Clinical Performance Study of (Legionella One-Step kit) Coris BioConcept test in comparison with an EIA test (Bartels Trinity,Biotech)

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BACKGROUND

Pneumonia still remains one of the most common types of infection managed by healthcare providers, and it is caused by a wide variety of microorganisms. Consequently, different antibiotic regimens are required, and in order to choose an optimal treatment, a correct etiologic diagnosis is necessary. Although *Legionella pneumophila* ranks among the most common microbial pathogens involved, diagnosis of *Legionella* pneumonia can be difficult. Clinical manifestations, and radiographic features are non-specific, and conventional laboratory tests present known drawbacks. Given that the rate of mortality by *Legionella* pneumonia increases significantly in incorrectly treated patients, particularly in immunocompromised patients, rapid diagnosis and early antibiotic treatment are needed.

The availability of commercial urinary antigen tests increased diagnosis of Legionnaires’ disease, and also decreased mortality, possibly as a result of obtaining an earlier diagnosis in the course of disease. Detection of the *L. pneumophila* serogroup 1 soluble antigen in urine by EIA has proven rapid, sensitive and specific in the diagnosis of legionellosis.

In the last years, rapid immunochromatographic assays (ICT) have been developed to detect *Legionella* in urine. ICT methods are technically less complex and require less equipment than the EIA, and this allowed a wide diffusion of *Legionella* urinary antigen for conventional laboratories. The use of concentrated urine by selective ultrafiltration improves the yield significantly. However, it is important to remark that selective ultrafiltration, although allows to obtain results within one working day, requires added processing time. Therefore, using this strategy to increase the sensitivity of the tests also reduces its utility as a rapid diagnostic test.

The value of urinary antigen-detection assays to diagnose Legionnaires’ disease would be enhanced if new tests that allow reach high sensitivities values in non-concentrated urine samples are developed.

Recently, a new version of lateral flow test based for detecting *Legionella* antigen in urinary samples has been developed.

OBJECTIVES

To determine the sensitivity and specificity of the *Legionella* One-Step kit (Coris BioConcept test) for the *L. pneumophila* serogroup 1 antigen detection, comparing the results with EIA (Bartels Trinity,Biotech, Ireland). The *Legionella* V-TestT will be tested to compare with the cassette format of test.
MATERIAL AND METHODS

Study design
The evaluation of the test accuracy was developed in a retrospective study.

Group of patients
Group 1: 50 urine samples from patients with clinical and radiological signs of pneumonia, and microbiologically confirmed as *Legionella pneumophila* etiology, were studied. The legionellosis diagnosis was made by serology and/or by isolation of *L. pneumophila* from respiratory samples. In all cases the patients were diagnosed by urinary antigen detection by means of EIA (Bartels *Legionella Urinary Antigen, Trinity*) in concentrated urine.

Group 2: 59 specimens from patients with pneumonia due to other etiologies; and urines samples from patients with no clinical or radiological evidence of pneumonia and with urinary tract infections, were included.

*Legionella* One-Step kit (Coris BioConcept test)
Both Coris tests (Coris K-Set and Coris V-Test) are a ready-to-use membrane test based on colloidal gold particles. No pre-dilution of urine specimen is required. Briefly, the urine were gently mixed before testing. 100 µL of urine sample was taken and deposited in the device. In the Coris K-Set the samples are dispensed in the sample well (placing the marked device horizontally); and in the Coris V-Test the samples are dispensed inside the device funnel on the top of the device (placing the marked device vertically in a rack with the aperture at the top. The reaction lasts for 15-min before reading results. The test was performed and the results interpreted following the recommendations of the manufacturer. After testing, the patient specimens were frozen at -80°C for further analysis according to results of tests obtained.

Reference immunological tests
The Coris test was compared with the EIA Bartels in non-concentrated urine. In case of discordant results, the urine samples were retested with the ICT Binax test.

The Bartels assay is a direct sandwich assay that uses polyclonal rabbit antibodies that react with *L. pneumophila* serogroup 1 antigen as the capture and detection antibody. Results were read using a microplate reader, although a visual interpretation could be performed.

