BIOSYNEX® Legionella

Ref: 1030001

Rapid test for the qualitative detection of *Legionella pneumophila* serogroup 1 antigens in urine specimens

1. INTENDED USE

BIOSYNEX® Legionella test cassette is a rapid chromatographic immunoassay for the qualitative detection of *Legionella pneumophila* serogroup 1 antigens in human urine.
It aims to detect *Legionella pneumophila* antigens in urine specimens from patients with symptoms of pneumonia to aid in the presumptive diagnosis of a Legionella infection (Legionnaires' Disease) caused by *Legionella pneumophila* serogroup 1 in conjunction with culture and other methods.
Each device is designed for professional and *in vitro* diagnostic.

2. INTRODUCTION

The Legionnaires' disease is a serious form of pneumonia that carries with it a mortality rate in the order of 10-15% in otherwise healthy individuals. Symptoms include a flu-like illness, followed by a dry cough and frequently progress to pneumonia. Approximately 30% of people infected may also present with diarrhea and vomiting and around 50% may show signs of mental confusion. The incubation period normally ranges from 2-10 days with 3-6 days the typical illness onset time after exposure. Legionnaires' disease may present as an outbreak of two or more cases following a limited temporal and spatial exposure to a single source, as a series of independent cases in an area in which it is highly endemic or as sporadic cases without any obvious temporal or geographical grouping. Outbreaks have occurred repeatedly in buildings such as hotels and hospitals.
The BIOSYNEX® Legionella test allows for early diagnosis of *Legionella pneumophila* serogroup 1 infection through detection of a specific soluble antigen present in the urine of patients with Legionnaires' disease. *Legionella pneumophila* serogroup 1 antigen has been detected in urine as early as three days after the onset of symptoms. The test is rapid, giving a result within 15 minutes, and utilizes a urine specimen which is convenient for collection, transport, and subsequent detection of early, as well as later, stages of disease.

3. PRINCIPLE

The BIOSYNEX® Legionella test is a qualitative lateral flow immunoassay for the detection of Legionella pneumophila serogroup 1 antigen in human urine.
A pair of antibodies against *Legionella pneumophila* serogroup 1 antigens is used for the antigens detection. One is immobilized on the nitrocellulose membrane at the level of the T test line: it corresponds to the capture antibody. Another one is labelled with particles for the subsequent revelation.
During the sample migration, *Legionella pneumophila* serogroup 1 antigens if present in the sample, will form antigen-antibody complexes with the labelled antibodies. These complexes will be captured by the capture antibodies on the T test line, creating one colored line generated by nanoparticles.
The presence of a colored internal control line in the upper part of the membrane indicates that the result is valid and that the followed procedure is correct. It serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

4. PRECAUTIONS

- For professional *in vitro* diagnostic use only
- For single use only
- Do not use components after stated expiration date (see pouch and box label)
- Do not use the test if pouch is damaged. The test device should remain in the sealed pouch until use
- The test must be carried out within 2 hours after opening the sealed bag.
- Do not eat, drink or smoke in the area where the specimens or kits are handled
- Handle all specimens as if they contained infectious agents
- Observe established precautions for microbiological risks throughout all procedures and standard guidelines for appropriate disposal of specimens
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested
- Bring all reagents to room temperature (15-30°C) before use
- Do not touch the reaction area of the device to avoid contamination
- Store and transport the test device always at 2-30°C
- Do not mix reagents from different lots. Do not interchange solution bottle caps.

