METHOD STATEMENT

Determinand:
Legionella Spp. including *Legionella pneumophila* Sg1 & Sg2-15

Matrix:
Domestic hot and cold water systems, Cooling towers and Process waters

Principle of Method:
This method relies on the concentration of bacteria including *Legionella* by filtration or centrifugation followed by elution followed by Sonication. Reduction of unwanted bacteria is undertaken by heat on one portion and acid treatment of a separate portion. Inoculation of these portions and an untreated portion are plated onto BCYE agar with antibiotic supplements, (GVPC), and incubated. Following incubation, an enumeration of morphologically characteristic colonies is made.

Legionella are then confirmed either by MALDI-TOF MS protein profiling, or as those, which show a growth requirement for L-cysteine[16]. Serological tests using commercially available latex kits are then used for species identification.

Sampling and sample preparation:
Samples for Legionella analysis only should be transported at ambient temperature (6-20°C) and can be stored at room temperature unless they have associated TVC analysis (2-8°C). They should also be protected from heat and sunlight. Prior to receipt at the laboratory, storage temperatures are beyond our control unless sample is sampled by ALSE

Samples should be delivered to the laboratory as soon as possible. The analysis of samples containing biocide should be commenced as soon as possible after receipt in the laboratory. The time interval between collection of the sample and its concentration should not exceed 2 days. The maximum time from sample to culture of the concentrate should not exceed 14 days[16].

Storing the sample at temperatures below 6°C may reduce the recovery of Legionella bacteria since they may be induced into a viable but non-culturable state.

Interferences:
Chlorine and chloramines. Neutralise by adding sodium thiosulphate which at a concentration of 18 mgl^{-1} should counteract up to 5 mgl^{-1} of free and combined residual chlorine.
Prolonged refrigeration of samples should be avoided.
Sodium Chloride in excess of 0.03% w/v, (300mg/l), is inhibitory for *Legionella*.

Performance of Method:
The method is capable of detecting Legionella spp as well as Legionella pneumophila at a concentration less that 100 colony forming units per litre. This is the minimum action limit suggested in the HSE guidance document for the control of Legionella bacteria in water systems.

Results are reported as colony forming units per litre of sample analysed. Samples are weighed on receipt (UKAS requirement) and results are calculated per litre equivalent, on the assumption that 1000g is equivalent to 1 litre.

Results for legionella pneumophila and legionella species are reported separately. Therefore results reported for L. species are counts for non-pneumophila L. species.

Where a species identification can be obtained, this will be reported as an analyst comment.

Detection limit

If 1 colony is found on the untreated or heat treated plates the theoretical detection limit is 20cfu/L where 1 litre is analysed.

This is calculated as follows:

$$\frac{(C \times V)}{(I \times O)} \text{ cfu/litre}$$

Where: -

C = Number of confirmed *Legionella* colonies on plate
V = Concentrate volume (normally 10mls).
I = Inoculums on plate in litres. Typically 0.0005L (500 □l).
O = Original Sample Volume in grams. This is approximately equivalent to the volume in millilitres (ml).

Inoculating 500ul (1000ml sample bottle) onto a GVPC plate:

Therefore based on 1 colony being isolated on untreated GVPC plate where 1000ml has been filtered and 500ul plated the calculation is as follows:

$$\frac{1 \times 10}{0.0005 \times 1000} = \frac{10}{0.5} = 20 \text{ cfu/L}$$

Inoculating 100ul (1000ml sample bottle) onto a GVPC plate:

Therefore based on 1 colony being isolated on untreated GVPC plate where 1000ml has been filtered and 100ul plated the calculation is as follows:

$$\frac{1 \times 10}{0.0001 \times 1000} = \frac{10}{0.1} = 100 \text{ cfu/L}$$

Inoculating 500ul (500ml sample bottle) onto a GVPC plate:

Therefore based on 1 colony being isolated on untreated GVPC plate where 500ml has been filtered and 500ul plated the calculation is as follows:

$$\frac{1 \times 10}{0.0005 \times 500} = \frac{10}{0.25} = 40 \text{ cfu/L}$$

References:

BS 6068 – 4.12 :1998 Water Quality – pt. 4: Microbiological Methods : section 4.12 :Detection & Enumeration of *Legionella*.

ISO 11731-2: Water Quality - Detection and Enumeration of Legionella - Part 2: Direct Membrane Filtration Method for Waters with low bacterial counts

BS 7592 : 2008 Sampling for Legionella bacteria in Water Systems – Code of Practice.