METHOD STATEMENT

Determinand:
Enterococci

Matrix:
Raw and Potable 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 48 hours (EA Report 2002). Some strains may produce colonies that are very small and/or pale in colour.

Confirmatory tests are then carried out on the presumptive colonies. The colonies are then sub cultured 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. As an alternative to confirmation, colonies may be identified directly using a MALDI-TOF MS system to conduct protein profiling.

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 or identify as Enterococcus species. 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:
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.
Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

Performance of Method:

Range of Application:	0 - 100 cfu/100ml without dilution
Limit of Detection:	1 cfu/100ml
Normal Reporting Level:	0 cfu/100ml = Not Detected

References:

Environment Agency – The Microbiology of Drinking Water (2012) -Part 5– A Method for the Isolation and Enumeration of Enterococci by Membrane Filtration.