Immunochromatic kits Xpect Legionella and BinaxNOW Legionella for detection of Legionella pneumophila urinary antigen have low sensitivities for the diagnosis of Legionnaires’ disease

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Urinary antigen tests are the most widely used methods for diagnosing Legionnaires’ disease (LD). However, all available urinary antigen tests have the disadvantage that they lack sensitivity for serogroups (sgs) other than Legionella pneumophila sg 1. Recently, Oxoid introduced the Xpect Legionella test kit for detection of L. pneumophila sg 1 and sg 6. In this study, we have evaluated the Xpect kit together with the BinaxNOW kit and compared them with the Binax EIA kit. One hundred and fifteen urine samples from 91 patients with laboratory-confirmed LD were examined. Ninety-three samples were from 69 culture-proven cases of which 27 samples were from 23 non-sg 1 cases. At the patient level, the overall sensitivities for the three Legionella urinary antigen kits were 79 % for the Binax EIA, 47 % for the BinaxNOW and 32 % for the Xpect kit. None of the urine samples from the 10 L. pneumophila sg 6 cases were positive by the Xpect kit whereas samples from four of the patients were positive by the Binax EIA. Overall, the sensitivities for both immunochromatic assays were poor and they should not be used as the sole method for the diagnosis of LD.

INTRODUCTION

Urinary antigen tests are the most widely used methods for laboratory diagnosis of Legionnaires’ disease (LD). Compared to culture (the gold standard), urinary antigen tests are much faster, easier to perform, cheaper and more sensitive. In particular, the immunochromatic tests are fast and easy to perform and can be used without specialized equipment. PCR is used in some laboratories and has a high specificity and sensitivity (Jespersen et al., 2009), but specialized laboratory facilities are required and the method has not yet been approved by the European Centre for Disease Prevention and Control as a confirmatory test. Serology is in general sensitive and specific, but antibody levels considered as positive are often only seen late in the course of the disease.

However, all available urinary antigen tests have the disadvantage that they lack sensitivity for serogroups (sgs) other than Legionella pneumophila sg 1 (Olsen et al., 2009). It is claimed that the Xpect Legionella test kit, which Oxoid recently introduced, is able to detect L. pneumophila sg 1 and sg 6, which would be an improvement towards detecting more sgs. In the kit insert, it is claimed that sg 6 is the second most common cause of LD; however, based on European data for culture-confirmed cases reported to the European Working Group for Legionella Infections from 1995 to 2006, sg 6 is only the third most common cause of LD (approx. 2 %) while sg 3 is the second most common cause (approx. 4 %) (Joseph & Ricketts, 2010).

In this study, we have examined 115 urine samples from 91 patients with laboratory-confirmed LD using three Legionella urinary antigen kits. Two kits were immunochromatic assays: one was the new Xpect kit (introduced in 2009) and the other was the BinaxNOW kit, which has been on the market for several years. The Binax EIA was partly used as a reference method in the study.

METHODS

Culture and sero- and subgrouping of isolates. Lower respiratory tract specimens were cultured for Legionella by the standard method on Modified Wadowsky-Yee Oxoid (MWY-O) medium (SSI Diagnostica) or Buffered Charcoal Yeast Extract x-ketoglutarate (BCYE) (SSI Diagnostica) and BCYE agar supplemented with BMPA-Selective Supplement (BMPA) (Oxoid).
All *L. pneumophila* isolates were further investigated with the Dresden panel of monoclonal antibodies, including mAb 3/1 of the international panel (Helbig et al., 2002), to determine the sg and subgroup if applicable.

**Urine samples.** In this study, we analysed 115 urine samples from 91 patients with pneumonia caused by *L. pneumophila*. Ninety-three of the urine samples were from patients with culture-confirmed LD. All except two samples were collected within 2 months of onset of symptoms, most of them within 14 days. The two exceptions were samples from two patients with *L. pneumophila* sg 6 infection. All the samples had been stored frozen before they were analysed.

The 115 samples were divided in four groups, A–D.

Group A contained 27 samples from 23 patients with culture-confirmed *L. pneumophila* non-sg 1 infection, distributed as follows: sg 3 (n=9), sg 4 (n=1), sg 5 (n=1), sg 6 (n=12), sg 10 (n=1) and sg 12 (n=3).

Group B contained 35 samples from 23 patients with *L. pneumophila* sg 1 subgroup Pontiac (mAb 3/1-positive) infection, distributed in the following subgroups: Philadelphia (n=13), Benidorm (n=11), Knoxville (n=6) and Allentown/France (n=5).

