Clinical utility of a *Legionella pneumophila* urinary antigen test in a large university teaching hospital

Michel Dionne MD^{1,2}, Todd Hatchette MD, FRCPC^{3,4,5}, Kevin Forward MD, FRCPC^{1,3,4,5}

M Dionne, T Hatchette, K Forward. Clinical utility of a *Legionella pneumophila* urinary antigen test in a large university teaching hospital. Can J Infect Dis 2003;14(2):85-88.

OBJECTIVE: To determine the clinical utility of diagnosing Legionella pneumonia by urinary antigen testing (LPUAT) in a low prevalence centre.

DESIGN: The results of LPUATs were abstracted and analyzed from the authors' laboratory information system. Medical records were reviewed in detail for all positive tests and a random sample of 50 negative tests.

SETTING: The Queen Elizabeth II Health Sciences Centre, a large university hospital complex.

POPULATION STUDIED: Patients who were admitted from the emergency room with pneumonia or who had developed pneumonia in hospital and who had a LPUAT performed between April 1998 and October 2000.

MAIN RESULTS: One thousand one hundred fifty-four tests were performed on 1007 patients. Seven patients had nine positive LPUATs. Three of these patients had confirmed *Legionella pneumophila* pneumonia. Three others had probable or possible *L. pneumophila* pneumonia. There was one probable false positive. Six of the seven patients were already on empirical anti-*L. pneumophila* therapy. Of the 50 negative tests reviewed in detail, 31 patients were on one of the antibiotics of choice for *L. pneumophila* at the time the test was ordered; in 21 (68%) of these patients the negative result did not lead to a change in therapy.

CONCLUSIONS: The cost to diagnose each case of Legionella pneumonia by LPUAT was approximately \$5,770 and most patients were already on appropriate antibiotics. In patients with negative tests, antibiotics were often not changed in response to the test result. Rigorous screening of patients is required to increase pretest probability for LPUAT to be justified.

Key Words: Clinical utility; *Legionella species*; Urinary antigen testing

L'utilité clinique d'un examen des antigènes urinaires au *Legionella pneumophila* dans un grand hôpital universitaire

OBJECTIF : Déterminer l'utilité clinique de diagnostiquer un *Legionella pneumophila* par examen des antigènes urinaires (EAULP) dans un centre à faible prévalence

MÉTHODOLOGIE : Les résultats des EAULP ont été abstraits et analysés à partir du système d'information de laboratoire des auteurs. Les dossiers médicaux ont été examinés en détail afin de déceler tous les examens positifs et d'obtenir un échantillon aléatoire de 50 examens négatifs.

LIEU : Queen Elizabeth II Health Sciences Center, un grand complexe universitaire en milieu hospitalier.

POPULATION ÉTUDIÉE : Patients hospitalisés par l'urgence en raison d'une pneumonie ou qui avaient développé une pneumonie nosocomiale et qui avaient subi un EAULP entre avril 1998 et octobre 2000.

PRINCIPAUX RÉSULTATS : Mille cent quarante-quatre examens ont été effectués auprès de 1 007 patients. Sept patients présentaient neuf EAULP positifs. Trois de ces patients présentaient une pneumonie à *L. pneumophila* confirmée. Trois autres en avaient une probable ou possible. Un faux cas positif était probable. Six des sept patients prenaient déjà un traitement empirique contre le *L. pneumophila*. Sur les 50 examens négatifs ayant subi une évaluation approfondie, 31 patients prenaient l'un des antibiotiques de choix contre le *L. pneumophila* au moment de l'examen, et chez 21 (68 %) de ces patients, le résultat négatif n'a pas donné lieu à une modification du traitement.

