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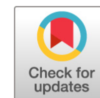

Evaluation of the bioNexia *Legionella* Test, Including Impact of Incubation Time Extension, for Detection of *Legionella pneumophila* Serogroup 1 Antigen in Urine

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ABSTRACT In this study, we compared the bioNexia test (bioMérieux, Marcy-l'Étoile, France), a new immunochromatographic assay for the detection of *Legionella pneumophila* serogroup 1 in urine, with the BinaxNOW urinary antigen test (Alere, Waltham, Massachusetts, USA). After 15 min of incubation (in accordance with the manufacturers' instructions), the sensitivities and specificities were, respectively, 76.5% and 97.2% for the bioNexia test and 87.1% and 100% for the BinaxNOW test. After a prolonged incubation time of 60 min, the sensitivities and specificities increased to, respectively, 89.4% and 97.2% for the bioNexia test and 91.8% and 100% for the BinaxNOW test. When the tests were read after 15 min, the concentration of discrepant urine samples increased the sensitivities to 94.1% for both tests. In conclusion, we found that although the bioNexia test showed lower sensitivity for the detection of *L. pneumophila* antigen in nonconcentrated urine compared to the BinaxNOW test, a prolonged incubation time as well as the use of concentrated samples showed comparable sensitivities for both tests.

KEYWORDS *Legionella*, Legionnaires' disease, urinary antigen test

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Legionnaires' disease (LD) is a severe pneumonia caused by *Legionella* spp., a Gram-negative bacillus found in many environments, including (man-made) aquatic systems and soil. *Legionella* spp. are responsible for 2 to 15% of all community–acquired pneumonias (1, 2). More than 90% of LD cases are caused by *Legionella pneumophila*, and 70 to 80% of these belong to serogroup type 1 (3, 4).

In both Europe and the United States, the detection of *Legionella* antigen in urine is the most requested laboratory test for diagnosing legionnaires' disease (4). Most frequently used are immunochromatographic tests (ICTs). These tests are easy to use and are known for their relatively high sensitivity (70 to 90%) and specificity (95 to 100%), especially for *L. pneumophila* serogroup 1 (5–7). In addition, concentration of urine samples can increase sensitivity without affecting the specificity (3), although this preanalytical process requires some additional efforts. Heat treatment may be used to remove false-positive results caused by interfering antibodies (8). The aim of this study was to evaluate the bioNexia test (bioMérieux, Marcy-l'Étoile, France), a newly developed ICT for the qualitative detection of *L. pneumophila* serogroup 1 antigen in urine. When *L. pneumophila* serogroup 1 antigen is present in the sample, it binds to an anti-*Legionella* antibody that is conjugated to purple colloidal gold particles. The antigen-antibody gold particle complex migrates up the membrane to the test line by capillary action. There it binds to the second anti-*Legionella* antibody present in the test line region. A purple-colored line is formed in the test line region. We compared the

TABLE 1 Sensitivity results of the bioNexia and the BinaxNOW tests for nonconcentrated urine samples after 15 min and 60 min[a]

	No. of positive LD samples (cases)		No. of positive non-LD samples (controls)	
Test	15 min	60 min	15 min	60 min
bioNexia	65/85 (76.5%)	76/85 (89.4%)	2/72 (2.8%)	2/72 (2.8%)
BinaxNOW	74/85 (87.1%)	78/85 (91.8%)	0/72 (0%)	0/72 (0%)

[a]LD, legionnaires' disease.

bioNexia test with the BinaxNOW test (Alere, Waltham, Massachusetts, USA), a widely used ICT. The impact of a prolonged incubation time (from 15 to 60 min) and the effect of concentration of discrepant urine samples were also investigated.

RESULTS

A total of 157 urine samples were tested. As shown in Table 1, after 15 min of incubation (the time specified by the manufacturers), the sensitivity and specificity were, respectively, 76.5% (65/85) and 97.2% (70/72) for the bioNexia test and 87.1% (74/85) and 100% (72/72) for the BinaxNOW test. When the incubation time was extended to 60 min, the sensitivity and specificity were, respectively, 89.4% (76/85) and 97.2% (70/72) for the bioNexia assay and 91.8% (78/85) and 100% (72/72) for the BinaxNOW assay.

After concentration of the urine samples that showed a discrepancy between the test results (Table 2), the sensitivities, when read after 15 min, increased to 94.1% (80/85) for both tests. Two samples of non-LD cases showed a bioNexia-positive result and a BinaxNOW-negative result. These samples remained positive after the nonconcentrated urine was heated and were classified as false positive. The laboratory results for these two non-LD cases were as follows: detection of pneumococcal antigen in urine and *S. pneumoniae* cultured from blood for one of the non-LD cases and detection of pneumococcal antigen in urine, *S. pneumoniae* cultured from blood, and *S. pneumoniae* cultured from sputum for the other non-LD case. Four samples of LD cases showed BinaxNOW- and bioNexia-negative results that remained negative after concentration and prolonged incubation. These urine samples were classified as false negative. One sample came from an LD case with cultured *L. pneumophila* serogroup 6. This sample showed negative results for both tests.

