LEGIONELLA PNEUMOPHILA PLUS - CARD

For in *Vitro* diagnostic use only

Immunochromatographic CARD test for the qualitative detection of Legionella Pneumophila serogroup 1 antigen in urine specimens

I. INTENDED USE

Legionella pneumophila Plus Card Mascia Brunelli is an in vitro rapid immunochromatographic assay for the qualitative detection of Legionella pneumophila serogroup 1 in urine specimens from patients with symptoms of pneumonia. It is intended to aid in the presumptive diagnosis of Legionella infection (Legionnaires' Disease) caused by Legionella pneumophila serogroup 1 in conjunction with culture and other methods. The kit including a positive control for Legionella.

II. SYNTHESIS

The species most frequently involved in human cases is Legionella pneumophila. 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, which can reach up to 30-50% in the case of hospital infections. It is presented as acute pneumonia indistinguishable from other forms of acute respiratory infections of the lower airways. The incubation period normally ranges from 2-10 days with influenza-like disorders such a illness, myalgia and headache followed by high fever, cough, wheezing and common symptoms to other forms of pneumonia. Sometimes there may be complications such as lung abscess and respiratory failure. In addition, extra-pulmonary symptoms may appear useful, in aid to diagnose such neurological, renal and gastrointestinal manifestations.
Pontiac Fever is a mild flu-like illness, is presented as a self-limiting acute illness that not affected the lung: after incubation time of 24-48 hours appear fever, malaise, myalgia, headache and sometimes cough and sore throat. The first outbreak of Pontiac fever was caused by serogroup 1 L.pneumophila while subsequent outbreaks have been attributed to L. feeleii, L.anisa and L.micdadei.
Infection can also occur in subclinical form, ie without clinical symptoms, and the infection is evident only with the detection of antibodies to Legionella spp, in the absence of episodes of pneumonia and/or flu-like forms. 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 Legionella pneumophila Card Mascia Brunelli 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.

III. PRINCIPLE OF THE TEST

The Legionella pneumophila Plus Card Mascia Brunelli is an immunochromatographic membrane assay to detect Legionella pneumophila serogroup 1 soluble antigen in human urine. Anti-Legionella pneumophila serogroup 1 antibody, the test line, is adsorbed onto nitrocellulose membrane. Antibodies of the control line were adsorbed onto the same membrane as a second band. Anti-Legionella pneumophila serogroup 1 antibodies are conjugated to visualizing particles that are dried onto an inert absorbent support.
During testing the sample is allowed to react with the conjugate which was pre-adsorbed on the CARD test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. A positive test result is read visually in 10-15 minutes or less depending on the concentration of antigen present in the urine specimen. A negative Legionella pneumophila result, read in 15 minutes, indicates that L. pneumophila serogroup 1 antigen was not detected in the urine sample.
The test is interpreted by the presence or absence of visually detectable redish colored lines. A positive result will include the detection of both a patient and a control line, while a negative assay will produce only the control line. Failure of the control line to appear, whether the patient line is present or not, indicates an invalid assay.

IV. STORAGE AND STABILITY

Store as packaged in the sealed pouch the devices and the reagents tightly capped, either at refrigerated or room temperature (2-30ºC). The test is stable through the expiration date printed on the sealed pouch/labels. Do not use after expiration date.

V. REAGENTS AND MATERIALS

Each kit contains:
1. **Legionella pneumophila card** (25 devices): the devices are in a sealed pouch containing a dissecant and a plastic dropper.
2. **Extraction buffer** (1 bottle x 2,5 mL)
3. **Positive Control**: N.1 vial with dropper containing non-infectious components, sodium azide (NaN_3) as preservative (1x0.5 mL).
4. **Negative control**: use the extraction buffer
5. **Test tubes**
6. **Instruction for use** (1 item)

Required materials (not supplied)

- Urine specimen collection container, Disposable gloves, Timer

VI. SPECIMENS COLLECTION

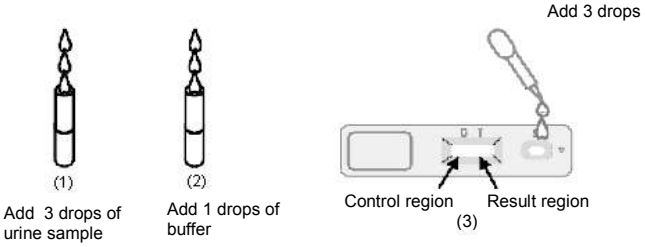
Urine specimens should be collected in standard containers. The samples can be stored at room temperature (15-30°C/59-86°F) if assayed within 24 hours of collection. Alternatively, specimens may be stored at 2-8°C for up to 14 days or at -10°C to -20°C for longer periods before testing.
When necessary, 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.

