Water Testing

# iQ-Check *Legionella*

## Fact Sheet

BIO-RAD

## 1. SIGNIFICANCE OF *Legionella* TESTING

Legionellosis is an infection caused by *Legionella* bacteria; they are the cause of an acute pneumonia, Legionnaire's disease and a milder form of pulmonary infection, Pontiac fever. In Europe, the number of declared cases of Legionnaire's disease increases by 25% per year. In the United States, the CDC (Centers for Disease Control and Prevention) estimates the number of cases of Legionellosis at between 10,000 and 20,000 per year. In Canada, there were 332 cases in 2018 which represents 64% increase compared to 2017. In Quebec an estimate of 200 cases were reported last year. The actual number of cases, however, is thought to be much higher, as many people with pneumonia may not be tested for *Legionella* infection. The *Legionella pneumophila* species is responsible for approximately 90% of the clinical cases. Regular control of the presence of *Legionella* in water supply systems is the only way of preventing the disease.

One of the most sensitive and specific methods developed for *Legionella pneumophila* testing is qPCR (quantitative Polymerase Chain Reaction). To ensure accuracy of data, commercially available detection methods must comply with specific requirements and validations recommended by government agencies and professional organizations that have published guidelines not only for the design, but also the commissioning requirements for cooling towers and water systems.

Bio-Rad's iQ-Check method has been used for over 10 years to monitor *Legionella* in cooling towers and has been demonstrated to be a very powerful tool for the industry and government agencies.

## 2. ISO TECHNICAL SPECIFICATION 12869

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. ISO/TS 12869 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*. The aim of this standard is to provide proof of performance of a *Legionella* PCR system that meets the highest level of quality management, from manufacturing of the product to the results analyses. This helps build vendor credibility and buyer confidence by providing the marketplace with the assurance that performance claims are valid, credible and supported by high-quality, independent test data.

This technical specification describes a method for the detection and quantification of *Legionella* spp. and *L. pneumophila* using a quantitative polymerase chain reaction (qPCR). It specifies general method requirements, performance evaluation requirements, and quality control requirements including inclusivity/exclusivity analyses, limit of detection, limit of quantification of PCR step, calibrating function, link to primary standard, and efficiency and robustness of extraction with Aquadien kit. The guidelines, minimum requirements and performance characteristics are intended to guarantee that the results are reliable and reproducible between different laboratories.

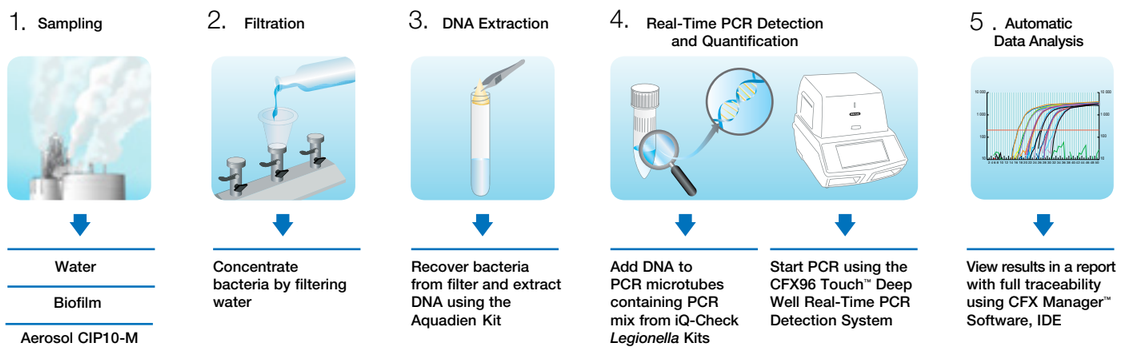
**iQ-Check *Legionella* spp. and iQ-Check *Legionella pneumophila* kits are NF VALIDATION certified according to NF T90-471 and ISO/TS 12896. The MD 15161-2013 Control of *Legionella* in Mechanical Systems Standard published by Public Works and Government Standards Canada (PWGSC) recommends the use of methods that are in accordance with the ISO TS 12869.**

## 3. iQ-CHECK METHOD

iQ-Check *Legionella* is an ISO/TS 12869 validated method based on amplification and detection of genomic sequences by the real-time PCR method. The Aquadien kit is also an ISO/TS 12969 method that allows an optimal DNA extraction and purification from bacteria present in water samples for PCR detection. The principle of the extraction is based on alkaline lysis of bacteria in presence of thermal shock and DNA purification using ultrafiltration. The Aquadien W2 wash solution is optimized for dirty samples including those containing PCR inhibitors such as additives and chemicals commonly used for water treatment. The kits contain ready-to-use reagents required to perform the analysis of samples: PCR amplification solutions including *Taq* DNA Polymerase and internal control, specific fluorescent probes and primers, negative and positive controls. Additional standards are provided for quantification.

