Laboratory Tests for Legionnaire's Disease

W. Michael Dunne Jr, PhD[a], Nathalie Picot, MSc[b], Alex van Belkum, PhD[c,*]

KEYWORDS

• *Legionella pneumophila* • Detection • Characterization • Diagnostics

KEY POINTS

- Despite the fact that *Legionella* is a recently discovered pathogenic genus, many clinically useful culture-based, immunologic, and molecular tests have been developed, although there still is a need for further optimization of the diagnostic process.
- Despite disadvantages including low sensitivity and long incubation times, the excellent specificity of culture of *Legionella* species makes it a valuable addition to any diagnostic algorithm and facilitates downstream antibiotic susceptibility testing.
- Both urine antigen testing and molecular assays for the detection of *Legionella pneumophila* are important but still in need of improvement in terms of sensitivity.
- Combining urine antigen testing with culture or molecular assays currently provides the best algorithm for diagnosis of *Legionella* disease.
- Whole genome sequencing facilitates the epidemiologic association of isolates of *Legionella* species potentially involved in outbreaks of infection.

INTRODUCTION

In 1977, McDade and colleagues[1] first reported on the isolation of a gram-negative, non–acid-fast bacillus from the lung tissue of 4 patients who died of a severe respiratory illness acquired while attending an American Legion convention in Philadelphia in 1976. The tissue from those patients was inoculated intraperitoneally into Guinea pigs, several of which became moribund 3 to 6 days after the onset of fever. Tissue (liver, lung, spleen) taken at necropsy of the animals was then inoculated into embryonated

Disclosure Statement: All authors are employees of bioMérieux and have a business implication in the work presented here. bioMérieux develops and sells a wide variety of diagnostic tests in the field of clinical microbiology and infectious diseases. However, the study was designed and executed independently by the authors and the company had no influence on the overall outcome of the literature studies presented here.

[a] Scientific Office, BioMérieux, 100 Rodolphe Street, Durham, NC 27712, USA; [b] Innovation Group, Info Doc, BioMérieux, Chemin de l'Orme, Marcy-l'Étoile 69280, France; [c] Scientific Office, BioMérieux, 3, Route de Port Michaud, La Balme Les Grottes 38390, France
* Corresponding author.
E-mail address: alex.vanbelkum@biomerieux.com

Infect Dis Clin N Am ■ (2016) ■–■
http://dx.doi.org/10.1016/j.idc.2016.10.012 **id.theclinics.com**

hen's eggs which, in turn, caused death of the embryos 4 to 7 days after inoculation. Smears of the yolk sacs from those embryos found pleomorphic gram-negative rods that did not grow on routine microbiologic culture media, but viable colonies could be cultivated using Mueller-Hinton agar supplemented with 1% hemoglobin and Isovitalex. Using the cultured organisms as a source of antigen, the group found increasing antibody titers or a single elevated titer from patients with this newly described entity called Legionnaire's disease using an indirect immunofluorescent assay. The same was observed for Guinea pigs who survived inoculation with the cultured organisms. Therefore, the first diagnostic assay for Legionnaire's disease was defined as a 4-fold increase in indirect immunofluorescent assay titer to a level $\geq$64 or a single antibody titer of 128. Using this assay, 2 previously unidentified but similar outbreaks of febrile illness that occurred in a psychiatric hospital in Washington, DC in 1965 and in a county public health office in Pontiac, Michigan in 1968 were found to have been instigated by the same or similar bacterial species. Within 1.5 years of the initial outbreak, a diagnostic tool and a means of recovering the etiologic agent of Legionnaire's disease was developed, although the true width and breadth of illness caused by the rapidly expanding *Legionella* species was yet to be appreciated. Testing for and characterization of *Legionella pneumophila* is not straightforward, as the bacteria have an intracellular lifestyle within alveolar macrophages and monocytes. One can only wonder how quickly the diagnostic process would have developed had metagenomic sequencing and whole genome analysis been available at the time.

Seven years after this publication, Edelstein,[2] in the *Proceedings of the 2nd International Symposium on Legionella*, reported on the state-of-the-art laboratory diagnosis of Legionnaire's disease. He indicated that one of the major advances was made courtesy of improvements in media formulation, which allowed for improved recovery of *Legionella* from clinical samples and the recognition of new species and recognition of serogroups within the species *L pneumophila*. Among culture media advancements, the successive development of charcoal-yeast extract agar, buffered charcoal-yeast extract (BCYE) agar, and BCYE with α-keto-glutarate with antibiotic inhibitors made recovery of *L pneumophila* and other *Legionella* species possible—even from highly contaminated specimen types. Classical bacteriology played an important role in the first phases of having to identify a novel pathogen and develop methods for its rapid detection. The diversity of *Legionella* spp infection was also beginning to be appreciated at this time, especially extrapulmonary sites including systemic disease with concomitant positive blood cultures. However, in 1984, diagnosis still relied heavily on culture and, more importantly, serologic means or detection in respiratory secretions using direct or indirect immunofluorescent staining. Dr. Edelstein's astute recognition of the potential for urine as a source of soluble bacterial antigen testing generated speculation that "once commercially available, it may replace other immunologic means of diagnosis."[2]

Initially, both radio immunoassays and enzyme immunoassays were developed to detect soluble *L pneumophila* antigens in urine.[3] These were then converted to latex agglutination[4] and lateral flow immune-chromatographic assays[5] for ease of use, the latter of which are currently used today.

FIRST-GENERATION MOLECULAR TESTING SYSTEMS

The fledgling laboratory tool of polymerase chain reaction (PCR) amplification entered *Legionella* diagnostics in the early 1990s for detection of the genus in environmental sources[6] and directly from clinical (broncho-alveolar lavage [BAL]) samples.[7,8] Using the *mip* gene as a target, Jaulhac and colleagues[7] could detect *L pneumophila*