METHOD STATEMENT

Determinand:
Clostridium perfringens including Sulphite Reducing Clostridia

Matrix:
Raw and Potable waters

Principle of Method:
A known volume of the water sample is filtered through a membrane filter with 0.45µm pores upon which the bacteria are retained. The filter is then placed on a selective growth medium containing sulphite, iron (III) and D-cycloserine (which inhibits other bacteria and reduces the size of colonies that develop). The medium is incubated under anaerobic conditions at 37°C + 1°C for 21 + 3 hours or 44°C + 1° C for 21 + 3 hours for Sulphite-reducing Clostridia (SRC) or *Clostridium perfringens* respectively. Black colonies may be produced as a result of the reduction of Sulphite to Sulphide, which reacts with the iron(III) salt

SRC do not require confirmation however *Clostridium perfringens* must be confirmed. *Clostridium perfringens* may be confirmed by direct identification using a MALDI-TOF MS system to perform protein profiling.

Alternatively, *Clostridium perfringens* produces the enzyme acid phosphatase, which is a diagnostic characteristic for this species amongst the Clostridia. The traditional confirmation method of identifying production of acid phosphatase, following subculture of the presumptive colonies onto Columbia Agar, is therefore also an acceptable confirmation technique.

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 (2021) – Part 6 –Methods for the Isolation and Enumeration of Sulphite-reducing Clostridia and Clostridium Perfringens by Membrane Filtration.