INSTRUCTIONS FOR USE

LEGIONELLA PNEUMOPHILA CARD

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR THE QUALITATIVE DETECTION OF *LEGIONELLA PNEUMOPHILA SEROGROUP 1* ANTIGEN IN URINE SPECIMENS

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

Legionnaires' Disease, named after the outbreak in 1976 at the American Legion convention in Philadelphia, is caused by *Legionella pneumophila* and is characterized as an acute febrile respiratory illness ranging in severity from mild illness to fatal pneumonia. 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. Known risk factors include immunosuppression, cigarette smoking, alcohol consumption and concomitant pulmonary disease. The young and the elderly are particularly susceptible. *Legionella pneumophila* is responsible for 80-90% of reported cases of Legionella infection with serogroup 1 accounting for greater than 70% of all legionellosis. Current methods for the laboratory detection of pneumonia caused by *Legionella pneumophila* require a respiratory specimen (e.g. expectorated sputum, bronchial washing, transtracheal aspirate, lung biopsy) or paired sera (acute and convalescent) for an accurate diagnosis.

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.

2 - PRINCIPLE OF THE METHOD

Legionella Pneumophila Card is an non-invasive, simple to perform, rapid and very accurate immunochromatographic method for the determination of *Legionella pneumophila* serogroup 1 in urine samples.

The strip consists of a nitrocellulose membrane pre-coated with polyclonal antibodies on the test line (T), in the results window, against *Legionella pneumophila* (*L. pneumophila*) and with rabbit polyclonal antibodies, on the control line (C), against a specific protein. The label/sample absorbent pad is sprayed with test label solution (polyclonal antibodies anti-*L. pneumophila*) conjugated to red polystyrene latex and control label solution (specific binding protein) conjugated to blue polystyrene latex, forming coloured conjugate complexes.

If the sample is positive, the antigen of the diluted sample reacts with the red-coloured conjugate complex (anti-*L. pneumophila* polyclonal antibodies-red polystyrene microspheres) which was previously pre-dried on the absorbent pad. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the binding conjugate complexes migrate. The anti-*L. pneumophila* antibodies present on the membrane (test line) capture the coloured conjugate and the red line will be visible. This band is used to interpret the result.

If the sample is negative, there is no *L. pneumophila* antigens presence and yet, the antigen may be present in a concentration lower than the detection limit value, for which the reaction will not take place with the red-coloured conjugate complex. The anti-*L. pneumophila* antibodies present on the membrane (test line) will not capture the antigen-red-coloured conjugate complex (not formed), for which the red line will not appear. Whether the sample is positive or not, the mixture continues to move across the membrane to the immobilized specific antibodies placed in the control line. The anti-specific protein antibodies present on the membrane will capture control blue-conjugate complex and the control line will always appears. The presence of this blue line serves as: 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) an internal control for the reagents.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Legionella Pneumophila Card	Immunochromatographic test	VQ84100 (25 tests)	25 sealed in foil pouch containing the device, with dessicant and one plastic dropper. 1 glass bottle with dropper tip containing the diluent. (1 x 2,5 mL). 25 plastic testing tubes. Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container, Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Legionella Pneumophila Card is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- This product is not classified as dangerous according to current European legislation.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test device is for single use only.
- Never use reagents from another lot.
- The test should remain in the sealed pouch until use, and the test must be carried out within 2 hours of opening the sealed bag.
- Do not use the test if pouch is damaged.
- Wear gloves when handling the sample.
- Disposable gloves, diluent, test tubes, and used devices in a propre biohazard container.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- If the device contains raw materials of animal origin. The raw material involved is derived from animals that have been slaughtered in an authorized slaughterhouse and, following an antemortem inspection, which have not shown any sign of disease transmissible to humans or animals. In any case is recommended that the kit be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes.

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- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - Storage conditions and shelf life

Upon receipt, store the kit in their original pack at refrigerated or room temperature (2-30°C/36-89°F). If properly stored, the kit may be used up to the expiration date. The device test must remain in the sealed pouch until use. Do not use the device test after 2 hours of opening sealed-bag. Do not freeze.

7 – Specimens collection and Preparation

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.

