



DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



See external label

2°C



8°C



96 tests



8305-3

Legionella Urinary Antigen ELISA

REF 8305 –3

Test	Legionella Urinary Antigen ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Sandwich; Antibody Coated Plate
Detection Range	Qualitative Positive; Negative control
Sample	100 µL Urine Sample
Specificity	95%
Sensitivity	100%
Total Time	~ 100 min
Shelf Life	12 Months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended Use

DAI Legionella Urinary Antigen test is intended as an adjunct to culture for the presumptive diagnosis of past or current Legionnaires' disease by qualitative detection of *Legionella pneumophila* antigen in human urine.

Introduction

Legionella pneumophila was first isolated and characterized in 1976 after a major outbreak of pneumonia at an American Legion convention in Philadelphia, Pennsylvania; thus the name "Legionnaires' Disease". Traditional laboratory methods for the detection of pneumonia caused by *Legionella pneumophila* infection requires an adequate respiratory specimen (e.g. sputum, bronchial washings, transtracheal aspirate, lung biopsy) or paired serum specimens (acute and convalescent) for accurate diagnosis. Accurate diagnosis of *Legionella pneumophila* using these methods depends upon proper specimen collection and the level of technical expertise available. Patient compliance in obtaining the specimens can be poor, and serological methods are retrospective in nature. Approximately 80% of Legionella pneumonia patients excrete soluble Legionella antigen in their urine. This presents the opportunity for rapid detection of Legionella Urine Antigen (LUA) in a urine specimen. Urine is the preferred specimen for collection, transport, and detection in all phases of the disease. Specific soluble Legionella antigen is present in the urine of patients with Legionnaires' disease.

Principle

The DAI Legionella Urine Antigen ELISA is designed to detect Legionella antigen in Human Urine sample. This assay is a double antibody (sandwich) ELISA using an anti-*Legionella pneumophila* antibody to capture the antigen from the urine. A second antibody, conjugated to peroxidase (HRP), is then added which binds to the complex. This reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *Legionella pneumophila* antigens being bound by the anti-*Legionella pneumophila* antibodies.

Reagents / Materials

(A) Included with kit

Item	Description	Symbol
Test Strips	Microwells containing purified rabbit anti- <i>Legionella pneumophila</i> IgG antibodies.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11ml of purified rabbit anti- <i>Legionella pneumophila</i> IgG antibody conjugated to HRP with red dye and Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted <i>Legionella pneumophila</i> antigen in buffer with Thimerosal.	CONTROL +
Negative Control	One (1) vial containing 2 ml of dilution buffer with Thimerosal.	CONTROL -
Chromogen	One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Wash Concentrate (20X)	One (1) bottle containing 25 ml of concentrated buffer and surfactant.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

(B) Not included with kit

- Distilled or deionized grade water, graduated cylinder, Pipettes, Absorbent paper towel
- Squeeze bottle for washing strips (narrow tip is recommended)

(Optional)

- Microplate washer (*Diagnostic Automation Cat # DAX 50*)
- Plate reader Bichromatic readings 450 nm and 620-650 nm (*Diagnostic Automation Cat # DAX 800*)

Precautions

- Do not deviate from the specified procedures when performing this assay. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken* if applicable for this kit
- Controls and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipette with constant intervals, so that all the wells of the micro titer plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipette tips have to be used.
- No reagents from different kit lots have to be used; they should not be mixed among one another.
- All reagents have to be used within the expiry period.

- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipettes and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.
- All reagents should be stored at 2-8 °C

Specimen Collection and Handling

- Urine specimens should be collected in standard sterile containers stored at room temperature or refrigerated (2-8 °C) and assayed within 24 hours of collection.
- Alternatively, specimens may be stored at 2-8 °C for up to 14 days or frozen (-70 °C) for longer periods before testing.
- Whenever possible, urine specimens should be shipped at 2-8 °C or frozen
- Urine specimens containing excess urates, phosphates, or other dissolved salts may develop salt crystals when stored from at 2-8 °C or lower.
- Insure all samples are at room temperature before performing the assay. (15-25° C)

Storage Conditions

- Reagents, strips and bottled components should be stored at 2-8 °C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C).

Preparation

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- *(20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration. To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.*

Test Procedure

Ensure all samples and reagents are at room temperature (15-25 °C) before use. Frozen samples must be thawed completely before use.

- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided prediluted. **DO NOT dilute further.**

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in well holder. Return all unused strips to the pouch and reseal the zip lock closure.
2. Add **100 µl** of negative control to well # 1
3. Add **100 µl** of positive control to well # 2.
4. Add **100 µl** of the urine samples to each test well.
5. Incubate for **30 minutes** at room temperature (15-25 °C), then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops** of Conjugate to each well.
7. Incubate for **10 minutes**, then wash*. After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
8. Add **2 drops** of Chromogen to each well. Incubate for **5 minutes**. DO NOT WASH after this step.
9. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the sides of the plate with index finger.
10. Read results visually or using an ELISA plate reader (see instructions below).

* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

Interpretation of Results

Interpretation of Results – Visual (Manual)

Positive: Any sample well that is obviously more yellow than the negative control well.

Negative: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result.

Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using bichromatic reading with filters at 450 nm and 620-650 nm.

Positive: Absorbance reading of 0.15 OD and above indicates the sample contains *Legionella* antigen.

Negative: Absorbance reading less than 0.15 OD indicates the sample does not contain detectable levels of *Legionella* antigen.

Limitation of Procedure

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. This assay Kit is intended for use with human urine samples only. *It should not be used for non-human applications.*

A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for *Legionella*.

Excretion of *Legionella* antigen in urine may vary depending on the individual patient and the stage of the disease. Some individuals have been shown to excrete antigen for an extended period of time, so a positive ELISA reaction may reflect a recent but not active infection. Early treatment with appropriate antibiotics may also decrease antigen excretion in some individuals. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to a year afterwards.

DO NOT concentrate urine samples. Assay will not give accurate results on a concentrated sample.

Specific Performance Characteristics

		DAI	
		+	-
Reference Method	+	37	0
	-	2	234

Positive Agreement: 100% (37/37)

Negative Agreement: 99.2% (234/236)

*Reference Method refers to a commercially available ELISA.

Expected Values

Normal healthy individuals should be free of *Legionella* and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of *Legionella* antigen.

Quality Control

The positive and negative control must be included each time the kit is run. The use of a positive and negative control allows easy validation of kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

Troubleshooting

Problem: Negative control has excessive color after development.

Reason: Inadequate washings.

Correction: Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out.

Date Adopted	2016-10-04
REF 8305-3	DA-Legionella Urinary Antigen



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