# METHOD STATEMENT

**Determinand:**
Swabs (Total coliforms, Escherichia coli, Clostridium perfringens , Enterococci and TVC's).

**Matrix:**
Sample Type: Waters.

**Principle of Method:**
For membrane filtration methods a swab is placed into a universal containing Maximum Recovery Diluent (MRD) and then mixed. The MRD is then filtered onto a plate of selective growth medium.
For coliform and E.coli analysis the swab is filtered onto m-Lactose Glucuronide 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.
All 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 produce b-galactosidase and are oxidase-negative. Confirmed Escherichia coli produce b-galactosidase, are oxidase negative and produce indole from nutrient agar supplemented with 1% tryptone within 24 hours at 44°C.
For Enterococci analysis the swab is filtered onto Slanetz and Bartley agar. Enterococci will reduce 2,3,5-triphenyltetrazolium chloride to the insoluble red dye formazin to produce red, maroon or pink colonies
Plates are incubated at 37°C ± 1.0°C for 44 ± 4 hours. After which colonies characteristic to Enterococci are counted and picked of for confirmation if required.
Confirmatory tests are carried out on the presumptive colonies. The colonies are subcultured on to Kanamycin azide aesculin agar (KAAA) and incubated for up to 18 hours Enterococci will show hydrolysis of aesculin by producing a black halo. Small colonies are grown up on Brain Heart Infusion Agar at 37°C prior to subculture onto a confirmatory medium.
For Clostridium analysis the swab is filtered onto Tryptose-Sulphite-Cycloserine agar. Following anaerobic incubation at 44°C + 0.5°C for 21 + 3 hours presumptive Clostridium perfringens will produce black, grey, colourless or cream coloured colonies.
Confirmed Clostridium perfringens will produced a purple/brown colour when tested for the enzyme acid phosphatase. Clostridium perfringens is a gram-positive spore forming rod shaped bacterium.
For TVC analysis a swab is placed into a universal containing MRD and then mixed. 1ml of the the inoculum is then pipetted onto into an empty Petri dish per TVC determinand and incubated at 37°C for 44 ± 4 hours and 22°C for 68 ± 4 hours.

**Sampling and Sample Preparation:**
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. All samples must be analysed within 24 hours

**Interferences**
High numbers of non coliforms can inhibit the growth of target organisms (coliforms).

**Performance of Method:**

**Limit of Detection:**
N/A

**Uncertainty of measurement:**
N/A

**References:**
See methods WPM1, WPM2, WPM3 and WPM4