Rapid Check™

Rapid Check™ RSV Antigen Test

REF GCRSV-502Sa

INSTRUCTIONS FOR USE

CLIA Categorization: Waived

For *in vitro* diagnostic use.
For prescription use only.

INTENDED USE

The Rapid Check™ RSV Antigen Test is intended for the rapid, qualitative detection of respiratory syncytial virus fusion protein directly from nasopharyngeal swab, and nasal aspirate specimens in children less than 6 years and adults over the age of 60. The test is intended for use as an aid in the rapid laboratory diagnosis of acute respiratory syncytial virus infection in patients with symptoms consistent with RSV infection. It is recommended that negative test results be confirmed by cell culture.

SUMMARY

Respiratory syncytial virus is a highly contagious, acute, viral infection of the respiratory tract. The causative agent is a single stranded (negative strand) RNA virus of the *Paramyxoviridae* family. RSV is now recognized as the leading cause of hospitalization of children during the first year of life. It is also the major viral cause of nosocomial illness in children already hospitalized for other reasons. Half of all infants become infected during their first year of life. Virtually all have been infected by their second year.[1] Infection involving the lower respiratory tract carries an associated mortality rate of 0.5%, especially in premature infants or infants and children with underlying lung disease.

RSV antigens may be detected in clinical specimens by immunoassay. The Rapid Check™ RSV Antigen Test is a gold-labeled lateral flow immunoassay using monoclonal antibodies directed against RSV fusion (F) protein antigens.

PRINCIPLE

The Rapid Check™ RSV Antigen test is a lateral flow immunogold assay for the direct visual detection of RSV protein F in clinical samples. The basis for protein F detection is in the use of a red - colored gold labeled mouse monoclonal anti-RSV protein F antibody that after addition of extracted sample travels laterally along the strip test device membrane. This lateral flow carries the mixture of sample and gold labeled anti-RSV protein F through a membrane adsorbed monoclonal anti-RSV protein F Test Line (T) and then through a membrane adsorbed goat an anti-mouse immunoglobulin Control Line (C). When RSV protein F is present in clinical samples, the fluid phase mouse anti-RSV protein F binds this antigen and this formation of antigen - antibody complex is then in turn bound at the Test Line (T). The unbound or excess mouse anti-RSV protein F passes through the Test Line (T) and is bound at the Control Line (C) by goat anti- mouse immunoglobulin.

Therefore, in the presence of RSV protein F antigen, 2 red lines become visible: one at the Test Line (T) and a second at the Control Line (C). But when RSV antigen is absent only one red line appears at the Control Line (C).

MATERIALS PROVIDED

20 Individually packaged test devices
A membrane coated with mouse monoclonal antibody specific for RSV antigen (fusion protein, IgG, 150,000 Daltons) and control line antibody combined with gold labeled mouse anti-RSV antibody.

1 R1 (Extraction Reagent Solution)
2 mL: Surfactant in 0.5 molar MOPS buffer, pH 8.0

20 Extraction Tubes
Disposable vials for mixing 160 µL of patient sample with 4 drops of extraction reagent.

40 Disposable Pipettes
Only use the pipettes provided in this kit or a calibrated pipette capable of delivering 160 µL.
Calibrated transfer pipettes used to transfer approx. 160 µL of patient sample.

1 PC (Positive Control Reagent)
1.0 mL: Inactivated Long Strain of RSV at 3.8×10^5 PFU/mL in extraction reagent.

1 NC (Negative Control Reagent)
1.0 mL: Extraction reagent solution

1 Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

Timer, clock, or watch for specimen collection and test procedure.
Vortexer or other mixing device for preparing nasopharyngeal swab specimens. Manual mixing is optional.
Sterile nasopharyngeal swabs for specimen collection (see **SAMPLE TRANSPORT AND STORAGE** section).
Specimen containers for nasal aspirate specimen collection.
Sterile normal saline for nasal aspirate specimen collection.
Aspiration bulb for nasal aspirate specimen collection.

WARNINGS AND PRECAUTIONS

- The Rapid Check™ RSV Antigen Test is for *in vitro* diagnostic use.
- The positive control (PC) is made with Clorox inactivated RSV and should be handled as though it could transmit disease.
- Do not use the kit contents beyond the expiration date printed on the outside of the box and on the individual components.
- Use appropriate precautions against microbial hazards in the collection, handling, storage, and disposal of patient samples and used kit contents.[2] Discard used material in a proper biohazard or sharps container. Patient samples should be handled as though they could transmit disease.
- The test device must remain sealed in the protective foil pouch until use.
- The R1, PC, and NC contain a detergent solution. If the solution contacts the skin or eye, flush with copious amounts of water.
- To obtain accurate results, you must follow the test procedure in the package insert.
- To obtain accurate results, use appropriate nasopharyngeal swabs and recommended transport media.
- All transfer pipettes and test vials are single-use items. do not use more than once.

