

Bio-Rad's solution for *Legionella* risk management

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Introduction

Legionellosis prevention is an important part of Bio-Rad's R&D investment and commitment for industrial control. Regular testing for the presence of Legionella in water supply systems is the only way of preventing the disease. Bio-Rad Legionella testing products will help you find the best way to control Legionella levels in water, biofilm and aerosol samples and actually prevent damage to brand image. Bio-Rad provides a complete and integrated solution for Legionella risk management which is based on iQ-Check real-time PCR technology kits for detection and quantification of Legionella spp. and L. pneumophila.

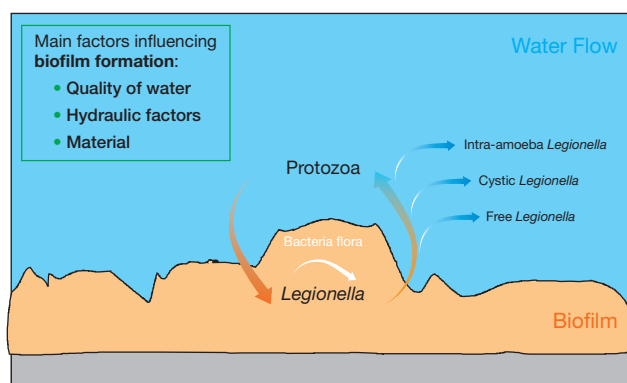
These products are completed by the Aquadien DNA extraction kit and a complementary range of culture media products for detecting and enumerating Legionella spp. or L. pneumophila. All iQ-Check Legionella real-time PCR kits comply with French standard (AFNOR T90-471). All culture media comply with ISO standards (ISO/DIS 11731-2 and ISO 11731).

Legionella bacteria

Legionella are small bacteria living in environmental water sources and in man-made water systems such as recreational waters or water distribution systems. Among the forty-eight species of *Legionella*, *L. pneumophila* is responsible for approximately ninety percent of infections.

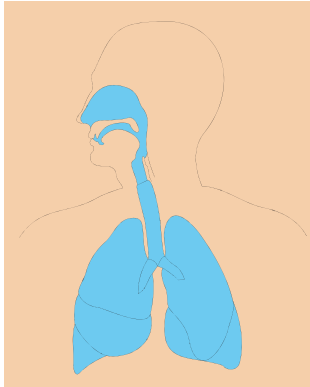
Owing to this public health issue, detection and enumeration of *Legionella* are mandatory or strongly recommended in most countries. Difficulty in monitoring this population rests on various factors. *Legionella* can survive in a wide range of temperatures, pH and oxygen partial pressure conditions. They can also proliferate inside biofilm or as parasites of other microorganisms, mainly protozoa. In stress conditions, this intracellular location protects *Legionella* against biocides, enabling them to recolonize the water network after treatment.

Biofilm is a slick coating produced by microorganisms and usually found in man-made water systems. This thin layer, which constitutes a complex ecosystem, provides bacteria with habitat, food and protection against biocides (scheme 1).



Scheme 1: Complexity of biofilm and presentation of the four ways that *Legionella* proliferates

Legionellosis disease



Legionella infections can lead to two types of disease, mainly Legionnaires' disease (legionellosis) and non-pneumonic Legionnaires' disease (Pontiac fever). Legionnaires' disease is a lung infection, an uncommon form of pneumonia. The majority of cases (>90%) reported in

the US are caused by *Legionella pneumophila*, with a mortality rate of approximately 13%. People become infected when they breathe in air that contains tiny droplets of water known as aerosols, inside of which are the *Legionella* bacteria.

About 10,000 to 20,000 cases are estimated in the United States per year. In Europe, Legionellosis were increased by 25% to 35% from 1999 to 2002 and by 25% from 2003 to 2004.

The degree of infection risk depends on four key factors:

- the density of the bacteria in the source;
- the extent of aerosol generation;
- the number of inhaled bacteria;
- the susceptibility of the exposed individual.

Legionella regulation

Regarding the evolution of Legionellosis over the past 15 years, governments have been pushed to set up measures for infection prevention. As *Legionella* is a very resistant bacteria, usual water treatments are ineffective, so routine control remains the only solution for Legionellosis prevention.

Regular analysis of water distribution systems is required for systems producing aerosols or having a temperature favorable to the proliferation of bacteria like cooling tower and sanitary hot water. This analysis aims to prevent contamination of population.

The threshold authorized by regulations differs according to the associated risk and differs with countries.

