

Utility of PCR, Culture, and Antigen Detection Methods for Diagnosis of Legionellosis

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The goal of this retrospective study was to evaluate the performance of different diagnostic tests for Legionnaires' disease in a clinical setting where *Legionella pneumophila* PCR had been introduced. Electronic medical records at the Cleveland Clinic were searched for *Legionella* urinary antigen (UAG), culture, and PCR tests ordered from March 2010 through December 2013. For cases where two or more test methods were performed and at least one was positive, the medical record was reviewed for relevant clinical and epidemiologic factors. Excluding repeat testing on a given patient, 19,912 tests were ordered (12,569 UAG, 3,747 cultures, and 3,596 PCR) with 378 positive results. The positivity rate for each method was 0.4% for culture, 0.8% for PCR, and 2.7% for UAG. For 37 patients, at least two test methods were performed with at least one positive result: 10 (27%) cases were positive by all three methods, 16 (43%) were positive by two methods, and 11 (30%) were positive by one method only. For the 32 patients with medical records available, clinical presentation was consistent with proven or probable *Legionella* infection in 84% of the cases. For those cases, the sensitivities of culture, PCR, and UAG were 50%, 92%, and 96%, respectively. The specificities were 100% for culture and 99.9% for PCR and UAG.

Legionnaires' disease is an atypical, respiratory illness associated with exposure to water colonized with *Legionella* species (1). In the United States, up to 18,000 hospitalizations occur each year for legionellosis, with the vast majority (70% to 92%) attributed to *Legionella pneumophila* serogroup 1 (Lp1) (2). This predominance of Lp1 disease is thought to be a reflection of virulence rather than environmental distribution (3–5). Besides Lp1, the strains most commonly associated with human disease are other *L. pneumophila* serogroups, *Legionella micdadei*, *Legionella bozemanii*, and *Legionella longbeachae* (2, 6). Risk factors for legionellosis include whirlpool spa exposure, recent overnight travel or plumbing repairs (two weeks prior to onset of symptoms), immunosuppression, alcoholism, diabetes, malignancy, hepatic or renal failure, chronic obstructive lung disease, smoking history, and patient age of >50 years (7). Patients with Legionnaires' disease often require intensive care unit (ICU) admission, have failed outpatient antimicrobial treatment, or may meet criteria for nosocomial pneumonia (8).

The urinary antigen (UAG) test is commonly used to diagnose Legionnaires' disease because sputum production is limited and *Legionella* culture requires special techniques. The Lp1 antigen is typically detectable in urine beginning 2 to 3 days after the onset of clinical symptoms until 2 months after clearance of disease but may persist for a much longer period of time (2). The widespread availability of rapid, FDA-cleared, Lp1 UAG tests in the United States coincided with a 76% decrease in mortality rate (34% to 8%) from Legionnaires' disease during 1985 to 2009 (9, 10). However, it has been suggested that non-Lp1 Legionnaires' disease is being missed due to an overreliance on UAG testing (2, 9, 10).

The purpose of this retrospective study was to compare the yield of different *Legionella* diagnostic methods in a clinical setting where a laboratory-developed *L. pneumophila* nucleic acid amplification test (NAAT) was added to the test menu. For patients within the health care system, the PCR order was only available with culture to ensure *Legionella* species other than *pneumophila* would not be missed. The utility of this approach and demographic, clinical, and epidemiologic factors were assessed.

(This study was presented in part at IDWeek 2014, Philadelphia, PA, 8 to 12 October, 2014 [11].)

MATERIALS AND METHODS

After approval was granted by the Cleveland Clinic institutional review board, the laboratory database at the Cleveland Clinic was retrospectively searched for *Legionella* UAG, culture, and PCR tests ordered from March 2010 through December 2013. Detection of UAG was performed using the Lp1-specific Binax *Legionella* urinary antigen enzyme immunoassay (Alere) according to the manufacturer's recommendations. Details of the laboratory-developed, real-time PCR targeting the *mip* gene for *L. pneumophila* were published previously (12).

