Comparison of Diagnostic Sensitivities of Three Assays (Bartels Enzyme Immunoassay [EIA], Biotest EIA, and Binax NOW Immunochromatographic Test) for Detection of Legionella pneumophila Serogroup 1 Antigen in Urine

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Received 24 March 2003/Returned for modification 28 May 2003/Accepted 14 October 2003

Laboratory diagnosis of Legionella pneumophila infection is performed on the basis of the results of culture growth, direct immunofluorescence, serologic testing, and antigen detection in urine. Since antigen detection in urine has proved to be a sensitive and rapid method for detecting L. pneumophila serogroup 1, this technique has become one of the most used tools for the diagnosis of Legionnaires’ disease. The urinary antigen test permits early diagnosis and initiation of appropriate antibiotic therapy (9), and it leads to the recognition of outbreaks of Legionnaires’ disease, allowing a rapid epidemiological investigation (10, 11, 13). Currently, there are several commercially available tests (the Binax Legionella urinary antigen enzyme immunoassay [EIA], the Biotest Legionella urine antigen EIA, and the Binax NOW Legionella urinary antigen immunochromatographic test [ICT]) for the detection of L. pneumophila antigen in urine. All of these tests have been previously evaluated, confirming their utility for the diagnosis of Legionnaires’ disease (6, 12). A wider range of sensitivity has been reported, however, although no research has demonstrated significant differences in sensitivity between these tests. Recently a new EIA (Bartels Legionella Urinary Antigen) for the detection of L. pneumophila serogroup 1 antigen in urine has been developed. We evaluated this new test by comparing its sensitivity to that of two widely used tests: the Biotest EIA, 61.5%; Bartels EIA, 71.3%; and Binax NOW, 75.8% (Bartels EIA). The differences in sensitivity levels for the EIAs: 61.5 versus 76.4% (Biotest EIA) and 71.3 versus 75.8% (Bartels EIA). The differences in sensitivity levels for the ICT (Bartels EIA, 71.3%; Biotest EIA, 65.1%; Binax NOW ICT, 37% [P < 0.001]). After concentration of the urine samples, no significant differences in sensitivity were found among the three tests.

A total of 178 urine specimens (obtained from patients with Legionnaires’ disease during an outbreak which occurred in Murcia, Spain, in July 2001) were included in the study. The samples were from patients who fulfilled the epidemiological criteria, suffered from symptoms compatible with pneumonia, and showed radiological signs of infiltration and for whom Legionnaires’ disease was confirmed either by culture (defined as isolation of L. pneumophila serogroup 1 from respiratory specimens) or serology (defined as a fourfold increase in L. pneumophila serogroup 1 antibody titer in paired sera or, in patients with clinically compatible illness, a high [≥ 256] stationary titer value).

During the outbreak, nonconcentrated urine samples were tested within a few hours after receipt by using Biotest EIA (182 were positive and 47 were negative); the samples were then frozen at −80°C. Samples were retested simultaneously using the Biotest Legionella urine antigen EIA (Biotest AG, Dreieich, Germany), Binax NOW Legionella urinary antigen test (Binax, Portland, Maine) and Bartels ELISA Legionella urinary antigen (Intracel, Frederick, Md.) after the samples were stored at −80°C for 3 months. All the tests were performed and the results were calculated following the manufacturers’ instructions. Urine samples were tested once. First, they were tested in nonconcentrated form. Nonconcentrated urines that had previously tested negative were concentrated 25-fold by selective ultrafiltration (Minicon B15; Millipore Corp., Bedford, Mass.) as described previously (3).

Data were analyzed with the statistical program Winstat 3.0. Sensitivity levels of the three tests were compared using a chi-square test.

