Diagnosis of *Legionella* Infection

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Legionellae, which are important causes of pneumonia in humans, continue to be incorrectly labeled as exotic pathogens. The ability to diagnose *Legionella* infection is limited by the nonspecific nature of clinical features and the shortcomings of diagnostic tests. Despite recent improvements, existing diagnostic tests for *Legionella* infection either lack sensitivity for detecting all clinically important legionellae or are unable to provide results within a clinically useful time frame. Understanding local *Legionella* epidemiology is important for making decisions about whether to test for *Legionella* infection and which diagnostic tests to use. In most situations, the use of both the urinary antigen test plus sputum culture is the best diagnostic combination. Polymerase chain reaction (PCR) is a promising tool, but standardized assays are not commercially available. Further work needs to focus on the development of urinary antigen tests assays that detect a wider range of pathogenic legionellae and on the development of standardized PCR assays.

Legionnaires disease continues to have the reputation of being an exotic infection. On the contrary, when systematically sought, *Legionella* species are consistently recognized as one of the common causes of pneumonia. Outside of the research setting, however, confirmed diagnoses of legionellosis are infrequent. This failure to diagnose legionnaires disease in routine practice is largely the result of 3 factors: the inability to clinically and radiographically distinguish legionnaires disease from other causes of pneumonia, the failure to order diagnostic tests for *Legionella* infection, and the shortcomings of available diagnostic tests. Legionnaires disease is more accurately described as an elusive diagnosis rather than an exotic infection.

Although diagnostic methods have improved during the 25 years since *Legionella pneumophila* was first described, no currently available test is able to diagnose legionnaires disease in a timely fashion with a high degree of sensitivity and specificity. Indeed, some authors have challenged the routine ordering of any microbiological tests for patients with pneumonia, supported by data suggesting that such tests do not significantly influence the choice of antibiotic therapy and patient outcome [1, 2]. Recently, Marrie [3] argued that this approach to the

diagnosis of legionnaires disease was wrong. Failure to test for *Legionella* infection may delay the recognition of outbreaks of legionnaires disease, may miss changes in the epidemiology of the disease (including the development of antimicrobial resistance), and may prevent persons from claiming compensation for occupationally acquired infection. Of importance, it is possible to select a subset of patients for whom the yield from diagnostic tests for *Legionella* infection is high and who would benefit from a specific diagnosis.

This review focuses on current diagnostic tests for *Legionella* infection, with a particular emphasis on the tests that provide a diagnosis in a time frame that will affect initial infection management. While reviewing the various diagnostic tests for *Legionella* infection, it is important to bear several factors in mind. First, specialized tests are required to diagnose *Legionella* infection, and these must be specifically requested by the clinician. Second, it is important to appreciate the distinction between the performance of a test in a research laboratory with what can be realistically achieved in a local diagnostic laboratory. Considerable interlaboratory variation has been documented for the ability to culture legionellae [4], and this is also likely to extend to other tests. Third, interpretation of the performance of diagnostic tests is hindered by the lack of a suitable "gold standard." Calculated sensitivity and specificity data will vary with different comparison standards. Fourth, the usefulness of diagnostic tests is influenced by local *Legionella* epidemiology. *L. pneumophila* serogroup 1 is the predominant cause of legionellosis in many, if not most, areas of the world,

and infection with this organism is easier to diagnose than is infection with other *Legionella* species and serogroups. In some regions, other species and serogroups are more important. For example, *Legionella longbeachae* is a major cause of legionellosis in Australia and New Zealand [5], where it is often associated with exposure to potting mix [6]. Infection with this species will not be detected by current urinary antigen tests and will be missed by laboratories that perform serological assays only for *L. pneumophila.*

CULTURE

Culture diagnosis requires special media, adequate processing of specimens, and technical expertise (table 1). Several days are required to obtain a positive result, with most *Legionella* colonies being detected within 3–5 days. The standard medium used to culture legionellae is buffered charcoal yeast extract (BCYE) agar supplemented with α-ketoglutarate, with or without antimicrobial agents. This medium provides iron and L-cysteine, both of which are essential for the growth of legionellae. Growth of some *Legionella* species (e.g., *Legionella micdadei* and *Legionella bozemanii*) is enhanced by supplementation of BCYE agar with bovine serum albumin [7], and addition of indicator dyes to the media may aid identification. Culture media containing cefamandole will inhibit the growth of *Legionella* species that do not produce β-lactamase, such as *L. micdadei* and *L. bozemanii* [8].

