Evaluation of the Binax and Biotest Urinary Antigen Kits for Detection of Legionnaires’ Disease Due to Multiple Serogroups and Species of *Legionella*

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The usefulness of urinary antigen detection for the diagnosis of Legionnaires’ disease has been well documented. The reported sensitivity of the Binax urinary antigen test was 56% for all cases classified as definitive in a study of community-acquired pneumonia (9) and 80% for culture-positive *Legionella pneu-
mophila* serogroup 1. In a limited number of studies, a positive result was obtained for patients with culture-con-

confirmed Legionnaires’ disease caused by strains other than *L. pneumophila* serogroup 1 (2, 8). However, there are no reports of positive urinary antigen results with Legionnaires’ disease caused by other species of *Legionella*. The Biotest urine anti-
gen enzyme immunoassay (EIA) (Biotest AG, Dreieich, Germany) has been recently introduced and according to the manufacturer has a wide range of cross-reactivity to *L. pneumo-
mophila* serogroups and other *Legionella* species (Biotest Le-

gionella urine antigen EIA instructions, Biotest AG). The broad-spectrum enzyme-linked immunosorbent assay (ELISA) antigen is an intragenus urine assay reported by Tang and Toma (12) to detect Legionnaires’ disease caused by numerous serogroups of *L. pneumophila* (13) and additional species of *Legionella* (11). We compared the abilities of the Binax Legio-

nella urine antigen radioimmunoassay (RIA) and/or EIA kit and the Biotest urine antigen kit to detect *Legionella* in urine samples that were positive in the broad-spectrum EIA and had been confirmed by either culture or the indirect immunofluo-

rescence assay.

Urine samples were submitted to the Laboratory Branch, Ontario Ministry of Health and Long Term Care, for testing between 1986 and 1998. If available, lower respiratory tract specimens were obtained for culture and direct fluorescent antibody staining by using standard procedures for *Legionella* detection (1). In addition, paired serum samples were obtained for antibody determination by the indirect immunofluorescence assay using formalized antigens (1). Forty-five samples that were positive in the broad-spectrum enzyme-linked immu-
osorbent assay were further evaluated by using the Binax Equate *Legionella* urinary antigen RIA and/or the EIA kit. A sufficient quantity of sample was available for 42 of these samples from 39 patients who were tested with the Biotest *Legionella* urine antigen EIA (Biotest AG). All samples were tested according to each manufacturer’s directions.

The results of testing the 45 urine samples with the Biotest and Binax RIA and/or EIA are shown in Table 1. Twenty-six of these samples were from patients with pneumonia caused by *L. pneumophila* non-serogroup 1. There were 19 samples from patients diagnosed with Legionnaires’ disease from a species other than *L. pneumophila*. Although the samples were tested with both the Binax RIA and EIA, the results of the EIA were used for comparison to the Biotest. The samples had been stored at −70°C for more than 6 months prior to testing with the EIA kit. Of the 45 samples that were previously positive with the broad-spectrum ELISA, 10 were positive with the Binax Equate *Legionella* urinary antigen EIA kit. Nine of the *L. pneumophila* non-serogroup 1 samples were positive. There was one patient with culture-confirmed disease due to *L. pneumo-

phila* serogroup 6 with a ratio of 2.5 or higher, which would have been considered probable by the criteria suggested by Hackman et al. (5). One of the 19 samples from patients with disease due to species of *Legionella* other than *L. pneumophila* were positive. This case was due to *Legionella hackeliae* sero-
group 2. There were eight samples which had been positive in the RIA which were negative in the EIA after storage.

The results of the Biotest are also listed in Table 1. Thirteen of 42 samples were positive. Twelve of these were from persons with Legionnaires’ disease due to *L. pneumophila* non-serogroup 1 and one was from a patient with disease caused by *L. hackeliae* serogroup 2. This sample was also positive in the Binax test. Two of the *L. pneumophila* non-serogroup 1 sam-

ples that were positive in the Biotest and negative in the Binax EIA were positive in the RIA. The Biotest identified all the samples which were positive in the Binax EIA except one.

The initial testing of urine samples was performed using the RIA kit. The samples were subsequently stored and retested using both the Binax and Biotest EIA kits. For comparison only the results of the EIA were used, because previous reports have shown that some urine samples can become negative after long-term storage (10). There were eight samples that were positive with the Binax RIA but negative in the EIA. The Binax kit was not as sensitive as the broad-spectrum ELISA, detect-
ing 10 of the 45 (22%) samples tested or 15 of 45 (33%) if a
ratio of >2.0 is used, as suggested by Domínguez et al. (3, 4). Previous reports have shown that all urine samples with a ratio above 2.0 were positive after concentration of the soluble antigen. Previous reports of positive results with the Binax RIA and/or EIA kit for non-serogroup 1 disease have been associated with other serogroups of L. pneumophila, specifically serogroups 4 and 10 (8) and serogroup 5 (2).

