METHOD STATEMENT

Determinand:
Enterobacteriaceae

Matrix:
Potable and Raw water

Principle of method:
A known volume of the water sample (normally 100ml) is filtered through a membrane filter with 0.45µm pores upon which bacteria are retained. The membrane is then placed onto pre-poured plates of violet red bile glucose agar (VRBGA).

The plates are then incubated aerobically at 37±1°C for 21±3 hours. Characteristic presumptive colonies are round and are pink to red or purple (with or without precipitation haloes). Presumptive colonies are counted and reported.

If further identification is required by the client for investigative purposes, the "Bacti ID" test may be requested. Identification by MALDI ToF MS may not be conclusive as the genus and species classification within the family Enterobacteriaceae is ever changing. Biochemical tests such as glucose fermentation and oxidase tests may give useful information. Confirmed Enterobacteriaceae have traditionally been regarded as oxidase negative and will make glucose agar turn yellow throughout the media when incubated at 37±1°C for 21±3 hours.

Sampling and Sample Preparation:
Once taken, microbial samples should be transferred directly to cold 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 between 2-8°C in dark conditions until the commencement of analysis.

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

Interferences:
Chlorine and chloramines are neutralised by adding sodium thiosulphate, which at a concentration of 18mg/l should counteract up to 5mg/l of free and combined residual chlorine.

The effectiveness of this method is affected by high turbidity samples. The filter may become blocked, limiting the maximum volume that can be examined. The accumulated deposits on the membrane may mask or inhibit the growth of target organisms. Dilutions may be carried out to reduce the impact of this but are only useful for samples containing high levels of Enterobacteriaceae.

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:	0 cfu/100ml
Normal Reporting Level:	0 cfu/100ml

References:

BS EN ISO 21528-2:2017 - Microbiology of the food chain. Horizontal method for the detection and enumeration of Enterobacteriaceae. Colony-count technique

Janda JM, Abbott SL. The Changing Face of the Family Enterobacteriaceae (Order: "Enterobacterales"): New Members, Taxonomic Issues, Geographic Expansion, and New Diseases and Disease Syndromes. Clin Microbiol Rev. 2021;34(2):e00174-20. Published 2021 Feb 24. doi:10.1128/CMR.00174-20