Legionellae can be isolated from a variety of sample types, although lower respiratory tract secretions (e.g., sputum and bronchoscopy samples) are the samples of choice. The major limitation of sputum culture is that fewer than one-half of patients with legionnaires disease produce sputum [9–12]. Other factors influence the sensitivity of culture once a sputum

sample is obtained. *Legionella* bacteria may survive poorly in respiratory secretions, and these samples should be processed promptly. Some patients with legionnaires disease produce sputum that has relatively little purulence; these samples may be rejected by laboratories that discard sputum samples containing few polymorphonuclear leukocytes [13]. Consequently, rejection criteria should not be applied to sputum samples sent for *Legionella* culture. The experience of laboratory staff is also important, and laboratories experienced at *Legionella* culture are more likely to recover the organism.

Estimated sensitivities of sputum culture range from <10% to ~80% and vary according to different comparison standards and by individual laboratories [9, 14–16]. In practice, the better results are likely to be achieved only by laboratories with a special interest in *Legionella* infection, and sensitivities are usually <50% when serological findings are used as the standard. Bronchoscopic samples are likely to produce a greater diagnostic yield than are expectorated sputum samples.

Legionella species can be isolated from blood cultures, but the yield is poor. Growth of legionellae is maintained by routine blood culture media but may not activate the alarm of commercial blood culture machines [17]. Consequently, blind subcultures onto solid media are required. Overall, the yield of blood cultures is low and is unlikely to influence clinical management.

DIRECT FLUORESCENT ANTIBODY (DFA) STAINING

DFA staining can detect legionellae in respiratory secretions and tissue samples. This technique has the advantage of providing a result within 2–4 h, but it is technically demanding and should be performed by experienced laboratory personnel.

Table 1. Diagnostic tests for *Legionella* infection.

Test	Turnaround time	Sample type	Sensitivity, %	Specificity, %	Comments
Culture	3–7 Days	LRT	<10–80	100	Detects all species and serogroups
		Blood	<10	100	Too insensitive for clinical use
Direct fluorescent antibody staining	<4 h	LRT	25–70	>95	Technically demanding
Antigen detection	<1 h	Urine	70–90	>99	Only reliable for detection of *Legionella pneumophila* serogroup 1
Serological testing	3–10 Weeks	Serum	60–80	>95	Must test both acute- and convalescent-phase serum samples; single titer results can be misleading
PCR	<4 h	LRT	80–100	>90	No commercially available assay for testing clinical samples; detects all species and serogroups
		Serum	30–50	>90	—
		Urine	46–86	>90	—

NOTE. LRT, lower respiratory tract.

Reported sensitivities of DFA staining vary, are consistently less than that of culture, and are less precisely known for species other than *L. pneumophila*. For lower respiratory tract secretions, sensitivities generally have a range of 25%–66%, with bronchoalveolar lavage fluid specimens having a higher yield than either transtracheal or sputum samples [15, 18, 19]. The specificity of DFA staining has been estimated to be ∼94% [18], although the test is likely to be less specific in inexperienced hands. False-positive results may occur because of cross-reactions with other bacteria, including *Bacteroides fragilis*, *Pseudomonas* species, *Stenotrophomonas* species, and *Flavobacterium* species. Cross-reactions may be less of a problem when monoclonal-antibody DFA agents are used. Problems with sensitivity and specificity have limited the use of DFA staining, and a positive DFA result in the absence of other supporting evidence is now generally not accepted as sufficient for the diagnosis of *Legionella* infection.

URINARY ANTIGEN DETECTION

Detection of soluble *Legionella* antigen in urine specimens is a rapid method that provides an early diagnosis of *Legionella* infection and has been a useful tool for the investigation of outbreaks of legionnaires disease [20]. Commercial kits that use both RIA and EIA methodologies have been available for several years and have similar performance characteristics. Recently, an immunochromatographic assay (NOW *Legionella* Urinary Antigen Test; Binax) has been developed that has similar sensitivity and specificity to EIA [21]. This test is easy to perform and can provide a result within 15 min.

