Legionella *K*-SeT

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IFU-5815/TB/03

***In vitro* rapid diagnostic test for the detection of *Legionella pneumophila* LPS antigen in urine samples**

FOR IN VITRO USE
FOR PROFESSIONAL USE ONLY
EN

References: K-1215, 20 tests per kit
K-1515, 20 tests per kit, positive control supplied

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I. INTRODUCTION

Legionellosis is a serious pneumonia caused by bacteria of the genus *Legionella* assigned to the family *Legionellaceae*. This family now includes 48 species and over 60 serogroups. Approximately 20 species are implicated in human disease. The overwhelming majority of *Legionella* infections are caused by *Legionella pneumophila*. Legionnaires' disease is the major clinical manifestation of *Legionella* infection although extra-pulmonary infection and non-pneumonic disease like Pontiac fever occur. The name *Legionella pneumophila* was derived from the dramatic outbreak at the 1976 American Legion Convention in Philadelphia. *Legionella pneumophila* is responsible for approximately 90% of infections, and of these, over 80% are due to a single serogroup, serogroup 1. *Legionella* bacteria are small faintly staining Gram-negative rods with polar flagella. *Legionella* bacteria have a widespread distribution in both natural and manmade aquatic habitats. They are readily found in fresh water, cooling towers and potable water systems. The organisms can survive in a wide range of conditions, and temperature is a critical determinant for *Legionella* proliferation. Nosocomial infection is particularly associated with colonization of hospital hot water system by *Legionella.*

The incubation period of Legionnaires' disease after being exposed to the bacteria is from two to ten days. Most patients who are admitted to the hospital develop high fever often higher than 39.5°C (103°F). Cough can be the first sign of a lung infection. Other common symptoms include headaches, muscle aches, chest pain, and shortness of breath. Gastrointestinal symptoms are common.

Legionnaires' disease (LD) is not contagious. The disease is transmitted by aerosol, and there is no evidence for direct person-to-person transmission. Person at risk are those whose immune system is compromised, including transplant recipients, the elderly, cigarette smokers, or those showing chronic obstructive pulmonary disease or chronic renal disease.

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 absorbant pad. Each strip is sensitized with anti- *Legionella* 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 stick. 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)
Sealed pouches containing one device and one desiccant. Each device contains one sensitized strip.

2. Instruction for use (1)

3. Disposable transfer pipettes (20)
Fixed volume (100 µL) transfer pipettes used for sample delivery into the device.

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.

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- Never use reagents from another kit.
- Green lines indicate immunoreagents adsorption sites. Green colour disappears during the test.
- Reagents' quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.

V. WASTE DISPOSAL

- Dispose of gloves, test tubes and used devices in accordance with GLP.
- Each user is 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.

Urine 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 (Miniplus) 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 sample).

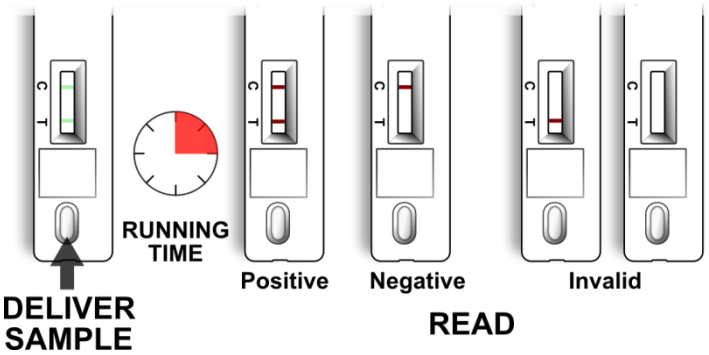
SPECIMEN PREPARATION PROCEDURE:

1. Swirl urine gently to mix before testing
2. Slowly dispense 100µL 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)
3. 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:



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.

LD status / Legionella *K*-SeT	Positive	Negative	Total
Positive	90	0	90
Negative	9	84	93
Total	99	84	183

		95 % Confidence Interval[1]
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 %	(82.0 to 95.2 %)
Agreement:	95.1 % (174/183)	

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

EIA / Legionella *K*-SeT	Positive	Negative	Total
Positive	40	0	40
Negative	1	68	69
Total	41	68	109

		95 % Confidence Interval[1]
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 have been 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 pneumonia, Nocardia asteroides, Parainfluenzae,* Rhinovirus, RSV, *Staphylococcus aureus*, *Streptococcus pneumonia, 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 enterocolitica* (types 3,9), HMPV, *Streptococcus (Group B, C, F, G), Streptococcus mutans, Vibrio parahemolyticus, Ureaplasma urealyticum, Mycobacterium avium, Mycobacterium intracellulare, 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

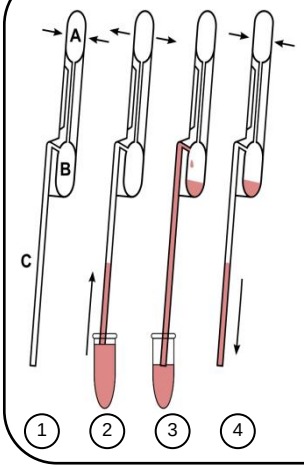
If you encounter a technical problem or if performances do not correspond with those indicated in this package insert:

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

A. **B. M.W. Diederen**; *Legionella spp. and Legionnaires'disease*; J. Inf. 2008 56:1-12, 2008
B. **J.H. Helbig et al.**; *Pan-European study on culture-proven Legionnaires' Disease*; Eur. J. Clin. Microbiol. Infect. Dis. 2002 21:710-716, 2002
C. **B.S. Fields et al.**; *Legionella and Legionnaires'Disease : 25 years of investigation*; Clin. Microbiol. Rev. 2002 15: 506-526, 2002

How to use the disposable transfer pipette



1) Squeeze firmly the upper bulb (A).

2) Insert the tip (C) of the transfer pipette into the urine sample and release the pressure on the upper bulb (A) in order to let the liquid be drawn into the tip C.

3) Be sure tip C is completely filled. Volume of tip C is 100 µL. All liquid in excess will move to into bulb B.

4) Move the transfer pipette above the device sample well and squeeze the upper bulb A to push the liquid present in tip C into the device. Make sure that the tip C is completely discharged and that all the excess liquid in bulb B remains in the bulb. Never press on bulb B

5) Discard the transfer pipette. Use only one transfer pipette per urine specimen.

Last update: NOVEMBER 2014

REF	Catalogue number		Manufactured by
IVD	In vitro diagnostic medical device		Temperature limitation
	Contains sufficient for <n> tests		Do not reuse
	Consult instructions for use		Use by
	Keep dry		

[1] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).