EVALUATION OF AN IMMUVIEW LEGIONELLA LONGBEACHAE URINARY ANTIGEN TEST FOR THE DIAGNOSIS OF PNEUMONIA

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INTRODUCTION

Over the last decade the majority of Legionella pneumonia's seen in New Zealand (NZ) have been caused by Legionella longbeachae. Fig 1. In the Christchurch area approximately 85% of Legionellosis is due to L. longbeachae.

The 2010 increase corresponds to when PCR was introduced to the testing regime predominantly in the Canterbury area. The 2015/2016 increase corresponds to when the testing regime in Canterbury was expanded to 17 other laboratories (96% of population) throughout the country for the LEGI NZ Study.

The Canterbury testing regime includes testing for Legionella by PCR on urinary specimens from patients where "pneumonia" is stated in the clinical details, where the patient is immunocompromised and where the patient has had a previous Urinary Antigen test (UAT) or Legionella is requested.

Because of the different epidemiology of Legionella in NZ, an immunochromatographic membrane assay for the detection of L. longbeachae pneumonia was sought. The SSI Diagnostica A/S (SSI), Denmark, developed the Prototype L. longbeachae UAT (LLVAL2), using cultures of L. longbeachae 1 and 2 from NZ.

AIM

To evaluate the ImmuView trial PROTOTYPE LLVal2 using stored urines from patients diagnosed with Legionellosis caused by L. longbeachae and other Legionella species.

The effect of concentrating the urines to increase sensitivity and heating of the urines to increase specificity were also evaluated.

METHOD

The 65 urine samples were tested as follows:

- Concentrated x 25 ensures that the maximum sensitivity is gained, the intensity of the reaction is greater. 48% of the unconcentrated specimens gave "faint" lines only, while in the concentrated urines a "faint" reaction was recorded in only 17%. When the intensity of the reaction line is 1+ or more in practice there is a greater level of confidence in the results, especially important for those laboratories who rely solely on UAT for a Legionella diagnosis.

- Of the 30 samples that were heated only 24% of the unconcentrated samples remained positive and 58% of the concentrated samples.

DISCUSSION

Concentrating the urine x 25 ensures that the maximum sensitivity is gained, increasing from 56% to 66%, without compromising the specificity. Another advantage to concentrating the urines is that the intensity of the reaction is greater. 48% of the unconcentrated specimens gave "faint" lines only, while in the concentrated urines a "faint" reaction was recorded in only 17%. When the intensity of the reaction line is 1+ or more in practice there is a greater level of confidence in the results, especially important for those laboratories who rely solely on UAT for a Legionella diagnosis.

On the small sample tested there is evidence that the L. longbeachae antigen in urines is not heat-stable and heating reduces the sensitivity of the test considerably.

Reasons for negative UAT results include the time of day the urines are taken (early morning urine is more concentrated), the urinary antigen may be bound to various proteins making it unavailable and the infection may be in the early stages or not severe and no urinary antigen is yet produced or not enough to be detectable (2).

CONCLUSION

The L. longbeachae Urinary Antigen Test developed by SSI is a useful test to exclude L. longbeachae as a cause of respiratory illness.

This ImmuView UAT has the advantage over other UAT’s in being able to detect both L. longbeachae and L. pneumophila.

Testing at European sites, planned later in the year may yield useful information as to the prevalence of L. longbeachae in other parts of the world.

REFERENCES


ACKNOWLEDGEMENTS

All the Staff at the Canterbury Health Laboratory who helped identify the patients so the urines could be stored. The Staff at the SSI Diagnostica especially Dr Perrelle Landebo Eiverdal and Dr. Jasper Sommen.

David Harris, ESR Wellington, NZ.

The 65 urine samples were tested as follows:

4 L. pneumophila sg 1, 1 L. pneumophila sg.6, 1 L. micdadei.

A cohort of 30 were tested unconcentrated and concentrated after heating to 100 degrees C for 10 minutes.

The real-time PCR used as the Gold Standard was a 16 s rRNA gene of Legionella spp. (Reischl et al 2002). To confirm the presence of L. longbeachae a specific, in-house, PCR is used.

RESULTS

Table 1. PCR vs LLVAL

<table>
<thead>
<tr>
<th>Year</th>
<th>Concentrated</th>
<th>Unconcentrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>24</td>
<td>2*</td>
</tr>
<tr>
<td>2008</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>2009</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>2010</td>
<td>29</td>
<td>2*</td>
</tr>
<tr>
<td>2011</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>2012</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>2013</td>
<td>90.5%</td>
<td>90.5%</td>
</tr>
<tr>
<td>2014</td>
<td>66%</td>
<td>66%</td>
</tr>
<tr>
<td>2015</td>
<td>56%</td>
<td>56%</td>
</tr>
<tr>
<td>2016</td>
<td>54%</td>
<td>54%</td>
</tr>
</tbody>
</table>

*1 Legionella longbeachae, 1 Legionella sg.6

The test has a PPV of 93.5 % on concentrated specimens.

Of the 30 samples that were heated only 24% of the unconcentrated samples remained positive and 58% of the concentrated samples.

The comparison between, culture positive, LLVAL2 positive and the Ct value of the PCR shows that at the higher Ct values i.e. >35 the L.longbeachae antigen test is more likely to confirm the diagnosis than the culture. (Figure 3)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct Cycles</td>
<td>23</td>
<td>25</td>
<td>27</td>
<td>29</td>
<td>31</td>
<td>33</td>
</tr>
</tbody>
</table>

Culture vs. LLVal2 vs. PCR Ct Value

44 L. pneumophila sg 1, 1 L. pneumophila sg.6, 1 L. micdadei.

A cohort of 30 were tested unconcentrated and concentrated after heating to 100 degrees C for 10 minutes.

The real-time PCR used as the Gold Standard was a 16 s rRNA gene of Legionella spp. (Reischl et al 2002). To confirm the presence of L. longbeachae a specific, in-house, PCR is used.