KIT STORAGE AND STABILITY

Store the kit refrigerated or at room temperature 2-30°C (36-86°F). Keep Dry and out of direct sunlight. Kit contents are stable until the expiration date printed on the package and kit contents. **DO NOT FREEZE**. Remove the Test Device from the foil pouch just prior to use.

QUALITY CONTROL

Built-in Procedural Control Features

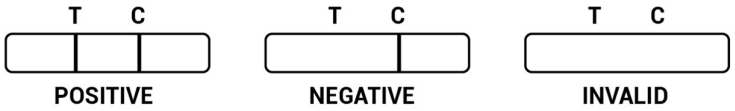
The Rapid Check™ RSV Antigen Test contains built-in procedural control features. The manufacturer's recommendations for daily control are to document these built-in procedural control features for each sample tested. The one- or two-line result format provides a simple interpretation for negative or positive results. The appearance of the one pink to red procedural control line (C) provides several forms of procedural control by demonstrating sufficient capillary flow has occurred and the functional integrity of the test device was maintained. If the pink to red procedural control line (C) does not develop at 15 minutes, the test result is considered invalid. Should this occur, review the test procedure, and repeat the test with a new test device.

External Quality Control

It is recommended that external controls be used to demonstrate that the reagents and assay procedure performed properly. External Positive and Negative Control Reagents (PC and NC) are supplied in the Test Kits and should be tested by adding six (6) drops of the PC or NC to the Sample Window (S) on the test device and allow the test to develop as described in the **TEST PROCEDURES** section. **The PC and NC bottles must be swirled or gently inverted twice before being used.** These controls **must** be tested with each new lot or shipment of materials. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. It is recommended that the user refer to NCCLS EP-12A8 and 42 CFR 493.1202 (c) for guidance on appropriate QC practices. The external positive and negative controls are intended to monitor substantial reagent failure. The positive control will not challenge the assay at the cutoff.

If the controls do not work as expected, a report should not be generated to the clinician.

SPECIMEN COLLECTION



Nasopharyngeal Swab Sample

To collect a nasopharyngeal swab sample, insert the sterile swab into the nostril that presents the most secretion under visual inspection. Using gentle rotation, push the swab until resistance is met at the level of the turbinate (less than one inch into the nostril). Rotate the swab a few times against the nasal wall. Nasopharyngeal specimens should be placed in a maximum of 3 mL normal saline or transport medium prior to processing. It is recommended that the viral transport medium or saline should be limited to no more than 3 mL total volume. For nasal swabs, Dacron™ polyester or rayon tipped swabs with an aluminum wire are recommended. See **SAMPLE TRANSPORT AND STORAGE** section for appropriate swabs.

Nasal Aspirate Sample

FOR OLDER CHILDREN AND ADULTS

Fill aspiration bulb or bulb syringe with 2.0 - 2.5 mL of sterile normal saline. Insert the tip of the bulb into the nostril until the nostril seals around the bulb and instill the saline into one nostril while the head is tilted back. Release the pressure on the bulb to aspirate the specimen back into the bulb. Transfer the specimen to a clean dry specimen container. Repeat for the other nostril and collect the fluid into the same specimen container.

FOR YOUNGER CHILDREN

The child should sit on the parent's lap facing forward, with the child's back against the parent's chest. The parent should wrap one arm around the child in a manner that will restrain the child's body and arms.
Fill aspiration bulb or bulb syringe with 1.5 - 2.0 mL of sterile normal saline (depending on the size of the child). Insert the tip of the bulb into the nostril until the nostril seals around the bulb and instill the saline into one nostril while the head is tilted back. Release the pressure on the bulb to aspirate the specimen back into the bulb. Transfer the specimen to a clean dry specimen container. Repeat the process for the other nostril and collect the fluid into the same specimen container.

Note: Nasal aspirate volumes of 3 to 5 mL are recommended. Excessive sample volumes may result in decreased test performance. If viral transport medium is added to the specimen, limit the total volume to no more than 5 mL. Process specimen as described in **TEST PROCEDURES** section.

SAMPLE TRANSPORT AND STORAGE

Samples should be tested as soon as possible after collection. Samples may be stored refrigerated, 2-8°C (36-46.4°F) in a clean, dry, closed container for up to 48 hours or frozen at - 70°C (-94°F) for up to one week prior to processing. Do not centrifuge specimen prior to use with the Rapid Test™ RSV Antigen Test Device, as removal of cellular material will adversely affect the sensitivity of the test. Because RSV is a very thermolabile organism, extreme caution should be used in collection and transport with regard to time, temperature and use of recommended transport medium. The following transport media have been tested and found to be compatible with the Rapid Check™ RSV Antigen Test:

