METHOD STATEMENT

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

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

Principle of Method:
This method relies on the concentration of bacteria including *Legionella* by filtration or centrifugation followed by elution using ultrasonication. Reduction of unwanted bacteria is undertaken by heat and acid treatments of separate sample portions. These portions and an untreated portion are inoculated onto BCYE agar with antibiotic supplements, (GVPC), and incubated. Following incubation, the agar plates are examined, and morphologically characteristic colonies are counted.

Legionella are then confirmed either by MALDI-TOF MS protein profiling, or requirement of L-cysteine for growth. Serological tests using commercially available latex kits are then used for species and/or serogroup identification.

Sampling and sample preparation:
Sample should be stored and transported in the dark.

For storage and transportation of samples prior to analysis, it is recognized that the count of bacteria by culture in water samples can change near to 0 °C or above 20 °C. There are contradictions on storage and transportation temperatures given in published guidance (for example "ambient", 6 °C to 20 °C or 5 ±3°C), therefore, ALS have verified that storage at ambient temperature in the laboratory is suitable. In addition, storage in controlled refrigerated conditions (2 – 8 °C) for the duration of sample transport in ALS vehicles is also suitable.

Upon receipt into the Microbiology laboratory, any Legionella samples that require associated TVC analysis from the same bottle must have the TVC analysis conducted as soon as possible after registration is complete. The sample will then be analysed for Legionella.

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

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. The method has been validated and accredited and is continually assessed using quantitative positive quality controls. A lower theoretical detection limit of 20 colony forming units per litre (CFU/L) of sample is generally achieved for the majority of samples submitted. This meets the requirements of HSE guidance documents such as L8 and the HSG 274 series, which recommends actions to be taken

when legionella is detected at levels of 100 CFU/L and 1000 CFU/L, and at levels lower than 100 CFU/L in healthcare facilities.

Reporting of Results:
Results are reported as colony forming units per volume (CFU/vol) of sample analysed. All sample volumes are measured on receipt in the laboratory. The sample volume is also included on the certificate of analysis. If a volume of less than 1 litre is supplied a calculated result in units of colony forming units per litre (CFU/L) is also included to allow comparison to HSE published action limits.

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.

Negative samples are reported as Not Detected (ND).

Theoretical Limit of Detection (tLOD)

Due to the necessary steps carried out in the method, portions of the samples are ultimately used to generate a colony count. Therefore, if a single Legionella colony is identified and the result calculated, this is deemed to be the tLOD. This value is stated in CFU/L. For the majority of samples analysed by this method (i.e. a 1-Litre sample), the tLOD will be 20cfu/L.

A rare exception to this may occur when there is overgrowth of non-target organisms on some of the agar plates, and the requirement to carry out a dilution on the acid treatment step. In this rare scenario, the tLOD is therefore 10x the standard tLOD for the sample (e.g. typically 200 cfu/L). Should this occur then a comment will be added to the sample.

If volumes of sample less than 1 litre are supplied, or due to the nature of the sample reduced volumes are analysed or dilutions carried out, the tLOD will increase.

tLOD is generally only reported on negative (ND) samples.

References:

BS EN ISO 11731:2017 Water quality. Enumeration of Legionella

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

HSG 274 Series (three parts) 2014; Legionnaire's disease, Technical Guidance, HSE

L8 (Forth Edition) 2013; Legionnaire's disease. The Control of Legionella Bacteria in Water Systems. Approved Code of Practice & Guidance. HSE