METHOD STATEMENT

Determinand:
Sulphite reducing clostridia (SRC), Clostridium perfringens, Enterococci, Pseudomonas aeruginosa, Pseudomonas species, Staphylococcus aureus, Listeria species, Bacillus cereus, yeasts and moulds, total coliforms, faecal coliforms, Enterobacteriaceae, Campylobacter, total viable counts, Nitrate reducing bacteria (NRB) and Sulphate reducing bacteria (SRB).

Matrix:
Sludge, soil and other solid material.

Principle of method:
The sample is homogenised and serially diluted using maximum recovery diluent (MRD). For most determinands the diluted sample is filtered through a 0.45µm membrane. The membrane filter is placed onto agar, selective to the target organism and incubated. The percentage dry mass of the sample is used to calculate the final result. For total viable count, the dilution series is pipetted and added to agar, forming pour plates. Test kits are employed for NRB and SRB testing

Sampling and Sample Preparation:
Samples should be taken in sterile plastic containers. A minimum of 100g should be provided to the laboratory.
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:
Interferences may include high levels of competing organisms or sample turbidity / debris.

Performance of method:

Range of Application –	Membrane filtration:	10 – 10,000,000 cfu/gram (wet weight)
	TVC:	10 – 100,000,000 cfu/gram (wet weight)
Limit of Detection:		10 cfu/gram
Normal Reporting Level:		<10 cfu/gram

References:
Microbiology of Sewage Sludge 2003, Part 2 - Practices and procedures for sampling and sample preparation

Method W24. Laboratory method for Escherichia Coli per Gram Dry Weight by Membrane Filtration. (Sludge, Soils and Other Solids). PSJ, SB 2007.

Method W7. Laboratory method for Isolation, Enumeration and Confirmation of Enterococci - Membrane Filtration Technique. EP 2006.

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Method W8. Laboratory method. for *Clostridium Perfringens* including Sulphite Reducing Clostridia – Membrane Filtration Technique. EP 2005.

Method W9. Laboratory method for *Pseudomonas* Spp – Membrane Filtration Technique. MJR 2005.

Method W11. Laboratory method for *Pseudomonas Aeruginosa* – Membrane Filtration Technique. PSJ, EP 2007.

Method W16. Laboratory method for Isolation and Confirmation of Listeria from Potable and Raw Water Samples. EP, MF 2005.

Method W20. Laboratory method for the Isolation and Enumeration of Yeasts and Moulds from Potable and Environmental Water Samples. MF 2006.

Method W21. Laboratory method for the Isolation and Enumeration of Staphylococcus Aureus from Potable and Environmental Water Samples. EP, MF 2006.

Method W26. Laboratory method for the Isolation and Enumeration of Faecal Coliforms, using MLSB. SB, PSJ 2007.

Method W10. Laboratory method for the Isolation and Enumeration of Total Coliforms and E.Coli. EP/MF 2008.

Method W1. Laboratory method for the Isolation and Enumeration of Heterotrophic Bacteria by Pour Plate Method. EP, PSJ, MF 2007.

Method W33. Laboratory method for the Isolation and Enumeration of Enterobacteriaceae. IM 2008.

Method W35. Laboratory method for the Isolation and Enumeration of Thermophilic Campylobacter by Selective Enrichment. SB 2008.

Method W18. Laboratory method for the Detection of Sulphate reducing bacteria in water systems. VD ED SB 2010

Method W19. Laboratory method for the Detection of Nitrate reducing Microorganisms in water samples. VD ED SB 2010