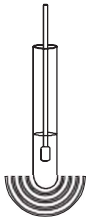
- Saline, Sterile Normal
- Phosphate Buffered Saline
- PBS plus 0.5% gelatin
- PBS plus 0.5% Bovine Serum Albumin (BSA)
- Viral Culturette™ (ideal for testing a negative result)
- Veal Infusion Broth (VIB)
- VIB plus 0.5% BSA
- Earle's Minimal Essential Medium (EMEM)
- EMEM with Lactalbumin Hydrolysate
- Tripticase™ Soy Broth plus 0.5% gelatin
- M5 media
- Microgent™/ Swab Combo in M4 media by Micro Test (ideal for testing a negative result)
    - *This swab and transport media kit was utilized in the clinical performance studies for the Rapid Check ™ RSV Antigen Test*

TEST PROCEDURES

Expiration date: Check expiration date on each individual test package (foil package or outer box) before using. ***Do not use any test past the expiration date on the label.***

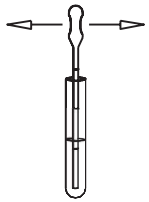
Nasopharyngeal Swab Procedure

1. Vortex or agitate the swab and transport medium or saline solution for 15-20 seconds to dislodge specimen from the swab.



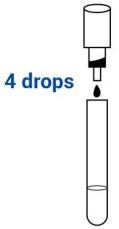
2. Roll the swab head against the inside of the transport tube or specimen container as you remove it. Dispose of the used swab in accordance with your biohazard waste disposal protocol.

3. Draw 160 µL (second notch) of the sample up into a disposable pipette.



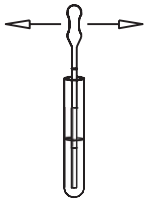
4. Add entire contents of the Pipette to the extraction tube.

5. Add four (4) **free falling** drops of R1 to the extraction tube while holding the bottle vertically over the tube.



6. Gently swirl the extraction tube to mix the sample and R1.

7. Draw 160 µL (second notch) of the sample up into a disposable pipette.



8. Squeeze entire contents of the pipette dropper onto the sample well of the test device.

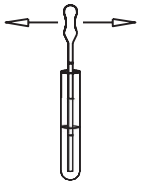
9. Read test result between fifteen (15) and twenty-five (25) minutes after applying the extracted sample to the test device.

Results read outside the recommended time range are considered invalid.

Nasal Aspirate Procedure

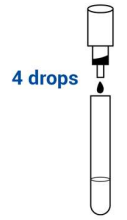
1. Gently agitate with swirling motion or vortex aspirate sample to suspend cellular material in sample.

2. Fill disposable pipette to the second notch (approximately 160 µL) with nasal aspirate sample.



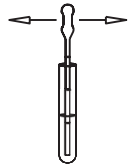
3. Add entire contents of the pipette to the extraction tube.

4. Add four (4) **free falling** drops of R1 to the extraction tube while holding the bottle vertically over the tube.



5. Gently swirl the Extraction Tube to mix the sample and R1.

6. Draw 160 µL (second notch) of the sample up into a disposable pipette.



7. Squeeze entire contents of the pipette dropper onto the sample well of the test device.

8. The test result should be read between fifteen (15) and twenty-five (25) minutes after applying the extracted sample to the test device.

Results read outside the recommended time range are considered invalid.

INTERPRETATION OF RESULTS

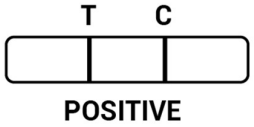
The test result should be read between fifteen (15) and twenty-five (25) minutes after applying the extracted sample to the test device.

If the results are read after 25 minutes, the result is invalid. Specimen should be retested.

Positive Result

For a positive specimen, the appearance of **TWO** pink to red lines, one at the procedural control line (C) and one at the test line (T) indicates the presence of respiratory syncytial virus antigen. Any pink to red test line, even if it is only slightly pink, is considered a positive test.

Results reported: Positive for the presence of RSV antigen. A positive result may occur in the presence of both viable and non-viable viruses.

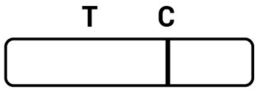


POSITIVE

Negative Result

For a negative specimen, the appearance of **ONLY ONE** pink to red line at the procedural control line (C) and no pink to red line at the Test Line (T) indicates that the sample is negative for RSV viral antigen.

Results reported: Negative for presence of RSV antigen. Infection due to RSV cannot be ruled out since the antigen present in the sample may be below the detection limit of the test. Cell culture confirmation of negative samples is recommended.



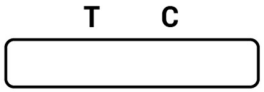
NEGATIVE

Invalid Result

If after 15 minutes, the pink to red procedural control line (C) does not appear, even if any shade of pink to red test line (T) appears, the result is considered invalid. If the test is considered invalid because a control line fails to appear, the test should be repeated with a new test device.