Type of installation	Pathogen	Target level CFU/L	Alert level CFU/L	Action level CFU/L
Sanitary hot water	<i>L. pneumophila</i>	<1000	>1000	>10,000
Cooling tower	<i>L. spp.</i>	<1000	>1000	>100,000
Healthcare establishments high risk patients	<i>L. pneumophila</i>	0	>1000	
Healthcare establishments: other	<i>L. pneumophila</i>	<1000	>1000	

Legionella norms

Today, reference methods in France, Europe and the US for *Legionella* detection and enumeration are cultured on selective media including antibiotics (GVPC), followed by confirmation on BCYE, with and without cysteine.

- 1 - ISO/DIS 11731-2** (November 2001): Water quality - Detection and enumeration of *Legionella* spp. and of *Legionella pneumophila* - Part 2: Direct membrane filtration technique for water with low bacteria content.
- 2 - ISO 11731** (May 1998): Water Quality - Detection and enumeration of *Legionella*.

Meeting analytical expectations in terms of delay of response for customers and administration faced with risk management linked to *Legionella*, it lets you use a method based on real-time PCR technology. This method for detection and quantification is a complement to the standard reference. This powerful and emerging method is already recognized in France as an AFNOR standard.

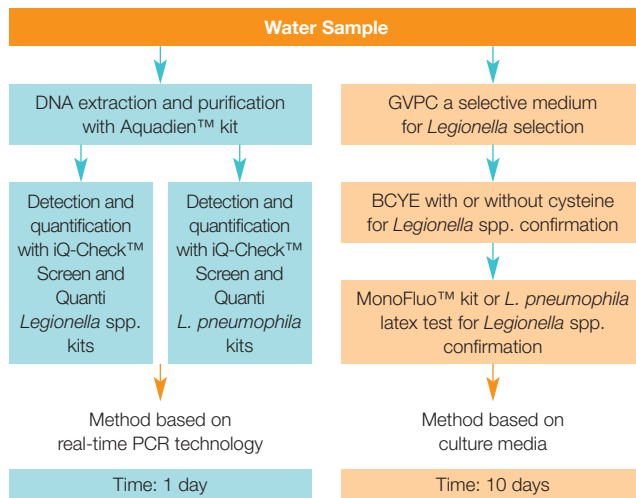
- 3 - AFNOR T90-471** (April 2006): Water quality standard - Detection and quantification of *Legionella* and/or *L. pneumophila* by concentration and gene amplification by polymerase chain reaction (PCR).

In accordance to this standard:

- In epidemiologic studies, the research of contaminated sites can be performed using the real-time PCR method, complementarily to culture analysis used to isolate the strains responsible for the outbreak.
- In on-site testing, the PCR technology can be used for regular installation follow-up for measuring the efficiency of biocide treatment.

Bio-Rad's product range for a complete risk assessment

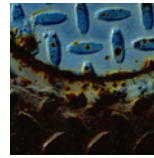
Two kinds of methods for accurate and complementary risk management.



Bio-Rad references for accurate and complementary risk management of *Legionella* bacteria.

357-8104 iQ-Check™ Screen <i>Legionella</i> spp.	96 tests	Real-time PCR technology
357-8105 iQ-Check™ Screen <i>L. pneumophila</i>	96 tests	
357-8102 iQ-Check™ Quanti <i>Legionella</i> spp.	96 tests	
357-8103 iQ-Check™ Quanti <i>L. pneumophila</i>	96 tests	
357-8121 Aquadien™ (DNA extraction kit)	100 tests	
357-8125 Aquadien™ lysis solution	34 tests	
356-3717 GVPC/Agar	90 mm x 20 dishes	Culture media
356-3719 GVPC/Agar 90 mm x 100 dishes	90 mm x 20 dishes	
356-3718 BCYE Biplate/Agar 90 mm x 20 dishes	90 mm x 20 dishes	
356-3720 BCYE with cysteine/Agar 90 mm x 20 dishes	90 mm x 20 dishes	
356-3722 BCYE without cysteine/Agar 90 mm x 20 dishes	90 mm x 20 dishes	
356-4760 <i>Legionella</i> Base/Agar	500 g	
356-4761 Suppl. for BCYE w/o cysteine/Freeze dried	Box of 10 vials	
356-4762 Suppl. for BCYE with cysteine/Freeze dried	Box of 10 vials	
356-4763 Suppl. for GVPC/Freeze dried	Box of 10 vials	
356-3804 Columbia + Horse Blood/Agar	90 mm x 20 dishes	
356-3784 Columbia + Sheep Blood/Agar 90 mm x 20 dishes	90 mm x 20 dishes	
356-3786 Nutrient Agar (Water testing) 90 mm x 20 dishes	90 mm x 20 dishes	
356-2790 <i>L. pneumophila</i> (1-15)/Latex	1 pack of 50 tests	
353-2514 MonoFluo™ kit <i>L. pneumophila</i>	1 pack of 24 tests	
359-3084 CIP-10 M	1 apparatus	Aerosol sample
359-3094 CIP-10 M US kit	1 apparatus	
359-1590 Chromo4™		Real-time PCR Instruments
359-1591 iQ5™		

