SAS™ Legionella Test

The SAS™ Legionella Test is a visually read, in vitro immunochromatographic rapid assay for the presumptive qualitative detection of *Legionella pneumophila* serogroup 1 antigens in human urine.

For *In-Vitro* Diagnostic Use

Store at 15° to 30° C

For Technical Assistance Call 800-272-2710
Outside the USA Call 210-699-8800

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INTENDED USE

The SAS™ Legionella Test is a visually read, in vitro immunochromatographic rapid assay for the presumptive qualitative detection of *Legionella pneumophila* serogroup 1 antigens in human urine. This test is intended to aid in the presumptive diagnosis of Legionnaires' disease in conjunction with culture and other methods for patients with signs and symptoms of pneumonia. This test is for prescription use only.

INTRODUCTION

Legionella pneumophila serogroup 1 is gram-negative bacillus that appears off-white in color and circular in shape. This bacterium is associated with Pontiac fever and Legionnaires' disease. An estimated 25, 000[2] to 10,000 [3] cases involving *Legionella pneumophila* infections occur in the United States annually. About 5% to 30% of people with Legionnaires' disease die.

Infections caused by this bacterium include the pulmonary disease of pneumonia. In addition, extra pulmonary infections may be spread through the bloodstream or the lymph system, infecting the heart, brain, kidney, liver, and/or spleen. Early onset symptoms include fever, chills, muscle aches, and lack of appetite. Respiratory symptoms such as coughing or congestion are usually absent. As the disease progresses, a dry, hacking cough develops and may become productive after a few days. Half of people who develop Legionnaires' disease suffer shortness of breath. In many cases[1], a fever as high as 104° F has been reported.

Common methods for the detection of Legionella pneumophila are culture, direct fluorescent antibody (DFA), indirect fluorescent antibody (IFA) [5], enzyme immunoassays (EIA) [5] and polymerase chain reaction (PCR) [6,8] using respiratory specimens of expectorated sputum, bronchial washing, transtracheal aspirate and lung biopsy. *Legionella pneumophila* serogroup 1 antigen has been detected in urine during the acute phase of the disease and has become the method by choice for laboratory diagnosis. [4,5] The SAS™ Legionella test is designed for the detection of *Legionella pneumophila* serogroup 1 soluble antigen in urine.

PRINCIPLE OF THE TEST

The SAS™ Legionella test utilizes a combination of polyclonal antibodies against the antigens of *Legionella pneumophila*. The SAS™ Legionella test begins with the addition of urine to the test device. The specimen is absorbed by the sample pad and then moves through the conjugate pad which contains dried gold conjugated antibodies which are specific for *Legionella pneumophila* antigens; if the Legionella antigens are present in the urine sample, a "half-sandwich" immunocomplex is formed. This immuno-complex then migrates via capillary action along a nitrocellulose membrane containing immobilized antibodies to *Legionella pneumophila* antigens. The immobilized antibodies bind the "half-sandwich" immuno-complex to form a "whole sandwich" immuno-complex. Thus, when the "whole sandwich" is formed, a visible, pink colored line develops in the specimen zone on the test device. In the absence of a Legionella antigen, a "sandwich" immuno-complex is not formed and a negative result is indicated. To serve as a procedural control, a pink colored control line will always appear in the control zone regardless of the presence or absence of Legionella antigen. The test is available in cassette format.

MATERIALS PROVIDED

1. Test Devices – A membrane coated with a combination of polyclonal antibodies against the antigens of *Legionella pneumophila* serogroup 1.
2. Disposable specimen dropper

MATERIALS NOT PROVIDED

1. Timer
2. Specimen collection containers

PRECAUTIONS

1. For *In-vitro* diagnostic use only.
2. Use pipette provided with kit only.
3. Use separate pipettes for each sample or control.
4. The test device should remain in the pouch until ready for use.
5. All specimens should be considered potentially hazardous, and should be handled in the same manner as an infectious agent.
6. Wear disposable gloves while handling samples and wash hands after the assay is complete. Warning: The user should refer to the relevant section of the CDC-NIH manual "Biosafety in Microbiology and Biomedical Laboratories," Fifth Edition, 2007.
7. Avoid any contact with eyes.
8. The test device and all materials should be discarded in a proper biohazard container after testing.
9. Do not use kits after the stated expiration date.
10. Do not reuse test devices or kit materials.

STORAGE CONDITIONS

SAS™ Legionella test devices should be kept at room temperature (15°-30°C) in sealed pouches with a desiccant. Do not freeze the test kit.

