METHOD STATEMENT

Determinand:
Total Coliforms and *Escherichia coli*

Matrix:
Raw, Potable and Surface 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 Glucoronide Agar which contains lactose, phenol red as an indicator of acidity and the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) to indicate the production of β-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 express β-galactosidase and are oxidase-negative. Confirmed *Escherichia coli* express β-galactosidase, β –glucoronidase and are oxidase negative. Confirmation is performed using either protein profiling, or defined substrate technology.

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 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 18 mg/l should counteract up to 5 mg/l of free and combined residual chlorine.
Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

Performance of Method:

Range of Application:	0 - 100 cfu/100ml without dilution
Limit of Detection:	1 cfu/100ml
Normal Reporting Level:	0 cfu/100ml = Not Detected

References:
DoE (Department of the Environment) (1994) The Microbiology of Water 1994 Part 1 - Drinking Water. Report on Public Health and Medical Subjects No 71. Methods for the Examination of Waters and Related Materials. Department of the Environment, HMSO, London. ISBN 011-753010 7

Environment Agency – The Microbiology of Drinking Water (2002) – Part 4 – Methods for the Isolation and Enumeration of Coliform Bacteria and Escherichia Coli (including E. Coli 0157:H7).