METHOD STATEMENT

Determinand:
Sulphite-reducing clostridia

Matrix:
Sample Types: Waters.

Principle of Method:
A known volume of the water sample is filtered through a membrane filter with 0.45mm 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 (The Microbiology of Drinking Water 2009, part 6).
Confirmed Clostridium perfringens are defined as those bacteria which produce colonies on tryptose-sulphite-cycloserine agar after anaerobic incubation at 44°C ± 1 °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 5 ± 3°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 5 ± 3 °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 2009, part 6).
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:

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:
Standing Committee of Analysts, The Microbiology of Drinking Water (2021) – Part 6 – Methods for the isolation and enumeration of sulphite-reducing clostridia and Clostridium perfringens by membrane filtration

Environment Agency: The Microbiology of Drinking Water (2010) – Part 2 –Practices and Procedures for Sampling.