The results of the three tests using nonconcentrated samples are shown in Table 1. The sensitivity levels were as follows: Biotest EIA, 61.5%; Bartels EIA, 71.3%; and Binax NOW, 57%. The differences between the sensitivity levels of the two EIAs were not significant (P = 0.2). By contrast, both EIAs showed sensitivity levels significantly higher than that of the ICT (P < 0.001). The results obtained after concentration of the negative-testing urine samples are shown in Table 1. The increase in sensitivity was statistically significant when using Binax NOW: 37 versus 69.6% (P < 0.001). A clear, but not statistically significant, increase in sensitivity levels was found for the EIAs: 61.5 versus 76.4% (Biotest EIA) and 71.3 versus 75.8% (Bartels EIA). The differences in sensitivity levels for the three tests were not significant (P = 0.2). Multiple com-

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parisons of the commercially available kits have been previously published (5, 7, 8). None of these previous studies were reported to have demonstrated significant differences in the abilities of the different tests to detect \textit{L. pneumophila} antigen in urine. When nonconcentrated urine samples were tested in our study, there were no significant differences between the results for the two EIAs, although the Bartels EIA gave a slightly higher level of sensitivity (Table 1). By contrast, the Binax NOW ICT was significantly less sensitive than the EIAs, detecting only 37\% of the cases. We did not expect to find such a large difference, since previous studies have shown that the sensitivity levels of both tests (the ICT and the EIAs) are approximately the same (8, 14). In spite of this, our findings are in agreement with those of Dominguez et al. (2), who observed that the sensitivity of the ICT assay using nonconcentrated samples was 55.5\% relative to the EIA results; after concentration of the urine, the sensitivity levels of the two methods (EIA and ICT) were the same.

Although it seems clear that concentration of urine increases the sensitivity of the tests, the reported increase shows great variations in the different studies (3, 4, 5, 14). Most of these previous reports were retrospective and used frozen samples. The different storage times of specimens and the different tests used could explain these variations. Chang et al. (1) reported that long-term storage of urine leads to a decrease in sensitivity for EIAs and radioimmunoassays. In our case, when nonconcentrated urine specimens were retested using the Biotest EIA after storage at $-80^\circ$C for 3 months, antigen was not detected in 11\% of previously positive specimens. It is possible that failure to detect specimens that had previously given positive results could be related to the reproducibility of detection by the Biotest EIA. Another explanation is the decay of the urinary antigen in the stored frozen specimens. This apparent instability of the antigen in frozen samples may partially decrease the sensitivity of the tests, and it is possible that this decrease is larger for the ICT assay. In our study the concentration of negative-testing samples increased the sensitivity of all three tests, but this increase (4, 11, and 32\% for the Bartels EIA, Biotest EIA, and Binax NOW ICT, respectively) was statistically significant only for the Binax NOW ICT.

Although the lack of specificity data for the three assays represents a major limitation, our study provides relevant data related to the clinical sensitivity levels of different commercial kits for the detection of \textit{L. pneumophila} antigen in urine. In conclusion, the Bartels EIA is comparable to the Biotest EIA in its ability to detect soluble antigen of \textit{L. pneumophila} in urine. The EIAs showed a level of sensitivity significantly higher than that of the ICT with nonconcentrated frozen urine specimens, but is possible that the level of ICT sensitivity might be higher at the time of the clinical diagnosis. Until prospective studies prove this fact, however, we suggest that urine samples be concentrated when an ICT is used.

REFERENCES


TABLE 1. Comparisons of sensitivity levels of the three \textit{Legionella} urinary antigen assays of urine samples in unconcentrated form and after concentration

<table>
<thead>
<tr>
<th>No. (%) of positive samples after storage at $-80^\circ$C for 3 mo$^a$</th>
<th>No. of samples tested</th>
<th>No. of samples with indicated Biotest EIA initial results</th>
<th>Biotest EIA</th>
<th>AC</th>
<th>UC</th>
<th>Bartels EIA</th>
<th>AC</th>
<th>UC</th>
<th>Binax NOW ICT</th>
<th>AC</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>131</td>
<td>47</td>
<td>116 (65.1%)</td>
<td>136 (76.4%)</td>
<td>127 (71.3%)</td>
<td>135 (75.8%)</td>
<td>66 (37%)</td>
<td>124 (69.6%)</td>
<td></td>
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$^a$ UC, unconcentrated urine samples; AC, urine samples after concentration.