CONCLUSION : Les coûts reliés au diagnostic de chaque cas de pneumonie à *Legionella* par EAULP étaient d'environ 5 770 \$, et la plupart des patients prenaient déjà les bons antibiotiques. La plupart du temps, chez les patients dont le résultat de l'examen était négatif, le type d'antibiotique n'était pas modifié à la réception des résultats de l'examen. Un dépistage rigoureux des patients s'impose pour accroître la probabilité que les examens préliminaires d'EAULP soient justifiés.

Legionella pneumophila is responsible for 1% to 4% of cases of community-acquired pneumonia requiring hospitalization (1). Although the clinical presentation and radiographic appearance may be suggestive of Legionella pneumonia, clinicians are not able to differentiate it from other bacterial causes of pneumonia on clinical grounds (1). In theory, early diagnosis of a suspected Legionella infection would permit appropriate antibiotic therapy in a timely fashion. In practice,

guidelines for the treatment of community-acquired pneumonia recommend the empirical use of macrolides and fluoroquinolones, which are the drugs of choice for *L. pneumophila* infections (2-4).

Several methods exist for diagnosing Legionella infection. Sputum culture is favoured by many because it has the ability to detect all species and subgroups of *Legionella* and has a specificity of 100% (1). High specificity is especially advantageous

¹Department of Pathology, Dalhousie University, Halifax; ²Division of Anatomical Pathology, Queen Elizabeth II Health Sciences Centre, Halifax;

³Department of Medicine, Dalhousie University, Halifax; ⁴Division of Microbiology, Queen Elizabeth II Health Sciences Centre, Halifax; and

⁵Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia

Correspondence: Dr Michel Dionne, Division of Anatomical Pathology, Queen Elizabeth II Health Sciences Centre, 5788 University Avenue, Halifax, Nova Scotia B3H 1V8. Telephone 902-473-7706, fax 902-473-7978, e-mail michel.dionne@cdha.nshealth.ca

Received for publication November 30, 2001. Accepted March 21, 2002

Figure 1) Patients with positive urinary antigen tests

Figure 2) Patients with negative urinary antigen tests. CXR Chest x-ray

for low prevalence diseases. However, the sensitivity is approximately 70% (1), and positive results typically take three to five days. Serological diagnosis is limited due to the time delay for seroconversion (5). Direct fluorescent antibody (DFA) testing has the ability to provide results in a time frame able to influence clinical management and has a specificity of close to 100% (5). However, DFA is technically demanding and insensitive. As with sputum culture, DFA has limited usefulness when patients cannot produce sputum.

L pneumophila by urinary antigen testing (LPUAT) is a rapid tool for early diagnosis of Legionella infection (6-14). An enzyme immunoassay (EIA) for detecting *L pneumophila* serogroup 1, which accounts for between 50% and 70% of cases of Legionella pneumonia (15,16), has been shown to be both sensitive and specific. In addition, some studies have shown that the test is capable of cross-reacting with several *L pneumophila* serogroups and even other *Legionella* species (6,9). Specificity is typically more than 99% (7-9).

Since the authors had been using a urinary antigen assay since 1998, they wished to determine how many additional cases of *L pneumophila* pneumonia had been diagnosed and whether negative test results influenced a change in the antimicrobial agent used or the duration of the treatment.

METHODS

The results of the Binax Legionella Urinary Antigen EIA tests (Binax Incorporated, USA), *L pneumophila* cultures and antibody studies, which were performed at the Queen Elizabeth II Health Sciences Centre between April 1998 and October 2000, were abstracted using the authors' laboratory information system. To determine the extent to which patient care was influenced by the results of the EIAs and whether negative test results led to appropriate changes in management, the charts of all patients who tested positive were reviewed, as well as a randomly chosen sample of 50 cases in which the patients tested negative. The selection was done by choosing the cases of every 21st patient on an alphabetically ordered list of those who had the test during the study period.

