Comparison of three *Legionella* urinary antigen assays during an outbreak of legionellosis in Belgium

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During an outbreak of legionellosis in Belgium, urine samples of 32 legionellosis patients were tested with three *Legionella* urinary antigen assays: the Biotest enzyme immunoassay (EIA) kit, the Binax EIA kit and the Binax NOW Immunochromatographic Test kit. The three tests were concomitantly compared. The test sensitivities on the first urine samples were 65.6 % for the Biotest EIA, 50.0 % for the Binax EIA and 56.3 % for the Binax NOW. Testing of a second urine sample increased the sensitivities to 71.9 %, 59.4 % and 65.6 %, respectively. The differences were not statistically significant. In outbreak settings, testing second samples from patients presenting with symptoms but initially testing negative and/or concentrating urine samples for testing might be valuable additions to the urinary antigen test to increase the sensitivities of the tests.

**INTRODUCTION**

Severe Legionnaires disease has a high mortality, and one of the most important determinants of outcome is a rapid diagnosis for early initiation of adequate antibiotic treatment (Breiman & Butler, 1998; Marston et al., 1994). Diagnostic tests that allow an early detection of *Legionella* infection are direct immunofluorescence, urinary antigen detection and nucleic acid amplification assays.

Although fluorescent-antibody staining of sputum is a rapid detection method, its routine use is limited, mostly due to the variable and rather low sensitivity reported (25–70 %) and the specificity, which greatly depends on the quality of the antibodies used (Edelstein, 1993; Fehrenbach et al., 1995; Stout & Yu, 1997; Waterer et al., 2001). Amplification of *Legionella* DNA by PCR (sensitivity reported 75 % to >90 %) seems promising, especially real-time PCR, but remains to be validated for the rapid diagnosis of *Legionella* infections (Ballard et al., 2000; Cloud et al., 2000; Templeton et al., 2003; Welti et al., 2003; Wilson et al., 2003).

During an outbreak of legionellosis after a trade fair in Kapellen, a small town in northern Belgium, we used, evaluated and compared three common urinary antigen assays, the Binax Legionella Urinary Antigen EIA, the Binax NOW Legionella Immunochromatographic Test (ICT) (Binax) and the Biotest Legionella Urine EIA (Biotest), for rapid diagnosis of visitors presenting to local hospitals with symptoms possibly suggesting legionellosis (De Schrijver et al., 2000, 2003).

**METHODS**

Patients. Of the 539 trade fair visitors presenting to local hospitals with symptoms suggesting legionellosis (from flu-like symptoms to severe pneumonia), 41 were confirmed by X-ray and in the laboratory, the latter through isolation of *Legionella* from respiratory secretions or normally sterile body fluids or tissue and/or through seroconversion and/or through detection of *Legionella* antigen in urine and/or through detection of specific IgM and/or IgG antibodies with the Serion ELISA classic *Legionella pneumophila* 1-7 IgM and IgG kit (Virion Serion) in combination with detection of *Legionella* DNA in respiratory specimens by PCR. Urine samples from 32 of these 41 confirmed cases of legionellosis were tested with three different urinary antigen assays.

Urine antigen assays

**Biotest Legionella EIA.** The Biotest Legionella EIA is a direct sandwich assay using polyclonal rabbit immunoglobulin G as capture and detection probes for antigens of all *L. pneumophila* serogroups as well
as for antigens of other *Legionella* species. The test was performed according to the manufacturer’s instructions and took less then 4 h. The cut-off value for a positive result was calculated from the mean OD405 value of two negative controls plus 0.200. Urine samples with an OD405 below the cut-off value were considered negative, samples with an OD405 equal to or greater than the cut-off were considered positive. Weak positive samples in the region of the cut-off±0.200 were tested again for confirmation and were considered positive if the repeat result was above the cut-off value.

