Legionella pneumophila Rapid Test Cassette (Urine)

Package Insert

REF ILEG-102	English

A rapid test for the qualitative detection of Legionella pneumophila antigen in human urine specimen.
For professional in vitro diagnostic use only.

INTENDED USE

Legionella pneumophila Rapid Test Cassette (Urine) is an *in vitro* diagnostic test based on immunochromatographic assay. It is designed for detection of soluble antigen from *Legionella pneumophila* serogroup 1 in human urine specimen.

SUMMARY

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.[1]

Legionella pneumophila is responsible for approximately 90% of infections, and of these, over 80% are due to a single serogroup, serogroup 1.[2] *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.[3]

Diagnosis of legionellosis can be difficult because signs and symptoms are nonspecific 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 pneumophila* Rapid Test Cassette (Urine) detects soluble antigen from L .pneumophila serogroup 1 in urine.[2]

PRINCIPLE

This is a ready-to-use membrane test based on colloidal gold particles. This test allows detection of *Legionella pneumophila* LPS in urine samples. The test sensitivity and specificity come from monoclonal and polyclonal anti-Legionella antibodies. mouse anti-Legionella antibodies are conjugated to colloidal gold particles and dried on a conjugate absorbent pad. Each strip is sensitized with goat anti-Legionella antibodies at the T-line region and with a control antibody at the C-line region 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.

REAGENTS

The test cassette contains mouse anti-Legionella particles and goat anti-Legionella coated on the membrane.

MATERIALS

Materials Provided

- Test Cassettes
- Droppers
- Package Insert

Materials needed but not provided

- Specimen Collection Containers
- Timer

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.
- Reagents' quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated in the insert.
- 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.

STORAGE AND STABILITY

An unopened pouch may be kept at between 2 - 30 °C and used until the shelf life date indicated on the packaging. Once the pouch is opened, run the test immediately. **DO NOT FREEZE.**

SPECIMEN COLLECTION AND PREPARATION

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 Proclin 300 as preservative has been validated on the *Legionella pneumophila* Rapid Test Cassette (Urine).

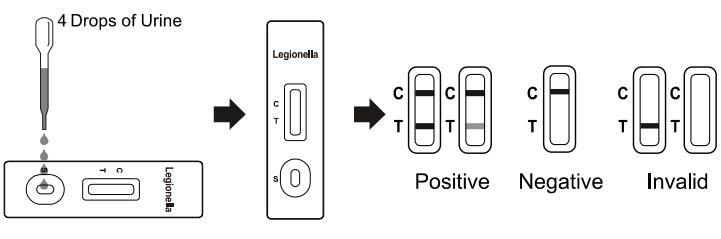
Urine sample specimens must be tested as soon as possible after they are collected. If necessary, they can be stored at 2-8 °C for up to 1 week or at -10°C to -20 °C for longer periods of time.

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

DIRECTIONS FOR USE

Allow the test, specimen and/or controls to reach room temperature (15-30 °C) prior to testing.

1. Open the pouch and remove the device. Once opened, run the test immediately.
2. Swirl urine gently to mix before testing.
3. Add **4 drops of urine sample**(Approx. 100 µL) to the sample well.
4. Wait for the color line to appear. **Read the results at 15 minutes**, do not interpret the results after 20 minutes.



RESULTS INTERPRETATION

(Please refer to the illustration above)

POSITIVE:* Two colored lines appear. One colored line should be in the control line region (C) and another colored line should be in the test line region (T). A positive result indicates that Legionella pneumophila was detected in the specimen.

***NOTE**: The intensity of the color in the test line region (T) will vary depending on the concentration of Legionella pneumophila present in the specimen. Therefore, any shade of color in the test line region (T) should be considered positive.

NEGATIVE: **One colored line appears in the control line region (C). No line appears in the test line region (T).** A negative result indicates that Legionella pneumophila antigen is not present in the specimen, or is present below the detectable level of the test.

INVALID: **Control line fails to appear**. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal valid procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

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.

PERFORMANCES

Sensitivity and Specificity

The kit was evaluated on 109 clinical samples in a National Reference Laboratory in Spain. 41 urine samples from patients with LD defined by clinical and radiological signs of pneumonia and microbiologically confirmed were studied. EIA method was used as laboratory evidence. 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.

Method		EIA		Total Results
Legionella pneumophila Rapid Test Cassette (Urine)	Result	Positive	Negative	
	Positive	40	0	40
	Negative	1	68	69
Total Results		41	68	109

Relative sensitivity: 97.6% (95%CI*: 87.1%~99.9%);
Relative specificity: >99.9% (95%CI*: 95.7%~100%);
Accuracy: 99.1% (95%CI*: 95.0%~99.9%). *Confidence Intervals

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.

Cross-reactivity

Cross-reactivity to urines spiked with the following pathogens was tested and found to be negative.

Adenovirus	Clostridium difficile	HMPV
Aspergillus niger	E.coli (different strains)	Streptococcus mutans
Candida albicans	Enterobacter cloacae	Vibrio parahemolyticus
Haemophilus influenzae	Enterococcus faecalis	Ureaplasma urealyticum
Influenza A	Escherichia hermanni	Mycobacterium avium
Influenza B	Helicobacter pylori	Mycobacterium intracellulare
Moraxella catarrhalis	Klebsiella pneumoniae	Mycobacterium tuberculosis
Mycoplasma pneumonia	Legionella bozemanii (sg1)	Serratia marcescens
Nocardia asteroides	Legionella longbeachae	Pseudomonas aeruginosa
Parainfluenzae	Neisseria meningitidis	Shigella sonnei
Rhinovirus	Proteus mirabilis	Campylobacter coli
RSV	Salmonella enteritidis	S. typhimurium
Staphylococcus aureus	Shigella flexneri	Vibrio parahemolyticus
Streptococcus pneumonia	Staphylococcus epidermidis	Neisseria meningitidis (sg C)
Streptococcus pyogenes	Yersinia enterocolitica (types 3,9)	Mycoplasma hominis
Campylobacter jejuni	Streptococcus (Group B, C, F, G)	

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.

BIBLIOGRAPHY

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

Index of Symbols

	Consult instructions for use or consult electronic instructions for use		Contains sufficient for <n> tests	30°C 2°C	Temperature limit
IVD	*In vitro* diagnostic medical device	LOT	Batch code	REF	Catalogue number
EC REP	Authorized representative in the European Community/European Union		Use-by date		Do not re-use
	Do not use if package is damaged and consult instructions for use		Manufacturer		Caution

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