Within 15 minutes the result area should be white to light pink and allow the clear interpretation of the test result. **If the background color persists and interferes with the interpretation of the test result, the result is considered invalid.**

Should this occur, review the test procedure and repeat the test with a new test device.
If the test result is still invalid after repeating with a new test device, then it is considered invalid.



INVALID

LIMITATIONS

*See **Table 9** for limits of detection of Rapid Check™ RSV Antigen Test.*

- The contents of this kit are to be used for the qualitative detection of respiratory syncytial virus antigen from nasopharyngeal swab and nasal aspirate specimens.
- Failure to follow the test procedure or Interpretation of test results instructions may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test, or from improper sample collection and storage.
- Fresh specimens are preferable to frozen for RSV testing because of the highly thermolabile and fragile nature of the virus. Sub-optimal test performance may result with the latter.
- A negative test result does not rule out other microbial respiratory tract infections.
- Cross-reactivity of this assay with human metapneumovirus has not been studied.
- Monoclonal antibodies may not detect all antigenic variants or new strains of RSV.
- Testing with both the sample and/or the kit should not be performed at 4°C (39.2°F). Kit performance could be diminished if either the sample and/or the kit are not brought to room temperature first.
- Testing low levels of RSV in high humidity at room temperature may reduce the test's ability to detect RSV.
- Testing with total sample volumes less than or greater than the recommended volume could reduce the ability of the test to detect low levels of RSV.
- Low-level positive results may not be properly visualized at <30% 'normal' bench lighting levels.

EXPECTED VALUES

The rate of positivity observed in RSV testing will vary depending on the time of year, age of the patient, geographic location, and local disease prevalence.[1] In temperate climates, RSV infections occur primarily during annual outbreaks, which peak during the winter months.[4] RSV is a common pathogen among infants and young children.[5] However, it may cause serious lower respiratory tract illness throughout life, especially among the elderly and those with compromised immune systems.[5-7] The following table summarizes the age and sex demographic data with the number of samples in each age group and the percent positivity for that group among the clinical samples tested by Rapid Check™ RSV Antigen Test. There are a total of 180 patients whose age was given with the sample included in the table. Of these, 92 had the sex of the patient recorded with the sample. See **Table 1** below:

Table 1: Patient Demographics

Patient Age	Male: Female	# Samples	% Positive Rapid Check™ Results
2d-1 M	2:2	12	5/12 = 41.6%
1-2 M	2:2	8	4/8 = 50%
2M-6 Y	22:12	112	30/112 =26.7%
51-60 Y	1:1	2	0/2 = 0%
61-70 Y	2:7	9	3/9 = 33.3%
71-80 Y	1:6	7	0/7 = 0%
81-90 Y	7:6	13	2/13 = 15.5%
91-100 Y	6:11	17	2/17 = 11.7%

Of the 17 patients whose age was not recorded, 68.7% were positive by Rapid Check™ RSV Antigen Test and 62.5% were positive by the reference method. Nine patients whose age was recorded as "greater than 60" were placed in the 61–70-year age group.

CLIA CONSIDERATIONS

This is a waived test under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) so long as it is used according to the instructions set in this package insert.
Any modification by the laboratory to the test system or the test system instructions will result in this test no longer meeting the requirements for waived categorization. A modified test is considered high complexity and is subject to applicable CLIA requirements. Further, the laboratory should notify Healgen Scientific of any performance, perceived or validated, that does not meet the performance specifications as outlined in the instructions.

Under CLIA, Consumer Precision Studies and Consumer Accuracy Studies were conducted to demonstrate that lay users with no formal laboratory training could read the instructions for use and perform the test with a high level of concordance with trained laboratorians. Consumer Precision Study testing was conducted using 90 swab samples at negative (0 pfu/mL RSV), LOD (1.9×10^5 pfu/mL RSV), and moderate positive (commercial kit positive control at 3.8×10^5 pfu/mL RSV) levels.
No significant differences were observed between the lay users and the trained laboratorians (Consumer Accuracy Study) or between the lay users within each site and among the three sites (Consumer Precision Study).
A summary table of the Consumer Accuracy Study and Consumer Precision Study is shown below (**Table 2**).

