

Eurofins Legionella PCR (Polymerase Chain Reaction) Technical Information Sheet

PCR versus Culture Technique

PCR is a molecular biology technique in which the DNA of micro-organisms is extracted and then amplified (multiple copies are made). This enables the laboratory to determine the presence and quantity of that organism's DNA in a water sample. As it is the DNA that is being assessed, the technique can detect live cells, cells that are alive but cannot be cultured (so-called Viable Non-Culturable) as well as dead cells.

In contrast the traditional culture technique for Legionella relies on the bacteria's ability to grow and multiply in order to produce visible colonies on an agar plate which can be counted by the laboratory. Any Legionella cells which do not grow and multiply therefore remain undetected.

Legionella bacteria are known to be difficult to recover from samples in a laboratory situation. Therefore a certain proportion in a sample will always remain undetected when using the culture technique.

Eurofins uses the BioRad Aquadien DNA Extraction Kits and iQ-Check Detection / Quantification *Legionella* spp. and *L. pneumophila* kits.

Advantages of Using the Legionella PCR Method Rather than the Culture Technique

The traditional Legionella culture technique takes 10 days to arrive at a confirmed negative result as Legionella bacteria are slow growing in the laboratory. A further 3 days are required for confirmation in the case of suspect colonies being identified on the agar plates. In contrast, the Legionella PCR technique is able to arrive at a confirmed positive or negative result in 24 hours. This includes being able to quantify *Legionella* species and *Legionella pneumophila* serogroup 1. This can be particularly useful when determining how successful a treatment has been in a water system.

Current action and alert levels for *L. pneumophila* in L8 are given in colony forming units (cfu)/L. For this reason whenever a Legionella test is conducted by PCR, this must be carried out alongside a test by traditional culture technique. Therefore 2 samples must be provided to the laboratory for this purpose.

Interpretation of Results

PCR gives results as genomic copies (GU) per litre of sample, whereas the traditional culture technique gives results in colony forming units (cfu) per litre of sample. As PCR can detect dead and viable non-culturable cells the quantitative results from PCR are expected to be higher than those obtained by culture.

Results from the two methods are therefore not directly comparable, though research suggests that action and alert levels can be determined for Legionella GU (Lee et al 2011).

Limit of Detection

The detection limit corresponds to the smallest quantity of genomic units that generate a positive result.

Eurofins is able to use its PCR system to perform screening for the presence or absence of *Legionella* spp. and *Legionella pneumophila* (Qualitative testing) or for enumerating genomic units of *Legionella* spp and *Legionella pneumophila* (Quantitative testing)

The limit of detection (LOD) for *Legionella* spp. and *Legionella pneumophila* for samples screened using Qualitative testing is 160 GU/L.

The LOD for *Legionella* spp. and *Legionella pneumophila* for samples enumerated using Quantitative testing is 80 GU/L.

Limit of Quantification

The quantification limit corresponds to the smallest quantity of genomic units required to generate an accurate result

The lower limit of quantification (LOQ) for both tests is 672 GU/L

If a result is greater than the LOD (80 GU/L or 160 GU/L depending on the method employed) but less than 672 GU/L then this will be reported as

“Detected, < limit of quantification”

Conversely the upper limit of quantification is the largest quantity of genomic units where an accurate result can be generated. If the sample is not further diluted, then the upper limit of quantification is 1.24×10^6 GU/L for both tests.

If a result is greater than 1.24×10^6 GU/L then this will be reported as

“Detected, > limit of quantification”

Further Questions

If you require any further information regarding PCR testing for Legionella then please contact either;

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