Group C contained 31 samples from 23 patients with *L. pneumophila* sg 1 non-Pontiac (mAb 3/1-negative) infection, distributed in the two subgroups Bellingham (n=9) and OLDX/Oxford (n=22).

Group D contained 29 urine samples from 29 patients randomly chosen from the Danish database of LD cases. The inclusion criteria were: a laboratory-confirmed case with a stored urine sample collected a maximum of 2 months after onset of symptoms. This group should mimic a panel of routine urine samples from LD patients and should be suitable for estimation of the real sensitivities for diagnosing LD with the two immunochromatographic tests. The cases were confirmed by culture (n=7), Binax EIA (n=17) or PCR (n=4) or by a significant rise in antibodies to *L. pneumophila* sg 1 (n=1) measured by ELISA (absorbance values rose from 0.48 to 1.67). It should be noted that seven samples from the seven culture-confirmed cases in group D were also included in the appropriate groups A–C.

Group E contained samples from patients considered not to have had LD. A collection of urine samples (n=94) randomly chosen from samples tested positive by pneumococcal urinary antigen test (Severin, 1972) (n=36), from patients positive by pneumococcal PCR (n=2) (Corless et al., 2001), from patients with positive *Mycoplasma pneumoniae* respiratory PCR (n=25) (Dumke & Jacobs, 2009; Jensen et al., 1989), from patients with positive *M. pneumoniae* serology (n=2) (Virotech), from patients with positive *Bordetella pertussis* PCR (n=2) (Kusters et al., 2002), *Pneumocystis* (n=2) respiratory PCR (Bandt & Monecke, 2007) and from patients with positive *Chlamydia pneumoniae* IgM serology (n=5) (Western blot; Mikrogen Immunodiagnostics) were examined.

**Legionella urinary antigen detection kits.** The urine samples were analysed with the OxoId Xpect Legionella test kit, BinaxNOW Legionella urinary antigen test (Binax) and the Binax Legionella Urinary Antigen EIA according to the manufacturers’ instructions with the exception that the samples were heat-treated (5 min at 95 °C) before analysis. None of the samples were concentrated before testing.

For the Xpect kit, a sample was considered positive when two lines were visible after 45 min. For the BinaxNOW kit, a sample was considered positive when two lines were visible after 15 min. For the Binax EIA kit, a sample was considered positive when the absorbance value was three times the value of the negative kit control.

**RESULTS**

Fig. 1 shows the absorbance values measured by the Binax EIA for all the samples from LD patients and the corresponding distribution of negative and positive results for the three assays. The broad absorbance range measured by the Binax EIA and the relatively high number of low absorbance values were noteworthy. From the figure, it is clear that there was correlation between absorbance values in the Binax EIA and positive reactions in the BinaxNOW test as samples with low absorbance values had a tendency to be false-negative by BinaxNOW. This correlation was not so obvious for the Xpect assay. This difference was not surprising as it is reasonable to expect that both Binax assays are based on the same preparation of capture antibodies. Table 1 shows the distribution of positive and negative results and the sensitivities at both the patient level and the sample level for the four groups, as well as the specificity calculated for urine samples in group E (non-LD). The overall sensitivity for the three *Legionella* urinary antigen kits evaluated with the 115 urine samples was 78 % for the Binax EIA, 45 % for the BinaxNOW and 29 % for the Xpect. Calculated at the patient level the sensitivities were 79 %, 47 % and 32 %, respectively. For group A, it was noteworthy that none of the 12 samples from the 10 sg 6 cases were positive by the Xpect kit, neither were they positive by the BinaxNOW kit. The urine samples of group E were used to evaluate the specificity of the kits. All except a few of the positive pneumococcal urinary antigen samples were negative in all three assays. The positive pneumococcal urinary antigen test samples (n=56) were all negative in BinaxNOW, but two were weakly positive (absorbance values from 0.300 to 0.350) in the Binax EIA and three other samples were positive in the Xpect assay.

![Fig. 1. Distribution of positive and negative results in the different kits according to absorbance value in the Binax EIA for 115 urine samples from 91 patients with LD.](image-url)
DISCUSSION

The calculated overall sensitivity was partly based on selected samples from culture-confirmed cases and might not be the sensitivity achieved in a routine setting. Group D, however, consisted of a panel of samples representing non-selected Danish LD cases; although only relatively few cases were investigated, we find that these samples represent routine samples received in a microbiological laboratory for diagnosing LD.