Table 2: CLIA Consumer Precision/Accuracy Studies

	Frequencies of Test Results (95% C.I)					
Participants	**Negative**		**LOD**		**Mod Positive**	
	%+ Test	%- Test	%+ Test	%- Test	%+ Test	%- Test
Lay Users (3 Sites)	0%	100%	94.4%	5.5%	100%	0%
	0/90	90/90	85/90	5/90	90/90	0/90
	(0.0 – 4.1%)	(95.9 – 100%)	(87.6 – 97.6%)	(2.4 – 12.4%)	(95.9 – 100%)	(0.0 – 4.1%)
Laboratorian (3 Sites)	0%	100%	96.7%	3.3%	100%	0%
	0/90	90/90	87/90	3/90	90/90	0/90
	(0.0 – 4.1%)	95.9 – 100%)	(90.7 – 98.9%)	(1.1 – 9.3%)	(95.9 – 100%)	(0.0 – 4.1%)

PERFORMANCE CHARACTERISTICS

The performance of the Rapid Check™ RSV Antigen Test was compared to Tissue Culture, EIA and Direct Fluorescent Antibody (DFA) in a prospective multi-center field clinical study in the Midwest and Ontario Canada during the 2002 flu season. In this trial, the Rapid Check™ RSV Antigen Test was compared to DFA at the two United States sites or Tissue Culture and EIA at one Canadian site. The study comprised a total of 197 nasal aspirate and nasopharyngeal swab samples obtained from patients symptomatic for RSV infection. All samples were split to allow testing of the same sample by Rapid Check™ RSV Antigen Test and the reference method (Tissue Culture and EIA at the Canadian site or DFA at the U.S. sites). The age of the patients ranged from 2 days to 100 years of age. The sample type in the U.S. consisted of nasopharyngeal swabs and aspirates and the sample type in Canada was entirely nasopharyngeal swabs.

United States Data

There was a **total of 104** samples tested in the United States. There were **90** nasal aspirates, **7** nasopharyngeal swabs, and **7** samples with no designation of sample type given. All 104 of the samples tested in the United States were compared to DFA as the reference method as shown in **Table 3**.

Table 3: United States: Rapid Check™ RSV Antigen Test Compared to DFA

		DFA Result	
		Positive	**Negative**
Rapid Check™ Result	**Positive**	42	2
	Negative	3	57

Positive Agreement: 42/45 = 93.3% (95% C.I. = 80.7 – 98.6%)
Negative Agreement: 57/59 = 96.6% (95% C.I. = 88.3 – 99.6%)

A subset of the U.S data, the **90** samples designated as nasal aspirates is shown in **Table 4**.

Table 4: United States: Rapid Check™ RSV Nasal Aspirate Samples Compared to DFA

		DFA Result	
		Positive	**Negative**
Rapid Check™ Result	**Positive**	35	2
	Negative	2	51

Positive Agreement: 35/37 = 94.6% (95% C.I. = 81.8 – 99.3%)
Negative Agreement: 51/53 = 96.2% (95% C.I. = 87.7 – 99.3%)

Note: The 7 samples with no sample type designation and the 7 nasopharyngeal swab samples in the U.S. have not been broken out into separate tables but are included in **Table 2**. Of the 7 samples designated as nasopharyngeal swabs in the U.S., 3 were positive and 4 were negative and there was 100% positive agreement and 100% negative agreement with DFA.

Canadian Data

There was a **total of 93** samples tested in Canada. All were nasopharyngeal swab samples. The reference test for **92** of these was **both** Tissue Culture and EIA and **one** sample had **only EIA** as the reference for a total of **93**. The performance data for Rapid Check™ RSV Antigen Test against the two reference methods is shown in **Tables 5 and 6**.

Table 5: Canada: Rapid Check™ RSV Antigen Test Compared to Tissue Culture (All Nasopharyngeal Swabs)

		Tissue Culture Result	
		Positive	**Negative**
Rapid Check™ Result	**Positive**	10	10
	Negative	0	72

Sensitivity: 10/10 = 100% (95% C.I. = 69.2 – 100%)
Specificity: 72/82 = 87.8% (95% C.I. = 80.7 – 94.9%)

Table 6: Canada: Rapid Check™ RSV Antigen Test Compared to EIA (All Nasopharyngeal Swabs)

		EIA Result	
		Positive	**Negative**
Rapid Check™ Result	**Positive**	20	1
	Negative	1	71

Positive Agreement: 20/21 = 95.2% (95% C.I. = 76.2 – 99.9%)
Negative Agreement: 71/72 = 96.6% (95% C.I. = 92.5 – 99.9%)

Summary of Clinical Data for All Sites

All Samples: There were a total of **197** samples, **104** in the U.S. and **93** in Canada:
Overall positive agreement = 93.9% (95% C.I. = 85.4 – 97.6%)
Overall negative agreement = 97.7% (95% C.I. = 93.5 – 99.2%)

For Nasopharyngeal Swabs: There were a total of **100** samples, **7** in the U.S. and **93** in Canada:
Overall positive agreement = 95.8% (95% C.I = 79.8 – 99.3%)
Overall negative agreement= 98.7% (95% C.I. = 92.9 – 99.8%)

For Nasal Aspirates: There were a total of **90** samples in the U.S.
Positive Agreement: = 94.6% (95% C.I. = 81.8 – 99.3%)
Negative Agreement: = 96.2% (95% C.I. = 87.7 – 99.3%)

Note: 7 samples in the U.S. had no designation of sample type and are not included in the breakdown of nasopharyngeal swab performance (N = 100) and nasal aspirate performance (N = 90). These 7 samples are included in the overall performance data for all samples at all sites (N = 197) and in the table for all of the U.S data (**Table 2**).