The Binax Legionella Urinary Antigen EIA 96 well test (Binax Incorporated, USA) was performed in accordance with the manufacturer's instructions. In brief, patient urine was added to microtitre wells coated with polyclonal rabbit antibody specific for *L pneumophila* serogroup 1 antigen. Captured antigen was detected with anti-Legionella horseradish peroxidase labelled conjugate. Unbound antigen and conjugate were decanted and the wells were washed. A colour developer was added and absorbance was read on a microplate reader. Samples with absorbencies greater than three times the negative controls were considered positive.

Respiratory secretions submitted for *Legionella* species cultures were planted onto buffer charcoal yeast extract agar and buffer

charcoal yeast extract agar with vancomycin and polymixin B. Plates were incubated at 37°C in carbon dioxide and examined daily for seven days. Organisms with colony morphology, Gram stain characteristics and that did not grow on sheep blood agar were further identified by DFA staining using *L pneumophila* group 1 to 6 antisera.

The Legionella indirect fluorescence antibody testing was performed by the Ministry of Health laboratory in Toronto, Ontario. A fourfold rise or a rise in titre to greater than 1:256 from the acute specimen in an acute-convalescent pair was considered evidence of recent infection.

Definitions

Risk factors for *L pneumophila* pneumonia: Transplant recipient; taking systemic steroids or cytotoxic chemotherapy; diagnosed with chronic obstructive pulmonary disease (COPD), end stage renal disease, cancer, diabetes mellitus or AIDS; and active cigarette smoker.

Proven case of *L pneumophila* pneumonia: Clinical picture compatible with pneumonia including appropriate radiographic changes and a positive culture for *L pneumophila* or a fourfold rise in convalescent anti-*L pneumophila* antibody.

Probable case of *L pneumophila* pneumonia: Clinical picture compatible with pneumonia including appropriate radiographic changes, presence of risk factors other than smoking for *L pneumophila* pneumonia, and no laboratory evidence of other infectious etiologies.

Possible case of *L pneumophila* pneumonia: Clinical picture compatible with pneumonia including appropriate radiographic changes and no laboratory evidence of other infectious etiologies.

RESULTS

One thousand one hundred fifty-four LPUATs were performed on 1007 patients during the study period. Seven patients had nine positive tests. Their breakdown into proven, probable or possible cases of *L pneumophila* pneumonia and whether they were already on anti-Legionella therapy at the time of the test result is shown in Figure 1. Of the seven patients, six were already on anti-Legionella therapy before the test result was available.

Information relating to the LPUAT's influence on therapy in the 50 cases with negative test results is shown in Figure 2. Of the 31 cases in which the patients were taking anti-Legionella medication, 21 (68%) had no change in therapy with the negative test result. It is of note that 28% of those with negative LPUATs had no evidence of pneumonia on chest radiograph when subsequently read by a radiologist. Thirty-six per cent of patients with negative LPUATs had no

TABLE 1
Patient demographics, presence or absence of recognized risk factors and final chest radiographic reports

	Positive LPUATs	Negative LPUATs
Number	7	50*
Median age (range)	64 (40-77)	70 (33-90)
Men	5/7 (71%)	17/47 (36%)
Suspected cases of community-acquired pneumonia [†]	5/7 (71%)	38/50 (76%)
Presence of risk factors for Legionella pneumonia	4/7 (57%)	32/50 (64%)
CXR suggestive of pneumonia	6/7 (86%)	36/50 (72%)

*Forty-seven patients, 50 pneumonia episodes; [†]The remaining cases were hospital acquired. LPUAT Legionella pneumophila urinary antigen test

recognized risk factors for *L pneumophila* pneumonia. Data on patient demographics, presence of recognized risk factors for *L pneumophila* pneumonia and final chest radiographic reports is listed in Table 1.

Details of the cases with positive LPUATs

Proven positive cases:

Case 1: A 53-year-old male smoker with COPD presented with symptoms and signs of community-acquired pneumonia. Bloodwork showed a slightly increased white blood cell (WBC) count and a sodium level of 125 mmol/L. A chest radiograph revealed bronchopneumonia. He was started on cefuroxime and erythromycin therapy. An LPUAT and Legionella sputum culture were performed on the second day. The culture grew *L pneumophila* on day 4 and the LPUAT was positive. Cefuroxime was discontinued on day 5. A repeat LPUAT was also positive. Serology was not performed.

