METHOD STATEMENT

Determinand:
Heterotrophic bacteria

Matrix:
Sample Type: Waters

Principle of Method:
A viable count is used to determine the number of cells in the sample capable of forming colonies on a suitable agar medium, with the assumption that each viable cell can yield one colony. For this reason, the viable count is often called the colony count or plate count.
There are two ways of performing a colony count: the spread plate method, an alternative technique not covered by this method, and the pour plate method. Pour plates are prepared by pipetting a known volume of sample water into a sterile Petri dish and then adding molten yeast extract agar and mixing gently by swirling the plate. Following incubation under the relevant conditions, the numbers of colony forming units are counted.

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.

Interferences
Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18 mg/l should counteract up to 5 mg/l of free and combined residual chlorine.
Yeast extract agar is a nutrient rich medium and is known to support the growth of only a small percentage of heterotrophic bacteria present in water.
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 (2020) Part 7. Methods for the enumeration of Heterotrophic Bacteria.
The Microbiology of Water and Associated Materials (2017) - Practices and Procedures for Laboratories
Investigation into the time taken for YEA bottles to achieve a temperature of 45-48°C :-In-house validation Nov 2010.

Environment Agency – The Microbiology of Drinking Water (2010) – Part 2 –Practices and Procedures for Sampling.
Investigation into the time taken for YEA bottles to achieve a temperature of 45-48°C: - In house validation December 03.
PWTAG Code of Practice for Swimming Pool Water – 2016.
BS EN ISO 8199:2018 Water quality. General requirements and guidance for microbiological examinations by culture.
BS EN ISO 6222:1999 (BS 6068-4.5:1999) - Water quality — Enumeration of culturable micro-organisms — Colony count by inoculation in a nutrient agar culture medium