**Binax Legionella EIA.** The Binax Legionella EIA is a direct sandwich assay, using polyclonal rabbit immunoglobulin G as the capture and detection probes, but specific for *L. pneumophila* serogroup 1 antigen. Following the manufacturer’s instructions this test took less than 4 h. A sample was considered positive when the ratio of the patient urine absorbency/mean absorbency of two negative controls was >3. Urine samples with a ratio < 3 were considered negative.

**Binax NOW Legionella ICT.** The Binax NOW Legionella ICT is an immunochromatographic assay using rabbit anti-*L. pneumophila* serogroup 1 antibody as the capture probe and rabbit anti-*L. pneumophila* serogroup 1 antibody conjugated to colloidal gold as the detection probe. Performed according to the manufacturer’s instructions the reaction could be read within 15 min, by the presence or absence of visually detectable pink-to-purple coloured lines. The appearance of both a control and a patient line corresponds to a positive result, whereas a negative assay reveals only a control line. Absence of any line indicates an invalid assay, requiring the test to be repeated.

**Second urine samples.** From 17 confirmed patients who were tested with all three urinary antigen assays, a second urine sample was available for additional testing with the three assays. Ten of these had symptoms suggesting legionellosis, but tested negative in their first sample. They were all retested within 2 weeks of the end of the fair. Patients whose health deteriorated after a first urine sample had been collected were also asked for a second urine specimen.

**Statistical analysis.** Data were analysed using the Epi-Info-software, version 6.0.

## RESULTS

### Comparison of the three urinary antigen assays

The first urine specimens of the 32 cases were collected within 2 to 15 days of the end of the fair, mostly within the first week. For the 17 cases where second urine specimens were available, the mean time between the collection of the first and the second specimen was 3 days (range 0–10 days).

Considering only the first urine specimens, the Biotest EIA detected 21, the Binax EIA 16 and the Binax NOW ICT 18 cases. The differences are not statistically significant (Biotest EIA vs Binax EIA, *P* = 0.21; Biotest EIA vs Binax NOW ICT, *P* = 0.44; Binax EIA vs Binax NOW ICT, *P* = 0.61) (Table 1).

Testing of second urine specimens resulted in two more cases being detected (after 3 and 9 days) with the Biotest EIA and three more cases (one after 2 days, two after 3 days) with the Binax EIA as well as the Binax NOW ICT. When these test results were included, the sensitivity of the Biotest EIA, the Binax EIA and the Binax NOW ICT improved slightly. The observed differences were not statistically significant (Biotest EIA vs Binax EIA, *P* = 0.29; Biotest EIA vs Binax NOW ICT, *P* = 0.59; Binax EIA vs Binax NOW ICT, *P* = 0.60) (Table 1).

### Discrepancies between the three urinary antigen assays

For six of the 32 patients (18.8 %) discrepancies between the three urinary antigen assays were observed after testing the first urine samples.

One patient tested positive with the Binax NOW ICT in the first urine sample, but remained negative with the other two tests. This patient tested negative with all three tests on the second urine sample. Another patient tested positive for Biotest EIA and Binax NOW ICT in the first urine sample, but negative for Binax EIA. No second urine sample was available for further testing. The remaining four patients tested positive with the Biotest EIA, but negative with the other two tests. For one patient no second urine sample was available. One patient tested positive with all three tests on the second urine sample. Two patients tested negative with all the three tests on the second urine sample. One of these patients later showed seroconversion.

## DISCUSSION

During the Kapellen outbreak we evaluated three commonly used assays for rapid detection of *Legionella* urinary antigens: the Biotest EIA, the Binax EIA and the Binax NOW ICT.

