JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1997, p. 954–956 Vol. 35, No. 4
0095-1137/97/$04.00+0


# Rapid Diagnosis of *Legionella pneumophila* Serogroup 1 Infection with the Binax Enzyme Immunoassay Urinary Antigen Test

D. KAZANDJIAN, R. CHIEW, AND G. L. GILBERT*

*Department of Clinical Microbiology, Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Sydney, New South Wales 2145, Australia*

Received 3 September 1996/Returned for modification 4 November 1996/Accepted 3 January 1997

**The Binax legionella urinary antigen (LUA) enzyme immunoassay (Binax, Portland, Maine) was evaluated in 159 patients with suspected or proven legionellosis and 209 controls. A positive LUA test was found in 37% of patients with suspected legionellosis overall and in 83% of those with proven *Legionella pneumophila* serogroup 1 infection. The sensitivity of the LUA test was significantly greater than that of the direct fluorescent-antigen test (83 versus 42%; $P < 0.0001$) but not significantly different from that of culture (85%) or serology (91%); specificity was at least 99.5%.**

The clinical and radiological features of legionellosis are nonspecific, and diagnosis depends on laboratory tests. These tests include culture and serology, which are moderately sensitive (>75%) and highly specific (>99%) but slow, and the direct fluorescent-antigen (DFA) test, which is rapid but insensitive (15). Diagnostic PCR for legionella has not yet been widely evaluated (5, 9). *Legionella pneumophila* serogroup 1 causes most cases of Legionnaires' disease, but the proportion varies in different populations and according to the diagnostic tests used.

Detection of a heat-stable soluble antigen of *L. pneumophila* serogroup 1 in urine was first reported in 1979 (1, 2, 12). The antigen is now believed to be a lipopolysaccharide (14). The presence of the antigen can be demonstrated by radioimmunoassay or enzyme immunoassay (EIA), both of which are sensitive (~80%) and specific (>99%) (3, 6, 7).

The aim of this study was to evaluate the clinical utility of a commercial EIA for *L. pneumophila* serogroup 1 soluble urinary antigen (LUA) (Binax, Portland, Maine). Urine samples from several groups of patients were tested.

Group 1 comprised 59 patients with *L. pneumophila* serogroup 1 infection confirmed by culture, defined as isolation of *L. pneumophila* serogroup 1 from respiratory specimens (of which 85% were sputa and the remainder were endotracheal aspirates, bronchoalveolar lavage fluids, and lung tissue) by conventional methods (15), and/or serology, defined as demonstration of a fourfold or greater rise in *L. pneumophila* serogroup 1 antibody titer in paired sera or, in patients with clinically compatible illness, high stationary *L. pneumophila* serogroup 1 antibody titers (≥256), measured by the indirect fluorescent-antibody test (13). Twenty-nine of these cases of *L. pneumophila* serogroup 1 infection occurred during three outbreaks of legionellosis, two of which were reported previously (4, 10).

Groups 2 and 3 were patients with suspected but unconfirmed legionellosis; group 2 comprised 37 patients with suspected legionellosis associated with the three recognized *L. pneumophila* serogroup 1 outbreaks, and group 3 comprised 51 sporadic cases. Cultures were done for 43 patients (49%) and paired sera were tested for 22 (25%) of 88 patients in these two groups, all with negative results.

Group 4 consisted of 12 patients with culture-proven legionellosis due to species or serogroups other than *L. pneumophila* serogroup 1 (*Legionella longbeachae*, 7 patients; *L. pneumophila* serogroup 2, 2 patients; and *L. pneumophila* serogroups 3, 4, and 10, 1 patient each). The diagnoses were also confirmed serologically in seven of eight cases in which paired sera were tested.

Controls (group 5) were urine specimens from 209 patients in whom legionellosis had been excluded or was not suspected. Diagnoses included bacteremia (23 patients), acute bacterial pneumonia not due to legionellosis (57 patients, including 55 of 106 instances previously described in detail by Lim et al. [8]), and suspected urinary tract infection confirmed by urine culture (63 patients). In addition, 66 urine specimens with no significant bacterial growth were randomly selected from those submitted to the laboratory for culture.

Specimens of urine were stored without preservative either at −20°C or, for longer periods, in liquid nitrogen and thawed immediately before testing. The Binax EIA method is a direct sandwich assay that uses polyclonal rabbit immunoglobulin G specific for *L. pneumophila* serogroup 1 as the capture and detection antibody. The test was performed according to the manufacturer's instructions, except that urine samples were boiled for 10 min and then centrifuged at 3,000 × *g* for 10 min, a procedure that has been reported to reduce nonspecific reactions (6, 11). Duplicate urine samples were incubated with horseradish peroxidase conjugated with immunoglobulin G in a single step. Tetramethylbenzidene and hydrogen peroxide were used as the substrates. Absorbances were read at 450 nm.

