METHOD STATEMENT

Determinand:
Male-specific (F+) bacteriophages and Somatic coliphages

Scope:
Water, Soil, Sludge & Sediments

Principle of Method:
The test sample is mixed with a small volume of semi-solid nutrient medium to which a culture of host strain is also added, Escherichia coli ATCC 700891 is used as the F+ host strain and Escherichia coli ATCC 700078 is used as the Somatic coliphage host strain. This is mixed and poured on to a solid plating medium which when set, is incubated under specified conditions. After incubation, the Male-specific (F+) bacteriophages and Somatic Coliphages will have infected the host strain to produce visible plaques (zones of clearance) which can be counted. Results are expressed as the number of plaque-forming units (pfu) per volume.

Sampling and Sample Preparation
Samples should be taken in sterile containers and transported to the laboratory under cool, dark conditions as quickly as possible.
On receipt, samples may be stored at 5°C ± 3°C and analysed within 24 hours. Alternatively, samples requiring F+ bacteriophages analysis may be frozen until the day of analysis when added to TYE broth. Add 1 volume of TYE broth to 4 volumes of well mixed sample and freeze at -70°C ± 10°C. Immediately prior to analysis, frozen samples must be defrosted at room temperature.

Interferences:
Once started, the speed of analysis is critical. If the sample and host bacteria remain in contact for too long prior to agar pouring, then the number of plaque forming units may be over-estimated. The entire plating process should be carried out in less than 20 minutes.

Clear plaques with smooth edges that appear larger and more definitive than F+ bacteriophages are more likely to reflect the presence of somatic coliphages and should be ignored during enumeration if only an F+ bacteriophage result has been requested.

Similarly, small bubbles may occasionally appear within the agar which must be distinguished from any plaque growth present. Small areas of solidified overlay should also be distinguished from any plaques that may have formed. High numbers of competing organisms may overgrow the host and mask plaque production.

Reporting of Results:
F+ Coliphage are expressed as plaque forming units, per volume of sample (usually 1ml) analysed.

References:
BS EN ISO 10705-1:2001 (BS 6068-4.11:1996) - Water quality - Detection and enumeration of bacteriophages – Part 1: Enumeration of F–specific RNA bacteriophages.

BS EN ISO 10705-2:2001 (BS 6068-4.13:2000) - Water quality - Detection and enumeration of bacteriophages – Part 2: Enumeration of somatic coliphages.

METHOD STATEMENT

Detection and quantitation of F-specific RNA bacteriophages by double agar layer assay using Escherichia coli Famp or Salmonella typhimurium WG49 as the host bacterium. ALS method COR-MM510. I. Bolch, 14/10/10.

US EPA Method 1602: Male-specific (F+) and somatic Coliphage in water by single agar layer (SAL) procedure. 2001.

The Microbiology of Recreational and Environmental Waters (2016) – Part 11 – Methods for the isolation and enumeration of somatic and F-specific bacteriophages and bacteriophages infecting Bacteroides fragilis.