For *Legionella* culture, specimens from nonsterile sites were diluted 1:10 with 0.2 M acid (KCl) buffer (pH 2.2), vortexed, and digested for 5 min prior to inoculation of 0.1 ml onto buffered charcoal yeast extract agar with α -ketoglutarate (BCYE). If the volume of bronchoalveolar lavage specimens was ≥ 10 ml, a centrifugation step ($2,013 \times g$ for 15 min) was employed to concentrate the specimen prior to acid treatment and plating on BCYE. Specimens from normally sterile sites were inoculated to BCYE without acid treatment. Plates were parafilmed to ensure a moist environment and incubated in ambient air at 35°C for 7 days. Gram-negative bacilli recovered on BCYE with no growth after subculture to blood agar were identified further using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry or *L. pneumophila* PCR. Isolates that were not identified using these methods were

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TABLE 1 Overview of *Legionella* test orders and results during the study period

Test	No. of tests ordered		No. (%) of unique results	
	Total	Unique ^a	Positive	Negative
Antigen	14,539	12,569	336 (2.7)	12,233 (97.3)
Culture	7,243	3,747	15 (0.4)	3,732 (99.6)
PCR	7,089	3,596	27 (0.8)	3,569 (99.2)
Total	28,871	19,912	378 (1.9)	19,534 (98.1)

^a A total of 3,104 of the unique culture and PCR requests were ordered together.

sent to the Ohio Department of Public Health Laboratory or a reference laboratory for further identification.

When at least two tests were performed on a single patient and there was at least one positive result, the electronic medical record was reviewed for relevant clinical and epidemiologic factors that have been associated with Legionnaires' disease. Patient management and clinical course were also assessed. Because the purpose of the study was to compare diagnostic methods, charts of patients with only one test method performed were not reviewed.

The likelihood of active *Legionella* infection was assigned based on modified criteria from Dionne et al. (13). *Legionella* infection was considered proven if the clinical and radiographic picture was compatible with pneumonia and two of the laboratory test methods had positive results. Probable cases of *Legionella* infection were those that had only one laboratory test method with a positive result in the setting of clinical and radiographic pneumonia and the presence of risk factors for *Legionella* pneumonia. Possible cases were those that had only one laboratory test method with a positive result in the setting of clinical and radiographic pneumonia, had no other infectious etiology identified, and lacked risk factors for *Legionella* pneumonia. Unlikely cases were those that had only one laboratory test method with a positive result in the setting of clinical pneumonia, and there was evidence suggesting an infectious etiology other than *Legionella*.

RESULTS

A total of 28,871 tests were ordered during the 4-year study period (Table 1). Excluding repeat testing on a given patient, 19,912 unique tests were ordered (12,569 UAG, 3,747 cultures, and 3,596 PCR). There were 378 unique positive results, 336 UAG, 15 cultures, and 27 PCR, with positivity rates of 2.7%, 0.4%, and 0.8%, respectively.

There were 37 cases where at least two different test methods were ordered on a single patient and at least one method yielded a positive result (Table 2). For these 37 cases, 10 (27%) cases were positive by all three methods, 12 (32%) were positive by UAG and PCR only (9 included a negative culture), 9 (24%) cases were positive by UAG only (7 included negative culture and 6 included negative PCR), 3 (8%) cases were positive by culture and PCR only (1 included negative UAG), 2 (5%) cases were positive by PCR only (each with negative culture, UAG not performed, and medical records unavailable for review), and 1 (3%) case was positive by UAG and culture only (PCR not performed).

Median and mean patient ages were 60 and 59 years, respectively (range 35 to 76 years old), and most were males (65%). All 37 patients were from the United States with 34 (92%) from Ohio and one patient each from Florida, Maryland, and West Virginia. Five patients did not have medical records available for further chart review. Clinical information for the 32 patients where sufficient data were available is summarized in Table S1 in the supplemental material. Eight (25%) of those patients were immunocom-

TABLE 2 Patterns for 37 patients with ≥ 2 methods and at least one positive result

No. (%) of cases	Test result ^a for:		
	Urine antigen	Culture	PCR
10 (27)	+	+	+
9 (24)	+	—	+
4 (11)	+	—	—
1 (3)	—	+	+
3 (8) ^b	+	—	NP ^c
1 (3) ^d	+	+	NP
3 (8)	+	NP	+
2 (5)	+	NP	—
2 (5) ^e	NP	+	+
2 (5) ^d	NP	—	+

^a +, Positive result; —, negative result.

^b Only 32 patient medical records were available for review. One of these 3 was not available.

^c NP, not performed.

^d Patient records not available for review.

