# METHOD STATEMENT

**Determinand:**
Escherichia coli

**Matrix:**
Sample Type: sludges and soils

**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 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 until analysis commences.
See the Microbiology of Sewage Sludge 2003, Part 2 - Practices and procedures for sampling and sample preparation (15.2) for more specific instruction on sampling.

**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.
This method is not suitable for sludge samples that have been lime-treated or where enhanced microbial reduction is expected. These samples should be examined using a MPN technique.
The maximum number of colonies that should be counted on a filter membrane is 100. Anything reported higher than this is an estimation.

**Performance of Method:**

**Range of Application:**
The number of *Escherichia coli* present in sludge is expressed on a wet or dry weight basis. Typically results are reported as cfu of *Escherichia coli* per g of dried sludge

**Limit of Detection:**
The Limit of Detection has not yet been determined for this method

**Uncertainty of measurement:**
The Uncertainty of Measurement has not yet been determined for this method

**References:**
The Microbiology of Sewage Sludge (2003) - Part 1 - An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health.

Environment Agency - Methods for the Examination of Waters and Associated Materials. (Downloadable pdf only - no ISBN assigned).
The Microbiology of Sewage Sludge (2003) - Part 2 - Practices and procedures for sampling and sample preparation. Environment Agency - Methods for the Examination of Waters and Associated Materials. (Downloadable pdf only - no ISBN assigned).
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).
The Microbiology of Sewage Sludge (2004) - Part 4 - Methods for the detection, isolation and enumeration of Salmonellae. Environment Agency - Methods for the Examination of Waters and Associated Materials. (Downloadable pdf only - no ISBN assigned).