ECOTEST **LEG-U23** **Legionella Antigen Rapid Test Device (Urine)**

For professional in vitro diagnostic us.

INTENDED USE

Legionella Antigen Rapid Test device is an in-vitro rapid immunochromatographic assay for the qualitative detection of Legionella pneumophila antigen (L. pneumophila antigen) 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 L. pneumophila in conjunction with culture and other methods.

INTRODUCTION

Legionnaires' disease is a serious form of pneumonia tat 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 diarrhoea 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 Legionella Antigen Device allows for early diagnosis of Legionella pneumophila infection through detection of a specific soluble antigen present in the urine of patients with Legionnaires' disease. Legionella pneumophila antigen has been detected in urine as early as three days after the onset of symptoms. The test is rapid, giving a result within 15minutes, and utilizes a urine specimen which is convenient for collection, transport, and subsequent detection of early, as well as later, stages of disease.

PRINCIPLE

The Legionella antigen Device is an immunochromatographic membrane assay to detect Legionella pneumophila soluble antigen in human urine. Anti-Legionella pneumophila 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 antibodies are conjugated to visualizing particles that are dried onto an inert absorbent support.

During testing the sample is allowed to react with conjugate which was pre-adsorbed on the strip 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. L. pneumophila urinary antigen captured by immobilized anti-.L. pneumophila antibody reacts to bind conjugated antibody.The other immobilized antibodies also captures visualizing conjugate, forming the control line. 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 pneumopila Device result, read in 15 minutes, indicates that .L. pneumophila urinary antigen was not detected in the urine sample.

The test is interpreted by the presence or absence of visually reddish color lines. A positive result will include the detection of both a test and control line, while a negative assay will produce only the control line. The control line does not appear, whether the test line is present or not, indicates an invalid assay.

MATERIALS

Materials Provided

- Individually packed test devices
- Disposable pipettes
- Package insert

Materials Required but Not provided

- Timer
- Specimen collection container

PRECAUTIONS

- 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 tests.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
- Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
- Read the entire procedure carefully prior to testing.
- Do not eat, drink or smoke in any area where specimens and kits 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 the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.
- Used testing materials should be discarded according to local regulations.

STORAGE AND STABILITY

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

SPECIMEN COLLECTION AND STORAGE

- The Legionella Antigen Rapid Test Device (Urine) is intended for use with human urine specimens only.
- Although urine specimens from any time of day can be used, first morning urine specimens are preferred as they contain the highest concentration of Legionella pneumophila.
- Turbid specimens should be centrifuged, filtered, or allowed to settle and only the clear supernatant should be used for testing.
- Urine specimens must be collected in clean, dry containers. Ensure that the volume of specimen collected is sufficient to submerge the dip region of the strip.
- Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 48 hours. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.

PROCEDURE

Do not remove device test from pack until test sample has reached room temperature.

Bring patient urine and/or liquid urine control(s) to room temperature (15-30℃ /59-86 °F)

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. For best results the assay should be performed within one hour.
2. Add 2 drops of specimen (approximately 80 µL) directly into the specimen well (S) and start the timer.

   Avoid trapping air bubbles in the specimen well (S), and do not add any solution to the result area.

   As the test begins to work, color will migrate across the result area in the center of the device.
3. Wait for the colored band(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 20 minutes.

NOTE: Low LEG concentrations may produce very weak T lines after a prolonged period of time. Therefore, do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

NEGATIVE: Only one colored band appears, in the control region (C). No apparent colored band appears in the test region (T).

INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test, and cannot determine the concentrations of analytes in specimens.
2. Insufficient specimen volume, incorrect operation procedure or expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. 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 OF THE TEST

1. Legionella antigen Device 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.
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.Culture is recommended for suspected pneumonia to detect causative agent other than L. pneumophila and to recover L. pneumophila 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 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.
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 antigen Device 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 antigen Device on diuretic urine has not been evaluated. The Legionella antigen Device has been evaluated on hospitalized patients only. An outpatient population has not been tested.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

The study was performed on 351 negative specimens (EIA confirmed) and 254 positive specimens (EIA confirmed) have been tested in the assays.

Relative Sensitivity:
98.8% (96.6%-99.6%) *
Relative Specificity:
98.6% (96.7%-99.4%) *
Overall Agreement:
98.7% (97.4%-99.3%) *
***95% Confidence Interval**

		Legionella Test		
		+	-	Total
EIA test	+	251	3	254
	-	5	346	351
Total		256	349	605

LITERATURE REFERENCES

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2. White. A, et al. Rapid diagnosis of Legionnaires' disease. Trans Am Clin. Climatol. Assoc. 1 1982;93:50-62
3. 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.
4. Tang, P.W and S. Toma. Broad-spectrum enzyme-linked immunosorbent assayfor detection of Legionella soluble antigens.J.Clin.Microbiol.1986;24:556-558
5. 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.

GLOSSARY OF SYMBOLS

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	In vitro diagnostic medical device		Use by
	Manufacturer		Contains sufficient for <n> tests
	Do not reuse	EC REP	Authorized representative in the European Community
CE	CE marking according to IVD Medical Devices Directive 98/79/EC		

ECOTEST

CE

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