Filtration Method S. Townsend. Mythe Laboratory, Severn Trent Water Limited.

A performance evaluation of Slanetz and Bartley agar, bought in and prepared in house. D Hard, Claire Peacock, (STL February 1999).

Protocol for the evaluation of subculture against membrane transfer for the confirmation of enterococci from S&B agar. E Parker (STL August 2006).