

## Detection of *Legionella* in Aerosols for Infection Risk Assessment

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### Abstract

*Legionella* bacterium is a pathogenic agent responsible for serious lung diseases. As aerosols are the unique vectors of these bacteria, regular air controls constitute the preliminary step for preventing outbreaks. This article describes an original solution for the quantification of *Legionella* in aerosols with the CIP10-M device and real-time PCR technology. Complete, sensitive and accurate, this method is a breakthrough for *Legionella* risk assessment for human health.

### Introduction

Thirty years ago, during an American Legion convention in Philadelphia, the so-called Legionellosis disease was identified for the first time due to an outbreak of pneumonia. Today, the number of cases is increasing, though still underestimated. In Europe, Legionellosis cases grew by 25 to 35% from 1999 to 2002 and by 25% from 2003 to 2004. In the United States, it is estimated that there are about 10,000 to 20,000 cases a year. Described as an uncommon form of pneumonia, Legionellosis is a lung infection triggered by inhaling aerosols carrying *Legionella* bacteria. During the inspiration phase, water spray or water droplets in suspension transport *Legionella* to lung alveoli. More than 90% of cases reported are caused by the *Legionella pneumophila* strain and the mortality rate of the disease is evaluated at 13%.

*Legionella* are small bacteria living in environmental water sources and in man-made water systems, like recreational water and hot water distribution systems. In the natural environment, bacteria are not generally Legionellosis vectors. On the other hand, man-made water systems for comfort in buildings, leisure or industrial activities are responsible for outbreaks and sporadic cases. Warm water distribution systems and cooling towers are namely



incriminated in *Legionella*'s proliferation. Cooling towers are common equipment used in refrigeration devices on industrial sites or large public buildings like hospitals, hotels or cruise ships. They are at the origin of the providing of *Legionella* in water, with an ideal environment for their proliferation.

The high volume of aerosols produced by these systems is known to be responsible for human contamination. *Legionella*'s detection is based on standard methods with culture media (AFNOR T90-431 and ISO 11731). Other more sensitive and faster methods, based on real-time PCR technology, are recognized for on site self-checks and epidemiologic studies (AFNOR T90-471). Methods for detecting *Legionella* in aerosol remain experimental.

Regulations on the threshold for *Legionella*'s presence exist but vary according to the regional regulations, WHO (World Health Organization) regulations and CDC (Centers for Disease Control, United-States) regulations.

Furthermore, all these thresholds concern the presence of *Legionella* in water, while Legionellosis can only be caused by aerosol inhalation.

Even though standard methods and regulations do not exist for detecting *Legionella* in aerosols, checking for its presence remains the only way to truly evaluate human exposure and infection risks. This risk assessment for human health is essential in the neighborhood of cooling towers where people could be exposed to aerosols produced and for the workers who maintain such systems.

The aim of this article is to present an original and efficient method for sampling, detecting and quantifying *Legionella* spp. and *L. pneumophila* in aerosols aforementioned.

This method uses on-site sampling of aerosols with a portable apparatus called Personal Microbiological Pollutant Sampler (CIP10-M), combined with real-time PCR technology for detecting and quantifying the *Legionella* bacteria, in compliance with the AFNOR T90-471 standard (for detection and quantification by real-time PCR technology).

The sampling protocol was kindly worked out and provided by Bouisson Bertrand Institute, France.



CIP10-M Device

## Material and Methods

### • Principle and main characteristics of the CIP10-M

The CIP10-M is a standalone unit designed for collecting microorganisms in aerosols in order to evaluate the quantity likely to be inhaled by workers on the job. This patented device was validated by INRS (National Institute for Security Research, France).

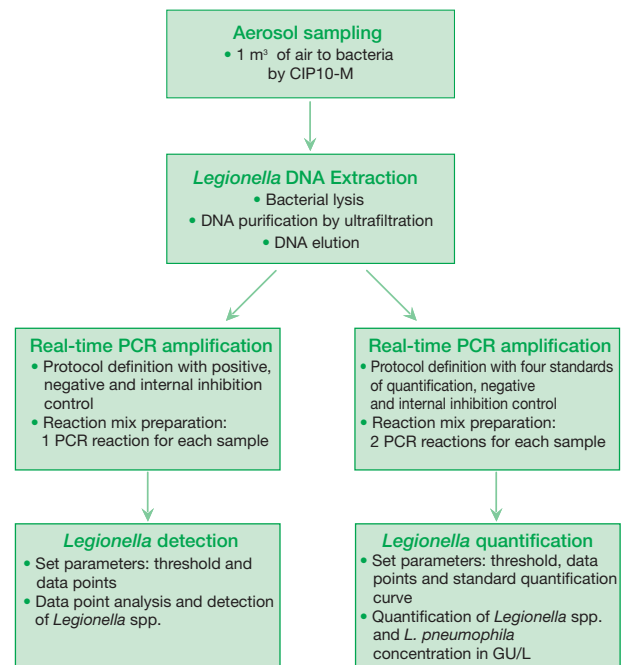
The CIP10-M measures a person's exposure to aerosol in the workplace or in a general environment. It is normally worn on the chest, but can also be placed in a fixed position in the work zone or in any other place for ambient measurement.

