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) and incubated under anaerobic conditions. Black colonies may be produced as a result of the reduction of Sulphite to Sulphide, which reacts with the iron(III) salt

Confirmed *Clostridium perfringens* are defined as those bacteria which produce colonies on tryptose-sulphite-cycloserine agar after anaerobic incubation at 44°C ± 0.5°C for 21 ± 3 hours. Colonies are non-motile, reduce nitrate, ferment lactose and liquefy gelatin. *Clostridium perfringens* also produce the enzyme acid phosphatase, which is a diagnostic characteristic for this species amongst the Clostridia.

As an alternative to traditional confirmation, colonies may be identified directly using a MALDI-TOF MS system to perform protein profiling.

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 (2002) – Part 6 –Methods for the Isolation and Enumeration of Sulphite-reducing Clostridia and Clostridium Perfringens by Membrane Filtration.