Diagnosis of legionellosis can be difficult because signs and symptoms are non-specific and do not distinguish L. pneumophila infections from other common causes of pneumonia. L. pneumophila infections are considered to be fairly common but they are probably underdiagnosed and under-reported. The underdiagnosis of legionellosis can in part be attributed to the need for rapid, specific and sensitive diagnostic testing methods.

The Legionella K-SeT detects soluble antigen from L. pneumophila serogroup 1 in urine.

II. PRINCIPLE OF THE TEST

This is a ready-to-use membrane test based on colloidal gold particles. This test allows detection of Legionella pneumophila LPS in urine samples. Legionella K-SeT sensitivity and specificity come from monoclonal and polyclonal anti-Legionella antibodies. Some antibodies are conjugated to colloidal gold particles and dried on a conjugate absorbent pad. Each strip is sensitized with anti-L. pneumophila antibodies at the upper line and with a control antibody at the bottom (migration control) line.

When the urine sample migrates, conjugate is rehydrated and migrates along with the sample. If L. pneumophila urinary antigens are present in the sample, a complex between the anti-L. pneumophila conjugates and the L. pneumophila antigens is formed that will be caught by the specific anti-L. pneumophila reagent coated on the strip. Results appear in 15 minutes in the form of a red line that develops on the strip.

The solution continues to migrate to encounter a control reagent that binds the control conjugate, thereby producing a second red (migration control) line.

III. REAGENTS AND MATERIALS

1. Legionella K-SeT (20)

2. Instruction for use (1)

3. Disposable transfer pipettes (20)

4. Materials supplied with K-1515

Positive control (0.7 mL; C-1095): Heat-inactivated L. pneumophila bacteria suspension

Materials to be ordered separately:

- Negative control (1 mL; CTR-1000): Heat-inactivated S. pyogenes bacteria suspension.

V. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).

- Each person responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30°C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.

- Avoid freezing devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

Specimens to be tested should be obtained and handled by standard methods for the collection of urine sample. Urine specimens should be collected in standard containers. The use of boric acid as preservative has been validated on the Legionella K-SeT.

Urinary sample specimens must be tested as soon as possible after they are collected. If necessary, they can be stored at 2-8°C for up to 1 week or at -10°C to -20°C for longer periods of time.

Although it requires added processing time, the antigens present in the urine can be concentrated with a disposable concentrator (Minipus) or a centrifugation system (Centricon).

VIII. PROCEDURE

PREPARATIONS OF THE TEST:

- Allow kit components, in unopened packaging, and specimens to reach room temperature (15-30°C) before performing a test.

- Open the pouch and remove the device. Once opened, run the test immediately. Indicate the patient’s name or specimen number on the device (one device per specimen).

SPECIMEN PREPARATION PROCEDURE:

- Slowly dispense 100 µL of urine sample into the sample well of the device as illustrated below (Use provided disposable transfer pipette or use a lab pipette to take 100 µL).

- Leave to react for 15 minutes. The results are observed in the reading window. Positive results may be reported sooner the moment the test and control lines become visible.

- The result must be read on still wet strip.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows:

DELIVER SAMPLE

Positive Negative Invalid

READ

Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other band is present.
Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the test line may vary according to the quantity of antigens found in the sample. Any reddish-purple line (T), even weak, should be considered as a positive result.

Invalid test result: The absence of a Control line indicates a failure in the test procedure. Repeat invalid tests with a new test device.

Note: during the drying process, after 60 minutes, a very faint shadow may appear at the Test line position. It should not be regarded as a positive result.

X. QUALITY CONTROL
In accordance with Good Laboratory Practices, we recommend to check the test’s performance regularly according to the laboratory’s requirements.

Positive and Negative Controls (provided in the kit K-1515) can be run as a quality control to demonstrate a positive or negative reaction in order to ensure that test reagents are working and the test is correctly performed. Positive and negative controls must be used as a urine sample (see VIII).

