METHOD STATEMENT

Determinand:
Total coliforms and Escherichia coli.

Matrix:
Sample Types: Waters.

Principle of Method:
A known volume of water sample is filtered through a membrane filter with 0.45 µm pores upon which bacteria are entrapped. The filter is then placed on a selective growth medium m-Lactose Glucuronide Agar which contains lactose, phenol red as an indicator of acidity and the chromogenic substrate 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (BCIG) to indicate the production of b-glucuronidase.
Plates are incubated at 30°C for 4 hours and at 37°C for a further 14 hours after which colonies characteristic of coliforms and Escherichia coli are counted and picked off for confirmation where necessary.
Presumptive total coliforms appear as yellow, green or blue colonies. Presumptive Escherichia coli appear as green colonies. Confirmed total coliforms produce b-galactosidase and are oxidase-negative. Confirmed Escherichia coli produce b-galactosidase, are oxidase negative and produce indole from nutrient agar supplemented with 1% tryptone within 24 hours at 44°C.
As an alternative to traditional confirmation, colonies may be identified directly 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 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.
Where an exceedance has occurred the customer should be informed or a statement reflecting this should be included with the report (except where the customer has been already made aware that this is occurring on a regular basis and requests this not to be applied).

Interferences
Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mgl-1 should counteract up to 5mgl-1 of free and combined residual chlorine.
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.

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