

POLYMERASE CHAIN REACTION (PCR)

Detection of Legionella by Real Time Polymerase Chain Reaction (PCR)

To provide customers with a rapid diagnosis, ALS Environmental offer an advanced PCR assay for the detection and quantification of Legionella.

Legionella are commonly occurring bacteria, found in both natural and artificial water systems. In favourable conditions, the bacteria are able to rapidly multiply, potentially reaching hazardous levels. Favourable conditions for Legionella bacterial growth are water temperatures of 20-45°C and the presence of nutrients in the form of sediment, scale, sludge and biofilms, which often occur in man-made water systems.

Legionella pneumophila is the causative agent of Pontiac fever and Legionnaires disease (1). The bacteria is common to natural water sources and may be detected due to a random finding. Nevertheless, its finding is significant and indicates a potential health risk however small or variable this may be. Cell culture and PCR are the most commonly used methods for detection and identification of Legionella.

POLYMERASE CHAIN REACTION

Polymerase Chain Reaction (PCR) is the amplification of DNA in an exponential manner. ALS Environmental use the latest real-time PCR technology from Genesystems to apply a rapid, sensitive and specific technique that has been used in the detection of many infectious pathogens (3).

The method consists of several steps, the first involves concentrating viable cells through membrane filtration. The concentrated cells are then subjected to sonication and heating procedures, which lyse the cells and free the DNA. The final stage is the purification of the DNA for the Polymerase Chain Reaction. PCR is undertaken using the Genedisc system. The actual PCR run lasts for 55 minutes with the results expressed as genomic units per sample volume.

ADVANTAGES OF PCR

The PCR method is an important tool for establishing risk under emergency or outbreak situations.

- Real-Time PCR is a rapid process that provides results in 24 hours compared to the 10 day turnaround of the culture method.
- Real-time results demonstrate the Legionella status of the water system at the time of sampling.
- The limit of detection is far greater than that of the culture method due to the higher sensitivity of the PCR process. PCR can detect Legionella in a 10^{-10} dilution whereas the culture method can only detect Legionella up to a 10^{-5} dilution.
- The process detects at the level of DNA so the barriers caused by Legionella trapped in biofilms, and within other organisms such as amoeba, are overcome.
- PCR primers are Legionella specific. Therefore the presence of non target organisms is eliminated.
- PCR will often detect Legionella that traditional culture methods can fail to pick up.
- Problems of high levels of non-target organisms are eliminated in PCR.
- Identification at the level of DNA means PCR is more accurate than serological kits which can produce inaccuracies due to cross-reactions with non-target organisms.
- Viable and non-culturable Legionella can be detected by PCR.
- Legionella are bacteria that are contained within biofilms or even organisms such as amoebas.

POLYMERASE CHAIN REACTION (PCR)

Rapid detection PCR assay therefore offers accurate, rapid results, often detecting Legionella that traditional methods can fail to pick up.

	Culture	PCR
Matrix Type	Water (both process, including cooling towers and treated), Sludge	Water (both process, including cooling towers and treated), Sludge
Enumeration	Colony Forming Units	DNA based
Units	CFU/litre	GU/litre
Turn Around	10 days	<6 HRS
Qualitative result	Yes	Yes
Typing Available	Yes	Yes
Detect Viable Organisms	Yes	Yes
UKAS status	Yes	No
Real Time Analysis	No	Yes
LOD	20 CFU/litre	80 GU/litre
Intended Use	Routine Monitoring Outbreaks	Investigative Tool Outbreaks Monitoring/ Efficiency of Treatment Rapid same day turn around.
MALDI-TOF Compatible	Yes	No

ALS Environmental are members of the Legionella Control Association (LCA) and have numerous members of the Water Management Society (WMSoc) throughout our business. With laboratories in Coventry and Wakefield we are your ideal partner for Legionella and Microbiological analytical services.

REFERENCES

Lai, S., Surman-Lee, S. B., Fry, N. K., Afshar, B., Lee, J. V. Rapid Assessment of Water Systems for Legionella pneumophila Using Real Time PCR Hybridisation Assay. 2008.