

EZER™ Legionella Antigen Rapid Test Device

This kit is designed for testing freshly collected urine samples.

INTENDED USE

The EZER™ Legionella Antigen Rapid Test Device is an *in vitro* diagnostic test based on the principle of immunochromatography for the qualitative detection of *Legionella pneumophila* serogroup 1 LPS antigen in urine specimens. It is intended to aid in the rapid differential diagnosis Legionella infection.

BACKGROUND

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 extrapulmonary 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. *Legionella pneumophila* is responsible for approximately 90% of infections, and of these, over 80% are due to a single serogroup, serogroup 1. *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.

Diagnosis of legionellosis can be difficult because signs and symptoms are non-specific 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

This product is a test plate consisting of three parts: the lower part of the sample, the reagent part and the expansion part, and has a long square-shaped carrier. The reagent part comprises a latex microspheres-labeled anti *Legionella pneumophila* serogroup 1 monoclonal antibody A (mouse) (hereinafter referred to as colloidal gold-labeled antibody A); and the expanded part comprises an immobilized monoclonal antibody B (rat) of *Legionella pneumophila* serogroup 1 (hereinafter referred to as Anti-*Legionella pneumophila* serogroup 1 antibody B) and murine IgG polyclonal antibody (hereinafter referred to as anti-mouse immunoglobulin antibody).

After the sample is dropped from the lower portion of the sample plate of the test plate, the latex microspheres - labeled antibody A is dissolved to form an immune complex with the *Legionella pneumophila* serogroup 1 antigen in the sample. The immune complex moves due to the capillary phenomenon of the developing portion, and is captured by the anti-*Legionella pneumophila* serogroup 1 antibody B immobilized in the Testing portion [T], so that the Testing portion [T] forms a purple-red band due to the action of the latex microspheres. This purple-red band of the kit can be visually determined to determine the presence of *Legionella pneumophila* serogroup 1. In the on the other hand, regardless of whether *Legionella pneumophila* serogroup 1 is present in the sample, the remaining latex microspheres-labeled antibody A continues to move in the developing portion, and is captured by the anti-mouse immunoglobulin antibody immobilized in the Control portion [C], and purple-red band is formed by latex microspheres. This shows the normal movement of the latex microspheres marker antibody.

CONTENTS

Test devices (40), Transfer pipette (40), Package insert (1)

STORAGE CONDITIONS

Test devices must be stored at 2~30°C. DO NOT FREEZE. Devices must be brought back to room temperature at time of testing.

WARNINGS AND PRECAUTIONS

1. For in vitro Diagnostic Use
2. Pathogenic microorganisms may be present in clinical specimens. All specimen and the related contaminated items need to be handled, stored and disposed following "Standard Precautions" and institutional guidelines.
3. Do not use kit components beyond the expiration date.
4. Apply universal precaution when performing the test.
5. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
6. Please do not touch the sample drop and the judgment part of the test board directly by hand.
7. Do not reuse the device.
8. Use a clean, sterile pipette tip for each sample.
9. Please ensure that an appropriate amount of samples are used for testing. Too much or too little sample size may lead to deviation of results.
10. If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
11. Read the result at 15 min, or up to 30 min. Do not read the result longer than 30 min, because the result might be altered if the device is dry out.
12. All samples and used accessories should be treated as infectious and discarded according to local regulations.

SAMPLE COLLECTION AND PREPARATION

1. Applicable samples: Urine.
2. Urine
 - Take the mid-stream urine. Note: When taking a urinary sample, first remove the previous urination and leave the mid-stream urine. The last urination should not be retained. Collect urine in a standard container.
 - After collection, if measured within 24 hours, store at room temperature (15~30 °C). In addition, the urine is stored at 2~8°C or stored frozen for 14 days. Boric acid can be used as a preservative. If necessary, urine samples can be transported in leak proof containers at 2~8 °C or in frozen conditions.
3. Note on sample processing:

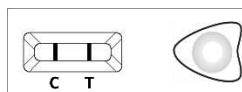
- Since removing the cellular components will reduce the sensitivity, do not centrifuge before performing this test.
- All samples are at risk of infection, please be careful when handling.

