METHOD STATEMENT

Determinand:
Legionella Spp. including *Legionella pneumophila*.

Matrix:
Drinking Water and Process Water – this includes hot and cold sanitary water from within-premise water systems and cooling tower waters. Recreational water, wastewater and surface water can also be tested.

Principle of Method:
The PCR method should be used in conjunction with the standard methodology or as an investigational tool to monitor water treatment following a positive result using the standard method, particularly if early indication is required on the effectiveness of treatment. Two sample bottles should therefore be supplied for PCR and culture analysis.

The results can be available in 24 hrs for emergency analysis.

The method relies on the capture of the target organism via membrane filtration followed by extraction and purification of DNA. PCR (polymerase chain reaction) analysis of the purified DNA is then carried out which amplifies the target DNA to a threshold level in order for it to be detected by the PCR instrument. The PCR result will be presence, absence, or inhibition. If present, a quantification can also be expressed as Genomic Units /Litre.

The result is therefore an indication of target DNA content, not an expression of colony forming units as determined by the standard method. There is no current strong evidence to suggest any correlation between a PCR results and the result generated by the standard, culture-based methodology.

The PCR method uses the Bio-Rad Aquadien extraction protocols and iQ Check Legionella PCR kits.

Sampling and Sample Preparation:
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. They should however be protected from heat and sunlight, and samples taken from hot and cold water systems should be stored/transported in separate containers to avoid the samples influencing each other. Samples may arrive in separate bottles for PCR and culture analysis.

Interferences & Limitations:
There are a number of chemicals that can interfere with the PCR reaction in the different sample matrix types such as fluvic and humic acids in surface water. There may also be some chemical by-products of the treatment process that may inhibit the PCR reaction but have no impact on the standard culture method.

Although steps are taken to remove the free DNA from dead bacteria (sample filtration and the optional addition of free-DNA removal solution), the DNA from dead bacteria may be detected. Bacteria known as viable but not culturable (VBNC) can be detected by PCR analysis but not by culture analysis.

Highly turbid samples may make membrane filtration difficult. Smaller volumes of sample can be filtered but this may impact the detection limit and/or limit of quantification.

Performance of Method:
The Bio-Rad kits hold certification by an independent and recognized third-party validation body (AFNOR) that they have been validated according to the protocols in ISO/TS 12869. ALS have also conducted a verification evaluation of the performance of a third-party validated method according to ISO/TS 12869.

The detection limit ("limit of detection" or LOD) of the method is typically as follows when translated to a 1 litre sample:

- 80 GU/L for quantitative analysis.
- 160 GU/L for qualitative (presence/absence) analysis.

The limit of quantification (LOQ) of the method will vary per analysis carried out but will typically be in the region of 480 GU/L. A comment will be included on certificates of analysis if a sample shows detection below the LOQ and will include the LOQ applied.

The values and examples above assume a 1L samples is analysed and no dilutions were required. Where other volumes and/or dilutions are used, the LOD and LOQ for the sample will change.

References:
PD ISO/TS 12869:2019 - Water quality – Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).

Lee J. V. et al, An international trial of quantitative PCR for monitoring Legionella in artificial water systems, Journal of Applied Microbiology, Volume 110, Issue 4, 1 April 2011, Pages 1032–1044, https://doi.org/10.1111/j.1365-2672.2011.04957.x.

Collins S. et al, Evaluation of Legionella real-time PCR against traditional culture for routine and public health testing of water samples, Journal of Applied Microbiology, Volume 122, Issue 6, 1 June 2017, Pages 1692–1703, https://doi.org/10.1111/jam.13461.

Young, C.; Smith, D.; Wafer, T.; Crook, B. Rapid Testing and Interventions to Control Legionella Proliferation following a Legionnaires' Disease Outbreak Associated with Cooling Towers. Microorganisms 2021, 9, 615. https://doi.org/10.3390/microorganisms9030615.

Health and Safety Executive, Legionnaire's disease, Frequently Asked Questions (FAQs), "Testing/monitoring Legionella" - https://www.hse.gov.uk/legionnaires/faqs.htm.