Case 2: A 40-year-old male smoker presented with symptoms and signs of severe community-acquired pneumonia. Bloodwork showed a normal WBC count but a left shift and a sodium level of 130 mmol/L. A chest radiograph revealed multilobar pneumonia. He was initially treated with levofloxacin, which was later changed to ciprofloxacin to cover a second organism. An LPUAT ordered on day 1 was positive. *L pneumophila* sputum culture and a repeat LPUAT ordered on days 7 and 8, respectively, were both negative, but acute and convalescent antibody titres revealed a fourfold rise.

Case 3: A 71-year-old male smoker with COPD and ischemic heart disease developed right-sided airspace disease evident on a chest radiograph the day after an emergent coronary artery bypass graft. He was started on ceftriaxone therapy for nosocomial pneumonia. A chest radiograph on postoperative day 3 showed progression of the pneumonia and the patient required reintubation. A positive LPUAT on the sixth postoperative day resulted in a discontinuation of the ceftriaxone. Treatment with erythromycin, ciprofloxacin and rifampin was started. Sputum samples collected on the sixth and seventh postoperative day to confirm the LPUAT result grew *L pneumophila*. Serology was not performed.

Probable positive cases:

Case 4: A 64-year-old male heart transplant recipient with COPD presented with symptoms and signs of pneumonia. He had been discharged from hospital three days before, after a two-day admission for flu-like symptoms. Bloodwork showed an increased WBC count ($30 \times 10^9/L$) and a chest radiograph

revealed multifocal airspace disease. Treatment with levofloxacin was started. An LPUAT was positive, but three sputum samples for Legionella culture collected on days 4 and 5 were negative. Serology was not performed. Sputum culture for respiratory viruses was negative. He improved and was discharged after 17 days. Four weeks later he was readmitted with right upper lobe pneumonia and was started on levofloxacin. An LPUAT was positive, but *Streptococcus pneumoniae* susceptible to levofloxacin was isolated from a blood culture. The levofloxacin was continued. The patient improved and was discharged after five days of hospitalization.

Possible positive cases:

Case 5: A 49-year-old male cigar smoker with no risk factors for Legionella pneumonia was started on oral cefuroxime therapy for a right lower lobe community-acquired pneumonia two days before admission. He was admitted with worsening symptoms. Bloodwork showed an elevated WBC count ($16 \times 10^9/L$) and a sodium level of 130 mmol/L. A chest radiograph was not repeated. He was started on intravenous erythromycin and cefuroxime. An LPUAT ordered on admission was positive. The cefuroxime was discontinued and the dose of erythromycin was increased. He recovered quickly and was discharged after four days. No other tests for Legionella were ordered.

Case 6: A 67-year-old female smoker with no other risk factors for Legionella pneumonia developed a left upper lobe segmental nosocomial pneumonia during a hospital stay for psychiatric illness. She was started on levofloxacin, which was then changed to azithromycin on the following day. An LPUAT was positive. She remained on azithromycin and recovered. No other tests for Legionella were ordered.

Probable false positive case:

Case 7: A 77-year-old woman with type 2 diabetes mellitus was admitted to a community hospital and treated for congestive heart failure and a possible community-acquired pneumonia with cefuroxime and azithromycin. She developed acute renal failure and was transferred to the Queen Elizabeth II Health Sciences Center on day 5. A chest radiograph performed on arrival revealed congestive cardiac failure, but no evidence of pneumonia. The patient was afebrile and her serum WBC count was normal. Antibiotics were discontinued. An LPUAT was ordered on day 7. The LPUAT was positive but it was felt that this was a false positive. The patient improved without antibiotic treatment. A repeat LPUAT performed three weeks later was negative. Serology was performed 24 days after her admission to the community hospital and was also negative. Cultures for Legionella were not performed.