For the detection of *L. pneumophila* serogroup 1, urinary antigen tests have sensitivities in the range of 70%–100% and specificities approaching 100% [22]. Sensitivities can be increased by as much as 20% by 25-fold concentration of urine samples before testing [23]. The major disadvantage with these tests is their inability to reliably detect organisms other than *L. pneumophila* serogroup 1. Broad-spectrum assays that detect soluble antigens from a wide variety of *Legionella* species have been developed but are not commercially available. The Biotest *Legionella* Urine Antigen EIA (Biotest) is intended to detect legionellae other than *L. pneumophila* serogroup 1, but it does so less reliably than it detects *L. pneumophila* serogroup 1 [24]. False-positive urinary antigen results have occurred in patients with serum sickness [25].

Legionella antigenuria can be detected as early as 1 day after onset of symptoms and persists for days to weeks. In one instance, excretion of antigen was documented to occur for >300 days [26]. Soluble *Legionella* antigens have also been detected in samples other than urine, including samples of sputum, lung tissue, serum, and pleural fluid, although the use of such samples has not been fully evaluated.

Urinary antigen testing is now an established and valuable tool for the diagnosis of legionnaires disease, particularly in regions where *L. pneumophila* serogroup 1 is the most common cause of the disease. In locations where only a minority of infections are caused by *L. pneumophila* serogroup 1, currently available urinary antigen assays contribute less to existing laboratory tests.

SEROLOGICAL TESTING

Serological testing for *Legionella* infection is a valuable epidemiological tool but has little impact on clinical decision making because of the time delay before a result is available. The antibodies produced in response to infection are generally a mixture of IgA, IgM, and IgG, and tests should detect all types for optimal sensitivity. The measurement of specific IgM is an unreliable marker of acute infection, because IgM antibodies can persist for long periods of time. Seroconversion may take several weeks, which is a major limitation of serological testing. In most cases, a 4-fold increase in antibody titer is detected within 3–4 weeks, but in some cases, this may take >10 weeks [27]. Obtainment of convalescent-phase serum samples too early undoubtedly results in many false-negative results and probably partially accounts for the 20%–30% of patients with legionnaires disease who supposedly do not develop a detectable antibody response [18]. However, it is clear that a proportion of people with proven *Legionella* infection do not have detectable seroconversion [15]. In practice, clinicians should be encouraged to obtain convalescent-phase serum samples 3 weeks after the onset of illness for testing in parallel with serum obtained during the acute phase. If there is no seroconversion after this time period and *Legionella* infection is still suspected, an additional convalescent-phase sample should be obtained.

Of the various antibody detection methods that are available, indirect immunofluorescence is the standard reference test. A 4-fold or greater increase in reciprocal antibody titer to ≥128 is considered diagnostic. Acute-phase reciprocal antibody titers of ≥256 in the presence of pneumonia were once considered sufficient for a presumptive diagnosis, but this has been shown to be unreliable [28], especially given the high prevalence of *Legionella* antibody positivity among some people without clinical evidence of legionellosis. There is virtually no role for testing single serum samples.

Another disadvantage of serological testing is the inability to accurately detect all *Legionella* species and serogroups. Although seroconversion to *L. pneumophila* serogroup 1 is generally regarded as being highly predictive of disease, the sensitivity and specificity of seroconversion to other species and serogroups has not been rigorously confirmed. Furthermore, cross-reactive antibody formation among members of the family Legionellaceae can make it difficult to determine the infecting species

or serogroup. Some patients with non–*L. pneumophila* serogroup 1 infection will have seroconversion to *L. pneumophila* serogroup 1 [29]. Conversely, sequence-based identification of *Legionella* PCR products from patients who have had seroconversion to *Legionella* species other than *L. pneumophila* indicate that some were likely to have been infected with *L. pneumophila* (unpublished observations). Cross-reactive antibodies are also occasionally found in patients with infections caused by non-*Legionella* bacteria, including pseudomonads, mycobacteria, *Bacteroides* species, and *Campylobacter* species. An interesting cross-reaction occurs between *L. bozemanii* and *Rickettsia typhi* as a result of a shared antigen [30].

NUCLEIC ACID AMPLIFICATION

Recently, DNA detection techniques have shown promise for the rapid diagnosis of *Legionella* infection. PCR enables specific amplification of minute amounts of *Legionella* DNA, provides results within a short time frame, and has the potential to detect infections caused by any *Legionella* species and serogroup. Currently, *Legionella* PCR is only available in a limited number of laboratories that use a variety of in-house assays.

