METHOD STATEMENT

Determinand:
Escherichia coli

Matrix:
Sludge, Soil and other solids

Principle of method:
The sample is homogenised and serially diluted with maximum recovery diluent (MRD) and filtered through a membrane. The membrane filter is placed on an MLGA plate and *E.coli* are enumerated after incubation at 30 ± 1.0°C for 4 ± 0.5 hours, followed by 44 ± 0.5°C for 14 hours.

The MLGA contains lactose, an acidity indicator–phenol red and the chromagenic substrate BCIG (5-bromo-4-chloro-3-indolyl- β-glucoronide) either as a sodium or cyclohexylammonium salt. When hydrolysed BCIG indicates the presence of β-glucuronidase. Colonies that are β-glucuronidase positive are regarded as *E.coli*.

Sampling and Sample Preparation:

Samples should be taken in sterile plastic containers. A minimum of 100g of sample should be provided to the laboratory.

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:
Interferences may include high levels of competing organisms or sample turbidity / debris.

Limitations - this method excludes a proportion of strains of *Escherichia coli* that are unable to grow at 44°C, do not express β-glucuronidase activity on primary isolation or are β-glucuronidase negative.

Performance of method:

Range of Application:	10 – 10,000,000 cfu/gram (wet weight)
Limit of Detection:	10 cfu/gram
Normal Reporting Level:	<10 cfu/gram

References:
The Microbiology of Sewage Sludge (2003) - Part 3 - Methods for the isolation and enumeration of *Escherichia coli*, including verocytotoxigenic *Escherichia coli.* Environment Agency - Methods for the Examination of Waters and Associated Materials. (Downloadable pdf only - no ISBN assigned).