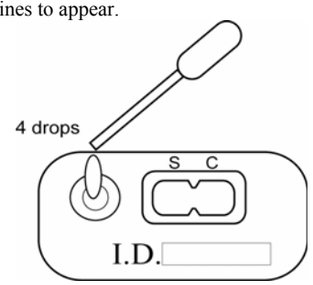
SPECIMEN COLLECTION AND PREPARATION

The urine specimen must be collected in a clean, sterile container. Urine specimens may be refrigerated (2°–8°C) and stored up to 72 hours prior to assay. Specimens may be stored frozen for up to 2 years. If specimens are refrigerated, they must be equilibrated to room temperature before testing.

TEST PROCEDURE

Allow specimen and/or controls to reach room temperature (15° – 30°C) prior to testing.

1. Remove test device from pouch and lay on a flat surface. Label the device with the patient ID.
2. Dispense 4 drops (approximately 0.15 ml) of specimen into the round sample well (see illustration below). Wait for colored lines to appear.

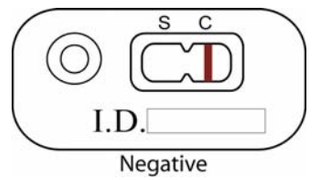


3. Read results at 15 minutes or less. The presence of the control line is not indicative of the test being completed. Interpretation after 15 minutes may be inaccurate.

INTERPRETATION OF RESULTS

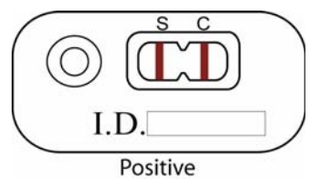
Negative Results

The test is negative if a colored line appears in the C area (control) only.



Positive Results

The test is positive if one colored line appears in the S (specimen) area and one colored line appears in the C (control) area. Any colored line in the specimen area should be considered positive. Colored lines may appear lighter or darker than one another. Specimens with Legionella antigen levels near the threshold of the test may develop color (faint lines) overtime after the 15 minute reading; however, a line that appears after 15 minutes is not diagnostic and should be ignored.



Invalid Results

The test is invalid if no colored line appears in the C (control) area even if a colored line appears in the S (specimen) area. If a colored line does not appear in the C area, the test is invalid and

should be repeated. Colored lines that appears in the S (specimen) area after 15 minutes are not diagnostic and should be ignored.

Quality Control

Internal Controls

The appearance of a Control line in the C region of the device is a positive procedural control. Correct procedural technique, specimen flow and device perf ormance is conf irmed when a colored line appears in the C (control) area of the membrane. If the colored line fails to appear i n the C (control) area, the test result is invalid.

A clear background is an internal negative procedural control. The background colo r should be white to light p ink and should not interfere with the reading of the test result. If any result is diff icult to interpret, the test should be re peated with the sa me sample to eliminate the pot ential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.

It is recommended that the internal quality controls be recorded for each sample run.

External Controls

Quality control should be perfor med on each ne w test kit box opened. Additional testing of the appropriat e controls of *Legionella pneumophila* may be performed in accordance with federal, state an d/or local regulations, accrediting requirements, and your laboratory's quality co ntrol procedures. Positive and negative external controls are supplied separately.

LIMITATIONS OF THE PROCEDURE

1. A negative antigen result does not rule out the possible infection from *Legionella pneumophila* serogroup 1. The antigen concentration may fall bel ow the detectabl e limit o f the test. C ulture is recommended f or patients w ith s uspected pneumonia to determine if other c ausative agents other than *Legionella pneumophila* serogroup 1 are responsible.
2. The test provides a presumptive diagnosis for only *Legionella pneumophila* serogroup 1 infection. In order to make an accurate diagnos is, culture results, serology testing and antigen detection methods in conjun ction with clinical findings should be used.
3. Sensitive immun oassays may demonstrate positi ve results with specimens containing heterophilic antibodies. If the qualitative interpretation is inconsistent with clinical findings, then further tes ting by an alternate method should be performed.
4. Excretion of Legionella antigen may be seen as early as three (3) days after the onset of symptoms. However, antigen may continue to be e xcreted up to on e (1) year af terwards[7]. A positive result w ith the SA S™ Legionella Test may occur during a current and past infection; therefore, in order to make an acc urate diagnosis, culture results, serology testing and antigen dete ction methods in conjun ction with clinical findings should be used.