DISCUSSION

Although certain risk factors, clinical features and laboratory abnormalities may suggest a diagnosis of *L pneumophila* pneumonia, clinical differentiation from common bacterial pneumonias is usually not possible (17,18). The diagnosis of *L pneumophila* pneumonia is often not considered and, in one large study, it was suggested that only 3% of sporadic cases of Legionnaires' disease are correctly diagnosed (19). In some centres, *L pneumophila* is second only to *S pneumoniae* as a cause of community-acquired pneumonia requiring admission to a critical care unit (17,20). A prompt diagnosis by the detection of *L pneumophila* antigen in urine would, therefore, seem highly desirable in view of its sensitivity and specificity by immunoassay. Legionella antigen testing can be rapidly performed on urine, is usually detectable at the time of presenta-

tion and is highly specific. On the other hand, these same characteristics may lead to its overuse in patients with a low pretest probability of *L pneumophila* infection. It could be argued that, because current guidelines for the empirical treatment of community-acquired pneumonia suggest the use of macrolides or respiratory fluoroquinolones, a specific diagnosis is less important than it once was. Although macrolides and fluoroquinolones are likely to be continued when no diagnosis is forthcoming, the dose and duration may be altered or a second agent added if Legionnaires' disease is diagnosed.

The cost to diagnose each case of Legionnaires' disease using the LPUAT was approximately \$5,770 (materials and supplies only). Six of the seven patients with a positive LPUAT were already on, or, in one case, had already been, treated with antibiotics active against *L pneumophila* when the tests were ordered. The patient who was not taking an appropriate antibiotic did have a change in antibiotic because of the test result. For both cases in which the patient had been started on dual therapy (cephalosporin and macrolide), the cephalosporin was discontinued when the diagnosis of *L pneumophila* pneumonia was made, but in one of these cases, a positive Legionella culture was available. It is of note that 28% of patients with negative LPUATs did not have evidence of pneumonia on chest radiograph when the test was ordered. In these cases the diagnosis of pneumonia was usually made by

housestaff before the chest radiograph was seen or before their interpretation of the chest radiograph was confirmed by the radiologist. In 62% (31 of 50) of the cases, the patient was started on empirical therapy for *L pneumophila* and in 68% (21 of 31) of these cases the treatment was not influenced by the negative LPUAT. During the period studied 448 specimens from 343 patients were submitted for Legionella culture. Only three specimens, from two patients, were positive. Both of these patients had positive LPUATs. No other *Legionella* species were recovered during the study.

In summary, the authors feel that the LPUAT may have some clinical utility when it is used to confirm a diagnosis of *L pneumophila* pneumonia. However, the pretest probability of *L pneumophila* pneumonia should be reasonably high and results should alter patient care or have an impact on infection control measures. Criteria to identify patients at high risk for *L pneumophila* pneumonia should be developed in the context of local epidemiology. There were too few positives to recommend what those criteria might be, however, the literature would suggest advanced age, cigarette smoking, chronic lung disease, recent surgery and immunosuppression (14,16). Unfortunately, most patients admitted to hospital with pneumonia will have at least one risk factor. Perhaps only the most severely ill patients, and those with suspect single source pneumonia, should be tested.

