METHOD STATEMENT

Determinand:
Legionella Spp. including *Legionella pneumophila*.

Matrix:
Raw and Potable waters, Cooling Waters, Waste Waters, Sludges, Compost and Solid samples.

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.
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 of DNA from intact bacterial/ protozoan/ algal cells. A PCR (polymerase chain reaction) run of the purified DNA to amplify the target DNA to a threshold level in order for it to be detected by the instrument is then carried out. The results are displayed graphically as real time curves on the VDU display of the instrument if a positive or negative result, giving the quantified target DNA result. The PCR result will be presence, absence or inhibition. If presence, the quantification is expressed as Genomic copies/Litre

Thus the result is an indication of target DNA content not an expression of colony forming uints 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 methodology.

This standard method relies on the concentration of bacteria including *Legionella* by filtration or centrifugation followed by elution. Decontamination 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 as those, which show a growth requirement for L-cysteine. 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 and can be stored at room temperature. Cooling should be avoided, but they should also be protected from heat and sunlight. Samples may arrive in separate bottles for PCR and culture analysis. The 2 bottles should be mixed by the PCR analyst prior to analysis, by pouring 500ml from each into a new sterile 1L bottle. PCR samples and associated culture samples should be analysed by the same analyst(s).

Interferences:
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.

Performance of Method:
The Limit of Detection has not yet been determined for this method.

References:

Genesystems 2006. Molecular Diagnostics; Polymerase Chain Reaction. Genesystems information brochure.

Rapid assessment of water systems for Legionella phumophila using real time PCR hybridisation assay. Sandra Lai, Susanne B Surman-Lee, Norman K Fry, Baharak Afshar, John V Lee.

ISO/TS 12869 Water quality — Detection and quantification of Legionella spp. and/or Legionella pneumophila by concentration and genic amplification by quantitative polymerase chain reaction (qPCR).

Legionnaires' disease CIBSE TM13: 2002. The Chartered Institution of Building Services Engineers 222 Balham High Road, London SW12 9BS. Minimising the risk of Legionnaires' disease.