5. STORAGE AND STABILITY

Store as packaged in the sealed pouch at either refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

6. MATERIAL PROVIDED

- Devices
- Plastic pipettes
- Instructions for use
- Positive Control Swab: Inactivated L. pneumophila swab + Reagent Control (+) vial + testing tube

7. MATERIAL REQUIRED BUT NOT PROVIDED

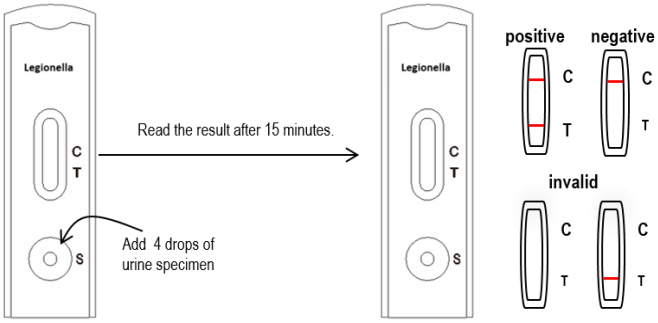
- Specimen collection container
- Disposable gloves
- Timer

8. SPECIMEN COLLECTION AND STORAGE

- Urine samples should be collected in standard containers.
- The samples 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 14 days or at -10°C to -20°C for longer periods before testing.
- Boric acid may be used as a preservative (in this case, the sample will be totally thawed, and brought to room temperature before testing).
- When necessary, urine specimens should be shipped in leak-proof containers at 2-8°C or frozen.
- Allow all specimens to reach room temperature before testing.

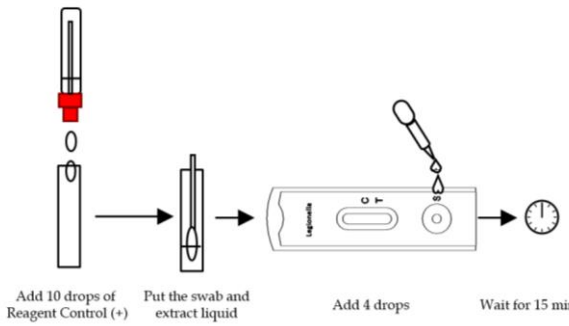
9. PROCEDURE

1. Bring the complete kit and urine samples to be tested to room temperature (15-30°C) prior to testing.
2. Remove the test from its pouch just before use. Use a separate test for each sample.
3. Using the disposable plastic pipette, dispense exactly 4 drops of the urine sample into the sample well. Start the timer.
4. Read the result at 15 minutes.



Procedure for Positive Swab Control. See illustration below:

1. Hold Reagent Control (+) vial vertically.
   Add slowly 10 drops of Reagent Control (+) is the testing tube
2. Immediately remove swab control from the pouch and put the swab into the testing tube with reagents.
3. Mix 1 minute and extract as much liquid possible from the swab, squeezing the sides of the tube as the swab is withdrawn. Discard the swab.
4. Remove the device test from its sealed bag just before using.
5. Place the test on a flat surface. Dispense exactly 4 drops from the testing tube, into the circular window. Start the timer. Read the result at 15 minutes.



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10. INTERPRETATION OF RESULTS

Caution: Do not take into account any appearance of colored trace below the level of the letter T. Consider only positive any pink colored cross line located at the level of the letter T.

NEGATIVE: Only one colored line appears at the level of the control line (C). No line appears at the level of the test T zone.

POSITIVE: Presence of 2 distinct colored lines: A control line appears at the level of the C zone and one colored line **(even of weak intensity)** appears at the level of the test T zone.

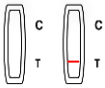
CONFIRMATION OF POSITIVE RESULTS

Any positive result must be confirmed according to the following protocol:

1. Heat the sample for 5 minutes at 100°C.
2. Centrifuge for 15 minutes at 1,000 g.
3. Take out a new test cassette. Using the disposable plastic pipette, collect the supernatant and transfer 4 drops into the sample well (S). Start the timer.
4. Read the result at 15 minutes.

- If the result is positive again, with the presence of C and T colored lines (even of weak intensity), then the positive result is confirmed.
  See the chapter "Recommended report" below for the presentation of results

- If the result is negative, with only the presence of the C colored line, then the result is in favour of a non-specific reaction on fresh urine.
  See the chapter "Recommended report" below for the presentation of results.



INVALID: No visible colored line at the level of control line C (whatever test line T apparition).
Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents 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 and contact your local distributor.