Analytical Specificity and Cross-Reactivity

The bacterial and viral organisms listed below were used to assess cross reactivity in the Rapid Check™ RSV Antigen Test. No cross reactivity was noted with any of the organisms tested. The testing was done in replicates of three. (**See Table 7 and 8**)

Table 7: Bacterial Panel

Organism Tested	Strain/Reference	Final Concentration	Results	
			Interference	Cross-Reactivity
Candida albicans	10231	5.0×10^6 org/mL **	3/3 pos	3/3 neg
Chlamydia psittaci	VMLCPS0427	$2.0\times\times10^6$ inc/mL **	3/3 pos	3/3 neg
Chlamydia trachomatis	VML CT 092801	2.0×10^6 inc/mL	3/3 pos	3/3 neg
Haemophilus influenzae	10211	5.0×10^7 org/ml	3/3 pos	3/3 neg
Klebsiella pneumoniae	13883	5.0×10^7 org/mL	3/3 pos	3/3 neg
Mycoplasma pneumoniae	15531	2.6×10^3 CFU/ml*	3/3 pos	3/3 neg
Neisseria meningitidis	13090	5.0×10^7 org/mL	3/3 pos	3/3 neg
Pseudomonas aerugenosa	9027	5.0×10^7 org/mL	3/3 pos	3/3 neg
Serratia liquifans	27592	5.0×10^7 org/mL	3/3 pos	3/3 neg
Staphylococcus aureus	25923	5.0×10^7 org/mL	3/3 pos	3/3 neg
Staphylococcus aureus	Cowan	3.0×10^8 org/mL	3/3 pos	3/3 neg
Staph epidermidis	12228	5.0×10^7 org/mL	3/3 pos	3/3 neg
Strep pneumoniae	Wild strain	5.0×10^7 org/mL	3/3 pos	3/3 neg
Streptococcus sp gr A	12384	5.0×10^7 org/mL	3/3 pos	3/3 neg
Streptococcus sp gr F	12392	5.0×10^7 org/mL	3/3 pos	3/3 neg
Streptococcus sp gr G	12394	5.0×10^7 org/mL	3/3 pos	3/3 neg

* The Cowan Strain of Staphylococcus aureus is a Protein A producing strain and did not show cross reactivity with Rapid Check™ RSV Antigen Test.
** org/mL = organisms/mL
*** inc/mL = inclusions/mL

Table 8: Viral Panel

Organism Tested	Strain/Reference	Final Concentration	Results	
			Interference	Cross-Reactivity
Adenovirus 5	VR-5	1.0×10^5 PFU/mL	3/3 pos	3/3 neg
Adenovirus 7	VR-7	1.0×10^5 PFU/mL	3/3 pos	3/3 neg
Adenovirus 2	VR-846	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Coxsackie A19	VR-1015	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Coxsackie B1	VR- 28	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Coxsackie B3	VR-30	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Cytomegalovirus	AD-169	8.8×10^4 PFU/mL	3/3 pos	3/3 neg
Poliovirus	VR-1004	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Echovirus 6	VR-36	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Echovirus 11	VR-41	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
HSV Type 1	VR-6143	3.3×10^5 PFU/mL	3/3 pos	3/3 neg
HSV Type 2	VR-15671	3.3×10^5 PFU/mL	3/3 pos	3/3 neg
Influenza A	N1H1	9.2×10^2 PFU/mL	3/3 pos	3/3 neg
Influenza B	Beijing	5.9×10^4 PFU/mL	3/3 pos	3/3 neg
Parainfluenza 1	VR-105	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Parainfluenza 2	VR-92	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Parainfluenza 3	VR-93	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Rhinovirus 1B	VR-1366	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Rhinovirus 39	VR-340	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Rhinovirus 16	VR-1126	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Rhinovirus 14	VR-284	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Rhinovirus 37	VR-1147	1.0×10^5 $TCID_{50}$/mL	3/3 pos	3/3 neg
Varicella	Ellen	6.5×10^3 PFU/mL*	3/3 pos	3/3 neg

These viral strains were obtained from American Type Culture Collection (ATCC) and the titers were established by the independent laboratory where cross reactivity testing was performed.

Analytical Sensitivity

The Rapid Check™ RSV Antigen Test limit of detection (LOD) for RSV antigen subgroup A (Long and A2 strains) and antigen subgroup B (18537 and WV/14617/85, B-1 strains) are shown in **Table 9**.