Although the bioNexia test showed a lower sensitivity (76.5%) compared to the BinaxNOW test (87.1%) for nonconcentrated samples read at 15 min (McNemar's test, $P = 0.012$), extension of the incubation time to 60 min (for nonconcentrated samples) showed an increase in sensitivity to 89.4% (McNemar's test, $P < 0.001$) for the bioNexia test and to 91.8% (McNemar's test, $P = 0.125$) for the BinaxNOW test. Concentration of the urine samples also increased the sensitivities of both tests to 94.1% when read at 15 min.

DISCUSSION

Our results showed that although the bioNexia test had a lower sensitivity compared to the BinaxNOW test for nonconcentrated urine samples read after 15 min, the sensitivities of both tests were relatively high after 60 min of incubation (bioNexia

TABLE 2 Sensitivity results of the bioNexia and the BinaxNOW tests for concentrated urine samples after 15 min and 60 min[a]

	No. of positive LD samples (cases)		No. of positive non-LD samples (controls)	
Test	15 min	60 min	15 min	60 min
bioNexia	80/85 (94.1%)	80/85 (94.1%)	2/72 (2.8%)	2/72 (2.8%)
BinaxNOW	80/85 (94.1%)	81/85 (95.3%)	0/72 (0%)	0/72 (0%)

[a]LD, Legionnaires' disease.

89.4% and BinaxNOW 91.8%) and after concentration of the samples (94.1% for both tests). The specificity of each test was not impacted after reading time was extended from 15 to 60 min. These results demonstrate a reading time stability of both the bioNexia and the BinaxNOW tests.

In a previous study, the bioNexia test was evaluated and compared with the BinaxNOW test and the SOFIA (FIA) test (Quidel, San Diego, USA) for the detection of *L. pneumophila* serogroup 1 antigen in urine samples (9). This study showed a high overall percent agreement (OPA) between the bioNexia and BinaxNOW tests when they were performed on both nonconcentrated and concentrated urine samples (OPA 99.6%), which is not in line with our results. One of the possible explanations for this difference is the selection of urine samples from the LD cases in our study. In contrast to the study of Congestrì et al. (9), our LD cases were selected based on a positive result in any (combination) of the following diagnostic tests for *Legionella*, including culture, PCR, serology, and urinary antigen detection, and not solely on a positive (SOFIA FIA) ICT. This may have resulted in a selection of urine samples that included more low-positive urine samples, allowing the detection only with a more sensitive assay (BinaxNOW) when nonconcentrated urine was used.

In addition, the urine samples for LD cases used in our study were frozen samples that had been collected between 2005 and 2014. The storage of urine samples for a relatively long period may lead to decay of the urinary antigen in the samples, as was previously reported by Chang et al. (10), and could influence the performance of urinary antigen tests. This apparent instability of the antigen in frozen urine samples may have decreased the sensitivity of the tests (11) and may have had a greater impact on the performance of the bioNexia test compared to the BinaxNOW test. The results of our study showed an increase in sensitivities of both the bioNexia (from 76.5%, to 89.4%, to 94.1% after prolonged incubation time and concentration) and the BinaxNOW assays (from 87.1%, to 91.8%, to 94.1%), which supports the hypothesis that the majority of false-negative results after 15 min of incubation were based on samples with a low concentration of *Legionella* antigen or potentially deteriorated samples. The detection of low concentrations of *Legionella* antigen by prolongation of the reading time, resulting in an increased sensitivity, was described in previous studies (12).

The majority of LD cases that were included in our study were probably infected by *L. pneumophila* serogroup 1, which makes it difficult to generalize our results on the performance of the tested assays for infections caused by other *Legionella* species or other *L. pneumophila* serogroups. We know of one sample that belonged to an LD patient with cultured *L. pneumophila* serogroup 6. This sample was negative in both tests. It is known that the sensitivity for non-serogroup 1 strains is low (13). Several studies suggest that the predominant use of urinary antigen tests as a diagnostic tool for LD in recent years may have led to an increased number of undetected LD cases (caused by non-*L. pneumophila* serogroup 1) (7, 14). It is therefore recommended that clinicians be encouraged to perform additional diagnostic testing for LD in a patient with an initial negative urinary antigen test but in whom suspicion of LD remains (7, 15). A limitation in this study was the number of available urine samples that could be used. A larger number of urine samples derived from LD patients infected by non-*L. pneumophila* serogroup 1 and non-LD patients infected by an even broader range of other pathogens than the non-LD patients included in our study would strengthen the evaluation of *Legionella* urinary antigen tests.