Sample: water, biofilm, amoebae and aerosol



- **Water samples** from 1 liter to 100 ml of water, even dirty samples.
- **Amoebae:** MPN culture method.
- **Biofilm samples** on a 25-cm² surface (cf. Bio-Rad's technical note - Ref. 16995: Analysis of *Legionella* bacteria in the biofilm for complete risk management).
- **Aerosol samples** are obtained with a personal dust sampler apparatus or biocollector for capturing *Legionella* in aerosol.

- Principle: the liquid contained in the metal cup spins at the same speed as the metal cup, causing aspiration of the aerosol and collection of bacteria (cf. Bio-Rad's technical note - Ref. 16186: Quantification of *Legionella* in aerosols for infection risk assessment).

DNA Extraction: Aquadien kit

The Aquadien kit provides an optimal DNA extraction and purification from the bacteria present for real-time PCR detection. It has shown excellent real-time PCR analysis results for *Legionella* spp. and *Pseudomonas aeruginosa*.

The Aquadien kit contains sufficient reagents for 100 extractions.

The Aquadien kit's performance, robustness and yield extraction have been tested in 3 different laboratories.

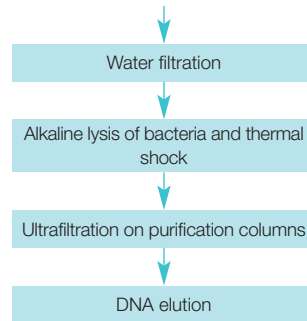
Results confirmed our expectations even on dirty samples.



Advantages

- Simple protocol
- Minimum hands-on time
- Extremely good extraction yield
- Reagents
 - Ready-to-use
 - All necessary reagents are included
 - Guaranteed free of *Legionella* contamination

Four-step protocol



iQ-Check™ Screen and Quanti *Legionella* spp. and *L. pneumophila*

Especially developed to meet the needs for rapid *Legionella* detection and quantification using real-time PCR technologies, iQ-Check *Legionella* kits give Bio-Rad a strong position in *Legionella* testing.

These kits are part of the *Legionella* detection range products, composed of ready-to-use or dehydrated culture media for *Legionella* enumeration.

Bio-Rad is a relevant partner for *Legionella* risk assessment.

Reference number	Kits
357-8104	iQ-Check™ Screen <i>Legionella</i> spp.
357-8105	iQ-Check™ Screen <i>L. pneumophila</i>
357-8102	iQ-Check™ Quanti <i>Legionella</i> spp.
357-8103	iQ-Check™ Quanti <i>L. pneumophila</i>

iQ-Check *Legionella* kits: general features

The detection limit of the PCR step (LOD PCR) for a *Legionella* PCR detection and quantification method is defined as the lowest number of Genomic Units generating a positive result at a 90% confidence limit. The quantification limit of the PCR step (LOQ PCR) for a *Legionella* PCR quantification method is defined as the lowest number of Genomic Units providing accurate quantification (maximum interval of 0.5 decimal logarithm between the high and low value of the confidence interval at 95%). NF T90-471 (April 2006): Detection and quantification of *Legionella* and/or *Legionella pneumophila* by concentration and genic amplification by polymerase chain reaction (PCR).

- Inclusivity validated on 40 strains out of 27 species of *Legionella*.
- Exclusivity validated on 35 non-*Legionella* strains tested.
- LOD PCR and LOQ PCR according to the protocols established in the AFNOR standard NF T90-471.
- LOD for all kits is 5 GU/PCR.
- LOQ for iQ-Check Quanti *Legionella* spp. kits is 10 GU/PCR.
- LOQ iQ-Check Quanti *L. pneumophila* is 15 GU/PCR.