PROCEDURE

1. Return the reagent plate to room temperature 15~30 °C before use.
2. Open the foil pouch and take out the reagent plate. Once opened, test immediately. Write down the patient's name or number on the reagent plate. (One patient per reagent plate)
3. Mix the samples first.
4. Slowly aspirate 80 µL or more of the specimen in a single collecting action using the quantitative transfer pipette. Dispense the full volume of the specimen onto the sample well of the cassette in a single pipetting action. After dispensing, excess specimen remains inside the pipette.
5. After 15 minutes, observe the judgment section of the test board.

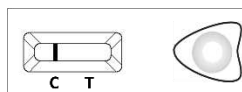
INTERPRETATION OF RESULTS

Allow the samples to react according to the procedure and read the red purple lines that appear in the reading area.



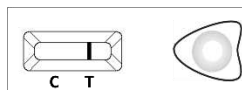
Positive

Two red lines appear. One red line appears in the control region (C), and one red line in the test region (T). This indicates that the specimen contains detectable amount of *Legionella pneumophila* serogroup 1 antigen. The shade of color may vary, but it should be considered positive whenever there is even a faint line.



Negative

Only one reddish band appears on control region of the device. No reddish line is visible next to the Test "T". This indicates that there is no detectable *Legionella pneumophila* serogroup 1 antigen in the sample.



Invalid Result

No red line appears in the control region (C). The test is invalid even if there is a line in the region (T). Review testing procedures and repeat the test using a new rapid test device.

REPORTING OF RESULTS

Positive Test Positive for the presence of *Legionella pneumophila* serogroup 1 antigen.

Negative Test Negative for the presence of *Legionella pneumophila* serogroup 1 antigen. Infection due to *Legionella pneumophila* serogroup 1 cannot be ruled-out because the antigen present in the sample may be below the detection limit of the test. Culture confirmation of negative samples is recommended.

Invalid Test Test result is inconclusive. Do not report results.

PRECAUTIONS FOR ASSESSMENT

1. Assessment must be conducted exactly 15 minutes after starting the reaction. Given the nature of the measurement (immunochromatography), the reaction and color development may slightly continue and progress even after 15 minutes.
2. The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid as long as a red line is present. Occasionally, broken lines may appear, but the test result is valid as long as a red line is present.
3. If the line is not red at all (e.g., black), the test result is invalid and another test should be performed.
4. If the specimen is dark-colored, it may stain the membrane and affect the assessment.
5. A highly viscous specimen may affect sample migration and/or the reaction, resulting in weak coloration, delayed or no formation of the line, or a nonspecific reaction because of specimen retention.
6. If invalid results are repeatedly obtained for the same specimen, centrifuge the specimen (15,000g, 10 minutes) and use 100 µL of its supernatant as the sample. This may facilitate obtaining valid test results.

QUALITY CONTROL

Internal control:

Each *EZER*TM *Legionella* antigen rapid test device contains internal/procedural controls. The appearance of a control line at the Control "C" position validates the proper reagent function and assures that the correct test procedure was followed.

PERFORMANCE CHARACTERISTICS

Minimum detection limit

This kit shows positive results when the tests are performed following the specified procedures with positive quality standard solution containing extract of *Legionella pneumophila* serogroup 1, ATCC33152 (5.0x10⁴ CFU/mL).

Cross-reactivity evaluation

1. Fungi and bacteria, with the exception of *Legionella* spp.

No crossreactivity was observed with the following bacteria and fungi:

Escherichia coli, *Candida albicans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Streptococcus viridans*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus equi*, *Streptococcus dysgalactiae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Bordetella pertussis*, *Salmonella enterica*, *Filobasidiella neoformans*, *Staphylococcus epidermidis*, *Streptococcus bovis*, *Enterococcus faecium*.