The sensitivities for both the BinaxNOW and the Xpect kits were disappointing, and both assays are probably inappropriate for routine diagnosis of LD, or at least the tests should be supplemented with other methods. The only kit with an overall satisfactory sensitivity of 80–90 % was the Binax EIA, but even for this kit several urine samples had low absorbance values near the cut-off value (Fig. 1).

Helbig et al. (2003) found an overall sensitivity of 80.6 % for the Binax EIA, which is in accordance with our result. Yzerman et al. (2002) found a sensitivity of 69 % for Binax EIA and 72 % for BinaxNOW for uncentrurized urine samples, but an increase in sensitivity of approximately 10 % for concentrated samples. Also, Dominguez et al. (1998) found a low sensitivity (55.5 %) for BinaxNOW using uncentrurized urine samples and an increase in sensitivity to 97.2 % with concentrated samples. Guerrero et al. (2004) found an increase from 37 % to 69.6 % for BinaxNOW by concentrating the urine samples. These results (uncentrurized samples) are in accordance with our overall sensitivity and the sensitivity for group D for the BinaxNOW kit. Concentration of samples was not performed in this study. It is not recommended by the manufacturer, and our experiences (unpublished data) with concentrated samples indicated that heat treatment is necessary to avoid non-specific reactions.

Table 1. Number of positive and negative samples and patients as well as sensitivities for the three urinary antigen tests

<table>
<thead>
<tr>
<th>Group of samples</th>
<th>Kit</th>
<th>No. of positives (sample/patient)</th>
<th>No. of negatives (sample/patient)</th>
<th>Sensitivity (%) (sample/patient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Non-sg 1 (27 samples/23 patients), sg 6 (12 samples/10 patients)</td>
<td>Binax EIA</td>
<td>11/11</td>
<td>16/12</td>
<td>41/48</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>0/0</td>
<td>27/23</td>
<td>0/0</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>0/0</td>
<td>27/23</td>
<td>0/0</td>
</tr>
<tr>
<td>(B) sg 1 Pontiac (35 samples/23 patients)</td>
<td>Binax EIA</td>
<td>31/21</td>
<td>4/2</td>
<td>89/91</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>19/15</td>
<td>16/8</td>
<td>54/65</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>20/16</td>
<td>15/7</td>
<td>57/70</td>
</tr>
<tr>
<td>(C) sg 1 non-Pontiac (31 samples/23 patients)</td>
<td>Binax EIA</td>
<td>27/19</td>
<td>4/4</td>
<td>87/83</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>20/15</td>
<td>11/8</td>
<td>65/65</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>3/3</td>
<td>28/20</td>
<td>10/13</td>
</tr>
<tr>
<td>(D) Randomly chosen (29 samples/29 patients)</td>
<td>Binax EIA</td>
<td>26/26</td>
<td>3/3</td>
<td>90/90</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>15/15</td>
<td>14/14</td>
<td>52/52</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>11/11</td>
<td>18/18</td>
<td>38/38</td>
</tr>
<tr>
<td>Overall (groups A–D) (115 samples/91 patients)</td>
<td>Binax EIA</td>
<td>90/72</td>
<td>25/19</td>
<td>78/79</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>52/43</td>
<td>63/48</td>
<td>45/47</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>33/29</td>
<td>82/62</td>
<td>29/32</td>
</tr>
<tr>
<td>(D–) Randomly chosen minus culture (22 samples/22 patients)</td>
<td>Binax EIA</td>
<td>21/21</td>
<td>1/1</td>
<td>95/95</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>13/13</td>
<td>9/9</td>
<td>59/59</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>10/10</td>
<td>12/12</td>
<td>45/45</td>
</tr>
<tr>
<td>(E) Non-Legionella infection samples (n=94)</td>
<td>Binax EIA</td>
<td>2</td>
<td>92</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>0</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>3</td>
<td>91</td>
<td>97</td>
</tr>
</tbody>
</table>

Specificity (%)

<table>
<thead>
<tr>
<th>Group of samples</th>
<th>Kit</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (groups A–D) (115 samples/91 patients)</td>
<td>Binax EIA</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>BinaxNOW</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Xpect</td>
<td>97</td>
</tr>
</tbody>
</table>
confirm this on real samples. It is questionable whether it is sufficient to evaluate a kit with spiked samples, as they are not necessarily comparable with urine samples from patients with real infections where the antigen is processed. It can be assumed that antigen delivered in broth or other liquids from a cultured strain is different from antigen excreted in urine. Though this study had some limitations in terms of relatively low numbers of samples, we believe that the Xpect kit has some problems in detecting antigen in urine from patients with sg 6 infections.