Study: Biocide treatment follow-up in a water distribution system

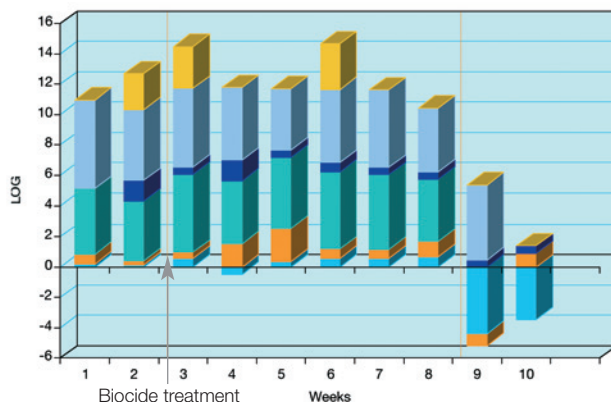
Method

- Samplings are performed once a week, over a period of 10 weeks in several points: process water box, hot water tank, upstream water and emergency pump. We decide to show data for hot water tank and process water box.
- After two weeks, a biocide treatment is performed and is followed up for 8 weeks.
- Six water quality indicators are measured.
- One indicator (named CFU) is based on *Legionella*'s quantification by culture media.
- Three indicators are based on *Legionella*'s quantification by real-time PCR technology:
 - in the Biofilm (named Biofilm),
 - in the water (named water PCR),
 - between the point of sampling and the make-up water (named Δ PCR). This one measures the variation of *Legionella* between the two points.
- The indicator Δ ATP measures the variation of total flora between the point of sampling and the make-up water.
- The amoebae indicator measures the quantity of amoebae which help *Legionella*'s proliferation.

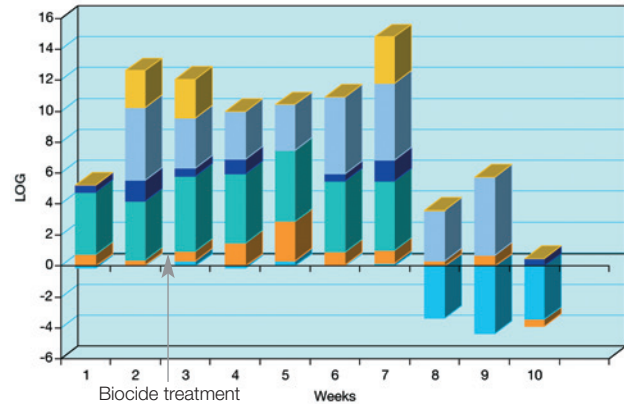
Results

Indicators	Assessment method
CFU	ISO 11731 in log (CFU/l)
BIOFILM	PCR <i>Legionella</i> spp. in log (GU/25 cm ²)
AMOEBAE	MPN plate count method
WATER PCR	PCR <i>Legionella</i> spp. XP T 90-471 in log (GU/l)
Δ ATP	Point - Make-up water in log (RLU)
Δ PCR	Point - Make-up water in log (GU/l)

Process Water Box



Hot Water Tank



Interpretation

- Results were consistent, but differences were observed from one point on the circuit to another.
- Reduction of the *Legionella* spp. PCR signal within a facility is possible.
- PCR signals are significantly and homogeneously reduced over time.
- Significant effects are detected after 5 weeks' treatment.
- The cultivability of *Legionella* was heterogeneous throughout the facility and over time.
- Eukaryotic flora reduction is difficult to measure.
- The biofilm remains the most difficult to reduce.
- Amoebae seem to be resistant to the current treatment.
- Make-up water quality gets a significant impact. At the end of the sampling period, circuit load is lower than the make-up water.
- None of the 6 indicators, when used alone, would take into account the complexity of facility evolution.

Conclusion

Biocide treatment follow-up must be performed in several points of water distribution system and monitored over the time. This follow up must also concern the make-up water which can bring a part of *Legionella* in the water distribution system and be at the origin of the contamination.

Among the indicators used to assess the water microbiological quality, real-time PCR technology appears as a new and accurate method for quantifying *Legionella* not only in water but also in biofilm. This innovative technology is both an efficiency measurement tool for various treatments follow-up and a relevant indicator for *Legionella* risk management.

For further information on:

- iQ-Check™ *Legionella* kits
- CIP10-M
- Aquadien™ DNA Extraction kit
- Culture media
- Study shown: Biocide treatment follow-up in a water distribution system
- Bio-Rad's technical notes:
 - Analysis of *Legionella* bacteria in the biofilm for complete risk management - Ref.: 16995
 - Quantification of *Legionella* bacteria in aerosols for infection risk assessment - Ref.: 16186
 - Detection of *Legionella* bacteria within amoebae (in progress)

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