^e One of these 2 patient records was not available for review.

promised. Initial presentation included pneumonia in 31 (97%) of the 32 patients, diarrhea in 14 patients (44%), and hyponatremia in 5 patients (16%). Additional findings included septic shock, acute kidney injury, and altered mental status. All 32 cases were believed to be community acquired, and none had a recent history of travel. Four patients (13%) had a history of water or soil exposure, including pool-water aspiration, conference room air conditioning, and landscaping. Multiple, broad-spectrum antimicrobial agents were used for initial therapy in all 32 cases. After laboratory test results were available, 23 patients (72%) were switched to a single antimicrobial agent (a fluoroquinolone or macrolide). Nineteen patients (59%) were intubated, and 11 (34%) required pressors during the course of their treatment. Three patients (9%) expired despite aggressive management; two were receiving broad-spectrum antibiotics, and one had antibiotic treatment narrowed to levofloxacin alone.

The majority (75%) of cases were categorized as proven *Legionella* infections. The remaining 8 (25%) cases were categorized as probable ($n = 3$), possible ($n = 4$), and unlikely ($n = 1$). The unlikely case had a positive UAG test but negative culture and PCR for *Legionella*; respiratory cultures grew methicillin-resistant *Staphylococcus aureus* (MRSA).

If defining only proven cases of *Legionella* infection as true positives, the sensitivities were 96% for UAG (22 positive UAG tests of 23 cases where UAG was performed and the medical record was available for review), 57% (12 of 21) for culture, and 100% (24 of 24) for PCR. If probable and proven cases are considered true positives, the sensitivities would be 96% for UAG (25 of 26), 50% (12 of 24) for culture, and 92% (24 of 26) for PCR, while the specificities would be 99.9% for PCR and UAG and 100% for culture. If a true positive is defined as confirmation with a second method, the sensitivities of UAG, culture, and PCR were 96%, 61%, and 100%, respectively.

DISCUSSION

This retrospective study showed variable ordering patterns and yield for the three types of *Legionella* tests during the 46-month study period. The UAG test, ordered twice as frequently as PCR and culture, had the highest positivity rate (2.7%) and the lowest

cost to diagnose one case (Medicare reimbursement of $\$16.32 \times 14,539$ tests/336 unique positives = $\$706.18$). This was a much higher UAG positivity rate than that reported in a 1998 to 2000 Canadian study of 0.6% where the cost (based on material and supplies only) of diagnosing a case by UAG was approximately $\$5,770$ ($\$30 \times 1,154$ tests/6 unique positives) (13).

The reported sensitivity of the UAG test for Legionnaires' disease caused by Lp1 is 70% to 90% (8, 14). The sensitivity of UAG in the current study was higher (96%), but the actual clinical performance depends on the context of testing. Variable sensitivity of the UAG test has been reported for travel-associated, community-acquired, and nosocomial infections (93.7%, 86.5%, and 44.2%, respectively) (15). In our cohort of community-acquired pneumonias, the UAG test was positive in all proven, probable, and possible *Legionella* infections except for one case. The *L. pneumophila* in this case may have been a serogroup other than 1 since culture and PCR were positive, and the reported sensitivity of the UAG test for non-Lp1 is low (less than 50%) (1, 15).

While UAG testing is convenient and specific, culture is recommended to ensure detection of serogroups other than Lp1 and species other than *L. pneumophila* (2) as well as dual *Legionella* infections (16). In the current study, the utility of that approach was not confirmed since no species other than *L. pneumophila* were isolated from the 7,243 cultures performed. It is difficult to determine if there were *Legionella* isolates missed by culture due to technical issues or if there was simply an absence of non-*pneumophila* species causing disease among this cohort of patients. *Legionella feeleyi* was recovered from the bronchoalveolar lavage of a bone marrow transplant patient with pulmonary infiltrates 1 year after this study period, but the infectious disease physician caring for the patient concluded the isolate was not clinically significant. There have been 14 reports of *L. feeleyi* as the etiologic agent of pneumonia since 1984 (17).

Culture demonstrated the lowest sensitivity of the three methods compared in our study and did not contribute any unique results that were not already provided by the UAG or PCR tests (i.e., 14 of the 15 positive culture results had PCR and/or UAG ordered that was also positive). Based on Medicare reimbursement of $\$9.02$, the cost to recover each positive culture was $\$4,355$. The sensitivity of culture for the current study (50% to 61%) falls near the middle of the 20% to 80% range reported by others (18).

