METHOD STATEMENT

Determinand:
Clostridium perfringens including Sulphite Reducing Clostridia

Matrix:
Waste 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 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.

Where confirmation has been requested by the customer, isolation of colonies is followed by identification 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:
Samples with high turbidities may not be suitable for this method. The particulates may block the filter and limit the volume that can be examined. Accumulated deposits on the membrane may mask or inhibit the growth of the target organisms. High numbers of competing organisms may also mask or inhibit the growth of the target organisms.

Accumulated deposits on the filter membrane may also inhibit the growth of indicator organisms. For samples containing a lot of debris, the debris should be allowed to settle before filtration. More than one sample volume/dilution should also be filtered to enable easy reading of the final plates.

Chlorine and chloramines are neutralised 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:
The Limit of Detection has not been determined for this method.

References:

Standing Committee of Analysts - The Microbiology of Recreational and Environmental Waters (2015) – Part 1 – Water quality, epidemiology and public health

Standing Committee of Analysts - The Microbiology of Drinking Water (2002) - Part 1 -Water Quality and Public Health

Standing Committee of Analysts - The Microbiology of Recreational and Environmental Waters (2015) – Part 5 – Methods for the isolation and enumeration of sulphite-reducing clostridia and Clostridium perfringens

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