

# METHOD STATEMENT



## Determinand:

Heterotrophic Bacteria at 22°C, 37°C and at 30°C

## Matrix:

Clean and lightly polluted waters

## Principle of 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 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:

Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mg l<sup>-1</sup> should counteract up to 5mg l<sup>-1</sup> of free and combined residual chlorine.

## Performance of Method:

Range of Application:	0 - 300 cfu/ml without dilution
Limit of Detection:	1 cfu/ml
Normal Reporting Level:	0 cfu/ml = Not Detected

## References:

Environment Agency - The Microbiology of Drinking Water (2020) - Part 7 - Methods for the enumeration of heterotrophic bacteria.

Microbiology of Water and Associated Materials (2017) - Practices and Procedures for Laboratories.

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.

Investigation into the time taken for YEA bottles to achieve a temperature of 45-48°C - In-house validation April 03.

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.