Comparative Evaluation of the Novel bioNexia *Legionella* Test with the BinaxNOW *Legionella* Card Assay and the Sofia *Legionella* FIA Assay for Detection of *Legionella pneumophila* (Serogroup 1) Antigen in Urine Samples

Francesco Congestri,^a Elisabetta Crepaldi,^a Marina Gagliardi,^a Maria Federica Pedna,^a Vittorio Sambri^{a,b}

Unit of Microbiology, The Greater Romagna Hub Laboratory, Pievesestina di Cesena, Italy^a; DIMES, University of Bologna, Bologna, Italy^b

A new immunochromatographic test (bioNexia *Legionella*; bioMérieux) for the detection of *Legionella pneumophila* urinary antigen was evaluated in 255 urine samples. The results were compared with those obtained by the BinaxNOW and Sofia *Legionella* tests. The novel test compared well with those currently in use.

Legionnaires' disease (LD) is an acute pneumonia caused by *Legionella* species. Clinical manifestations and radiological signs cannot discriminate LD from other bacterial pneumonias; therefore, a rapid laboratory diagnosis is of paramount importance in suspected LD patients (1). To confirm a case of legionellosis (http://ecdc.europa.eu/en/healthtopics/legionnaires_disease/surveillance/Pages/EU-case-definition.aspx), at least one of the following laboratory criteria must be met: isolation of *Legionella* spp. from respiratory samples, detection of *Legionella* antigen in urine, seroconversion, and/or significant increase in serum anti-*Legionella* antibodies (2). The case is probable when *L. pneumophila* DNA is detected by PCR (3, 4, 5). The qualitative detection of *L. pneumophila* serogroup 1 antigen in urine is widely used for the diagnosis of LD (6, 7) due to the simplicity of sample collection and the rapidity and ease-of-use of the method. In addition, sputum production is generally scarce in LD (8). One disadvantage of the urine antigen (Ag) test is its inability to detect legionellae other than *L. pneumophila* serogroup 1 and the persistence of positive results after clinical resolution of LD (9, 10, 11).

The aim of this study was to evaluate a new immunochromatographic assay (bioNexia *Legionella*; bioMérieux, Marcy l'Etoile, France) with the BinaxNOW *Legionella* (Alere, Scarborough, ME, USA) and Sofia *Legionella* (Quidel, San Diego, CA, USA) assays, which are routinely used in our laboratory.

(These data were presented in part at the 2015 European Study Group for *Legionella* Infections [ESGLI] Conference in London, United Kingdom, and at the 44th Associazione Microbiologi Clinici Italiani [AMCLI] National Congress in Rimini, Italy.)

A total of 255 (231 fresh and 24 frozen) urine samples (US)

submitted for *Legionella* urinary antigen testing between February and August 2015 were evaluated. The 24 frozen native (not boiled and nonconcentrated) US were collected from September 2014 to August 2015 and stored at -80°C , and all were *Legionella* positive with the Sofia assay. All of the samples were collected from patients suffering from pneumonia, as confirmed by a clinician interview, and for positive samples, LD was highly suspected.

bioNexia *Legionella* is a rapid immunochromatographic assay for the qualitative detection of *L. pneumophila* antigen in nonconcentrated or concentrated urine specimens. In the case of a positive result, the *L. pneumophila* antigen is revealed by antibody conjugated to specific colloidal gold nanoparticles generating a deep purple test (T) line and is validated by the presence of a deep purple control (C) line.

The study was performed in two different steps: the three assays were first performed simultaneously on nonconcentrated US,

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Address correspondence to Francesco Congestri, francesco.congestri@auslromagna.it.

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TABLE 1 Comparison of results obtained with the novel bioNexia *Legionella* assay and with the BinaxNOW *Legionella* and Sofia *Legionella* FIA tests on nonconcentrated US

	Nonconcentrated US					
	BinaxNOW		Sofia before heat treatment		Sofia after heat treatment	
bioNexia nonconcentrated US result	+	–	+	–	+	–
Positive	38	0	38	0	38	0
Negative	1	216	17	200	13	204
OPA (% [95% CI]) ^a	99.6 (97.8–99.9)		93.3 (89.5–96.1)		94.9 (91.4–97.3)	

^a OPA, overall percent agreement.

