

METHOD STATEMENT

Determinand:

Total coliforms and *Escherichia coli*.

Matrix:

Sample Type: 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.

Interferences:

Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18mg^l⁻¹ should counteract up to 5mg^l⁻¹ of free and combined residual chlorine.

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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Validation of counting an MLGA plates Dr D Hard and E Parker December 2000.



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