L. pneumophila Ag FIA Rapid Test Device (Urine)

FI2-LEG-001

A rapid test for measuring Legionella pneumophila Antigen in urine with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000). For professional in vitro diagnostic use only.

INTENDED USE

The Biopanda Legionella pneumophila Antigen (L. pneumophila Ag) FIA Rapid Test Device is based on Fluorescence Immunoassay for the quantitative determination of L. pneumophila Ag in urine.

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.

PRINCIPLE

The L. pneumophila Ag FIA Rapid Test Device detects antigen of *Legionella pneumophila* in urine specimen based on Fluorescence Immunoassay. The sample moves through the strip from sample pad to absorbent pad. If the specimen contains *Legionella pneumophila* antigens, it attaches to the fluorescent microspheres conjugated *Legionella pneumophila* antibodies. Then the complex will be captured by the capture antibodies coated on the nitrocellulose membrane (Test line). The concentration of *Legionella pneumophila* antigens in the sample correlates with the fluorescence signal intensity captured on the T line, which can be calculated by the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) to show L. pneumophila Ag concentration in specimen.

REAGENTS

The test contains *Legionella pneumophila* antibody conjugated particles and *Legionella pneumophila* antibody coated on the membrane.

PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse.
3. Avoid cross-contamination of specimens by using a new specimen collection container for each specimen obtained.
4. Do not eat, drink or smoke in the area where the specimens and tests are handled. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
5. Do not interchange or mix reagents from different lots.
6. Humidity and temperature can adversely affect results.
7. Used testing materials should be discarded in accordance with local regulations.
8. Read the entire procedure carefully prior to any testing.
9. The Biopanda L. pneumophila Ag FIA Rapid Test Device should only be used with the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000) by approved medical professionals.

STORAGE AND STABILITY

1. The kit should be stored at 4-30°C until the expiry date printed on the sealed pouch.
2. The test must remain in the sealed pouch until use.
3. Do not freeze.
4. Care should be taken to protect the components of the kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipment, containers or reagents can lead to false results.

KIT COMPONENTS

- 25 x foil wrapped L. pneumophila Ag test devices
- 1 x ID card (L. pneumophila Ag)
- Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Centrifuge
- Pipette
- Biopanda Fluorescence Immunoassay Device (BR-FIA-2000)

SPECIMEN COLLECTION AND PREPARATION

The L. pneumophila Ag FIA Rapid Test Device can be performed using human urine. Urine specimens should be collected in standard containers. The sample can be stored at room temperature (15-30°C) if assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8°C for up to 3 days. When necessary, urine specimens should be shipped in leak-proof containers at 2-8°C or frozen. Allow all specimens to equilibrate to room temperature before testing.

DIRECTIONS FOR USE

Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for the complete instructions on use of the test. The test should be conducted at room temperature. Cold buffer solution or moisture condensation on the membrane can lead to invalid test results.

Note: There are two test modes for the Biopanda Fluorescence Immunoassay Device: Standard Test mode and Quick Test mode. *Standard Test* mode is a 'set and forget' method where the test will automatically be read after 15 minutes. *Quick* Test mode provides an instant result but the user must monitor the 15 minute test time themselves. It is suitable when running multiple tests concurrently.

Refer to the Biopanda Fluorescence Immunoassay Device Operation Manual for further details.

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

1. Turn on the Analyser. Then according to the user requirement, select "Standard test" or "Quick test" mode.
2. Take out the ID card and insert it into the Analyser port.
3. Pipette 75 µl of urine specimen into the specimen well of the test device. Start the timer at the same time.
4. Test results should be read at **15 minutes** with the use of the Biopanda Fluorescence Immunoassay Device (BR-FIA-2000).

INTERPRETATION OF RESULTS

The result of tests for L. pneumophila Ag is calculated by the Biopanda

Fluorescence Immunoassay Device and displays the result on the screen. For additional information, please refer to the user manual of the Biopanda Fluorescence Immunoassay Device.

NOTE: The test result of each specimen is given as Pos (+) or Neg (-) with a Reference Value. This Value is calculated that a measured signal is divided by an appropriate cutoff value.

- Test results of a Value ≥ 1.00 are considered positive for *Legionella pneumophila*.

- Test results of a Value < 1.00 are considered negative for *Legionella pneumophila*.

The Reference Value is not a quantitative value or the rate of antigens to *Legionella pneumophila* concentration. This is only a qualitative test.

QUALITY CONTROL

Each Biopanda L. pneumophila Ag FIA Rapid Test Device contains an internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by the Biopanda Fluorescence Immunoassay Device. An invalid result from the internal control causes an error message on the Biopanda Fluorescence Immunoassay Device indicating that the test should be repeated. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control 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.

LIMITATIONS

1. The L. pneumophila FIA Rapid Test Device is for *in vitro* diagnostic use only. The test should be used for the detection of *Legionella pneumophila* antigens in urine specimens only. The quantitative value of *Legionella pneumophila* antigens cannot be determined by this qualitative test.
2. The L. pneumophila FIA Rapid Test Device will only indicate the presence of *Legionella pneumophila* antigens in the specimen and should not be used as the sole criteria for the diagnosis of *Legionella pneumophila* infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of *Legionella pneumophila* infection.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The kit was evaluated on 96 clinical samples confirmed by commercial method. The results are tabulated as below:

		Other Rapid Test		
		Positive	Negative	**Total**
L. pneumophila FIA Rapid Test Device	Positive	33	0	33
	Negative	1	62	63
Total		34	62	96

Relative Sensitivity: 97.1% (95%CI*: 84.7%-99.9%)
Relative Specificity: >99.9% (95%CI*: 94.2%-100%)
Accuracy: 99.0% (95%CI*: 94.3%->99.9%) *Confidence Interval

Inter-Assay

Between-run precision has been determined by 3 independent assays on the same specimens: negative, low positive and high positive specimens. Three different lots of the L. pneumophila FIA Rapid Test Device have been tested using these specimens. The specimens were correctly identified >99% of the time.

Repeatability

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.

Cross-reactivity

Cross reactivity to urine spiked with the following pathogens has been tested and found 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)*	

REFERENCES

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

	Attention, see instructions for use		Tests per kit		Do not reuse
IVD	For in vitro diagnostic use only		Use by	REF	Catalogue #
	Store between 4-30°C	LOT	Lot Number		Consult instructions
	Do not use if package is damaged		Manufacturer		

Thank you for purchasing Biopanda's L. pneumophila Ag FIA Rapid Test Device. Please read this manual carefully before operating to ensure proper use.

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