The iQ-Check method is based on the following steps illustrated below:

**Fig. 1.** iQ-Check protocol




## 4. LIVE/DEAD DIFFERENTIATION WITH FREE-DNA REMOVAL SOLUTION (FDRS)

One of the primary challenges when performing PCR for environmental water testing is the potential presence of free DNA, which can lead to an overestimation of the level of targeted DNA sequences. When quantifying *Legionella*, culture and PCR levels can differ from each other by up to 2 logs. To improve *Legionella* testing in water, a supplemental step using the iQ-Check Free DNA Removal Solution (FDRS) can be introduced into the Aquadien DNA Extraction and Purification Kit. FDRS can be easily integrated in the Aquadien DNA Extraction Kit workflow and provides a non-carcinogenic and safe way to remove dead cell DNA or free DNA from environmental water samples prior to PCR analysis. According to the principle, free DNA is degraded by an enzymatic treatment of the sample prior to DNA extraction, and inactivated by the Aquadien Extraction Kit R1 solution allowing for the extraction of DNA from intact and living cells. The inclusion of FDRS in the iQ-Check workflow drastically eliminates the occurrence of false positives.

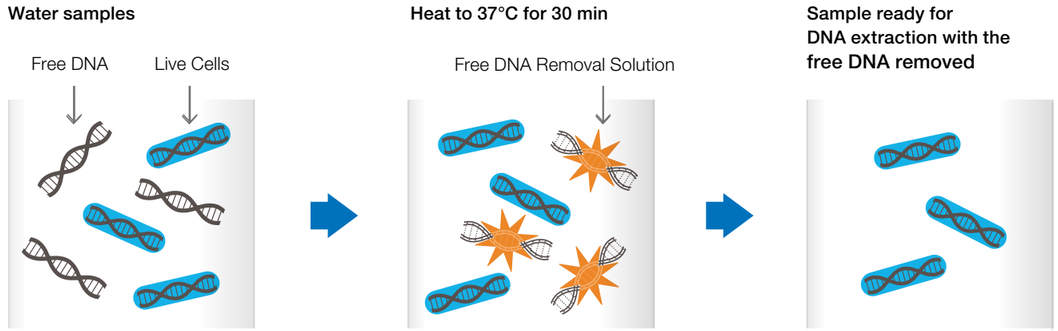
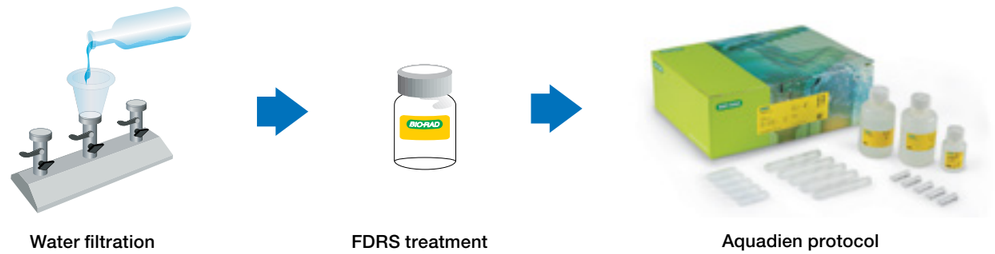



**Fig. 2.** The mechanism of action of FDRS

**Fig. 3.** FDRS protocol



## 5. iQ-CHECK PERFORMANCE CRITERIA

| PARAMETER | FEATURES |
|---|---|
| **Specificity** | *Legionella pneumophila* and *Legionella* spp. |
| **Sensitivity** | 5 GU (genomic units) |
| **Genetic Target** | Conserved *Legionella*-specific gene |
| **Internal Amplification Control** | Yes |
| **Assay Format** | Qualitative and quantitative |
| **Reagent shipping & storage** | Ambient temperature; +2 to +8°C |
| **Reagent Shelf Life** | 2 years |
| **Compatible Matrices** | Potable and non-potable including complex water, biofilm, aerosol |
| **Inclusivity** | *L.* spp : 35 isolates correctly identified<br>*L.pneumophila*: 15 isolates correctly identified |
| **Exclusivity** | *L.* spp :16 isolates correctly excluded<br>*L.pneumophila*: 25 isolates correctly excluded |
| **Time to Results** | <4 hr |
| **Viability Differentiation** | Yes (Free DNA Removal Solution) |
| **Validations** | NF T90-471; ISO/TS 12869 |

## 6. iQ-CHECK VS PORTABLE qPCR TECHNOLOGY

| FEATURES | iQ-Check *Legionella* | Portable *Legionella* qPCR |
|---|---|---|
| **Target Microorganism** | *Legionella* spp. and *Legionella pneumophila* | *Legionella pneumophila* only |
| **Throughput** | 96 samples | 1-3 samples |
| **Extraction Kit** | Validated protocols adapted for all water types | No validated protocols |
| **Live/Dead Differentiation** | Yes | No known live/dead differentiation |
| **Results** | Qualitative and quantitative | Quantitative |
| **Accuracy of Results** | High; Assay performed by trained technicians at reference labs lowers the risk of user error | Low; On-site analyses in non-accredited labs and inexperienced operators with lack of PCR skills may increase the risk of cross contamination and user error |
| **Validations** | NF Validation according to T-90 471 and ISO/TS 12869 | None |

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