8 - Test procedure

Allow the tests, samples and diluent to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

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 Diluent into the testing tube or vial and mix. (Fig. 1 and 2)
2. Remove the test card from the protective pouch. Identify the plastic cassette with the patients data.
3. 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)
4. Read the result at 15 minutes after dispensing the sample. Do not exceeded *15* minutes.

9 - Reading and interpretation

Interpret the results as follow:

	Legionella	Interpretation of results
1.	-	There is no *Legionella pneumophila* presence. No infection caused by *L. pneumophila* serogroup 1. Negative result.
	BLUE	
2.	+	There is *Legionella pneumophila* presence. Infection caused by *L. pneumophila* serogroup 1. Positive result.
	BLUE-RED	
3.	ANY OTHER RESULTS	Invalid result, we recommend repeating the assay using the same sample with another test.

INVALID: Total absence of any control coloured line (BLUE) regardless the appearance or not of the test line (RED). Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are mostly the main reasons for control lines failure. Review the procedure and repeat the assay with a new test. If the symptoms or situation still persists, discontinue using the test kit and contact your local distributor.

Notes on the interpretation of results

The intensity of the red coloured band in the test line (T) in the results windows will vary depending on the concentration of antigens present in the specimen. However, neither the quantitative value nor the rate of increase in antigens can be determined by this qualitative test.

10 - Internal quality control

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

11 – Expected values

More than 50 *Legionella* species have been characterized, and 25 species are known to cause human disease. Most human infections are caused by *Legionella pneumophila*, which is responsible for approximately 90% of the identified clinical cases, and the predominant serogroup is serogroup 1.
In recent years, hot springs have been recognized as a major source of outbreaks of legionellosis. Several outbreaks and a number of cases of *Legionella* infection that were associated with hot springs have been reported in Japan, Spain and France.

12 - Performances characteristics

A. Analytical sensitivity (detection limit)

Detection limit value is: 12.5 ng/mL. (pool of several serovars of *Legionella pneumophila*).

B. Clinical sensitivity and specificity

An evaluation was performed, with urine samples (149 samples), comparing the results obtained by an immunochromatographic test (Legionella Pneumophila Card Mascia Brunelli) and other commercial rapid test (Binax Now ® Legionella Urinary Antigen, Alere).
The results were as follows:

Sensitivity 100,0% (88,8-100,0%) **Specificity 99,2% (95,4-100,0%)** **PPV 96,9% (83,8-99,9%)** **NPV 100,0% (96,9-100,0%)**

C. Cross reaction

An evaluation was performed to determine the cross reactivity of Legionella Pneumophila Card Mascia Brunelli; no cross reactivity against other pathogens occasionally present in urine: *Streptococcus pneumoniae*.

D. Reproducibility Study

Evaluation studies were performed to determine reproducibility of the Legionella Pneumophila Card Mascia Brunelli, including inter-day, inter-laboratory, inter and intra lot, showing high reproducibility in all cases.

13 - LIMITATIONS OF THE METHOD

- Legionella Pneumophila Card should only be used with human urine samples. The use of other samples (e.g. plasma, serum or other body fluids or environmental samples (water)) has not been established.
- A positive result determine the presence of *L. pneumophila* serogroup 1 in urine samples. A positive result should be followed up with additional laboratory techniques (culture or serology) to confirm the results. A confirmed infection should only be made by a physician after all clinical and laboratory findings have been evaluated and must be based in the correlation of the results with further clinical observations.
- A negative result is not meaningful because of it is possible the antigens concentration in the urine sample is lower than the detection limit value. If symptoms or situation still persist, a *L. pneumophila* serogroup 1 determination should be carried out from a culture.
- 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 result with this test can occur due to current or past infection and therefore is not definitive for infection without other supporting evidence.
- It has been proven that 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.
- Performance of the Legionella Pneumophila Card Mascia Brunelli on diuretic urine has not been evaluated. The Legionella Pneumophila Card Mascia Brunelli has been evaluated on hospitalized patients only. An outpatient population has not been tested.

14 - REFERENCES

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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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TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 4	Updated layout and content	2023/03

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.