Our results do not correspond well with the results of Diedereren et al. (2009), which showed that the Xpect kit had a sensitivity of 89%. It must be presumed, in the light of our results, that only samples from patients with \textit{L. pneumophila} sg 1 Pontiac infection were investigated in that study. Also, no sg 6 infections were investigated by Diedereren et al. (2009); thus there was no conclusion about the sensitivity for cases of this infection. It should be emphasized that none of the kits had expired when used in this study.

**Group B**

The Pontiac group (B) is considered to include the most virulent \textit{L. pneumophila} strains, so a high sensitivity for this group is essential for a reliable diagnosis of LD. In Denmark, this group accounts for 40–50\% (Uldum et al., 2010) and in Germany up to 75\% of the culture-confirmed cases.

Only the Binax EIA kit had a satisfactory sensitivity of 91\% for this group. The sensitivity of the Xpect kit for this group was relatively low (70\%), but it is clear from the results that this kit almost exclusively had sensitivity for this subgroup of sg 1. The BinaxNOW kit showed a disappointing low sensitivity of 65\% for this group of samples. It is noteworthy that Helbig et al. (2001) found a similar sensitivity, 97.3\%, for the Binax EIA kit but a higher sensitivity, 98.6\%, for the BinaxNOW kit for this kind of sample (Pontiac). This difference in sensitivity for the BinaxNOW kit cannot readily be explained; the kit could have changed over the years or the timing of collection of urine samples could be different in the two studies.

The low sensitivity of 52\% for group D (randomly chosen urine samples) for BinaxNOW is, however, in accordance with results of other studies (Domínguez et al., 1998; Guerrero et al., 2004; Yzerman et al., 2002). Our experience from local Danish laboratories where BinaxNOW is often used as the preferred \textit{Legionella} urinary antigen kit is that a generally low sensitivity relative to PCR is seen (Olsen & Uldum, 2009).

**Group C**

A sensitivity of 13\% for the Xpect kit for the \textit{L. pneumophila} sg 1 non-Pontiac (mAb 3/1-negative) cases (group C) was especially disappointing. Although the non-Pontiac subgroup is not the most common cause of LD, it accounts for approximately 20\% of all culture-confirmed cases in Denmark, and strains belonging to this subgroup are important causes of nosocomial LD in particular (Helbig et al., 2002). The Binax EIA had a sensitivity for this subgroup of sg 1 of 83\%. A similar sensitivity of 81.8\% for this kit was found in a previous study (Olsen et al., 2009).

**Group D**

We acknowledge that the samples in the above-mentioned groups were selected; therefore, we also included a group of samples from ‘unselected’ LD patients (Table 1). We found a patient-based sensitivity of 95\% for the Binax EIA, followed by 59\% for the BinaxNOW and 45\% for the Xpect kit. These results are between the values for group B and C, which could be expected. This reflects that most cases are caused by \textit{L. pneumophila} sg 1. The group is, however, biased by the high portion of patients primarily diagnosed by detection of urinary antigen (Binax EIA).

**Group E**

It was a surprise that false-positive reactions were detected by the Binax EIA kit; it is generally assumed that samples are not false-positive in \textit{Legionella} urinary antigen assays after heat treatment, and we do not think that pneumococcal antigen cross-reacts with \textit{Legionella}. The samples were re-examined several times to exclude accidental background reactions. Real \textit{Legionella} infection can, however, not be excluded but for now we have no satisfactory explanation. The three samples positive by the Xpect kit were all different samples, and we have no explanation for these reactions either.

The study showed that both the Xpect and BinaxNOW kits had lower sensitivity than proposed by the manufacturer and in most previous publications especially for some subgroups of sg 1. The kits cannot generally be blamed for low sensitivity for non-sg 1 infections, except that it seems that the Xpect kit is not sensitive for sg 6 at all, although it is claimed to be so by the manufacturer. We cannot recommend any of these immunochromatic kits for the sole laboratory diagnosis of LD, especially not for laboratories capable of running ELISA. As was shown in several other studies, concentrating urine samples before testing can improve the sensitivity of the BinaxNOW kit and other urinary antigen kits (Domínguez et al., 1998; Guerrero et al., 2004; Yzerman et al., 2002), but it is important to be aware that concentration of urine samples can change the specificity.

In light of the results, we would recommend that urinary antigen examination should not stand alone, but should be supplemented with other methods such as PCR and culture of respiratory samples for a reliable diagnosis of LD.

**REFERENCES**

Low sensitivity of L. pneumophila urinary antigen kits


