

Evaluation of the Oxoid Xpect Legionella Test Kit for Detection of *Legionella pneumophila* Serogroup 1 Antigen in Urine[∇]

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We evaluated a new immunochromatographic assay (Oxoid Xpect Legionella test kit) for the ability to detect *Legionella pneumophila* serogroup 1 antigen in urine. The results were compared with those obtained with the Binax NOW urinary antigen test by following the manufacturers' instructions. The sensitivities and specificities were estimated to be 89 and 100%, respectively, for the Oxoid Xpect Legionella test kit and 86 and 100%, respectively, for the Binax NOW test.

Since the initial description of Legionnaires' disease (LD) in 1976, *Legionella pneumophila* has been increasingly recognized as a pathogen causing community-acquired, travel-associated, and nosocomial pneumonias (2, 4). LD is an acute pneumonia caused by *Legionella* spp., which are responsible for 2 to 5% of community-acquired pneumonias (6). More than 90% of LD cases are caused by *L. pneumophila*, and 70 to 80% of these belong to serogroup type 1 (2, 4).

The aim of our study was to evaluate a newly developed commercially available immunochromatographic urine antigen test (IC test), the Oxoid Xpect Legionella test kit (Thermo Fisher Scientific), for the detection of *L. pneumophila* serogroup 1 in nonconcentrated urine samples.

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We studied a panel of frozen nonconcentrated urine samples collected between 1995 and 2005 from 86 patients with pneumonia caused by *L. pneumophila* (cases) (3). LD patients were admitted with pneumonia, had radiological signs of infiltration, and showed laboratory evidence of infection with *L. pneumophila*. At least one of the following criteria constituted laboratory evidence of infection with *L. pneumophila*: isolation of *L. pneumophila* from a lower respiratory tract sample, a positive PCR result with a lower respiratory tract sample by a 16S rRNA-based assay, or seroconversion to positivity for specific immunoglobulin M (IgM) and/or IgG antibodies with the Serion enzyme-linked immunosorbent assay classic *L. pneumophila* 1-7 IgM and IgG kit (Virion Serion). The laboratory results for these patients were as follows [number of patients positive/number of patients tested (percent positive)]: serology, 61/69 (88%); isolation, 11/21 (53%); PCR, 43/46 (93%).

Urine samples from 87 patients with respiratory tract infections other than *Legionella* infections were tested in a similar

manner to test the specificity of the assays. The laboratory test results for these patients were as follows: *Streptococcus pneumoniae* (total, 57 patients; bacteria cultured from blood [blood], pneumococcal antigen [PAG; Binax NOW; Binax, Portland, ME] detected in urine, and bacteria cultured from sputum [sputum], 8 patients; blood and PAG, 23 patients; blood, 18 patients; sputum and PAG, 2 patients; sputum, 4 patients; PAG, 2 patients), *Haemophilus influenzae* (total, 8 patients; blood, 2 patients; sputum, 6 patients), *Moraxella catarrhalis* (sputum, 1 patient), *Staphylococcus aureus* (total, 4 patients; blood and sputum, 2 patients; sputum, 2 patients), *Escherichia coli* (total, 2 patients; blood and sputum, 1 patient; sputum, 1 patient), *Acinetobacter baumannii* (blood and sputum, 1 patient), *Streptococcus pyogenes* (blood and sputum, 2 patients), *Mycobacterium tuberculosis* (sputum, 1 patient), and *Pneumocystis jirovecii* (Giemsa and silver stain positive, 1 patient). Ten patients who had a fourfold or greater rise in (complement-fixing) antibodies against influenza A virus ($n = 2$), adenovirus ($n = 1$), *Chlamydia psittaci* ($n = 2$), *Mycoplasma pneumoniae* ($n = 4$), and parainfluenza virus ($n = 1$) were included.

Nonconcentrated urine samples were investigated for the presence of *L. pneumophila* antigen by using the Oxoid Xpect Legionella test kit, a qualitative IC test. We compared the sensitivities and specificities of these assays to those of a widely used IC test, the Binax NOW urinary antigen test (Binax NOW; Binax). Both tests were performed simultaneously and according to the manufacturers' instructions. All *Legionella*-positive and -negative urine samples were read at 15 and 60 min. Subsets of *Legionella*-positive samples ($n = 54$) and *Legionella*-negative samples ($n = 69$) were also read at 30 and 45 min. The clinical sensitivity and specificity of the assays were determined by using two-by-two contingency tables. Diagnostic sensitivity was defined as the fraction of the patients correctly identified by the IC test as having LD compared to determination by the standard (patients with LD). Diagnostic specificity was defined as the fraction of patients correctly identified by the IC test as not having LD compared to determination by the standard (patients with respiratory tract infections other than *Legionella* infections). The overall percent agreement represents the proportion of samples similarly classified by the

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TABLE 1. Results of Binax NOW and Oxoid Xpect tests after 15 min and 1 h of incubation

Test and incubation time (min)	% Sensitivity	% Specificity
Xpect		
15	81 (69/85) ^a	100 (0/86)
60	89 (76/85)	98 (2/86)
NOW		
15	86 (74/86)	100 (0/87)
60	93 (80/86)	100 (0/87)

^a The values in parentheses are the number of samples positive/total.

standard Binax NOW test and the Oxoid Xpect Legionella test. Samples that gave nonvalid results were not included in the calculations. Fisher's exact test was used to compare categorical data. A result with a *P* value of <0.05 was considered statistically significant.