The Biotest kit identified 13 of 42 (31%) samples tested. If a lower cutoff were used based on personal observation of the EIA plate, 20 (48%) samples would have been positive. There was agreement on nine of the samples, and the Biotest identified four samples that were negative in the Binax test. All of these samples were from patients with culture-confirmed disease due to L. pneumophila serogroup 6.

Previous comparison of the two tests by Domínguez et al. (3) was performed with urine samples from patients with pneumo-
nia due to L. pneumophila confirmed by culture (no serogroup was given) or seroconversion and patients with a prior positive result using the Binax kit. Their evaluation included one sample from a patient with pneumonia due to L. longbeachae that was negative in both tests. However, both tests were able to detect soluble antigen from culture extracts from all L. pneumo-
phila serogroups tested and L. bozemanii. These authors stated the major advantage of the Biotest kit over the Binax kit was the broad spectrum of cross-reactivity with antigens of other serogroups and species. Our evaluation showed that both kits were capable of detecting multiple serogroups of L. pneumo-
phila.

In a multicenter evaluation of the Biotest compared to the Binax and an in-house-developed assay, the Biotest was nega-
tive for eight urine samples from culture-proven cases of non-
serogroup 1 L. pneumophila. The eight samples were positive by using either the Binax or in-house assay. However, the number that were positive with the Binax kit alone was not provided (6). The Biotest was positive for two of three urine samples from patients with serological evidence of infection to L. pneumophila non-serogroup 1. In an evaluation of the Binax EIA kit, Kazandjian et al. (7) tested 12 samples from culture-confirmed non-serogroup 1 L. pneumophila legio-
nellosis, and all were negative. They concluded that the Binax EIA can detect only L. pneumophila serogroup 1 antigen. Our results show that this is not the case, which has importance for surveillance and epidemiology. Currently most, if not all, investigators using the Binax test report a positive urine result as being positive for L. pneumophila serogroup 1. Considering all urine antigen-positive cases as being due to L. pneumophila serogroup 1 would result in a higher percentage of cases being reported as possibly being caused by this particular serogroup.

The broad-spectrum assay is more sensitive for detecting urine antigen both from L. pneumophila of different sero-
groups and from other Legionella species than either of the commercial assays. The broad-spectrum assay was previously reported to have a sensitivity of 70% and a specificity of nearly 100% (11). Further evaluations performed between 1992 and July 1996 for 1,397 patients with both a respiratory tract spec-
imen and a urine specimen resulted in a specificity of 99.4%, a sensitivity of 77%, and a positive predictive value of 82%.

We did not have sufficient amounts of urine samples available to perform concentration experiments with the Binax and Biotest assays. This procedure has not been evaluated with the broad-spectrum ELISA. The enhanced sensitivity of both the Binax and Biotest when using concentrated urine from patients with L. pneumophila serogroup 1 has been shown (3, 4), and it is likely that this would apply to urine samples from cases caused by other Legionella species.

These results demonstrate that neither the Binax nor Biotest assay is as sensitive as the broad-spectrum ELISA; however, both are capable of detecting Legionnaires’ disease due to non-serogroup 1 L. pneumophila and other Legionella species.

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**TABLE 1. Results for Binax RIA and EIA and Biotest urine antigen test**

<table>
<thead>
<tr>
<th>Organism (no. of urine samples)</th>
<th>No. of samples positive/negative/not tested</th>
<th>RIA</th>
<th>Binax</th>
<th>Biotest</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. pneumophila SG 2 (2)</td>
<td></td>
<td>NT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>L. pneumophila SG 4 (1)</td>
<td></td>
<td>NT</td>
<td>1/0</td>
<td>1/0</td>
</tr>
<tr>
<td>L. pneumophila SG 5 (1)</td>
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<td>0/1</td>
<td>0/1</td>
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<td>L. pneumophila SG 6 (9)</td>
<td>3/1/5</td>
<td>1/8</td>
<td>5/4</td>
<td>0/1</td>
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<tr>
<td>L. pneumophila SG 7 (1)</td>
<td>NT</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>L. pneumophila SG 8 (4)</td>
<td>3/0/1</td>
<td>0/4</td>
<td>0/4</td>
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<td>L. pneumophila SG 10 (1)</td>
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<tr>
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<td>1/1</td>
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<td>0/0/2</td>
<td>0/2</td>
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<tr>
<td>L. hackeliae SG 1 (1)</td>
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<td>0/1</td>
<td>0/1</td>
</tr>
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<td>1/3</td>
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<td>L. longbeachae SG 1 (1)</td>
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<tr>
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<td>0/2</td>
<td>0/1/1</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> SG, serogroup.
<sup>b</sup> NT, not tested.
<sup>c</sup> Unable to distinguish species.

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References:


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