

METHOD STATEMENT

Determinand:

Enterococci

Matrix:

Sample Type: 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 for 48 hours after which characteristic colonies are counted and picked off for confirmation.

Confirmatory tests are then carried out on the presumptive colonies. The colonies are then subcultured onto a confirmatory medium and incubated for up to 18 hours to demonstrate the growth in the presence of bile salts and sodium azide and the hydrolysis of aesculin. Small colonies may be grown up on Brain Heart Infusion Agar at 37°C prior to subculture onto a confirmatory medium. A catalase test is performed on colonies which produce blackening of the agar. From the results of the confirmatory tests, the number of presumptive Enterococci and confirmed Enterococci present can be determined.

Interferences:

Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mg^l⁻¹ should counteract up to 5mg^l⁻¹ of free and combined residual chlorine (The Microbiology of Drinking Water 2002, part 5).

Process waters may contain different biocides and the use of sodium thiosulphate may not appropriate under these conditions. Customers should provide guidance when obtaining quotes.

Performance of Method:

Limit of Detection,

Estimated every 5 years.

Limit of detection = the number of organisms known to be added in the inocula at the end point dilution.

Uncertainty of measurement:

Recalculated every 6 months

References:

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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.

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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).

The Microbiology of Recreational and Environmental Waters (2015) – Part 4 – Methods for the isolation and enumeration of enterococci

