# METHOD STATEMENT

**Determinand:**
Enterobacteriaceae

**Matrix:**
Potable and Raw water

**Principle of method:**
A known volume of the water sample (normally 100ml) is filtered through a membrane filter with 0.45µm pores upon which bacteria are retained. The membrane is then placed onto pre-poured plates of violet red bile glucose agar (VRBGA).

The plates are then incubated aerobically at 37±1°C for 21±3 hours. Characteristic presumptive colonies are round, purplish-red and often surrounded by halos. Presumptive colonies are counted and identified using MALDI-TOF MS. Alternatively they may be picked off for confirmation by glucose fermentation and oxidase tests. Confirmed *Enterobacteriaceae* are oxidase negative and will make glucose agar turn yellow throughout the media when incubated at 37±1°C for 21±3 hours.

**Sampling and Sample Preparation:**
Once taken, microbial samples should be transferred directly to cold 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 between 2-8°C in dark conditions until the commencement of analysis.

Samples should be analysed as soon as practicable on the day of collection. If there is a delay, storage under the above conditions should not exceed 24 hours before the commencement of analysis.

**Interferences:**
Chlorine and chloramines are neutralised by adding sodium thiosulphate, which at a concentration of 18mg/l should counteract up to 5mg/l of free and combined residual chlorine.

The effectiveness of this method is affected by high turbidity samples. The filter may become blocked, limiting the maximum volume that can be examined. The accumulated deposits on the membrane may mask or inhibit the growth of target organisms. Dilutions may be carried out to reduce the impact of this, but are only useful for samples containing high levels of Enterobacteriaceae.

Plasticisers can interfere in the mass spectrometry analysis. Only certified, non-plasticising plastic materials should be used.

**Performance of method:**

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| Range of Application: | 0 – 100 cfu/100ml without dilution |
| Limit of Detection: | 0 cfu/100ml |
| Normal Reporting Level: | 0 cfu/100ml |

**References:**

Health Protection Agency (2004). Enumeration of Enterobacteriaceae by the Colony Count Technique. National Standard Method F 23. Issue 1.

Health Protection Agency (2004). Glucose Agar. National Standard Method MSOP 35. Issue 3.

Health Protection Agency (2007). Identification of Enterobacteriaceae. National Standard Method. BSOP ID 16. Issue 2.