VII. PROCEDURE

Allow the tests, samples and reagents to reach to room temperature prior to testing.

1. Use a separate testing tube or vial for each sample. Add 100-150 µL (3 drops) of urine sample, add 50 µL (1 drops) of Buffer into the testing tube or vial and mix. (Fig. 1 and 2)
2. With the plastic dropper dispense 3 drops of the extracted sample (100-150 µL) into the sample well (S) of the card. Start the timer. (Fig. 3)
3. Wait 15 minutes and observe the colored lines in the windows, indicated with " T" and "C".

VIII. PROCEDURE FOR CONTROLS: for the Positive and Negative Control use the same procedure (from step 2 onwards).



IX. INTERPRETING THE RESULTS

Positive: two colored bands appear across the central window. One blue in the control region (C) and one red in the test region (T). This means that the antigen was detected. Presumptive positive for L. pneumophila serogroup 1 antigen in urine, suggesting current or past infection.
Negative: only one blue colored band appears across the central window in the control region (C). No apparent colored band in the test region (T). Presumptive negative for L. pneumophila serogroup 1 antigen in 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 urine in early infection, and the level of antigen present in the urine may be below the detection limit of the test.
Invalid: a total absence of the control coloured band (C) across the central window, regardless the appearance or not of the test line (T). 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.

Positive **Negative** **Invalid**

X. 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.

XI. PRECAUTIONS

- The kit is for in vitro diagnosis only
- The card is in sealed pouch. Do not use the test if pouch is damaged. The test should remain in the sealed pouch until use. Do not touch the reaction area on the strip
- Do not use components past its expiration date
- Do not mix components from different kit lots
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. Observe established precautions against microbial hazards.

XII. PERFORMANCE

Sensitivity and specificity
The Legionella pneumophila Plus Card has a sensitivity > 99% of concordant result with Binax Now ® kit present on the market, on a casuistry of 63 frozen urine specimen and 9 fresh specimen coming from European Hospitals. The sensibility obtained on strain ATCC 33152 is of approximately 3000 CFU/mL.
Specificity > 99% of concordant result with kit Binax Now ®
Cross-reaction
No cross reaction was observed with positive urine specimen for Klebsiella pneumonia, Enterobacter and E.Coli.
Precision
Precision was determined by using 5 replicates of Legionella p. controls either negative and positive. The negative and positive values were correctly identified 100% of time.

XIII. LIMITS OF THE KIT

1. Legionella pneumophila Plus Card Mascia Brunelli 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. potable water).
2. 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 L. pneumophila serogroup 1. Culture is recommended for suspected pneumonia to detect causative agents other than L. pneumophila serogroup 1 and to recover L. pneumophila serogroup 1 when antigen is not detected in urine.
3. The diagnosis of Legionnaires' disease cannot be based on clinical or radiological evidence alone. There is no single satisfactory laboratory test for Legionnaires's disease. Therefore, culture results, serology and antigen detection methods should be used in conjunction with clinical findings to make an accurate diagnosis.
4. 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 Legionella pneumophila Card result can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.
5. Performance of the Legionella pneumophila Plus Card Mascia Brunelli on diuretic urine has not been evaluated.
6. The Legionella pneumophila Plus Card Mascia Brunelli has been evaluated on hospitalized patients only. An outpatient population has not been tested.
7. Although the test is compatible with boric acid, its presence increases the reactivity and can lead to false positives, so it is not recommended as a preservative.

XIV. REFERENCES

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2. Berdal, B.P., C.E. Farshy, and J.C. Feeley. Detection of Legionella pneumophila antigen in urine by enzyme-linked immunospecific assay. J. Clin. Microbiol. 1979;9:575-578.
3. White A., et al. Rapad diagnosis of Legionnaires' disease. Trans Am Clin. Climatol. Assoc.l 1982;93:50-62
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.
6. Kohler, R.B., W.C. Winn, Jr., and L.J. Wheat. Onset and duration of urinary antigen excretion in Legionnaires' disease. J. Clin. Microbiol. 1984;20:605-607.
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IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (25 tests)	**REF. VQ84100P**
Legionella pneumophila card	25 Devices (Card)
Extraction buffer	1 x 2,5 mL
Positive control	1x0.5 mL
Test tubes	25 items
Instruction for use	1 item

EDMA (EDMS) CODE 1501050100 CE IVD

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