



Report Supporting Document

**DETECTION AND QUANTIFICATION OF *LEGIONELLA PNEUMOPHILA*
USING QUANTITATIVE POLYMERASE CHAIN REACTION (QPCR)**

Introduction

Legionella are gram-negative bacteria and ubiquitous natural inhabitants of fresh water. In homes and commercial buildings, *Legionella* species are often found in wet warm places where biofilms form, such as water storage, heating systems, sinks, faucets, shower heads, humidifiers, air conditioning systems, water fountains, and water cooling towers. Improperly maintained swimming pools, whirlpool spas, and hot spas have also been reported as sites supporting *Legionella* populations.

Legionella contamination of building water systems can lead to illness and in some instances the death of building occupants; especially those having lung problems or compromised immune systems. *Legionella pneumophila* has been reported as the causal agent of over 90% of Legionnaires' disease and nearly half of diagnosed Pontiac fever cases. Conditions favorable for growth of this bacterium in water systems include temperatures of 77-108°F, stagnation, scale and sediment, bio-film, and the presence of Amoebae. *Legionella* growth may also be influenced by certain materials: natural rubber, wood, and some plastics may support growth, while materials such as copper inhibit growth of the bacteria. The growth and dissemination of *Legionella* can be effectively controlled by good engineering design and proper maintenance practices.

The QPCR Technology

Quantitative Polymerase Chain Reaction (QPCR) is employed for the detection and quantification of *Legionella pneumophila* specific DNA fragment in the sample provided. QPCR employs the DNA amplification technology of PCR using target specific DNA primers (targeted to specific DNA gene fragment) and the fluorescence detection technology allowing amplification to be read in real time. For instance, if the sample contains the target organism DNA, then specific primers will bind to it and amplify the DNA fragment. Quantification is then carried out depending on the number of starting molecules of DNA.

Sample Analysis and Reporting

On arrival, the sample is subjected to total DNA extraction of all organisms present in the sample. In the case of water samples, membrane filtration is performed to concentrate organisms present in the sample. The concentrate on the filter is then subjected to DNA extraction.

At Aemtek laboratory, the *Legionella* specific QPCR protocol uses species-specific primers to detect and quantify the presence of *Legionella pneumophila*. The QPCR is performed on the ABI 7700 Sequence Detection System (SDS) using elaborate standards and controls and known standard concentrations of *Legionella pneumophila* for quantification ranging from 10^1 to 10^8 colony forming units (CFUs)/ml. Standards, positive and negative controls, and spiked samples are always included with every analysis. Quantification in samples is obtained by comparison of sample DNA with standard DNA, automatically computed by the SDS software system.

Because QPCR runs are measured based on DNA extraction of the sample and the standards are measured in CFU/ml, the result can be measured as DNA equivalents of CFU/ml. In this case, if data suggests that the sample contains DNA equivalent of 20 CFU/ml, it can be interpreted that if 1 ml of the sample was to be cultured, assuming 100% viability, approximately 20 CFU of the bacteria would form. However, because QPCR detects DNA from both viable and non-viable organisms, we normally use number of **cells/ml** in the QPCR report to differentiate the QPCR method from culture method.

Limit of Detection (LOD) of *Legionella pneumophila* by QPCR

The LOD for *L. pneumophila* in the QPCR method is approximately DNA equivalent of 10 CFUs/100 ml or 10 cells/100 ml. In some cases the LOD may be affected by some external factors including inappropriate sampling (sample size, use of non-sterile container, use of container without preservative, etc. -see sampling guide-), and presence of PCR inhibitors.

Qualitative PCR

As a quality control measure, qualitative PCR is sometimes performed along with QPCR to give a quick analysis of the presence or absence of the target DNA fragments in the sample using genus-specific primers. This method detects other species of *Legionella* as well as *L. pneumophila* (Note: Most Legionnaires' disease is caused *L. pneumophila* while Pontiac fever can be caused by any species in the genus of *Legionella*). The results are analyzed after completion of the run by gel electrophoresis.

Amplification of the target DNA fragment is reported as a positive result with a detection limit of <10 cells/100 ml of sample.

Data Interpretation

Detection and quantification of *Legionella pneumophila* by QPCR (or conventional PCR) is a very powerful tool to obtain quick results on levels of contamination. However PCR yields qualitative data, only providing information regarding presence or absence of the target organism within its detectable limit and not the viability. As the organism must be viable to cause infection, there are situations where the organisms have been killed and will still yield a positive PCR result, usually in water systems that have undergone a recent disinfection treatment. The PCR and QPCR methods, however, provide invaluable information to identify potential sources and initiate immediate disinfection treatments, to prevent further exposures and possible disease.

Conventional culturing techniques require nearly two weeks for completion, during which time numerous susceptible individuals may be exposed to viable bacteria, thus posing a potent human health risk.

QPCR provides a numerical value measuring the load of the organism in the sample provided. While positive results in either analysis are significant, negative results do not necessarily indicate that there are no target organisms present in the sample. It is possible that the target organism may be present, but in a numbers below the detection limit of the method employed.

The EPA has suggested an acceptable level of *Legionella* in drinking water to be zero colony forming units per milliliter (i.e., 0 CFU/ml). Regulatory agencies and scientific community agree that any species or serotype of *Legionella* that is detected in a building water distribution system above 1 CFU/ml is unacceptable and that some measure of response is required.

The following is a summary of the risk levels associated with *Legionella* in various water systems.

<i>Legionella</i> (CFU/ml)	Cooling Water	Potable Water	Humidifier Water
1 – 9	Low	Moderately Low	Moderately High
10-99	Moderately Low	Moderately High	High
100-999	Moderately High	High	High
≥1,000	High	High	High

About Aemtek

Aemtek, Inc. is an environmental microbiology laboratory serving the indoor air quality industry. Its mission is to provide *accurate, fast, and reliable* expert services for detection, identification, analysis of fungi and bacteria in human environments. Aemtek also provides contracted research, technical training, and expert testimony services. Aemtek is committed to the highest level of quality, scientific integrity, and customer service.

Aemtek, Inc. implements a series of quality assurance procedures. All analytical protocols are developed based on scientific merits and are validated regularly. Aemtek is accredited by the American Industrial Hygiene Association (AIHA) Environmental Microbiology Laboratory Accreditation Program (Lab No.: 167620)

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