SAS™ RSVAlert

A Rapid Visual Assay for the Qualitative Detection of Respiratory Syncytial Virus Antigen in Nasopharyngeal Specimens

For *In-Vitro* Diagnostic Use

CLIA Complexity: Waived

Store at 15° to 30°C

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

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READ ALL INSTRUCTIONS BEFORE BEGINNING THE ASSAY

INTENDED USE
SAS™ RSVAlert antigen test kit is a visual and rapid assay for the qualitative detection of Respiratory Syncytial Virus (RSV) antigen directly from nasopharyngeal specimens in neonatal and pediatric patients. The test is for in-vitro diagnostic use only. It is recommended that negative test results be confirmed by cell culture.

BACKGROUND
Respiratory syncytial virus is a member of the *Paramyxoviridae* family and is the most significant respiratory pathogen for infants and children.[1, 7] Infection usually causes mild to moderate severe upper respiratory illness that may lead to life threatening pneumonia or bronchiolitis. RSV infections are seasonal and are most prominent from December to March in the northern hemisphere. The virus is spherical in shape with a lipoprotein envelope synthesized from the plasma membrane of the infected host cell. The virus is spread rapidly through droplets dispersed in the air or secretions from the respiratory tract of infected individuals. The incubation period is 3-7 days.[1] Specimens from patients are obtained by using nasopharyngeal aspiration, washes and swabs.[2]

Several methods have been developed for the detection of RSV. This includes Direct and Indirect Immunofluorescence on exfoliated cells, Enzyme Immunoassay (EIA) from nasopharyngeal samples, and isolation of the virus from Cell Culture. Cell Culture has remained historically the "gold standard" used for diagnosis, but requires specialized equipment, highly trained personnel, specialized care in specimen collection and transportation, and long periods of time to obtain results. Rapid immunodetection methods have provided a cost effective detection option, which allows for timely patient treatment to prevent possible nosocomial spread.[3, 5,6]

PRINCIPLE OF THE TEST
The SAS™ RSVAlert test utilizes a pair of Respiratory Syncytial Virus (RSV) specific antibodies in an immunochromatographic sandwich assay. The reaction between a positive sample and the colored particle-conjugated antibody forms 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.

MATERIALS & REAGENTS PROVIDED
1. Test Devices.
2. SAS™ RSVAlert Extraction Buffer (Contains mucolytic agent and 0.1% sodium azide as a preservative).
3. Disposable extraction tubes with filtered caps.
4. Disposable pipettes (150µl ea).
5. Package insert.

MATERIALS NOT PROVIDED
1. Timer.
2. RSV positive control.
3. RSV negative control.
4. Disposable Transfer Pipettes (1ml ea).

PRECAUTIONS
1. For in vitro diagnostic use only.
2. In accordance with the principles of Good Laboratory Practice, it is strongly recommended that all specimens be treated as potentially infectious and handled with all necessary precautions.
3. Discard all used devices into a biohazard container.
4. Do not use kits after the stated expiration date, and do not mix kit components from different lots.

5. Users are cautioned against over reading of membrane immunoassays. Only a clearly visible line in the S area should be considered a positive result.
6. Follow test procedure for each specimen type as written. Extraction tube and dropper tips should only be used with bloody or mucoid samples.
7. Do not expose test to extreme temperatures. Test performance may be affected.
8. If the laboratory modifies the test system instructions, then the test is considered high complexity and subject to all applicable CLIA requirements.
9. A Certificate of Waiver is required to perform the test in a waived setting. This waiver may be obtained from your local state agency or by completing Form CMS-116 available at www.cms.hhs.gov/clia.
10. Laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15 (e) (1).

STORAGE CONDITIONS
SAS™ RSVAlert Test devices should be kept at room temperature (15-30°C) in the sealed pouches. Do not freeze the test kit or kit reagents.

