METHOD STATEMENT

Determinand:
Enterococci

Matrix:
Waste water & Environmental 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 membrane filter is then placed on a selective growth medium containing triphenyltetrazolium chloride and incubated at 37°C for 48 hours after which characteristic colonies are counted and picked off for confirmation.

Presumptive enterococci bacteria are defined as those bacteria that reduce 2,3,5-triphenyltetrazolium chloride to the insoluble red dye formazin to produce red, maroon or pink colonies on Slanetz and Bartley agar after incubation at 37°C for 4 hours followed by 44°C for 44 hours. Some strains may produce colonies that are very small and/or pale in colour.

Where confirmation has been requested by the customer, isolation of colonies is followed by identification using a MALDI-TOF MS system to perform protein profiling.

The Microbiology of Drinking Water and the Microbiology of Recreational and Environmental Water do not provide a comprehensive list of reportable enterococci. The reference methods do not differentiate between the potential sources of enterococci. Indeed no distinction is made between intestinal enterococci and those found in environmental habitats. Therefore, all enterococci colonies identified on the MALDI-TOF MS system may be considered confirmed enterococci.

As an alternative confirmation, the membrane filter containing presumptive colonies is transferred from S&B plates to a confirmatory medium (Kanamycin azide aesculin agar) and incubated at 44°C for 4 hours. Alternatively the colonies are sub cultured onto the confirmatory medium and incubated for up to 18 hours to demonstrate growth in the presence of bile salts and sodium azide and the hydrolysis of aesculin. Confirmed enterococci are characteristic colonies from Slanetz and Bartley agar plates, which either produce a black or brown colour (hydrolysis of aesculin) when inoculated onto Kanamycin azide aesculin agar and incubated at 44°C for up to 18 hours.

From the results of the confirmatory tests, the number of presumptive enterococci and confirmed enterococci present can be determined.

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.

Interferences:
Samples with high turbidities 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.

Chlorine and chloramines are neutralised by adding sodium thiosulphate, which at a concentration of 18mgl-1 should counteract up to 5mgl-1 of free and combined residual chlorine.

Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

Reporting of Results:
Results will be reported as greater than 100 (>100) for any samples where the dilution used gives colonies in excess of this maximum number. Some customers will not accept results of greater than 100 and in these circumstances any necessary dilutions must be agreed between the customer and the laboratory prior to analysis.

Presumptive enterococci numbers are quoted as the number of colonies per 100ml. Calculate the enterococci count as follows: -

$$\text{Presumptive Enterococci / 100ml} = \frac{\text{No. of colonies} \times 100}{\text{Volume of sample filtered (ml)}}$$

Confirmed enterococci are calculated by multiplying the number of presumptive enterococci by the proportion of the isolates that confirmed.

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

References:
Standing Committee of Analysts - The Microbiology of Recreational and Environmental Waters (2015) – Part 1 – Water quality, epidemiology and public health

Standing Committee of Analysts - The Microbiology of Drinking Water (2002) - Part 1 - Water Quality and Public Health

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

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