The CIP10-M's rotating cup has horizontal blades on the upper part, which generate a flow by a centrifugal fan effect. The friction of the air with the vertical surfaces of the liquid contained in the liquid cup and with the other surfaces generates a low-pressure area to direct the airflow towards the collection liquid. Thus, the aspirated airflow follows a helicoidal movement to gently place the living cells on the fluid.

### • Material

- CIP10-M (Bio-Rad ref. 359-3084) or CIP10-M US kit (Bio-Rad ref. 359-3094).
- Aquadien™ lysis solution (Bio-Rad ref. 357-8125).
- Aquadien™ kit for DNA extraction (Bio-Rad ref. 357-8121).
- iQ-Check™ Screen *Legionella* spp. or *pneumophila* kits (Bio-Rad ref. 357-8104 and ref. 357-8105) or iQ-Check Quanti *Legionella* spp. or *pneumophila* kits (Bio-Rad ref. 357-8102 and 357-8103).

### • Flow chart



### • Aerosol sampling with CIP10-M

#### Preliminary step

Before starting the sampling step, and between samplings, the cup and the collection head must be sterilized with an autoclave.

**Note:** to perform the first autoclaving step, the snap-in alveolar nozzle has to be clipped on the cup chamber

to avoid any morphing due to high temperature. Bio-Rad recommends 15 minutes at 120°C.

### On-site steps

1. Fill the rotating cup with 3 ml of the R1 reagent supernatant (Aquadien™ kit or Aquadien™ lysis solution).
2. Place the cup on the CIP10-M apparatus.
3. Cover the cup with the protective cap and lock it with the side screw.

**Note:** these operations must be carried out when the CIP10-M is upright to keep the R1 reagent from spilling.

4. Start the CIP10-M by briefly passing the provided magnet against the instrument's case in the area marked by the I/O acronym. The LED "working" indicator lights up.
5. Switch on the CIP10-M. The instrument stays on for hundred minutes for collecting 1 m<sup>3</sup> of air. The CIP10-M can operate in any position. The collection solution is effectively flattened against the inside walls of the rotating cup by centrifugal force.
6. Put the CIP10-M on the air flow.
7. Stop the sampling by briefly passing the magnet. The CIP10-M must be stopped when upright. It should be noted that while the rotating cup mounted on the motor shaft starts turning quickly, it takes about 20 seconds to stop because of its kinetic energy of rotation.
8. Remove the CIP10-M's head from the instrument in an upright position, and carefully take out the cup using a cup extractor.
9. Pipette the reagent remaining up and down ten times to rinse the cup wall and remove all of the microorganisms. The volume is generally lower, about 1 ml depending on hygrometry.
10. Put the reagent in a cryotube provided with the Aquadien kit.
11. Store and transport the sample at 4°C.

### Special case of collecting in a dry atmosphere

Low ambient relative humidity limits the collection interval. In fact, with a very dry atmosphere, the collection fluid (depending on its vapor pressure) can evaporate in the air being sampled, thus reducing the standalone collection time.

**Step 8:** If the cup is empty, the sample does not comply and must be repeated. In this case, the level of the reagent must be checked after 45 minutes of utilization. If the level is too low, the cup may be filled again.

### Laboratory steps

12. Measure the volume of the R1 reagent supernatant pipetted into the cryotube.
13. Write down the volume. Top off this volume to 2 ml if it's lower than this value.
14. Perform direct extraction with the Aquadien kit.

### Cup Cleanup

- Clean with water and soap
- Rinse with water
- Clean with DNAway
- Rinse with sterile water
- Autoclave 15 minutes at 121°C.

The clean cup is stored in aluminium until the next use.

### • DNA extraction

Use Aquadien kit for the DNA extraction. Begin at the step 3-part 3 of the Aquadien protocol; bacteria are directly collected in the R1 reagent supernatant without membrane.

### • Detection and quantification

Detection and quantification of all samples are performed with iQ-Check™ Screen *Legionella* spp. (ref. 357-8104) and iQ-Check Quanti *Legionella* spp. (ref. 357-8102).

For specific detection of *L. pneumophila*, tests can be carried out with iQ-Check Screen *L. pneumophila* (ref. 357-8105) and iQ-Check Quanti *L. pneumophila* (ref. 357-8103). These kits are based on real-time PCR technology which provides accurate, sensitive, one-day detection and quantification of *Legionella*.

