METHOD STATEMENT

Determinand:
Enterococci

Matrix:
Sample Types: 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 using the following techniques:
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 subcultured onto a 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. Small colonies may be grown up on Brain Heart Infusion Agar at 37°C prior to subculture onto a confirmatory medium. From the results of the confirmatory tests, the number of presumptive Enterococci and confirmed Enterococci present can be determined.
As an alternative confirmation, colonies may be identified directly using a MALDI-TOF MS system to conduct 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. The confirmation by MALDI-TOF was validated as equivalent to the traditional method. Therefore, all enterococci colonies identified on the MALDI-TOF MS system may be considered confirmed enterococci.

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
Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mgl-1 should counteract up to 5mgl-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:

Range of Application:

Results will be reported as greater than 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 and confirmed Enterococci are expressed in colony forming units (cfu) per 100ml of sample.
Confirmed enterococci results are calculated by multiplying the number of presumptive enterococci by the proportion of the isolates that confirmed
Reporting counts >100
If the presumptive Enterococci result is >100, but only 6 out of 10 isolates confirm as Enterococci, the result must be reported as >60 Enterococci. If 7 out of 10 isolates confirm as Enterococci the result must be reported as >70 Enterococci etc.
If the alternative confirmation method is employed, a comment stating this should be entered into progress chaser.

Limit of Detection:

The Limit of Detection for this method is calculated as detailed in internal Procedure GOP7.2B.

Uncertainty of measurement:

The Uncertainty of Measurement for this method is calculated as detailed in internal Procedure GOP7.6C and the results are recorded on GQF7.6.3.

References:

Environment Agency – The Microbiology of Drinking Water (2012) -Part 5– The Isolation and Enumeration of Enterococci by Membrane Filtration.
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.
The Microbiology of Recreational and Environmental Waters (2015) – Part 4 – Methods for the isolation and enumeration of enterococci