

Sure-Vue® RSV Test

CLIA Complexity: Waived

READ ALL INSTRUCTIONS BEFORE BEGINNING THE ASSAY

INTENDED USE

Sure-Vue® RSV 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. The test is for health professional use only.

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 Sure-Vue® RSV 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. Sure-Vue® RSV Extraction Buffer. (*Contains mucolytic agent and 0.1% sodium azide as a preservative*)
3. Disposable extraction tubes with filtered caps.
4. Disposable pipettes (150 µl 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.

STORAGE CONDITIONS

Sure-Vue® RSV 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 Sure-Vue® RSV 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®
EMEM with Lactalbumin hydrolysate	EMEM

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

Acceptable specimens for evaluation with the Sure-Vue® RSV 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 Sure-Vue® RSV 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 Sure-Vue® RSV 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:

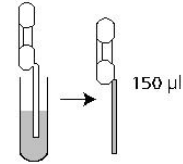
1. Nasopharyngeal aspirates should be collected in volumes between 0.5 and 1ml.
2. Samples then should be dispersed in 2 or 4 ml of viral transport medium or physiological saline up to 4 ml, depending on volume of aspirate received.
3. If specimen is mucoid or bloody see note above.

Procedure for Use with Nasopharyngeal Swabs:

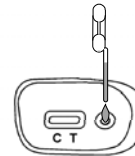
1. Place swab specimen into 0.75-3 ml of transport medium or saline.
2. Mix the swab and transport media or saline vigorously.
3. Express excess liquid from swab.
4. Dispose of swab into appropriate container.

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 Sure-Vue® RSV 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 (T) area is a negative result.



Positive Result

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



Invalid Result

No pink colored band in the control (C) area of the test is an invalid result. If the test is invalid, repeat the test or call Technical Assistance.

LIMITATIONS

1. The Sure-Vue® RSV 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 Sure-Vue® RSV 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.

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 Sure-Vue® RSV Test was performed.

Sure-Vue® RSV Test	Cell Culture		
	+	-	
+	57	0	57
-	*1	5	6
*Confirmed positive by EIA	58	5	63

Percent Positive Agreement: $(57/58) \times 100 = 98.3\%$
(95% CI, 90.8% to 99.9%)

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

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

Ninety-four (94) frozen patient samples were obtained from several laboratories. Each sample was thawed and a Sure-Vue® RSV Test and Other Commercial test were performed.

		Other Commercial Test		
		+	-	
Sure-Vue® RSV Test	+	83	0	83
	-	4	7	11
		87	7	94

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

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

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

Percent Agreement: $(90/94) \times 100 = 95.7\%$
(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 Sure-Vue® RSV Test and compared to Cell Culture. The results are shown in the table below.

		Cell Culture		
		+	-	
Sure-Vue® RSV Test	+	5	0	5
	-	0	126	126
		5	126	131

Sensitivity: $(5/5) \times 100 = 100\%$ (95% CI, 56.6 to 100%)

Specificity: $(126/126) \times 100 = 100\%$ (95% CI, 9.1 to 100%)

Correlation: $(131/131) \times 100 = 100\%$ (95% CI, 97.2 to 100%)

Retrospective Study

Three clinical sites tested one hundred twenty four (124) clinical samples blindly and retrospectively using the Sure-Vue® RSV 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		
		+	-	
Sure-Vue® RSV Test	+	86	2	88
	-	4	32	36
		90	34	124

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

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

Relative Correlation: $(118/124) \times 100 = 95\%$
(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 Sure-Vue® RSV Test and the Other Commercial RSV Test. The results are shown below.

		Other Commercial RSV Test		
		+	-	
Sure-Vue® RSV Test	+	6	0	6
	-	5	17	22
		11	17	28

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

Percent Negative Agreement: $(17/17) \times 100 = 100\%$

(95% CI, 80.5% to 100%)
Percent Agreement: $(23/28) \times 100 = 82.1\%$
(95% CI, 63.1% to 93.9%)

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)

The limit of detection (LOD) of the Sure-Vue® RSV 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 Sure-Vue® RSV 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 $1 \times 10^{6.5}$ TCID₅₀/0.2 ml. All yielded negative results.

To confirm noninterference of the Sure-Vue® RSV Test, RSV whole virus 9320 at titer 1.11×10^3 TCID₅₀ /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 $1.0 \times 10^{6.5}$ TCID₅₀/0.2 ml. All yielded positive results.

Bacterial Cross Reactivity

<i>Candida albicans</i>	<i>Streptococcus sp gr. A</i>
<i>Chlamydia trachomatis</i>	<i>Streptococcus sp gr. F</i>
<i>Corynebacterium diphtheriae</i>	<i>Streptococcus sp gr. G</i>
<i>Haemophilus influenzae type A</i>	<i>Streptococcus pneumoniae</i>
<i>Klebsiella pneumoniae</i>	<i>Mycoplasma pneumoniae</i>
<i>Serratia marcescens</i>	<i>Neisseria meningitidis</i>
<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>
<i>Staphylococcus aureus</i>	

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 Sure-Vue® RSV Test was evaluated at three physician offices. The SAS™ RSV 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 Sure-Vue® RSV Test was 100%.

Lay Person User Study

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

REFERENCES

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