TRANSPORT MEDIA
The following transport media have been tested and found to be compatible with SAS™ RSVAlert Test:

0.9% Saline	PBS
PBS 0.5% Gelatin	PBS 0.5% BSA
Tripticase Soy Broth	Todd Hewitt Broth
Viral CULTURETTE™	M4 VTM
M4-RT VTM	M5 VTM
EMEM with Lactalbumin hydrosylate	EMEM

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION
Acceptable specimens for evaluation with the SAS™ RSVAlert Test include nasopharyngeal washes; aspirates and swabs.[4] Specimens should be transported to laboratory immediately after collection. Specimens may be stored at 2-8°C for up to 48 hours or at -20°C for up to one week.

SPECIMEN PREPARATION
Acceptable specimens include nasopharyngeal washes, aspirates, and swabs.

Note: Mucoid or bloody specimens may fail to flow properly on the SAS™ RSVAlert Test causing an inconclusive test result (see Test Procedure). For excessive mucoid or bloody specimens, it may be helpful to treat the specimen with extraction buffer, followed by brief sonication, prior to addition to the SAS™ RSVAlert Test.

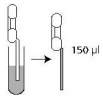
Procedure For Use with Nasopharyngeal Washes:
1. Nasopharyngeal wash volumes of 2 to 4 ml are recommended. Excess wash volume may decrease test performance.
2. If specimen is mucoid or bloody see note above.

Procedure for Use with Nasopharyngeal Aspirates:
1. Nasopharyngeal aspirates should be collected in volumes between 0.5 and 1ml.
2. Samples then should be dispersed in 2 or 4 ml of viral transport medium or physiological saline up to 4 ml, depending on volume of aspirate received.
3. If specimen is mucoid or bloody see note above.

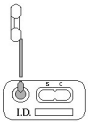
Procedure for Use with Nasopharyngeal Swabs:
1. Place swab specimen into 0.75-3 ml of transport medium or saline.
2. Mix the swab and transport media or saline vigorously.
3. Express excess liquid from swab.

4. Dispose of swab into appropriate container.

TEST PROCEDURE FOR SPECIMENS
1. Remove the test from the pouch and lay it on a flat surface.
2. Label test with the specimen type and ID.
3. Squeeze and fill the entire pipette with sample.



4. Squeeze and dispense the entire contents of the pipette into test device.



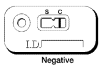
5. Read results at 15 minutes. Do not read results after 30 minutes.

Note: For Mucoid or Bloody Samples: Add 250µl of the nasopharyngeal wash specimen to extraction tube. Add 2 drops of SAS™ RSVAlert Extraction buffer. Insert filter cap, mix, and dispense 3-4 drops of extracted specimen from extraction tube into a fresh test device. Some positive results may be seen in as short as 30 seconds depending on the concentration of the antigen. Do not read results after 30 minutes.

TEST PROCEDURE FOR EXTERNAL CONTROLS
1. Remove test device from pouch and lay on flat surface. Label device with specimen type and ID.
2. Pipette 150µl of the external control into test device.
3. Read results at 15 minutes. Some positive results may be observed in as briefly as 30 seconds depending on the concentration of the antigen. Do not interpret results after 30 minutes.

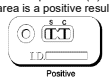
INTERPRETATION OF RESULTS

Negative Result
A pink colored band in the control (C) area without a pink colored band in the specimen (S) area is a negative result.



Negative

Positive Result
Any pink colored band in the specimen (S) area **with** a pink colored band in the control (C) area is a positive result.



Positive

Invalid Result
No pink colored band in the control (C) area of the test is an invalid result. No colored band in both the control (C) area and specimen (S) area is an invalid result. If the background interferes with the reading of the test, the test is considered invalid. If the test is invalid, repeat the test or call Technical Assistance.