TABLE 2 Comparison of results obtained with the novel bioNexia *Legionella* assay with the results of the BinaxNOW *Legionella* assay using concentrated US and with the results obtained by the Sofia *Legionella* FIA using nonconcentrated (heated and not heated) US

	Concentrated US		Nonconcentrated US			
	BinaxNOW		Sofia before heat treatment		Sofia after heat treatment	
bioNexia concentrated US result	+	–	+	–	+	–
Positive	49	0	49	0	49	0
Negative	1	205	6	200	2	204
OPA (% [95% CI]) ^a	99.6 (97.8–99.9)		97.6 (95.0–99.1)		99.2% (97.2–99.9)	

^a OPA, overall percent agreement.

according to the manufacturers' instructions. The US yielding discrepant results were boiled (100°C for 5 min) and centrifuged (15 min at 1,000 × g), and the tests were repeated in order to eliminate potential interference (12, 13). In the second stage of the study, the BinaxNOW and bioNexia assays were repeated after US concentration. This was performed due to evidence that this procedure enhances the sensitivity of the tests (14, 15). The Sofia assay was not included in this second stage of the study, since its specificity is decreased after US concentration (16). The concentration of individual US was achieved by centrifuging 4 ml of sample for 15 min at 1,500 × g in an Amicon Ultra-4 Ultracel-10k device (Millipore Corporation, Bedford, MA, USA) (17).

The overall percent agreement (OPA) between the bioNexia and the BinaxNOW and Sofia assays was determined by using two-by-two contingency tables. This measure represents the proportion of samples identically classified by the three methods. The results obtained are summarized in Tables 1 and 2. The 255 nonconcentrated US were identified as negative in 217 cases by the bioNexia test, in 216 cases by the BinaxNOW test, and in 200 cases by the Sofia test. As far as the positive results are concerned, 38, 39, and 55 positive samples were identified by the bioNexia, BinaxNOW, and Sofia methods. A total of 17 discrepant (positive when tested by the Sofia assay but negative with the bioNexia assay) samples were found (Table 1). Of these, 4 samples turned negative when retested with the Sofia assay after boiling, and 11 samples yielded a positive result when evaluated with the bioNexia assay after concentration. The remaining 2 discrepant samples were positive with the Sofia test after boiling but yielded a negative result when tested with the other two methods, either on native and concentrated samples (Table 2). The first discrepant result was likely a Sofia false-positive result, even after urine boiling. This conclusion is driven by the fact that the second sample obtained 24 h later was negative with all three methods. Furthermore, a sputum sample collected 48 h after the first US was negative by PCR for *Legionella* species.

Another discrepant sample was finally false negative by the bioNexia test; this sample remained positive with the Sofia test after boiling and positive with the BinaxNOW test after concentration (Table 2). Unfortunately, it was not possible to collect additional US or sputum samples from this patient.

The evaluation performed on concentrated US ($n = 255$) yielded 205 negative and 49 positive concordant results (see Table 2). The discrepancy of one sample was evaluated, as this specimen was positive when tested with the BinaxNOW assay and negative with the bioNexia test. Of the 6 discrepant results (Table 2) between bioNexia concentrated-US negatives and Sofia nonconcentrated-US positives, 4 US turned negative with the Sofia assay after heat treatment, and 1 US was defined as a false positive by the Sofia

assay. The last US was false negative by the bioNexia test, as detailed above. On nonconcentrated and concentrated US, the bioNexia test showed performance equivalent to that of the BinaxNOW test. The bioNexia test proved to be easier to use than the BinaxNOW *Legionella* test, since it does not require the use of any swab or reagent, being based on just the simple addition of 3 drops of US in the sample well on the device.

These findings confirmed that the sensitivities of the bioNexia and BinaxNOW tests are increased after urine concentration and are equivalent to the sensitivity of the Sofia test. The specificity of the Sofia test is increased after urine heating. This procedure is recommended for Sofia *Legionella* tests. The bioNexia *Legionella* test shows an excellent OPA (99.6%) on both concentrated and nonconcentrated urine compared to the BinaxNOW test. The bioNexia *Legionella* test on concentrated urine shows an excellent OPA (99.2%) compared to the Sofia test. It is not necessary to heat positive samples with the bioNexia *Legionella* test, whereas it is recommended for the Sofia *Legionella* test.

In conclusion, the performance of the novel bioNexia test on concentrated US is proven to be comparable to that of the routinely used assays, making this a suitable method for the routine diagnosis of *Legionella* species infection.

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