PCR has been successfully used to detect *Legionella* DNA in a range of environmental and clinical samples. When testing samples from the lower respiratory tract, PCR has repeatedly been shown to have a sensitivity equal to or greater than culture [31–33]. Indeed, PCR could be considered the test of choice for patients who produce sputum. The role of PCR for testing other sample types is less clear. *Legionella* DNA can be detected in urine, serum, and leukocyte samples obtained from patients with legionnaires disease with sensitivities of 30%–86% [34–37]. The sensitivity of PCR is likely to increase when testing samples that are obtained early in the course of illness and when testing >1 sample type from each patient [34]. Throat swabs may also be a suitable sample for PCR testing, but this application has only been evaluated in a single study [38].

Further work is needed to establish a standard PCR method that will be robust enough to be used outside the setting of a research laboratory. Its application to nonrespiratory samples is particularly attractive, because this will circumvent the problem of patients who do not produce sputum samples. The development of an optimal PCR assay has been complicated by the intermittent contamination of some commercial DNA extraction kits with *Legionella* DNA [39]. Use of these kits to process samples may result in false-positive results and highlights the importance of including appropriate controls in each assay run.

TESTING STRATEGY

Because legionnaires disease cannot be clinically or radiographically distinguished from other causes of pneumonia, the de-

cision to test for *Legionella* infection can be difficult and is often made erratically. It is important to be familiar with local epidemiology. Some laboratories in areas where *Legionella* species are a common cause of pneumonia have elected to routinely culture all sputum samples obtained from patients with pneumonia on *Legionella* media. Few laboratories, however, would be able to justify the additional cost associated with routine *Legionella* culture. In most locations, the incidence of *Legionella* infection is unknown, and the decision to order diagnostic tests for *Legionella* infection is usually limited to at-risk patients, to patients with severe pneumonia, and to outbreak scenarios. It is certainly possible to select a subset of patients for whom the yield from *Legionella* diagnostic tests is likely to be relatively high. This group includes elderly persons, smokers, immunosuppressed individuals, those with chronic lung disease, patients who reside in hospitals with *Legionella*-colonized water supplies, and individuals exposed to potting mix. The incidence of legionnaires disease is higher among patients with severe pneumonia, and all patients with pneumonia who are admitted to an intensive care unit should be tested for this infection.

In locations where *L. pneumophila* serogroup 1 is the predominant cause of *Legionella* infection, or during an outbreak of infection with *L. pneumophila* serogroup 1, the urinary antigen test is a particularly valuable diagnostic tool. The development of a commercial urinary antigen test that also reliably detects other human *Legionella* pathogens would be a major advance and would likely make this the diagnostic test of choice in almost all settings. In geographic locations where legionellae other than *L. pneumophila* serogroup 1 are numerically important pathogens, current urinary antigen tests are still useful but should not be used as the sole diagnostic tool. If available, *Legionella* PCR combined with urinary antigen testing is likely to be the best initial testing strategy that will detect all *Legionella* species and provide results within a time frame that will affect clinical management.

Where PCR is unavailable, the urinary antigen test combined with culture of lower respiratory tract samples is the best test combination. Culture remains an important diagnostic tool, but its relatively low sensitivity and the reliance on the availability of a lower respiratory tract sample make it inadequate as a sole diagnostic test. Although serological testing has no impact on initial management, it can be useful if a specific diagnosis is not made during the acute phase of infection, but both acute- and convalescent-phase samples must be tested in parallel. During a suspected outbreak of legionnaires disease, an aggressive approach to diagnosis is warranted, which would involve the use of a combination of testing methods.

Legionella species may occasionally cause Pontiac fever, an acute, febrile, nonpneumonic illness characterized by a high attack rate, short incubation period, and rapid recovery. The

diagnosis of Pontiac fever usually relies on the recognition of typical clinical features during an outbreak situation, and the diagnosis is confirmed by serological testing of affected persons. The use of culture is largely limited to testing environmental samples to determine the source of the outbreak, although *L. pneumophila* serogroup 1 has been isolated from a tracheal aspirate from a child with Pontiac fever [40].

CONCLUSIONS

Legionella infection is undoubtedly underrecognized. Diagnosis relies on the use of specialized tests, often in combination. Urinary antigen tests, sputum culture, and PCR testing of lower respiratory tract samples are the most important diagnostic tools for detection of *Legionella* infection early in the course of illness. The development of urinary antigen test assays that detect a wider range of pathogenic legionellae and the development of standardized PCR assays will be major advances in *Legionella* diagnostics. The increased availability and use of improved diagnostic tests will help better characterize the epidemiology of legionnaires disease, including the true incidence and geographic variation.

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