Results were calculated as the ratio of the absorbance values of the positive control urine (supplied by the manufacturer) compared with that of the patient's urine sample, provided that the mean absorbance of the positive control was at least three times that of the negative control (also supplied by the manufacturer). Patient samples with ratios of ≥3.0 were read as positive for LUA. Results are summarized in Table 1. Group 1 was used to calculate the sensitivity and group 5 (controls) was used to calculate the specificity of the Binax EIA.

In group 1, 46 of 59 (78%) LUA tests, 22 of 52 (42%) DFA

* Corresponding author. Mailing address: Department of Clinical Microbiology, Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Sydney, New South Wales 2145, Australia. Phone: (612) 9845 6255. Fax: (612) 9893 8659. E-mail: lyng@cidm.wh.su.edu.au.

TABLE 1. Binax EIA urinary antigen test, culture, and serology results for patients with suspected legionellosis and controls

| Group | *n* | No. of positive tests/no. performed (%)[a] | | |
|---|---|---|---|---|
| | | Culture | Serology | Binax EIA |
| 1. Proven *L. pneumophila* serogroup 1 infection | 59 | 46/54 (85) | 31/34 (91) | 46/59 (78) |
| 2. Suspected *L. pneumophila* serogroup 1 infection (outbreak associated) | 37 | 0/15 | 0/12 | 12/37 (32) |
| 3. Suspected legionellosis (sporadic) | 51 | 0/28 | 0/10 | 1/51 (2) |
| 4. Proven non-*L. pneumophila* serogroup 1 legionellosis | 12 | 12/12 | 7/8 | 0/12 (0) |
| Total | 159 | 58/109 (53) | 38/64 (59) | 59/159 (37) |
| 5. Controls[b] | 209 | ND | ND | 1/209 (0.5) |

[a] Differences in sensitivity of culture, serology, and Binax EIA results for group 1 are not significant. The sensitivity of the LUA test was significantly less than that of either culture ($P = 0.01$) or serology ($P = 0.005$) for all cases of legionellosis; differences were not significant if non-*L. pneumophila* serogroup 1 infections (group 4) were excluded. Note that the proportion of cases in which serological testing was performed was significantly lower than for culture or the LUA test ($P < 0.0001$). ND, not done.

[b] Patients with other bacterial infections ($n = 143$) in whom legionellosis was not suspected and patients with negative urine cultures ($n = 66$).

tests, and 46 of 54 (85%) cultures were positive. Paired sera were tested for only 34 (58%) of the 59 patients, and the diagnosis was confirmed serologically for 31 patients (91%) by demonstration of a fourfold or greater rise in antibody titer in 29 and a high stationary titer (both ≥1,024) in 2. Both sputum DFA and LUA tests were performed for 52 patients and were positive for 22 (42%) and 43 (83%), respectively ($P < 0.0001$). The sensitivities of LUA tests, culture, and serology were not significantly different.

Of 13 patients in groups 2 and 3 with positive LUA tests, only 2 had had both culture and paired sera tested. The one urine specimen in group 5 which was positive by Binax EIA (specificity, 99.5%) was from an 82-year-old man admitted to the hospital with rapidly progressive respiratory failure requiring ventilatory support. He died 2 days later from septic shock, pneumonia, and acute renal failure. Routine cultures were negative, but culture and serology for *Legionella* were not performed. His illness was coincident with a recognized outbreak, and he lived in a suburb adjacent to where the outbreak occurred.

The sensitivity of the Binax EIA LUA test in group 1 was comparable with that reported previously for *L. pneumophila* serogroup 1 urinary antigen tests (3, 6, 7). In patients with proven *L. pneumophila* serogroup 1 infection (group 1), rapid confirmation of the diagnosis would have been possible in nearly twice as many patients with the Binax EIA LUA test as with the DFA test. The sensitivity of the DFA test is highly dependent on the quality of the respiratory specimen, which is variable; bronchoalveolar lavage fluid is preferred but was submitted for culture in only 5% of cases in this study. Urine is easier to collect, and specimen quality is more consistent.

It is possible that some of the 13 positive Binax EIA tests in groups 2 and 3 were falsely positive. However, the facts that patients had clinical illnesses consistent with legionellosis and that patients in group 2 had been exposed to *L. pneumophila* serogroup 1 suggest that the results were true positive ones. The low yield for group 3 illustrates both the imprecision of clinical diagnosis of legionellosis and an important limitation of the Binax EIA, namely, that it can detect only *L. pneumophila* serogroup 1 antigen. The fact that all LUA tests for patients with legionellosis that was not due to *L. pneumophila* serogroup 1 were negative confirms the specificity of the test. There was one apparently false-positive Binax EIA test in the control group. However, a review of the patient's medical record indicated that his illness was consistent with legionellosis.