### • Results

PCR result must be multiplied by the Z value to obtain the final quantity of bacteria contained in the initial air sample (Genomic Unit per m<sup>3</sup> or UG/m<sup>3</sup>). Z represents the fraction of initial air sample analysed in each PCR well. If you perform the DNA extraction step with 2 ml of R1 reagent supernatant, the Z value is obtained by the following way:

- DNA Extraction/Purification: 1 ml is processed through the column out of 2 ml R1 supernatant. Purified fraction = 1/2 (The result should be multiplied by 2).
- PCR: 5 µl of the purified DNA solution are submitted to PCR out of 100 µl total extracted DNA. Analyzed fraction in PCR = 1/20 (The result should be multiplied by 20).
- The Z value for this protocol is: 2 x 20 = 40.

**Note:** If the filtered air volume is different from 1 m<sup>3</sup>, or if the volume of R1 supernatant collected is different than 2 ml, take into account this volume in the calculation.

## Conclusion

*Legionella* bacteria are frequently present in our environment. The difficulty of eradicating *Legionella* in water distribution systems leads to regular water treatment to limit their proliferation. Regular follow-up of *Legionella*'s presence is necessary to monitor the effectiveness of the treatments. Usually, tests are performed in water or, more commonly, in the biofilm. However, aerosols are the sole vector for human diseases. As only aerosol inhalation leads to Legionellosis, a complementary control of aerosol is thus necessary near cooling towers or other devices producing it. This assessment is the only way to evaluate *Legionella*'s dissemination in aerosol and to accurately quantify the number of bacteria inhaled. With the CIP10-M, Bio-Rad offers an original, complete and effective method of assessing *Legionella* risks to human health. The CIP10-M constitutes the first step in detecting and quantifying *Legionella*. Small, compact and robust, the CIP10-M apparatus is a milestone in *Legionella* risk management and in the prevention of Legionellosis outbreaks.

## References

- P. Görner, J-F. Fabriès, P. Duquenne, O. Witschger. Bioaerosol sampling by a Personal Rotating Cup Sampler CIP10-M. 2006. Journal of Environmental Monitoring, 8, pp. 43-48.
- P. Görner, J-F. Fabriès, P. Duquenne, O. Witschger, R. Wrobel, G. Greff-Mirquet. 2006. Echantillonner les bioaérosols sur les lieux de travail - Salles propres n°42, pp. 36-41.
- P. Duquenne, G. Greff-Mirquet. 2005. L'échantillonnage et l'analyse des aérosols microbiens. Hygiène et sécurité du travail, Cahier des notes documentaires, 198, pp. 23-28.
- P. Görner, J-F. Fabriès, R. Wrobel, O. Witschger, P. Duquenne. The Personal Bioaerosol Sampler CIP10-M. 2004. Journal of Aerosol Science, 35, Suppl. 1, pp. 607-608.
- R. Rapp, P. Görner. 2004. Métrologie des bioaérosols et ventilation. Maîtrise de la qualité de l'air dans les salles de tri de traitement des ordures ménagères. Actes du congrès INRS Actualités en prévention, Nantes, 28-29 juin.
- K. Willeke, X. Lin, S.A. Grinshpun. 1998. Improves Aerosol Collection by Combined Impaction and Centrifugal Motion. Aerosol Science and Technology, 28, pp. 439-456.
- J-F. Fabriès, P. Görner, P. Martin, J.P. Paquin, P. Hartemann. 1988. Développement d'un échantillonneur pour la mesure des aérosols biologiques. Actes des 5<sup>es</sup> Journées d'études sur les aérosols. Paris 8-9 décembre, pp. 35-40.
- Tyagi S, Kramer FR. 1996. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol.14, pp. 303-308.
- Wolters J, Erdmann VA. 1986. Compilation of 5S rRNA and 5S rRNA gene sequences. Nucleic Acids Res. 16 Suppl:r1-70.
- MacDonell MT, Colwell RR. 1987. The nucleotide sequence of the 5S rRNA from *Legionella pneumophila*. Nucleic Acids Res. 15:1335.
- Engleberg NC, Carter C, Weber DR, Cianciotto NP, Eisenstein BI. 1989. DNA sequence of mip, a *Legionella pneumophila* gene associated with macrophage infectivity. Infect Immun. 57, pp.1263-1270.
- Fields B, Benson R, and Besser R. 2002. *Legionella* and Legionnaires' disease: 25 years of investigation. Clinical microbiology reviews.
- NF T90-471 (April 2006). Water quality - Detection and quantification of *Legionella* and/or *Legionella pneumophila* by concentration and gene amplification by polymerase chain reaction (PCR).
- NF T90-431/A1 (April 2006). Water quality - Detection and enumeration of *Legionella* spp and *Legionella pneumophila* - Method by direct inoculation and after concentration by membrane filtration or centrifugation.



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