Table 9: Rapid Check™ RSV Antigen Test Limits of Detection (LOD)

RSV Strain	Antigen Subgroup	LOD (pfu/mL)	Frequency Positive Test	95% Confidence Interval
Long	**A**	1.9×10^5	90%	68.3 – 98.7%
A2	**A**	$>1.12 \times 10^5$	80%	56.0 – 94.2%
18537	**B**	$>1.9 \times 10^5$	80%	56.0 – 94.2%
WV14617/85	**B**	1.9×10^5	95%	75.0 – 99.9%

All concentrations of RSV are those found in original samples before manipulation and placement on the Rapid Check™ RSV Antigen Test device.

Interfering Substances

Whole blood, mucin and several over the counter (OTC) products, common chemicals, storage media and transport media were evaluated and did not interfere with the Rapid Check™ RSV Antigen Test at the levels tested. The testing was done in replicates of three. (**See Table 10**)

Table 10: Interfering Substances Panel

Test Substance	Final Concentration	Results	
		Interference	Cross-Reactivity
Scope Mouthwash	25%	3/3 positive	3/3 negative
Listerine Mouthwash	25%	3/3 positive	3/3 negative
CVS Mouthwash	25%	3/3 positive	3/3 negative
Ludens Cough Drops	25%	3/3 positive	3/3 negative
Hall Cough Drops	25%	3/3 positive	3/3 negative
Ricola Cough Drops	25%	3/3 positive	3/3 negative
Afrin Nasal Spray	10%	3/3 positive	3/3 negative
Neosynephrine N S	10%	3/3 positive	3/3 negative
CVS Nasal Spray	10%	3/3 positive	3/3 negative
4-Acetomidrophenol	10 mg/mL	3/3 positive	3/3 negative
Acetylsalicylic Acid	20 mg/mL	3/3 positive	3/3 negative
Chlorpheniramine	5 mg/mL	3/3 positive	3/3 negative
Dextromethorphan	10 mg/mL	3/3 positive	3/3 negative
Diphenhydramine	5 mg/mL	3/3 positive	3/3 negative
Ephedrine	20 mg/mL	3/3 positive	3/3 negative
Guiacol Glycerol Ether	20 mg/mL	3/3 positive	3/3 negative
Oxymetazoline	10 mg/mL	3/3 positive	3/3 negative
Albuterol	10 mg/mL	3/3 positive	3/3 negative
Mucin	4 mg/mL	3/3 positive	3/3 negative
Whole Blood	50% w/v	3/3 positive	3/3 negative
Saline	67%	3/3 positive	3/3 negative
Phosphate Buffered Saline	67%	3/3 positive	3/3 negative
PBS/0.5% Gelatin	67%	3/3 positive	3/3 negative
PBS/0.5% BSA	67%	3/3 positive	3/3 negative
2.5% Veal Infusion Broth	67%	3/3 positive	3/3 negative
VIB/0.5% BSA	67%	3/3 positive	3/3 negative
MEM/Earles Salts	67%	3/3 positive	3/3 negative
MEM/Lactalbumin Hydriste	67%	3/3 positive	3/3 negative
2.5% Trypticase Soy with 0.5% Gelatin	67%	3/3 positive	3/3 negative
M4 Media	67%	3/3 positive	3/3 negative
M5 Media	67%	3/3 positive	3/3 negative

All concentrations of RSV are those found in original samples before manipulation and placement on the Rapid Check™ RSV Antigen Test device.

Reproducibility Studies

NCCLS document EP 12, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline[8] defines critical values for evaluation of performance of a qualitative test. These critical values include the "cutoff" or the test response above which the test result is determined to be positive and below which the test response is determined to be negative, and the LOD at which the test response gives 95% positive results and 5% negative results. The cutoff is the level of virus that gives 50% positive results and 50% negative results. Levels at 10% and 20% above the cutoff should give rates of positivity between 50% and 95%. For levels of RSV at 10% and 20% below the cutoff the rate of negative test results should be between 50% and 95%.

Over 3 consecutive days, at numerous test sites, and by multiple operators, the Rapid Check™ RSV Antigen Test performance was evaluated by both in-house masked reproducibility trials (**Table 11**) and physician office laboratory masked reproducibility trials (**Table 12**). As shown, these trials define the values critical to qualitative function and include:

1. The cutoff at 9.3×10^4 pfu/mL of virus where 50% of replicate samples are found to be positive and 50% are found to be negative.
2. The limit of detection (LOD) at 1.9×10^5 pfu/mL of virus where positive test results approach 95% and negative test results approach 5%.
3. The region of virus concentration (pfu/mL) above the cutoff where positive test values range between 50% and 95%.
4. The region of virus concentration (pfu/mL) below the cutoff where negative test results range between 50% and 95% respectively.