2. *Legionella*

No cross-reactivity was observed with *Legionella* spp., with the exception of *Legionella pneumophila* serogroup 1

Sample	<i>Legionella</i> concentration (CFU/mL)	
	1.0x10 ⁸	1.0x10 ⁵
<i>Legionella pneumophila</i> SG 1	Positive	Positive
<i>Legionella pneumophila</i> SG 3	Negative	Negative
<i>Legionella pneumophila</i> SG 4	Negative	Negative
<i>Legionella pneumophila</i> SG 5	Negative	Negative
<i>Legionella pneumophila</i> SG 6	Negative	Negative
<i>Legionella pneumophila</i> SG 7	Negative	Negative
<i>Legionella pneumophila</i> SG 8	Negative	Negative
<i>Legionella pneumophila</i> SG 9	Negative	Negative
<i>Legionella pneumophila</i> SG 10	Negative	Negative
<i>Legionella pneumophila</i> SG 11	Negative	Negative
<i>Legionella pneumophila</i> SG 12	Negative	Negative
<i>Legionella pneumophila</i> SG 13	Negative	Negative
<i>Legionella pneumophila</i> SG 14	Negative	Negative
<i>Legionella pneumophila</i> SG 15	Negative	Negative

Clinical Studies

The performance of the *EZER*TM *Legionella* antigen rapid test device was compared to another immunochromatographic assay method (IMMUNOCATCHTM *Legionella* from EIKEN CHEMICAL CO., LTD.) at one clinical site. A total of 123 clinical specimens from suspect *Legionella* infection patients were evaluated with the two tests.

Table 1: Performance Summary of the *EZER*TM

Legionella antigen rapid test device test compared to IMMUNOCATCHTM *Legionella* with Percent Agreements

		IMMUNOCATCH TM <i>Legionella</i>		
		+	-	Total
<i>EZER</i> TM <i>Legionella</i> antigen rapid test device	+	43	0	43
	—	3	77	80
	Total	46	77	123

Positive result consistency: 93.5% (43/46)

Negative result consistency: 100.0% (77/77)

Overall result consistency: 97.6% (120/123)

INTERFERING SUBSTANCES

Use the influences of glucose (4000 mg/dL), sodium ascorbate (1000 mg/dL), serum albumin (1000 mg/dL), urea (3000 mg/dL), which showed no influence on the assessment results. If the whole blood content in the hematuria sample is $> 0.5\%$, the reagent strip will not flow or affect the interpretation, resulting in incorrect results.

LIMITATIONS OF THE PROCEDURE

1. This kit is a qualitative test and cannot determine the amount of antigen in the sample.
2. *Legionella pneumophila* serogroup 1 LPS antigen excreted into the urine reaches the detection limit 3 days after the onset of symptoms, but several weeks may be required in some cases. The assessment results must be interpreted while considering clinical symptoms and history.
3. Do not use this kit to predict therapeutic effects because *Legionella pneumophila* serogroup 1 LPS antigen may be excreted into the urine even after healing in some cases.
4. If any bacteria possess a common antigen with *Legionella pneumophila* serogroup 1, such as *S. mitis*, or its antigen is present in the specimen, the assessment result may be false-positive.
5. In some cases, *Legionella pneumophila* serogroup 1 antigens are excreted into urine the day after vaccination, causing the assessment result to be false-positive. The test should not be performed within 6 days after vaccination.

AVAILABILITY

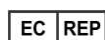
Product	Cat. No.	Contents
EZER™ Legionella antigen rapid test device	P241110	40 Test

REFERENCES

1. Quinn C., et al: Journal of Environmental Health. 78(5):8–13, 2015.
2. Kozak-Muiznieks NA, et al.: Journal of Clinical Microbiology. 52:201–211. 2014.

Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For <i>in vitro</i> diagnostic use only		Use by		Do not reuse
	Store between 2–30°C		Lot Number		Catalog #



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