PERFORMANCE DATA

Clinical Sensitivity and Specificity (Retrospective Study)

Three clinical sites (USA and Netherlands) tested three hundred twenty four (324) frozen specime ns using the SAS™ Legionella Cassette test. These samples w ere previously tested for Legionella by cell culture. Results are reported below.

		Cell Culture +	Cell Culture -	
SAS™ Legionella Test	+	95	11	106
	-	10	208	218
		105	219	324

Sensitivity: 95/105 x 100 = 90.5 % (95% CI: 83.4-94.8)
Specificity: 208/219 x 100 = 95.0 % (95%CI: 91.2-97.5)

Analytical Specificity

Forty-Nine (49) fresh patient urine s from healthy in dividuals were collected and assayed at one (1) clinical site. One hundred percen t (100%) of these were fo und to be neg ative b y the S AS™ Legionella test.

Cross-Reactivity with other Respiratory Tract Infections

Ninety-nine (99) urines from patients diagnosed for other etiological respir atory tract infect ions (84 culture confirmed, 15 suspected) were tested using the SAS™ Legion ella Test. The results showed a lack of reactivity in 98/99 samples (99.0%).

Bacterial Cross-Reactivity and Interference

To confirm the analytical specificity of the SAS™ Legionella Test, bacterial cultures likely to be found in the respiratory tract w ere tested. Bacterial cultures were tested at 1 x 10^8 cfu/ml. A ll yielded negative results.

To confirm a lack of interference by other bacterial species in the SAS™ Legionella Test, purified Legionella antigen was add ed to bacterial cultures likely to be found in the respira tory tract. The concentration of Legionella antigen was 1 x 10^5 cfu/ml. A ll tests yielded positive results.

Legionella pneumophila SG2	*Legionella gormanii*	*Proteus mirabilis*
Legionella pneumophila SG3	*Legionella longbeachae*	*Citrobacter freunii*
Legionella pneumophila SG4	*Legionella feeleii*	*Enterobactor clacae*
Legionella pneumophila SG5	*Staphylococcus aureus*	*Serratia marcesens*
Legionella pneumophila SG6	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
Legionella bozemanii	*Enterococcus faecalis*	*Candida albicans*
Legionella micdadei	*Escherichia coli*	*Streptococcus pneumoniae*
Legionella dumoffii	*Klebsiella pneumoniae*	*Heamophilus influenzae*

Common Urine Components and Drugs

The components and drugs listed below were tested to determine if they interfered or cross-reacted with S AS™ Legionella Test. Extracted *Legionella pneumophila* antigen was added at a concentration of 4.5 x 10^4 cfu/m L. In addition, each compound was tested in the absence of *Legionella pneumophila* antigen. None listed interfered or cross-reacted with the test results.

Glucose	2000mg/dL	Protein - BSA	500mg/dL
Urea	2000mg/dL	Bilirubin	20mg/dL
Ascorbic Acid	100mg/dL	Rifampicin	0.09mg/mL
Erythrocytes	10^6/mL	Erythromycin	0.067mg/mL
Leukocytes	10^6/mL	Ciprofloxacin	0.22mg/mL

Limit of Detection

The limit of detection of the SAS™ Legionella test w as determined to be 5 x 10^4. *Legionella pneumophila* serogroup 1 ATCC 33152 was prepared using BCYE agar. A dilution of the working concentr ation w as performed. The limit of detection of the SA S™ Legionella C assette Test w as determined from these concentrations. The test was p erformed in 2 replicates. Res ults were recorded at 15 minutes.

Reproducibility Study

The reproducibility of the SAS™ Legionella Test was evaluated at three clinical lab oratory sites. The SAS™ Legionella Test w as tested against a panel of six (6) specimens of which included four levels of positives and two negatives. The low and high positives were from the purified Legionella antigen. Negatives w ere comprised of ei ther urine or Legionella antigen below the detectable limit. Three (3) differe nt laboratory personnel assayed each specimen at each laboratory facility over 3 day s. The overall reproducibility for the SAS™ Legionella Test was 100%.

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KEY TO INTERNATIONAL SYMBOLS in ENGLISH

SYMBOLS	FOR
	Manufacturer
EXP	Expiration Date
IVD	In-vitro diagnostic device
EC REP	Authorized Representative in European Community
Σ	Contains sufficient for <n> tests
LOT	Batch Code
REF	Catalog Number
CE	Product conforms to IVD directive
	Temperature Limitation (15-30°C)

Authorised Representative:
MegaCor GmbH
Europaplatz 1
88131 Lindau
Germany

CE

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