Disadvantages of culture include technical difficulty with requirement of selective agars and pretreatment (heat or acid) and slow turnaround time. Careful examination of plates with a dissecting microscope is required. Selective BCYE may inhibit the recovery of *Legionella micdadei* or other species due to antimicrobial agents in the medium, while nonselective BCYE can be overgrown with bacteria that may obscure *Legionella* (1). Inoculation of respiratory specimens to nonselective BCYE only after a 5-min acid treatment may have reduced recovery of *Legionella* in the current study. Alternative approaches to culture include inoculating selective and nonselective BCYE media, plating specimens before and after acid treatment, only performing acid treatment if selective and nonselective BCYE plates are overgrown with bacteria other than *Legionella* after overnight incubation, and acid digestion for a shorter time period (e.g., 4 min) (1).

Legionella detection by PCR provides a more rapid turnaround time and a higher sensitivity than culture but at the highest cost. With Medicare reimbursement of $\$47.76$, the cost of a positive case was $\$12,540$ ($\$338,571/27$). At present, there are no FDA-

approved *Legionella* PCR assays available in the United States. The *mip* PCR assay utilized in the present study was 100% sensitive and 100% specific for *L. pneumophila*, including non-serogroup 1 isolates in a previously published evaluation conducted at our institution (12). The same 100% sensitivity was reported by two studies (19, 20), with specificities of 93% and 100% for a *Legionella* spp. PCR performed on bronchoalveolar lavage specimens. However, culture was observed to perform better than PCR on tissue (20). A retrospective study conducted in the Netherlands reported 92% sensitivity and 98% specificity for a PCR targeting the *mip* gene (21).

For the current study, there was good correlation between the UAG test and PCR. There were seven cases of either probable or possible *Legionella* infection where the UAG test was positive while culture and/or PCR were negative. This discrepancy between results may be attributable to the difference in specimen types, as an inadequate respiratory specimen would lead to negative results by culture and PCR. Unfortunately patient records were not available for the two cases with positive PCR and negative culture results (antigen not performed).

When considering all 32 cases where clinical data were available, *Legionella* diagnostic testing resulted in a change from multiagent broad-spectrum antibiotics to single-agent therapy in 72% of cases, reducing potential adverse effects, decreasing cost, and minimizing the selection for antibiotic-resistant organisms. Although the UAG assay may remain positive for up to a year after treatment, there are reports of PCR becoming negative soon after initiation of therapy (22, 23), making the latter helpful if the significance of a positive antigen result is unclear (see second patient in Table S1 in the supplemental material).

Although a large number of tests were ordered over the 46-month study period, only a small fraction of patients had two or more test methods performed where at least one test result was positive. There were 304 positive UAG test results where culture and PCR were not performed and, consequently, comparisons among tests were not made. The predominance of UAG tests reflects current Infectious Diseases Society of America (IDSA) practice guidelines for community-acquired pneumonia that recommends antigen testing as the primary diagnostic test for patients with risk factors for *Legionella* (8). However, compliance with IDSA guidance for culture follow-up to positive UAG tests was low in the current study.

Another limitation of the present study was that most of the samples came from Ohio patients, and all samples were from the United States. Theoretically, culture might show greater utility in other geographic regions where the prevalence of Lp1 is lower. However, in New Zealand where *L. longbeachae* represents 85% of *Legionella* spp. causing pneumonia, only 46% of specimens positive by an NAAT/probe assay optimized for *L. longbeachae* yielded isolates by culture (24).

In conclusion, our findings illustrate the difficulty of assessing the utility of diagnostic tests for a low prevalence disease. The UAG test was the most commonly used test with the highest sensitivity and lowest cost, but performance may be improved further by coverage of more *Legionella* serogroups and species (25). Although culture is recommended for detecting strains that would be missed by an Lp1-specific antigen and an *L. pneumophila* PCR, it is technically demanding with low sensitivity. The PCR was more sensitive than culture but was five times the cost. FDA-cleared NAATs targeting all *Legionella* spp. (alone or as part of

multiplex assays for other respiratory pathogens) are needed to replace culture. The current trend of increasing laboratory consolidation with delays in specimen processing may make recovery of *Legionella* by culture even more difficult. Although epidemiologic investigations are enhanced with the recovery of clinical and environmental isolates for strain typing, whole-genome sequencing may supplant culture-based techniques in the near future.

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