XI. PERFORMANCES
A. Sensitivity – Specificity
1°) An evaluation has been conducted on a panel of 183 clinical samples (The Netherlands). 99 urine samples from patients with LD defined by radiological signs and by laboratory evidence of infection with L. pneumophila (isolation of bacteria, PCR result or EIA methods) were used. Urine samples from patients with respiratory tract infections other than Legionella infections were tested in a similar manner to test the specificity of the kit.

<table>
<thead>
<tr>
<th>LD status</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>90</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>93</td>
<td>102</td>
</tr>
<tr>
<td>Total</td>
<td>99</td>
<td>94</td>
<td>183</td>
</tr>
</tbody>
</table>

95% Confidence Interval: Sensitivity: 90.9% (83.0 to 95.5%), Specificity: 100% (94.6 to 100%), Positive Predictive value: 100% (94.9 to 100%), Negative predictive value: 90.3% (62.0 to 95.2%), Agreement: 95.1% (174/183)

2°) National Reference Laboratory (Spain) has tested 109 urine samples by using EIA method.

<table>
<thead>
<tr>
<th>EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>68</td>
<td>109</td>
</tr>
</tbody>
</table>

95% Confidence Interval: Sensitivity: 97.6% (85.6 to 99.9%), Specificity: 100% (93.3 to 100%), Positive Predictive value: 100% (89.1 to 100%), Negative predictive value: 98.6% (91.1 to 99.9%), Agreement: 99.1% (108/109)

B. Repeatability and reproducibility
To check intra-batch accuracy (repeatability), same positive and negative urine samples were processed 15 times on kits of the same production batch in the same experimental conditions. The samples produced the expected results in 100% of cases.

To check inter-batch accuracy (reproducibility), same samples (positive and negative) were processed on kits from three different production batches. The samples produced the expected results in 100% of cases.

C. Interference
Cross-reactivity to urines spiked with the following pathogens was tested and found to be negative: Adenovirus, Aspergillus niger, Candida albicans, Haemophilus influenzae, Influenza A, Influenza B, Moraxella catarrhalis, Mycoplasma pneumoniae, Nocardia asteroides, Parainfluenzae, Rhinovirus, RSV, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Campylobacter jejuni, Clostridium difficile, E.coli (different strains), Enterobacter cloacae, Enterococcus faecalis, Escherichia hermanni, Helicobacter pylori, Klebsiella pneumoniae, Legionella bozemanii (sg1), Legionella longbeachae, Neisseria meningitidis, Proteus mirabilis, Salmonella enteritidis, Shigella flexneri, Staphylococcus epidermidis, Yersinia enterococolitica (types 3,9), HMPV, Streptococcus (Group B, C, F, G), Streptococcus mutans, Vibrio parahemolyticus, Ureaplasma urealyticum, Mycobacterium avium, Mycobacterium intracellularum, Mycobacterium tuberculosis, Serratia marcescens, Pseudomonas aeruginosa, Shigella sonnei, Campylobacter coli, S. typhimurium, Vibrio parahemolyticus, Neisseria meningitidis (sg C), Mycoplasma hominis.

The blood naturally present in urine (microhematuria conditions) doesn’t affect test performances. However, bloody specimens (at 0.1% whole blood) may fail to flow properly causing smears and inconclusive test results.

XII. LIMITS OF THE KIT
The test is qualitative and cannot predict the quantity of antigens present in the sample. Clinical presentation and other test results must be taken into consideration to establish diagnosis.

A positive test does not rule out the possibility that other pathogens may be present.

Kit test is an acute-phase screening test. Specimens that are collected after this phase may contain antigen titres below the reagent’s sensitivity threshold. If a sample is given a negative result despite the observed symptoms, a culture should be started to check the sample.

XIII. TECHNICAL PROBLEMS / COMPLAINTS
If you encounter a technical problem or if performances do not correspond with those indicated in this package insert:
1. Record the kit batch number
2. If possible, keep the clinical sample in the freezer during the complaint management
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor

XIV. BIBLIOGRAPHIC REFERENCES

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