It is our experience that appropriate investigations, especially convalescent-phase serological tests, often are not requested for patients with suspected legionellosis. For the four groups (159 patients) in this study with suspected legionellosis, cultures were requested for 109 patients (69%) and were positive for 58 (36%) and paired sera were tested for 64 patients (40%) and were positive for 38 (24%). By contrast, collection of urine specimens during the acute stage of illness is simple and noninvasive. The results are available rapidly, and, in this study, the proportion that was positive was similar to that obtained by culture, even when non-*L. pneumophila* serogroup 1 infections were included. Unlike culture, LUA remains detectable after antibiotic treatment has been started.

The Binax LUA EIA test is expensive compared with the DFA test and culture. The approximate cost of consumables for a single sample with three controls ($A66) is nearly twice that of consumables and labor for the DFA test ($A30) or culture ($A35). We recommend its use only for patients for whom suspicion of the disease is strong or during outbreaks when larger numbers of specimens can be tested in batches, allowing significant reduction in the cost per test. Detection of *Legionella* DNA in urine or respiratory specimens by PCR is likely to be the method of choice for rapid diagnosis of legionellosis in the future, but these methods are still not yet widely used (9).

We acknowledge the assistance of PanBio Pty Ltd., Australia, in subsidizing the cost of the Binax EIA kits; I. Lim and W. Winslow, Department of Clinical Microbiology, IMVS, Adelaide, Australia, for providing control urine samples; staff at the Western Sydney Area Public Health Unit, for providing convalescent-phase sera from patients associated with one outbreak; Department of Microbiology and Infectious Diseases, South West Area Pathology Service, for DFA test and culture results and urine samples from patients involved in another outbreak; and L. Hicks, Department of Clinical Microbiology, ICPMR, Westmead Hospital, for assistance with data analysis.

## REFERENCES

1. **Berdal, B. P., C. E. Farshy, and J. C. Feeley.** 1979. Detection of *Legionella pneumophila* antigen in urine by enzyme-linked immunospecific assay. J. Clin. Microbiol. **9:**575–578.
2. **Bibb, W. F., P. M. Arnow, L. Thacker, and R. M. McKinney.** 1984. Detection of soluble *Legionella pneumophila* antigens in serum and urine specimens by enzyme-linked immunosorbent assay with monoclonal and polyclonal anti-

bodies. J. Clin. Microbiol. **20:**478–482.
3. **Hackman, B. A., J. F. Plouffe, R. F. Benson, B. S. Fields, and R. F. Breiman.** 1996. 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. **34:**1579–1580.
4. **Jalaludin, B., I. Goldthorpe, C. Chow, J. Liddle, N. Shaw, and A. Capon.** 1995. Legionnaires' disease outbreak in Western Sydney. Communicable Diseases Intelligence **19:**114–115.
5. **Kessler, H. H., F. F. Reinthaler, A. Pschaid, K. Pierer, B. Kleinhappl, E. Eber, and E. Marth.** 1993. Rapid detection of *Legionella* species in bronchoalveolar lavage fluids with the EnviroAmp Legionella PCR amplification and detection kit. J. Clin. Microbiol. **31:**3325–3328.
6. **Kohler, R. B., and B. Sathapatayavongs.** 1983. Recent advances in the diagnosis of serogroup 1 *L. pneumophila* pneumonia by detection of urinary antigen. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. A **255:**102–107.
7. **Kohler, R. B.** 1986. Antigen detection for rapid diagnosis of *Mycoplasma* and *Legionella* pneumonia. Diagn. Microbiol. Infect. Dis. **4**(Suppl. 3)**:**47S–59S.
8. **Lim, I., D. R. Shaw, D. P. Stanley, R. Lumb, and G. McLennan.** 1989. A prospective hospital study of the aetiology of community-acquired pneumonia. Med. J. Aust. **151:**87–91.
9. **Maiwald, M., M. Schill, C. Stockinger, J. H. Helbig, P. C. Luck, W. Witzleb, and H. G. Sonntag.** 1995. Detection of *Legionella* DNA in human and guinea pig urine samples by the polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **14:**25–33.
10. **Munro, R., S. Neville, D. Daley, and J. Mercer.** 1994. Microbiological aspects of an outbreak of Legionnaires' disease in South Western Sydney. Pathology **26:**48–51.
11. **Ruf, B., D. Schurmann, I. Horbach, F. J. Fehrenbach, and H. D. Pohle.** 1990. Prevalence and diagnosis of *Legionella* pneumonia: a 3-year prospective study with emphasis on application of urinary antigen detection. J. Infect. Dis. **162:**1341–1348.
12. **Tilton, R. C.** 1979. Legionnaires' disease antigen detected by enzyme-linked immunosorbent assay. Ann. Intern. Med. **90:**697–698.
13. **Wilkinson, H. W.** 1986. Serodiagnosis of *Legionella pneumophila* disease, p. 395–398. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
14. **Williams, A., and M. S. Lever.** 1995. Characterisation of *Legionella pneumophila* antigen in urine of guinea pigs and humans with Legionnaires' disease. J. Infect. **30:**13–16.
15. **Winn, W. C., Jr.** 1995. *Legionella*, p. 533–544. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.