

## Legionella detection from clinical and environmental samples

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### Abstract

Several methods are used for diagnosis of legionellosis: culture, urinary antigen detection, serologic tests, PCR etc., characterised by different level of specificity, sensitivity and rapidity. Microbiological diagnosis is an essential tool to for the prompt adoption of an adequate antimicrobial therapy and to understand the real diffusion of legionellosis.

Likewise, the environmental samples analysis allows us to know the distribution of the *Legionella* in the environment and to detect the origin of the infection during an outbreak by comparing clinical and environmental strains.

### Introduction

#### *Clinical samples*

Laboratory diagnosis of Legionnaires' disease (LD) must be considered an essential tool in the clinical diagnosis and must be performed before the beginning of an antimicrobial therapy. In fact, due to the low prevalence of LD, the specificity of diagnostic tests should be close to 100%, to allow for the identification of sporadic cases.

The culture represents the gold standard for diagnosis of legionellosis from respiratory secretions and lung biopsy or autopsy specimens. This method is the most specific diagnostic procedure (99%) available and it is important for comparative studies with environmental strains. It requires a specific media such as Buffered Charcoal Yeast Extract enriched with  $\alpha$ -chetoglutarate (BCYE- $\alpha$ ) with and without selective agents. However, it is time-consuming and requires an experienced laboratory, specific media and, often, samples are not easy to find because patients with LD produce little or non purulent cough. Sensitivity, therefore, ranges between 10- 80%.

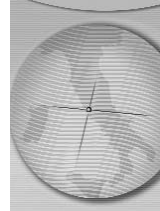
On the contrary, urinary *Legionella* antigen detection by immunochromatographic or enzyme immunoassay is a rapid and easily performed method for which samples are readily available, although it does not allow for the identification of *Legionella pneumophila* serogroups and species. Antigens are easily detectable in urine and persist for several weeks and their presence is independent from antimicrobial therapy. The specificity of this method is about 99% and the sensitivity ranges from 94% to 55% and 46%

depending respectively on community, travel or nosocomial legionellosis.[1]

Serology diagnosis, with a fourfold or more rise of antibody titre for *Legionella*, detected by indirect immunofluorescence between two sera at the disease onset and at the convalescent phase, is very useful for epidemiological purposes but less as a clinical diagnostic tool. In fact, specific antibodies are often absent, especially in immuno-compromised patients or they rise after the infection. Moreover, cross-reactivity reactions within *Legionella* genus and with other microorganisms are possible, and the positive result of a single serum has a presumptive assessment. The sensitivity of serologic methods is considered between 60-70% and the specificity > 95%.

Observation of biological samples by direct immunofluorescence of respiratory secretions, lung biopsy or autopsy specimens allows for rapid diagnosis with good specificity (> 95%), but with a low sensitivity (25-66%) and it is technically demanding.

DNA detection with PCR (polymerase chain reaction) based methods could be very useful for slow growth microorganisms like *Legionella*. PCR is carried out for many types of clinical samples (respiratory secretion, urine, serum, etc.), using prevalently 5S rDNA, 16S rDNA mip (macrophage infectivity potentiators) as target genes. However, they have been less utilised in clinical laboratories especially due to problems regarding their specificity, such as false positive results, and further work is necessary to establish and improve a standard PCR procedure. Recently, the use of a new type of PCR, the real-time PCR, has



been reported by some authors.[2] This method decreases the risk of cross-contamination, diminishes the time to process the samples, and eliminates the need to sample the analyses after PCR, because the reaction is visible during the amplification process. The last one is an innovative method that quantifies and selects live and dead microorganisms, but, as well as conventional PCR, needs more in-depth studies.

#### Environmental samples

Finding *Legionella* in the environmental samples has an important role for *Legionella* infection control. Environmental investigations could be carried out in hotels, hospitals, gym rooms, swimming pools for normal routine control procedures of both single case and epidemic clusters, to confirm or exclude the origin of infection.

It is important to perform environmental investigations before control measures are carried out, and all procedures should be performed by specialist laboratory staff. Intervention strategies should include the following points:

- Analysis of an updated map of the plant, if available, to focus the attention on the critical sites;
- Risk assessment for legionellosis according to:
  1. good environmental conditions for the replication of *Legionella* (temperature average: 20-50°C);
  2. good nutritional sources such as weeds, limestone, rust or other organic sources;
  3. aerosol producing devices (i.e. showers, whirlpools, cooling towers);
  4. minimum water flow in the piping;
  5. natural fibre or rubber strips;
  6. artificial respiration devices; aerosol and oxygen therapy;
  7. subjects sensitive to the acquisition of legionellosis.

The presence of subjects sensitive to the acquisition of legionellosis has to be considered as well as the number of sites sampled which should be representative of the water system and the major risk sites. Samples must be taken from:

1. hot water system (cold water system if temperature exceeds 20°C);
2. tank sediments or other wellhole sediments;
3. piping and tanks scale;
4. biofilms or other pipe scale (faucets, shower filters and cooling towers);
5. water-cooled condenser and air conditioning systems siphons waters and filters;
6. cooling tower basin waters;
7. whirlpools and fountains.

The methods used for detection and enumeration of *Legionella* in environmental samples are described in ISO 11731,[3] or in the protocol described in the Italian guidelines.[4] As for water sample analysis, it consists of a filtration or centrifugation procedure followed by the growth of *Legionella* on a specific media, such as buffered charcoal yeast extract enriched with  $\alpha$ -chetoglutarate (BCYE- $\alpha$ ) with and without selective agents at 37°C with 2.5% CO<sub>2</sub>.

#### Conclusions

Clinical samples need to be tested by more than one method, the gold standard culture method included, because the available tests do not have 100% sensitivity.

As far as environmental samples are concerned, it is important to emphasise that sampling procedures must be performed correctly to avoid erroneous results. An accredited laboratory should analyse the samples and the subsequent results used to suggest the control measures to be employed according to the Italian guidelines.[4] Environmental strains must be stored by the laboratory to support comparative studies with clinical strains and trace back to the infection sources.

#### Acknowledgements

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