The ICT Binax consists of a hinged test device, where rabbit anti-*L. pneumophila* serogroup 1 antibody is adsorbed onto nitrocellulose membrane (the patient line), and goat anti-rabbit IgG (the control line), is adsorbed onto the same membrane as a second stripe. The ICT was performed according to the manufacturer's instructions. The reaction was read visually in 15 min. The test was interpreted by the presence or absence of visually detectable pink-to-purple coloured lines. A positive result includes the detection of both a patient and a control line, while a negative assay produces only the control line.

The two reference tests were also performed following the recommendations of the manufacturers.
Sample treatment
Urine samples were tested directly as non-concentrated urine.

Safety procedures
In this study, all procedures were achieved using the safety laboratory recommended by the Centers for Disease Control and Prevention (CDC).

Statistical methods
Sensitivity and specificity of the Coris test for detection of urinary *Legionella* antigen were calculated, comparing the results with the reference methods. Sensitivity was calculated on base of results obtained in the group 1 of patients. Specificity was determined attending to the results obtained in patients group 2.

Concordance between the Coris test and the reference methods were determined by Cohen’s kappa coefficient. Kappa values below 0.4 indicate weak correlation, values of 0.41-0.60 indicate good agreement and values above 0.6 indicate strong agreement. We used the McNemar test to compare the results of the tests. Differences were considered significant when the P value was less than 0.05. All analyses were made with SPSS statistical software for Windows (SPSS version 15.0; SPSS, Chicago)
RESULTS

In Table 1 are shown the results obtained by Coris K-SeT and Coris V-Test in comparison with the results obtained by Bartels EIA. The Bartels EIA shows a higher sensitivity than both Coris tests, although the differences were not statistically significant (p=0.799 and p=0.461, respectively). The difference between both Coris tests was neither statistically significant (p=0.629).

Table 1. Sensitivity and specificity of the evaluated techniques in non-concentrated urines.

<table>
<thead>
<tr>
<th></th>
<th>Coris K-SeT</th>
<th>Coris V-Test</th>
<th>Bartels EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>80% (40/50)</td>
<td>80% (40/50)</td>
<td>82% (41/50)</td>
</tr>
<tr>
<td>Specificity</td>
<td>100% (0/59)</td>
<td>100% (0/59)</td>
<td>98% (1/59)</td>
</tr>
</tbody>
</table>

The concordance between the techniques was very high. The agreement between Coris K-Set and Bartels EIA results is 98% (κ=0.935), between Coris V-Test and Bartels EIA results was 98% (κ=0.935); and between Coris K-Set and Coris V-Test results was 96% (κ=0.767). In Table 2, Table 3 and Table 4 are shown the detailed concordance results between the techniques considering only the results obtained in the study group.

Table 2. Contingency table of the results obtained by Coris K-Set and Bartels EIA techniques.

<table>
<thead>
<tr>
<th></th>
<th>Bartels EIA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Coris K-Set</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3. Contingency table of the results obtained by Coris V-Test and Bartels EIA techniques.

<table>
<thead>
<tr>
<th></th>
<th>Bartels EIA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Coris V-Test</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4. Contingency table of the results obtained by Coris K-Set and Coris V-Test techniques.

<table>
<thead>
<tr>
<th></th>
<th>Coris V-Test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Coris K-Set</td>
<td>39</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>10</td>
</tr>
</tbody>
</table>

From the two discordant results between Coris K-Set and Coris V-Test, in both cases the EIA Bartels was positive, and the ICT Binax was negative. In the two cases the positivity was very weak, indicating that the amount of antigen was very low, being, probably, in the limit of detection of the Coris tests.
1. The sensitivity and specificity of Coris K-Set and Coris V-Test in detecting *Legionella* antigen have no statistical significant differences in comparison with the sensitivity obtained by Bartels EIA.

2. The three immunological tests (Coris tests and Bartels EIA) have a high concordance and agreement.

3. Two discordant results were obtained between Coris V-Test and Coris K-Set, although the positive results were very weak, indicating that the antigen concentration was very low, probably in the limit detection of the tests.

4. Coris One-Step tests are a good alternative for diagnosing *Legionella* pneumonia.
REFERENCES