Additionally, POL field studies at 3 independent sites showed expected results with values of percent positive test for the LOD at 1.9×10^5 pfu/mL that approach 95%. Levels of virus above the 95% interval at 7.7×10^5 pfu/mL that approach 100% positive test results are shown. Also, levels of virus at 10% (1.02×10^5 pfu/ mL) and 20% (1.12×10^5 pfu/mL) above the cutoff have expected positive test results between 61% and 74% respectively. At 20% below the cutoff (7.4×10^4 pfu/mL) the rate of negative test results is 77% and outside the 95% interval at 4.7×10^4 pfu/mL the rate of negative test results approach 100% (**See Table 12**).

Note: The positive control used in the reproducibility trials is the RSV Long Strain at the LOD (1.9×10^5 pfu/mL) of the device in order to better challenge this critical point of the assay. The control included in the kit is twice this LOD concentration.

In House Reproducibility

Table 11 below shows Rapid Check™ RSV Antigen Test positive and negative test results with 95% confidence intervals (C.I.) for 5 concentrations of RSV: (1) limit of detection (LOD) of 1.9×10^5 pfu/mL, (2) cutoff of 9.3×10^4 pfu/mL, (3) 20% above the cutoff at 1.12×10^5 pfu/mL, (4) 10% above the cutoff at 1.02×10^5 pfu/mL and, (5) 20 % below the cutoff at 7.4×10^4 pfu/mL. Testing was done with coded samples as daily runs over 3 consecutive days by 3 operators. All concentrations of RSV are those found in original samples before manipulation and placement on the Rapid Check™ RSV Antigen Test device.

Table 11: Rapid Check™ RSV In-House Reproducibility

RSV Long Strain (pfu/mL)	Number Replicates	Frequency (%) Positive (95% C.I.)	Frequency (%) Negatives (95% C.I.)
1.90×10^5	**30**	**83.3** (65.3 – 94.4)	**16.7** (5.64 – 34.7)
1.12×10^5	**90**	**67.8** (57.1 – 77.3)	**32.2** (28.8 – 42.9)
1.02×10^5	**90**	**58.9** (48.0 – 69.2)	**41.1** (30.8 – 51.9)
9.30×10^4	**90**	**50.0** (39.3 – 60.7)	**50.0** (39.3 – 60.7)
7.40×10^4	**90**	**23.3** (15.1 – 33.4)	**76.6** (66.6 – 84.4)

Physician Office Laboratory (POL) Reproducibility Studies

Table 12 shows Rapid Check™ RSV Antigen Test positive and negative test results with 95% confidence intervals (C.I.) for concentrations of RSV at the limit of detection (LOD) of 1.9×10^5 pfu/mL, above the 95% interval at 7.7×10^5 pfu/mL, three levels around the cutoff ranging from 7.40×10^4 pfu/mL to 1.12×10^5 pfu/mL and, below the 95% interval at 4.7×10^4 pfu/mL. Testing was done with coded samples by 6 operators with diverse educational backgrounds and work experiences in replicates of 3 at 3 different locations over 3 days. All concentrations of RSV are those found in original samples before manipulation and placement on the Rapid Check™ RSV Antigen Test device.

Table 12: Rapid Check™ RSV POL Reproducibility

RSV Long Strain (pfu/mL)	Number Replicates	Frequency (%) Positive (95% C.I.)	Frequency (%) Negatives (95% C.I.)
7.7×10^5	**54**	**100** (93.4 - 100)	**00.0** (0.0 - 6.6)
1.9×10^5	**54**	**100** (93.4 - 100)	**00.0** (0.0 - 6.6)
1.12×10^5	**54**	**74.1** (60.4 - 85.0)	**25.9** (14.9 - 39.65)
1.02×10^5	**54**	**61.1** (46.9 - 74.1)	**38.8** (25.9 - 53.12)
7.40×10^4	**54**	**22.2** (12.0 - 77.7)	**77.7** (64.4 - 87.96)
4.7×10^4	**54**	**00.0** (0.0 - 6.6)	**100** (93.4 - 100)

Note: The positive control used in the reproducibility trials is the RSV Long Strain at the LOD (1.9×10^5 pfu/mL) of the device in order to better challenge this critical point of the assay. The control included in the kit is twice this LOD concentration.

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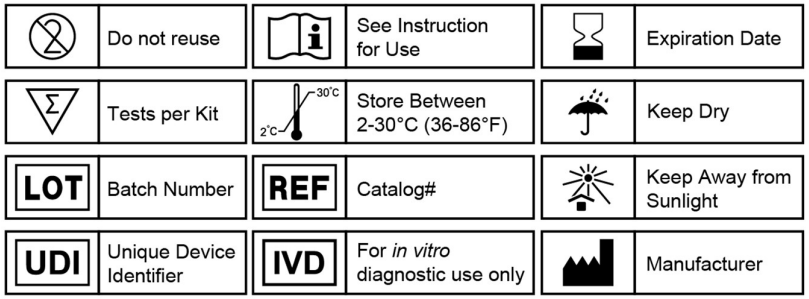
INDEX OF SYMBOLS



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