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# Prospective evaluation of RT-PCR on sputum versus culture, urinary antigens and serology for Legionnaire's disease diagnosis

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**Summary** *Objectives:* Legionnaires' disease (LD) is a severe disease associated with community and hospital-acquired pneumonia, frequently under diagnosed. The main aim of our study was to determine the value of PCR for the diagnosis of LD in routine clinical practice.
*Methods:* In a prospective study, from March 2007 to April 2010, the value of PCR on non-invasive respiratory specimens (NIRS) was compared to those of the other available tools for LD diagnosis in patients hospitalized for pneumonia.
*Results:* Among 254 consecutive cases of pneumonia included, 24 cases were LD (19 confirmed and 5 probable) representing the first documented microbiological etiology. Molecular diagnosis of LD was performed on NIRS by using 16S rRNA PCR, and secondarily *mip* PCR, with no discrepant results between the 2 methods: it was found positive in 14 cases and led to identify 2 supplementary probable cases of LD. Based on clinical and at least 2 positive LD tests, PCR yielded a better diagnostic value than antigen urinary test (12 vs 10 cases).

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*Conclusion:* These results revealed that molecular diagnosis of LD on NIRS is reliable and may contribute to better identify cases of LD.


## Introduction

Legionellosis mainly presents as Legionnaires' disease (LD), a severe multisystem disease involving pneumonia.[1] LD is a life threatening disease with a mortality rate of 10—40%, depending on the underlying diseases of the patient.[1—4] Among more than 50 species of *Legionellae* identified to date, *Legionella pneumophila* is the cause of LD in more than 90% of cases.[1,5] Clinical presentation alone fails to differentiate LD from other causes of pneumonia.[6]

*Legionellae* are Gram-negative bacteria found in freshwater environments; they are fastidious to growth and not easily detected.[5] The diagnosis of LD is challenging and probably underreported. Culture diagnosis was formerly the gold standard for diagnosis of legionellosis; however, it requires specific growth medium conditions, takes time to report the results, exhibits poor sensitivity and finally is rarely performed in routine practice.[1,4] Serologic diagnosis is helpful for epidemiologic investigations; however, for diagnosis of acute legionellosis, it needs the testing of paired serum samples collected 3—6 weeks apart to observe a fourfold increase of antibodies.[1] Moreover cross-reactions are possible with other pathogens such as *Pseudomonas* spp. Urinary antigen testing permits early diagnosis and rapid initiation of appropriate antibiotic therapy; it is now the most frequently used diagnostic test.[4] The capture antibody used in the majority of these assays is specific for *L. pneumophila* serogroup 1.[1,5] Therefore, even though most reported human cases of legionellosis are associated with this serogroup, total dependence on this diagnostic assay was described as missing as many as 40% of cases of LD,[1] notably in hospital-acquired LD.[7]

All respiratory samples such as sputum are suitable for the use of PCR which rapidly allows the diagnosis of LD including those due to non serogroup 1 *L. pneumophila*.[8,9] Molecular diagnosis in bronchoalveolar lavage has good specificity and sensitivity compared to other diagnostic methods such as culture or serology.[10] Currently, molecular diagnosis is based largely on detection of the 16S rRNA gene for identification of the *Legionella* genus and the *mip* gene for identification of the species *L. pneumophila*.[11,12] However, despite the development of molecular tools for the diagnosis of pneumonia, specific PCR for *Legionella* spp. remains rarely used in clinical practice, as based on a recently published European study reporting that only 2% of the 11,832 confirmed or probable cases of LD were ascertained by PCR.[4]

Here, in a cohort of hospitalized patients with pneumonia, we compared prospectively the use of real time PCR (16S rRNA PCR and *mip* PCR) on sputum to culture, urinary antigens and serology assays for the diagnosis of LD.

## Material and methods

The study took place at University Hospital of Saint-Etienne, France, from March 2007 to April 2010. At the time of the study, PCR did not belong to *Legionella* diagnostic criteria.[13]

### Inclusion criteria

The study was proposed to each patient, more than 18 year-old, hospitalized with a diagnosis of community- or healthcare-associated pneumonia. Pneumonia was suspected according to current definition.[14] Ventilator-associated pneumonia plus patients with absence of respiratory samples were excluded.

### Definition of legionellosis

According to the definitions of the French Institute for Public Health Surveillance (InVS)[15] and to the criteria used for reporting cases of Legionnaires' disease at the international level,[16] a confirmed case was associated with at least isolation of *Legionella* spp in a clinical specimen or increase ($\times$4) of the antibody titer or the presence of soluble urinary antigen. A case was considered probable if the antibody titer was equal or above 256, or the PCR test positive. In addition, as recently proposed by Chen et al., we considered "proven" cases of LD[17] as cases of pneumonia associated with at least two positive *Legionella* tests.

### Bacterial methods

#### Real-time PCRs

*Legionella* DNA was detected on respiratory samples (sputum with or without induction, nasopharyngeal aspirates or nasopharyngeal wash samples in 93% of cases) by 2 different real-time (RT) PCRs. A dual-color 16S rRNA PCR was performed on a Lightcycler 1.0 instrument (Roche) using the 2 primers and 4 FRET probes described by Reischl et al.[18] The method allows to detect all *Legionella* species and to differentiate *L. pneumophila* from other *Legionella* species by analyzing the melting curves of the amplicons. In brief, respiratory samples (200 $\mu$l) were pretreated with proteinase K before extraction on a Kingfisher apparatus using the Magnesil KF kit (Lifesciences). DNA was amplified by RT-PCR using 50 cycles of amplification. Positive and negative controls were included in each run. In addition, each PCR result was validated by the positive detection of a beta-globin gene control. During the study period, the efficiency of the 16S rRNA PCR was controlled by testing samples from the 2006—2007 QCMD panels. A *mip* PCR specific for *L. pneumophila* was done secondarily on a SmartCycler (Cepheid) using the primers and a *mip* Taqman probe previously described.[19]

#### Cultures

Respiratory samples were plated onto buffered charcoal yeast extract (BCYE) medium supplemented with alpha-ketoglutarate containing or not selective antibiotics (GVPC and BCYE plates, Oxoid) with and without a heat- (30 min at