When the results of the first urine samples of the confirmed patients were evaluated, the sensitivities of the Biotest EIA (65.6 %), the Binax EIA (50.0 %) and the Binax NOW ICT

### Table 1. Comparison of the different urinary antigen tests

<table>
<thead>
<tr>
<th>Urine antigen test</th>
<th>No. of positives/no. tested (%)</th>
<th><em>P</em>-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test using only first urine specimens</td>
<td>Test including second urine specimens</td>
</tr>
<tr>
<td>Biotest EIA</td>
<td>21/32 (65.6)</td>
<td>23/32 (71.9)</td>
</tr>
<tr>
<td>Binax EIA</td>
<td>16/32 (50.0)</td>
<td>19/32 (59.4)</td>
</tr>
<tr>
<td>Binax NOW</td>
<td>18/32 (56.3)</td>
<td>21/32 (65.6)</td>
</tr>
</tbody>
</table>

*Results, separed by commas, are shown for each pair of tests for: using only first specimens, including second specimens and the improvements resulting from the use of the second specimen.*
As the three assays primarily detect *L. pneumophila* serogroup 1 infections and this was the causative agent in the Kapellen epidemic, the low test sensitivities cannot be explained by the possibility of missing cases of Legionnaires disease caused by other serogroups and species. They could, however, be explained by a couple of other factors. It was suggested by Wever et al. (2000) and Yzerman et al. (2002) that there might be an association between the severity of the disease and test sensitivity. Yzerman et al. (2002) demonstrated that the test sensitivity for patients with mild disease is much lower than for severe cases (42 % compared to 75 %), which might explain our findings, as active case-finding probably brought more patients with mild legionellosis to the hospital. Another factor worth considering is the variation of antigen excretion in time. Most patients were detected by the examination of their first urine specimen, collected 2 to 15 days after the end of the fair. It is, however, possible that some first urine samples were collected before antigen appeared in the urine, usually within days after onset of illness, as cases were actively sought and many patients presented early. This might explain some discrepancies observed between negative first and positive second urine specimens. Dominguez et al. (1996, 1998) showed that the concentration of urine is important as well. Performing urinary antigen assays on concentrated urine samples improves sensitivity (Yzerman et al., 2002). Our limited experience seems to add to this, as four of six previously negative patients for whom we could concentrate urine gave positive results for the retest using the concentrated sample with the Biotest NOW ICT (increasing the sensitivity of the test by 12.5 %).

Examination of the second urine specimens increased the sensitivity considerably, to 71-9 %, 59-4 % and 65-6 %, respectively, for the Biotest EIA, the Binax EIA and the Binax NOW ICT, but the results remain at the low side of the range reported in the literature. The differences between the three tests were not significant. Again the factors mentioned above might explain the discrepancies observed between first and second urine samples.

Whenever an outbreak of legionellosis is suspected, we strongly recommend the examination of concentrated urine specimens and/or the testing of a second specimen a few days later for those patients presenting with symptoms but initially testing negative.

The results of both the Biotest EIA and the Binax EIA can be available within 4 h of receiving the urine specimen, whereas the Binax NOW ICT produces results within 15–20 min. Therefore, the Binax NOW ICT can be valuable when the time factor is important. Furthermore, it can be a valuable asset in those laboratories that are not familiar with the Legionella EIA kits as it is very easy to perform and does not require any additional equipment. On the other hand, the EIA kits may be preferred for reference laboratories, laboratories with a large demand for *Legionella* testing and during outbreaks, since larger numbers of specimens can be tested in a single batch, allowing a significant reduction in costs.

We conclude that, as long as PCR is not more widely evaluated and validated, the urinary antigen tests continue to play a very important role in the rapid diagnosis of outbreak-associated cases of legionellosis.

**ACKNOWLEDGEMENTS**

We thank all physicians and laboratory personnel from the regional hospitals and clinical laboratories for forwarding the specimens. We also thank Professor Sabine Lauwers and her team from the Department of Microbiology at the Academic Hospital of the Free University of Brussels for performing further identification of the *Legionella* isolates. Finally we thank the *Legionella* outbreak control team for helpful suggestions and dedicated assistance; among them, Dr E. Van Buwel, pneumologist, and Dr P. Van Rossum, clinical microbiologist, from the KLINa Hospital, Brasschaat, and Dr T. De Beukelaar, pneumologist, and Dr C. Vael, clinical microbiologist, from the Jan Palfijn Hospital, Merksem.

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