Increasing the sensitivity of the BinaxNOW Legionella urinary antigen immunochromatographic test by additional readings at later time points

It was with great interest that I read the article by Swarrer et al. (2012). The authors examined 115 urine samples from 91 patients with laboratory-confirmed Legionnaires’ disease and found sensitivities for the Xpect Legionella and the BinaxNOW Legionella urinary antigen immunochromatographic tests (ICTs) of 32% and 47%, respectively. By comparing the quantitative results of the BinaxNOW Legionella enzyme immunoassay (EIA) with the qualitative results of the BinaxNOW Legionella ICT, they found a correlation between the EIA absorbance and ICT results, meaning that urine samples with low absorbance values had a tendency to be falsely negative in the ICT. In their study, the BinaxNOW Legionella ICT was performed according to the manufacturer’s instructions and the results were visually read after 15 min.

We routinely use the BinaxNOW Legionella ICT as a means to obtain a rapid result whenever the Legionella EIA (Biotest Legionella urinary antigen EIA) is not immediately available (e.g. during the night). The Legionella EIA is routinely performed on the following day to increase sensitivity. However, by doing so, we noticed that in some cases with a negative reading after the recommended 15 min, a positive band was visible the following day. These ‘delayed positive’ tests were most frequently observed when the Legionella EIA absorbance was borderline or only slightly elevated. On the basis of these observations, we tested four archived urine samples with a borderline absorbance and one sample with a slightly elevated absorbance in the BinaxNOW Legionella ICT. In all cases, the first reading after 15 min was negative and it was only in the succeeding hours that a band appeared. All five patients had laboratory-confirmed Legionnaires’ disease, either by PCR from respiratory specimens or by a second urinary antigen EIA.

To further examine sensitivity in weakly positive samples, we prepared serial dilutions of a Legionella antigen positive urine and tested them simultaneously using both the Legionella EIA and ICT (Table 1). As expected, the undiluted and the 1:2 diluted urine sample had a positive Legionella EIA and both gave a band after 15 min of incubation. However, the 1:3, 1:4 and 1:6 dilutions, with a slightly elevated, borderline and negative absorbance, were negative in the first reading but became faintly positive after prolonged incubations of 30 min, 2 h and 4 h, respectively.

These preliminary results suggest that the sensitivity of the BinaxNOW Legionella urinary antigen ICT could be increased by additional readings up to 4 h post-testing. This approach is not recommended by the manufacturer, who instead discourages additional readings up to 4 h post-testing. One argument against prolonging the incubation time is that unspecific, false-positive reactions may occur. To investigate this possibility, we tested 60 urinary samples from patients with respiratory samples negative for Legionella by PCR or with negative results in the Legionella ICT. No false-positives were obtained even after 24 h of incubation.

Hellbig et al. (2001) described weakly positive bands in the BinaxNOW Legionella ICT when comparing the BinaxNOW Legionella EIA, the Biotest Legionella EIA and the BinaxNOW Legionella ICT. Of 535 urine samples, 10 (1.9%) showed a weakly positive band after 15 min of incubation. Five of these urine samples belonged to patients with Legionnaires’ disease and five to patients with pneumonia of another origin (Streptococcus pneumoniae) or to patients with urinary tract infection without pneumonia. Upon reassessing the weakly positive bands after 60 min, they observed that some bands increased in intensity while others did not. In their study, the bands showing an increased intensity belonged to patients with Legionnaires’ disease, whereas the bands that stayed weakly positive came from control patients.

In the following years, Diederen & Peeters (2006, 2007) and Diederen et al. (2009) noted the benefit of additional readings while studying the diagnostic performance of four different urinary antigen tests. Prolonging the incubation time from 15 to 60 min resulted, without exception, in an increased sensitivity (BinaxNOW Legionella: +4.2%; SAS Legionella: +14.2%; Rapid U Legionella: +9.6%; Xpect Legionella: +8%). However, the effect of additional readings on specificity was variable. While the specificities of the Rapid U Legionella Test and the BinaxNOW Legionella Test were not affected, the specificities of the SAS Legionella Test and the Xpect Legionella Test decreased by 5.7% and 2%, respectively. Remarkably, in none of the mentioned studies did the BinaxNOW Legionella ICT return any false-positive results.

In accordance with the findings of Diederen & Peeters (2006, 2007) and Diederen et al. (2009), our own experience shows that urine samples from patients with Legionnaires’ disease can be negative after 15 min, with a faint but Legionella-specific band appearing up to 4 h after starting the test. It may therefore be useful to conduct further readings at later time points in order to increase the sensitivity of the BinaxNOW Legionella ICT. Because of the possibility of false-positive results, it is mandatory that these ‘delayed positive’ tests are confirmed by additional diagnostic testing. Further studies are clearly necessary to clarify the impact of additional readings on diagnostic performance, in particular as regards the rate of false-positives.
Table 1. Semiquantitative band intensities in the BinaxNOW Legionella immunochromatographic test (ICT) as a function of time and absorbance (\textasciitilde Legionella antigen concentration)

The undiluted sample with a positive result in the Biotest Legionella enzyme immunoassay (EIA) showed a clearly visible band after the recommended 15 min of incubation. Only the 1:2 dilution was also positive at this time point with a just discernable band. All other dilutions became positive at later time points and would be missed if the manufacturer’s recommendations were followed. Band intensities: (+), very faint band that is just visible; +, weak, but clearly visible band; ++, clearly positive band; ++++, strength of the control band.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Biotest Legionella EIA</th>
<th>BinaxNOW Legionella ICT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A_{450})</td>
<td>Interpretation*</td>
</tr>
<tr>
<td>None</td>
<td>0.606</td>
<td>Positive</td>
</tr>
<tr>
<td>1:2</td>
<td>0.361</td>
<td>Positive</td>
</tr>
<tr>
<td>1:3</td>
<td>0.251</td>
<td>Positive</td>
</tr>
<tr>
<td>1:4</td>
<td>0.223</td>
<td>Borderline</td>
</tr>
<tr>
<td>1:6</td>
<td>0.121</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Interpretation: positive \(>0.231\); negative \(<0.131\).

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