Invalid

LIMITATIONS
1. The SAS™ RSVAlert Test is for the detection of viable and non-viable RSV particles. This test is not for confirmation of a respiratory infection caused by other microorganisms.
2. The SAS™ RSVAlert Test is dependent on antigen load and may not correlate with other methods used for the detection of RSV such as Cell culture performed on the same specimen.
3. Frozen specimens should be thawed and brought to room temperature before use.
4. False negatives may result from inadequate specimen collection, such as over dilution, improper handling or transport.
5. A negative test result does not rule out a possible RSV infection. Patient diagnosis should always include laboratory test results and all other clinical information available.

QUALITY CONTROL
Internal Controls
Each test device includes an internal procedural control. The appearance of a Control Line in the C region of the test device is a positive procedural control. Correct procedural technique, specimen flow and test device performance is confirmed when a colored line appears in the C (control) area of the membrane. If the colored line fails to appear in the C (control) area, 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 a more intensely red background color appears, it may interfere with the ability to read the test result; therefore, the test should be repeated.

External Controls
Negative and positive controls for RSV antigen should be tested and the appropriate results obtained. External quality controls should be performed on each lot, each new shipment, and as necessary by your established quality control procedures.

External controls must be purchased separately.

PERFORMANCE CHARACTERISTICS
Accuracy by Comparison:
Laboratory Studies
Sixty-three (63) frozen patient samples were obtained from several laboratories. An RSV viral culture was performed on each sample. Each sample was thawed and a SAS™ RSVAlert Test was performed.

		Cell Culture +	Cell Culture -	
SAS™ RSVAlert Test	+	57	0	57
	-	*1	5	6
*Confirmed Positive by EIA.		58	5	63

Percent Positive Agreement: (57/58)×100=98.3%
(95%CI, 90.8% to99.9%)

Percent Negative Agreement: (5/5)×100=100%
(95%CI, 47.8% to 100%)

Percent Agreement: (62/63)×100=98.4%
(95%CI, 91.5% to 99.9%)

Ninety-four (94) frozen patient samples were obtained from several laboratories. Each sample was thawed and a SAS™ RSVAlert Test and Other Commercial test were performed.

		Other Commercial Test +	Other Commercial Test -	
SAS™ RSVAlert Test	+	83	0	83
	-	*4	7	11
		87	7	94

*All four were confirmed positive by EIA and Cell Culture.

Percent Positive Agreement: (83/87)×100=95.4%
(95%CI, 88.6% to 98.7%)

Percent Negative Agreement: (7/7)×100=100%
(95%CI, 59.0% to 100%)

Percent Agreement: (90/94)×100=95.7%
(95%CI, 89.6 to 98.3%)

CLINICAL SPECIFICITY AND SENSITIVITY
Prospective Study
One hundred thirty-two (131) clinical samples collected over two (2) seasons were tested blindly and prospectively using the SAS™ RSVAlert Test and compared to Cell Culture. The results are shown in the table below.

		Cell Culture +	Cell Culture -	
SAS™ RSVAlert Test	+	5	0	5
	-	0	126	126
		5	126	131

Sensitivity: (5/5)×100=100%
(95%CI, 56.6 to 100%)

Specificity: (126/126)×100=100%
95%CI, 97.1 to 100%)

Correlation: (131/131)×100=100%
(95%CI, 97.2 to 100%)

Retrospective Study
Three clinical sites tested one hundred twenty four (124) clinical samples blindly and retrospectively using the SAS™ RSVAlert Test and compared the results to Cell Culture. Samples were stored frozen and thawed prior to testing. The results are shown in the table below.

		Cell Culture +	Cell Culture -	
SAS™ RSVAlert Test	+	86	2	88
	-	4	32	36
		90	34	124

Relative Sensitivity: (86/90)×100=95.6%
(95%CI, 89.0 to 98.8%)

Relative Specificity: (32/34)×100=94.1%
(95%CI, 80.3 to 99.3%)

Relative Correlation: (118/124)×100=95%
(95%CI, 89.8 to 97.8%)

CLINICAL COMPARISON
Nasopharyngeal Swabs
Two clinical sites tested twenty-eight (28) clinical swab specimens blindly and prospectively using the SAS™ RSVAlert Test and the Other Commercial RSV Test. The results are shown below.