11. RECOMMENDED REPORT

- In case of negative result with the first test or after completion of the sample heating protocol: the test is presumed negative for *Legionella pneumophila* serogroup 1 antigen in the urine, suggesting no recent or current infection. Infection due to *Legionella* cannot be ruled out since other serogroups and species may cause disease, antigen may not be present in the urine in early infection, and the level of antigen present in the urine may be below the detection limit of the test
- In case of positive result confirmed by the realisation of the heating protocol of the sample: the test is presumed positive for *Legionella pneumophila* serogroup 1 antigen in the urine, suggesting current or past infection

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the colored test line in the result line region (T) will vary depending on the concentration of antigens in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

12. QUALITY CONTROL

- Internal procedural controls are included in the test. A colored line appearing in the control zone (C) ensures that sufficient specimen volume has been loaded and that the correct procedure has been followed by the operator.
- Good laboratory practices recommend the use of control materials to ensure proper kit performance. Control sample (Positive control swab) specific for this product is available within the kit.
- To use liquid urine controls, simply process as you would a patient sample. Positive controls should be tested once for each new test kit opened and as otherwise required by your laboratory's standard quality control procedures.

13. LIMITATIONS

1. As with all diagnostic tests, the test result must be consistent with clinical findings.
2. The BIOSYNEX® Legionella test has been validated using urine samples only. Other samples (e.g. plasma, serum or other body fluids) that may contain Legionella antigen have not been evaluated. The test cannot be used on environmental samples (i.e. drinking water).
3. This test will not detect infections caused by other L. pneumophila serogroups and by other Legionella species. A negative antigen result does not exclude infection with *Legionella pneumophila* serogroup 1. Culture is recommended for suspected pneumonia to detect causative agents other than *Legionella pneumophila* serogroup 1 and to recover *Legionella pneumophila* serogroup 1 when antigen is not detected in urine.
4. The diagnosis of Legionnaires' disease cannot be based on clinical or radiological evidence alone. There is no single satisfactory laboratory test for Legionnaires' disease. Therefore, culture results, serology and antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.
5. Excretion of Legionella antigen in urine may vary depending on the individual patient. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to 1 year afterwards. A positive BIOSYNEX® Legionella test result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.
6. Performance of the BIOSYNEX® Legionella test on diuretic urine has not been evaluated. The BIOSYNEX® Legionella test has been evaluated on hospitalized patients only. An outpatient population has not been tested.

14. EXPECTED VALUES

The disease occurs in both epidemic and endemic forms and sporadic cases are not easily differentiated from other respiratory infections by clinical symptoms. An estimated 25,000 to 100,000 cases of Legionella infection occur in the United States annually. The resulting mortality rate, ranging from 25% to 40%, can be lowered if the disease is diagnosed rapidly and appropriate antimicrobial therapy is instituted early.

15. PERFORMANCE CHARACTERISTICS.

Sensitivity and specificity

An evaluation was performed using the BIOSYNEX® Legionella test with urine specimens in comparison with other immunoassay (Binax NOW® Legionella Urinary Antigen, Alere) A sensitivity >99% and a specificity >99% were obtained.

Cross reactivity

An evaluation was performed to determine the cross reactivity of the BIOSYNEX® Legionella test. There is no cross reactivity with other pathogens occasionally present in urine:

- *Streptococcus pneumonia*

16. REFERENCES

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4. Bibb, W.F., P.M. Arnow, L. Thacker, and R.M. McKinney. Detection of soluble Legionella pneumophila antigens in serum and urine specimens by enzyme-linked immunosorbent assay with monoclonal and polyclonal antibodies. J. Clin. Microbiol. 1984;20:478-482.
5. Tang, P.W., and S. Toma. Broad-spectrum enzyme-linked immunosorbent assay for detection of Legionella soluble antigens. J. Clin. Microbiol. 1986;24:556-558.
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SYMBOLS

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
2°C 30°C	Store between 2-30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
	Expiry		

IFU_1030001_EN_V04201902R01
Last date of revision : 02-2019

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