REFERENCES

1. Winn WC Jr. Legionella. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, eds. *Manual of Clinical Microbiology*, 7th edn. Washington: American Society for Microbiology, 1999:572-85.
2. Mandell LA, Marrie TJ, Grossman RF, Chow AW, Hyland RH. Canadian guidelines for the initial management of community-acquired pneumonia: An evidence-based update by the Canadian Infectious Diseases Society and the Canadian Thoracic Society. The Canadian Community-Acquired Pneumonia Working Group. *Clin Infect Dis* 2000;31:383-421.
3. Niederman MS, Mandell LA, Anzueto A, et al. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. *Am J Respir Crit Care Med* 2000;163:1730-54.
4. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Society of America. *Clin Infect Dis* 2000;31:347-82.
5. Waterer GW, Baselski VS, Wunderink RG. Legionella and community-acquired pneumonia: A review of current diagnostic tests from a clinician's viewpoint. *Am J Med* 2001;110:41-8.
6. Benson RF, Tang PW, Fields BS. Evaluation of the Binax and Biotest urinary antigen kits for detection of Legionnaires' disease due to multiple serogroups and species of *Legionella*. *J Clin Microbiol* 2000;38:2763-5.
7. Hackman BA, Plouffe JF, Benson RF, Fields BS, Breiman RF. Comparison of Binax Legionella Urinary Antigen EIA Kit with Binax RIA Urinary Antigen Kit for detection of *Legionella pneumophila* serogroup 1 antigen. *J Clin Microbiol* 1996;34:1579-80.
8. Kazandjian D, Chiew R, Gilbert GL. Rapid diagnosis of *Legionella pneumophila* serogroup 1 infection with the Binax Enzyme Immunoassay Urinary Antigen Test. *J Clin Microbiol* 1997;35:954-6.
9. Dominguez JA, Gali N, Pedrosa P, et al. Comparison of the Binax Legionella Urinary Antigen Enzyme Immunoassay (EIA) with the Biotest Legionella Urine Antigen EIA for detection of Legionella antigen in both concentrated and nonconcentrated urine samples. *J Clin Microbiol* 1998;36:2718-22.
10. Dominguez J, Gali N, Matas L, et al. Evaluation of a rapid immunochromatographic assay for the detection of Legionella antigen in urine samples. *Eur J Clin Microbiol Infect Dis* 1999;18:896-8.
11. Franzin L, Cabodi D. Comparative evaluation of two commercially available antigen immunoassays (EIA) for the detection of *Legionella pneumophila* urinary antigen in frozen non-concentrated urine samples. *N Microbiol* 2000;23:383-9.
12. Arakaki N, Higa F, Koide M, et al. Evaluation of urinary antigen detection methods for rapid diagnosis of Legionella pneumonia. *Kansenshogaku Zasshi* 1999;73:421-8.
13. Helbig JH, Uldum SA, Luck PC, Harrison TG. Detection of *Legionella pneumophila* antigen in urine samples by the Binax NOW Immunochromatographic Assay and comparison with both Binax Legionella Urinary Enzyme Immunoassay (EIA) and Biotest Legionella Urine Antigen EIA. *J Med Microbiol* 2001;50:509-16.
14. Horn J. Binax and Biotest legionella urinary antigen kits. *J Clin Microbiol* 2001;39:1682.
15. Marston BJ, Lipman HB, Breiman RF. Surveillance of Legionnaires' disease. *Arch Intern Med* 1994;154:2417-22.
16. Reingold AL, Thomason BM, Brake BJ, Thacker L, Wilkinson HW, Kuritsky JN. Legionella pneumonia in the United States: The distribution of serogroups and species causing human illness. *J Infect Dis* 1984;149:819.
17. Stout JE, Yu VL. Current concepts: Legionellosis. *N Engl J Med* 1997;337:682-7.
18. Sopena N, Sabria-Leal M, Pedro-Botet ML, et al. Comparative study of the clinical presentation of Legionella pneumonia and other community-acquired pneumonias. *Chest* 1998;113:1195-200.
19. Marston BJ, Plouffe JF, File TM Jr, et al. Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance study in Ohio. Community-Based Pneumonia Incidence Study Group. *Arch Intern Med* 1997;157:1709-18.
20. Potgieter PD, Hammond JM. Etiology and diagnosis of pneumonia requiring ICU admission. *Chest* 1992;101:199-203.