		Other Commercial RSV Test +	Other Commercial RSV Test -	
SAS™ RSVAlert Test	+	6	0	6
	-	5	17	22
		11	17	28

Percent Positive Agreement: (6/11)×100=54.5%
(95%CI, 23.4% to 83.3%)

Percent Negative Agreement: (17/17)×100=100%
(95%CI, 80.5% to 100%)

Percent Agreement: (23/28)×100=82.1%
(95%CI, 63.1% to 93.9%)

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)
The limit of detection (LOD) of the SAS™ RSVAlert Test was determined for five (5) RSV Strains. These strains included three (3) RSV B and two (2) RSV A strains.

Type	RSV Viral Strain	Limit of Detection ($TCID_{50}$/0.2 ml)
A	RSV (Long)	1.7×10^{3}
A	RSV (A-2)	9.9×10^{2}
B	RSV (9320)	5.5×10^{2}
B	RSV (Washington)	1.1×10^{3}
B	RSV (Wild-type)	8.9×10^{1}

CROSS REACTIVITY/INTERFERENCE STUDY
To confirm the analytical specificity of the SAS™ RSVAlert Test, bacterial and viral cultures likely to be found in the respiratory tract were tested. Bacterial cultures were tested at 1.0×10^{8} cfu/ml and the viral cultures at $1.0 \times 10^{3.5}$ to $1 \times 10^{6.5}$ $TCID_{50}$/0.2 ml. All yielded negative results.

To confirm noninterference of the SAS™ RSVAlert Test, RSV whole virus 9320 at titer 1.11×10^{3} $TCID_{50}$ /0.2 ml was added to bacterial and viral cultures likely to be found in the respiratory tract. Bacterial cultures were tested at 1.0×10^{8} cfu/ml and the viral cultures at $1.0 \times 10^{3.5}$ to $1.0 \times 10^{6.5}$ $TCID_{50}$/0.2 ml. All yielded positive results.

Bacterial Cross Reactivity

Candida albicans	*Streptococcus sp gr A*
Chlamydia trachomatis	*Streptococcus sp gr F*
Corynebacterium diptheriae	*Streptococcus sp gr G*
Haemophilus influenzae type A	*Streptococcus pneumoniae*
Klebsiella pneumoniae	*Mycoplasma pneumoniae*
Serratia marcescens	*Neisseria meningitides*
Staphylococcus epidermidis	*Pseudomonas aeruginosa*
Staphylococcus aureus	

Viral Cross Reactivity Panel

Adenovirus 5	Influenza A
Adenovirus 7	Influenza B-Hong Kong
Adenovirus 10	Parainfluenza 1
Coxsackie A9	Parainfluenza 2
Coxsackie B5	Parainfluenza 3
Coxsackie B6	Varicella zoster
Cytomegalovirus	Rhinovirus 1A
Echovirus 11	Rhinovirus 2
Echovirus 3	Rhinovirus 13
Echovirus 6	Rhinovirus 15
HSV Type-1	Rhinovirus 37
HSV Type-2	

REPRODUCIBILITY
Physician Office Lab Study
The reproducibility of the SAS™ RSVAlert Test was evaluated at three physician offices. The SAS™ RSVAlert Test was tested against a panel of five (5) specimens of which included three levels of positives and two negatives. The overall reproducibility for the SAS™ RSVAlert Test was 100%.

Lay Person User Study
Individuals having diverse educational backgrounds evaluated the SAS™ RSVAlert Test at three different sites. Each site tested a coded panel consisting of a negative, low positive and high positive. There was greater than ninety-eight percent (98%) agreement (221/225) of the expected results.

REFERENCES

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