TABLE 3 Number and results of laboratory tests performed on 85 LD samples

Laboratory test	No. of test results		No. of samples not tested
	Positive	Negative	
Urinary antigen	80	0	5
Culture	26	23	36
PCR	29	14	42
Serology	1	0	84

TABLE 4 Positive tests of the 85 LD samples

Laboratory test	No. of positive samples
Urinary antigen	49
Urinary antigen and culture	4
Urinary antigen and PCR	9
Urinary antigen and serology	1
Urinary antigen, culture, and PCR	17
Culture and PCR	3
Culture	2

In conclusion, we found that although the bioNexia test showed lower sensitivity for the detection of *L. pneumophila* antigen in nonconcentrated urine compared to the BinaxNOW test, a prolonged incubation time as well as the use of concentrated samples showed comparable sensitivities for both tests (without any impact on the specificity). The advantage of a prolonged incubation time over urine concentration is avoidance of additional costs and time and the fact that it could be recommended for low-positive samples.

MATERIALS AND METHODS

We evaluated the bioNexia test by using a panel of 157 nonconcentrated frozen urine samples. Of these samples, 85 urine samples (collected between 2005 and 2014) were obtained from LD cases, and 72 urine samples (collected between 2010 and 2014) came from patients with suspected respiratory tract infections who did not test positive for *Legionella*.

All LD cases were defined as patients with pneumonia who had laboratory evidence of LD, including at least one of the following criteria: detection of *L. pneumophila* antigen in urine (BinaxNOW; Alere, Waltham, Massachusetts, USA), isolation of *Legionella* spp. from a respiratory secretion sample, detection of *Legionella* spp. in a respiratory secretion sample using a 16S rRNA gene PCR (16), or seroconversion to positivity for specific IgM and/or IgG antibodies (EIA; Virion/Serion GmbH, Würzburg, Germany). The positive laboratory tests of the 85 samples from LD cases were as follows: urine antigen only, 49/85 (57.6%); urine antigen and culture, 4/85 (4.7%); urine antigen and PCR, 9/85 (10.6%); urine antigen and serology, 1/85 (1.2%); urine antigen, culture, and PCR, 17/85 (20.0%); culture and PCR, 3/85 (3.5%); and culture only, 2/85 (2.4%). In total, 26 *L. pneumophila* isolates were cultured. Of these 26 isolates, 25 were typed as serogroup 1, and 1 isolate was typed as serogroup 6. This LD isolate had no other positive laboratory test (Tables 3 and 4).

Urine samples from 72 non-LD patients with a suspected respiratory tract infection other than LD were tested in a similar way to assess the specificity of the two assays. The laboratory test results for samples from these patients were as follows: *Streptococcus pneumoniae* (in a total of 49 patients: bacteria cultured from blood [blood], pneumococcal antigen [PAG; BinaxNOW, Alere, Waltham, ME] detected in urine, and bacteria cultured from sputum [sputum], 2 patients; blood and PAG, 10 patients; blood, 5 patients; sputum and PAG, 2 patients; blood and sputum, 1 patient; sputum, 3 patients; PAG, 26 patients), *Staphylococcus aureus* (blood, 2 patients), *Moraxella catarrhalis* (sputum, 1 patient), *Haemophilus influenzae* (sputum, 1 patient), *Escherichia coli* (in a total of 3 patients: blood, 2 patients; sputum, 1 patient), *Enterobacter cloacae* (blood, 1 patient), *Pseudomonas aeruginosa* (blood, 1 patient), *Proteus mirabilis* (blood, 1 patient). For 13 patients, no pathogen could be isolated and no other positive laboratory test results were available.

The bioNexia test was compared with the BinaxNOW (Alere, Waltham, Massachusetts, USA), an ICT test that is widely used. The tests were performed simultaneously with nonconcentrated urine samples and read after 15 and 60 min. For the samples that showed a discrepancy between the test results, the urine was concentrated using a static ultrafiltration concentrator with a nominal molecular mass limit of 15 kDa (Minicon B15; Merck Millipore Ltd., Billerica, Massachusetts, USA) and tested again. The discrepant nonconcentrated urine samples were also heated at 95°C for 5 min and tested again.

The clinical sensitivities and specificities of the assays were determined using two-by-two contingency tables. Diagnostic sensitivity was defined as the proportion of LD cases that were correctly identified by each of the ICT tests; diagnostic specificity was defined as the proportion of non-LD cases that were correctly identified by the two ICTs. McNemar's test was used to compare sensitivities and specificities between the two ICTs.

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