

## METHOD STATEMENT

**Determinand:**

Sulphite-reducing clostridia

**Matrix:**

Sample Type: 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).

**Interferences:**

Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mg<sup>l</sup><sup>-1</sup> should counteract up to 5mg<sup>l</sup><sup>-1</sup> of free and combined residual chlorine (The Microbiology of Drinking Water 2009, part 6).

**Performance of Method:****Limit of Detection,**

Estimated every 5 years.

Limit of detection = the number of organisms known to be added in the inocula at the end point dilution.

**Uncertainty of measurement:**

Recalculated every 6 months

**References:**

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- SARTORY, D.P. (1986). Membrane filtration enumeration of faecal *Clostridia* and *Clostridium perfringens* in water. *Water Research*, 20, 1225-1260.
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- Collins and Lyne's *Microbiological Methods*. Sixth Edition 1989. Page 133, Membrane Filter Counts.
- Environment Agency: *The Microbiology of Drinking Water (2002) – Part 2 –Practices and Procedures for Sampling.*
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- Comparison of existing and proposed methodologies for the isolation of Sulphite reducing *Clostridia* from waters by membrane filtration by P. Merrikin, R. Williams and P. Holmes. Quality Assurance (Eastern). Severn Trent Water Limited. July 1991.
- Validation of acid phosphatase test by S Tharmaseelan. March 2009.
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