The results obtained are shown in Tables 1 and 2. A total of 173 samples were tested with the Oxoid Xpect and Binax NOW tests (Table 1). Two samples yielded nonvalid results in the Oxoid Xpect IC test; these samples were not included in the calculations. Sensitivity and specificity were estimated as, respectively, 81% (69/85; 95% confidence interval [CI], 71 to 88%) and 100% (0/86; 95% CI, 95 to 100%) for the Oxoid Xpect test and 86% (74/86; 95% CI, 77 to 92%) and 100% (0/87; 95% CI, 95 to 100%) for the Binax NOW urinary antigen test after 15 min of incubation. The sensitivities of the Oxoid Xpect and Binax NOW tests increased to 89% (76/85; 95% CI, 81 to 95%) and 93% (80/86; 95% CI, 85 to 97%), respectively, when the tests were examined after 60 min of incubation. The specificity of the Oxoid Xpect was 100% after 15 min of incubation and decreased to 98% (84/86; 95% CI, 91 to 100%) after 1 h of incubation. The difference in sensitivity between the Oxoid Xpect and Binax NOW tests did not reach statistical significance. Samples from 54 patients with proven LD and 69 with respiratory tract infections other than *Legionella* infections were read at 15, 30, 45, and 60 min of incubation to determine the optimal incubation time (Table 2). The optimal incubation time of the Oxoid Xpect test was determined to be 45 min; maximum sensitivity is observed without any loss of specificity. The two false positives obtained with the Oxoid Xpect test after 60 min of incubation were not observed after 45 min of incubation. Calculated agreement percentages of 96 and 97% (after 15 and 60 min of incubation, respectively) for the Oxoid Xpect test compared to the Binax NOW test were found.

Detection of *Legionella* antigenuria was already used shortly after the first outbreak in Philadelphia (1). It has revolutionized the laboratory diagnosis of LD, making it the most common laboratory test for diagnosis (4). Commercial kits that use both radioimmunoassay and enzyme immunoassay methodologies have been available for several years and have similar performance characteristics (2, 4). Agglutination assays have also been introduced, but they have not demonstrated acceptable sensitivity and specificity (4). In addition, immunochromatographic assays have been developed that have sensitivities and specificities similar to those of enzyme immunoassays (5). The majority are most sensitive for the detection of the Pontiac

TABLE 2. Results of Oxoid Xpect and Binax NOW IC tests with a subset of *Legionella*-positive urine samples at 15, 30, 45, and 60 min of incubation

Test	No. of urine samples with positive test result/total (% positive) after incubation for:			
	15 min	30 min	45 min	60 min
Oxoid Xpect	45/54 (83)	47/54 (87)	48/54 (89)	48/54 (89)
Binax NOW	46/54 (85)	51/54 (94)	51/54 (94)	51/54 (94)

monoclonal antibody type of *L. pneumophila* serogroup 1 (up to 90%), less sensitive for other monoclonal antibody types of *L. pneumophila* serogroup 1, and poorly sensitive for other *L. pneumophila* serogroups and other *Legionella* species (2). In most Western countries, the majority (about 90%) of cases of community-acquired LD are caused by the Pontiac subtype of *L. pneumophila* serogroup 1, and therefore the average sensitivity of this test is in the range of 70 to 80%. An important feature of these assays is their high specificity (>99%), which is a requirement when testing for a relatively rare disease.

We evaluated a new IC test, the Thermo Fisher Scientific (Oxoid) Xpect Legionella test kit, developed for the detection of *L. pneumophila* antigen in human urine. The data suggest that the Oxoid Xpect test has high degrees of sensitivity and specificity, with a performance comparable to that of the Binax NOW test. A limitation of this study was the relatively small group of patients that was evaluated, as this could influence both sensitivity and specificity. In addition, the included LD-positive patients were probably infected with *L. pneumophila* serogroup 1, making it impossible to reach conclusions concerning infections caused by other *L. pneumophila* serogroups or *Legionella* species.

By evaluating four different read time points, we showed that reading the Xpect test after 45 min of incubation returned results that gave optimal performance and this has now been adopted in the manufacturer's instructions. After 60 min of incubation, two false positives were detected, reducing the specificity and positive predictive value of the test. Although sensitivity is highest (94%) after 30 min of incubation, Binax recommends that the NOW test be read at 15 min of incubation. When comparing the two tests and using them according to the manufacturers' instructions, the relative sensitivity of the Xpect test was 89% (48/54; 95% CI, 77 to 95%) and that of the Binax NOW test was 85% (74/86; 95% CI, 77 to 93%).

In conclusion, the Oxoid Xpect test has performance comparable to that of the Binax NOW test and could be a good alternative for the detection of *L. pneumophila* antigen in urine from patients suspected of having LD.

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