METHOD STATEMENT

Determinand:
Total Coliforms, Faecal Coliforms and *Escherichia coli*

Matrix:
Waste water & Environmental 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 (MLGA), 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.

Where total coliform and *E. coli* analysis has been requested, 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.

Where faecal coliform analysis has been requested, plates are incubated at 30°C for 4 hours and at 44°C for a further 14 hours after which colonies characteristic of coliforms are counted and picked off for confirmation where necessary.

Presumptive coliforms appear as yellow, green, or blue colonies. Where confirmation has been requested by the customer, isolation of coliform colonies is followed by identification using a MALDI-TOF MS system to perform protein profiling. Alternatively confirmation using tests for β –galactosidase and β –glucoronidase activity can be used. This activity is observed as the ability of the organism to metabolise ortho nitrophenyl galactoside (ONPG) and 4-methylumbelliferyl glucuronide (MUG) respectively. Some bacteria (such as *Aeromonas spp*.) can give a false positive result with this confirmation technique. Therefore an Oxidase test is also carried out to exclude Oxidase positive colonies such as *Aeromonas spp*.

Escherichia coli appear as green colonies. Confirmation is not required. Blue colonies may be *E. coli* and should be confirmed.

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:
Samples with high turbidities may not be suitable for this method. The particulates may block the filter and limit the volume that can be examined. Accumulated deposits on the membrane may mask or inhibit the growth of the target organisms. High numbers of competing organisms may also mask or inhibit the growth of the target organisms.

Accumulated deposits on the filter membrane may also inhibit the growth of indicator organisms. For samples containing a lot of debris, the debris should be allowed to settle before filtration. More than one sample volume/dilution should also be filtered to enable easy reading of the final plates.

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

Reporting of Results:

Presumptive and confirmed coliforms are typically expressed in colony forming units (cfu) per 100ml of sample.

Escherichia coli are typically expressed in colony forming units (cfu) per 100ml of sample.

References:

Standing Committee of Analysts - The Microbiology of Recreational and Environmental Waters (2015) – Part 1 – Water quality, epidemiology and public health

Standing Committee of Analysts - The Microbiology of Drinking Water (2002) - Part 1 - Water Quality and Public Health

Standing Committee of Analysts - The Microbiology of Recreational and Environmental Waters (2016) – Part 3 – Methods for the isolation and enumeration of Escherichia coli (including E. coli O157:H7)

Standing Committee of Analysts - The Microbiology of Drinking Water (2016) – Part 4 – Methods for the isolation and enumeration of coliform bacteria and Escherichia coli (including E. coli O157:H7