CLSI SAS™ Legionella Test

Procedure	SAS™ Legionella Test

Prepared by	Date Adopted	Supercedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE OF THE TEST

SAS™ Legionella Test utilizes a pair of Legionella virus-specific polyclonal antibodies. Urine specimen is added to the device's sample well. The reaction between a positive sample and the colored particle-conjugated antibody will form a complex that migrates along the membrane. An immobilized capture antibody will form a colored line at the S (specimen) area upon reacting with the colored complex. An internal control line C (control) area is built in to assure that the test has been carried out correctly.

REAGENTS

Test device containing a membrane coated with a combination of polyclonal antibodies against the antigens of *Legionella pneumophila* serogroup 1.

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 AND STABILITY

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 & PREPARATION

The urine specimen must be collected in a clean, sterile container. Urine specimens may be refrigerated (2°-8°) 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.

MATERIALS PROVIDED

1. Test devices- A membrane coated with a combination of polyclonal antibodies against eh antigens of *Legionella pneumophila* serogroup 1. Disposable sample transfer pipettes
2. Disposable specimen dropper.

MATERIALS NOT PROVIDED

1. Timer
2. Specimen collection containers.

TEST PROCEDURE

Allow the pouch (test device), specimen and /or controls to reach room temperature (15º-30ºC) prior to testing.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with patient or control identifications.
2. Dispense 4 drops (approximately 0.15ml) of specimen into the round sample well. 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 only one colored line appears in the C (control) area.

Positive Results

The test is positive if two colored lines appear. One colored line will appear in the S (specimen) area and one in the C (control) area. A colored line of any intensity in the S (specimen) area should be considered positive. Colored lines may be lighter or darker than each other.

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 appear 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 procedural control. Correct procedural technique, specimen flow and device performance are confirmed when a colored line appears in the C (control) area of the device. If the line should fail to appear in the C area of the device, the test result is invalid. A clear background is an internal negative procedural control. The background color should be white to light pink and should not interfere with the reading of the test result. If any result is difficult to interpret, the test should be repeated with the same sample to eliminate the potential 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 performed on each new lot number opened. Additional testing of the appropriate controls of *Legionella pneumophila* may be performed in accordance with federal, state and/or local regulations, accrediting requirements, and your laboratory's quality control 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 below the detectable limit of the test. Culture is recommended for patients with suspected pneumonia to determine if other causative 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 diagnosis, culture results, serology testing and antigen detection methods in conjunction with clinical findings should be used.
3. Sensitive immunoassays may demonstrate positive results with specimens containing heterophilic antibodies. If the qualitative interpretation is inconsistent with clinical findings, then further testing 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 excreted up to one (1) year afterwards[7]. A positive result with the SAS™ Legionella Test may occur during a current and past infection; therefore, in order to make an accurate diagnosis, culture results, serology testing and antigen detection methods in conjunction 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 specimens using the SAS™ Legionella Cassette test. These samples were 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 urines from healthy individuals were collected and assayed at one (1) clinical site. One hundred percent (100%) of these were found to be negative by the SAS™ Legionella test.

Cross-Reactivity with other Respiratory Tract Infections

Ninety-nine (99) urines from patients diagnosed for other etiological respiratory tract infections (84 culture confirmed, 15 suspected) were tested using the SAS™ Legionella 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 were tested. Bacterial cultures were tested at 1 x 10^8 cfu/ml. All yielded negative results.
To confirm a lack of interference by other bacterial species in the SAS™ Legionella Test, purified Legionella antigen was added to bacterial cultures likely to be found in the respiratory tract. The concentration of Legionella antigen was 1 x 10^5 cfu/ml. All 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 SAS™ Legionella Test. Extracted *Legionella pneumophila* antigen was added at a concentration of 4.5 x 10^4 cfu/mL. 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 was determined to be 5 x 10^4. *Legionella pneumophila* serogroup 1 ATCC 323152 was prepared using BCYE agar. A dilution of the working concentration was performed. The limit of detection of the SAS™ Legionella Cassette Test was determined from these concentrations. The test was performed in 2 replicates. Results were recorded at 15 minutes.

Reproducibility Study
The reproducibility of the SAS™ Legionella Test was evaluated at three clinical laboratory sites. The SAS™ Legionella Test was 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 were comprised of either urine or Legionella antigen below the detectable limit. Three (3) different laboratory personnel assayed each specimen at each laboratory facility over 3 days. The overall reproducibility for the SAS™ Legionella Test was 100%.

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