

PROCEDURE

Title: RSV SOFIA-WAIVED METHOD ONLY AT HIGHLANDS REGIONAL MEDICAL CENTER

Procedure #: 2015WAIVED10

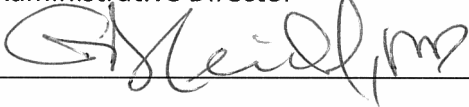
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Date: 6/5/2015

Title: Laboratory Administrative Director

Accepted by:  Date: 6-8-15

Title: Laboratory Medical Director

Date Patient Testing Implemented: _____

Review of procedure every two years

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the Package Insert. Any modifications to this document are the sole responsibility of the Laboratory.

Sofia RSV FIA

For use with the Sofia only

CLIA Complexity: Moderate for pediatric patients 7 to less than 19 years of age

CLIA Complexity: WAIVED for children less than 7 years of age

For *in vitro* use only.

A Certificate of Waiver is required to perform this test in a CLIA waived setting. To obtain a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA or from your state health department.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

INTENDED USE

The Sofia RSV FIA employs immunofluorescence for detection of respiratory syncytial virus (RSV) nucleoprotein antigen in nasopharyngeal swab and nasopharyngeal aspirate/wash specimens taken directly from symptomatic patients. This qualitative test is intended for use as an aid in the rapid diagnosis of acute RSV infections in pediatric patients. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or for other management decisions. A negative result is presumptive, and it is recommended these results be confirmed by virus culture or an FDA-cleared RSV molecular assay.

SUMMARY AND EXPLANATION

RSV is a causative agent of highly contagious, acute, viral infection of the respiratory tract in pediatric and elderly populations. Respiratory syncytial virus is a single-stranded RNA virus.¹ Nearly half of all children become infected with RSV in their first year of life. It is also the major viral cause of nosocomial illness in children already hospitalized for other reasons.² In the United States, RSV is estimated to be responsible for 73,400 to 126,300 hospitalizations annually for bronchiolitis and pneumonia alone among children younger than 1 year.³ In an analysis of U.S. viral surveillance and mortality data, respiratory syncytial virus (RSV) was reported as the most common viral cause of death in children younger than 5 years when compared to influenza A (H1N1), influenza A (H3N2), and influenza B.⁴ Among children hospitalized with RSV infection, the mortality rate is estimated to be as low as 0.3% to 1.0%^{3,5} and in the range of 2.5% to 4.0% for children with underlying cardiac or pulmonary disease.^{3,5,6}

PRINCIPLE OF THE TEST

The Sofia RSV FIA test employs immunofluorescence technology that is used with the analyzer, Sofia, to achieve the rapid detection of RSV antigens. The Sofia RSV FIA test involves the disruption of virus and detection of nucleoproteins inside the virus. The patient specimen is placed in the Reagent Tube, during which time the virus particles in the specimen are disrupted, exposing internal viral nucleoproteins. After disruption, the specimen is dispensed into the Cassette sample well. From the sample well, the specimen migrates through a test strip containing various unique chemical environments. If RSV viral antigens are present, they will be trapped in a specific location.

Note: Depending upon the user's choice, the cassette is either placed inside of Sofia for automatically timed development (Walk Away Mode) or placed on the counter or bench top for a manually timed development and then placed in Sofia to be scanned (Read Now Mode).

Sofia will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. Sofia will display the test results (Positive, Negative, or Invalid) on the screen. The results can also be automatically printed on an integrated printer if this option is selected.

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Cassettes (25): Mouse monoclonal anti-RSV antibodies
- Reagent Tubes (25): Lyophilized buffer with detergents and reducing agents
- Reagent Solution (25): Vials with salt solution
- Sterile Nasopharyngeal Swabs (25)
- Large (250 μ L), Pink Fixed Volume Pipettes (25)
- Small (120 μ L), Clear Fixed Volume Pipettes (25)
- RSV Positive Control Swab (1): Swab is coated with non-infectious RSV antigen
- Negative Control Swab (1): Swab is coated with heat-inactivated, non-infectious Streptococcus C antigen
- Package Insert (1)
- Quick Reference Instructions (1)
- QC Card (located on kit box)
- Printer Paper (1)

MATERIALS NOT SUPPLIED in kit

- Timer or watch for use in Read-Now Mode
- Sofia analyzer
- Sterile saline for the collection of Nasopharyngeal Aspirate or Wash Specimens
- Equipment used for collection of Nasopharyngeal Aspirate or Wash Specimens
- Calibration Cassette (supplied with the Sofia Installation Pack)

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.⁷
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.⁷
- Dispose of containers and used contents in accordance with Federal, State and Local requirements.
- Do not reuse the used Cassette, Fixed Volume Pipettes, Reagent Tubes, solutions, or Control Swabs.

- The user should never open the foil pouch of the test Cassette exposing it to the ambient environment until the Cassette is ready for immediate use.
- Discard and do not use any damaged Cassette or material.
- The Reagent Solution contains a salt solution (saline). If the solution contacts the skin or eye, flush with copious amounts of water.
- To obtain accurate results, the Package Insert instructions must be followed.
- The Calibration Cassette must be kept in the provided storage pouch between uses.
- Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
- Sample collection and handling procedures require specific training and guidance.
- To obtain accurate results, use the Viral Transport Medium (VTM) recommended in this Package Insert.
- When collecting a nasopharyngeal swab sample, use the nasopharyngeal swab supplied in the kit.
- Use the appropriate Fixed Volume Pipette in accordance with test procedures:
 - ▶ **Only the Small, Clear 120 µL Fixed Volume Pipette** is to be used for adding patient sample to the test Cassette.
 - ▶ **Only the Large, Pink 250 µL Fixed Volume Pipette** is to be used with the aspirate/wash or viral transport media test procedure when transferring the patient sample from the collection cup into the Reagent Tube.
- Do not pour samples from the Reagent Tube into the test Cassette sample well. Use the provided **Small, Clear 120 µL Fixed Volume Pipette** when adding the sample to the test Cassette.
- Do not write on the barcode of the Cassette. This is used by Sofia to identify the type of test being run and to identify the individual Cassette so as to prevent a second read of the Cassette by the same Sofia.
- Do not attempt to scan a Cassette more than one time. The barcode on the Cassette contains a unique identifier that will prevent Sofia from performing a second read on a previously scanned Cassette. An error message will be displayed if a Cassette is scanned more than once.
- As the detection reagent is a fluorescent compound, no visible results will form on the test strip. Sofia must be used for result interpretation.

KIT STORAGE AND STABILITY

Store the kit at room temperature, 59°F to 86°F (15°C to 30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

QUALITY CONTROL

There are three types of Quality Control for the Sofia and the Cassette: Sofia Calibration Check procedure, built-in procedural control features, and External Controls.

Sofia Calibration Check Procedure

Note: This is a “Calibration Check” procedure.

The Calibration Check Procedure should be performed every 30 days. Sofia can be easily set to remind the user to complete the Calibration Check Procedure.

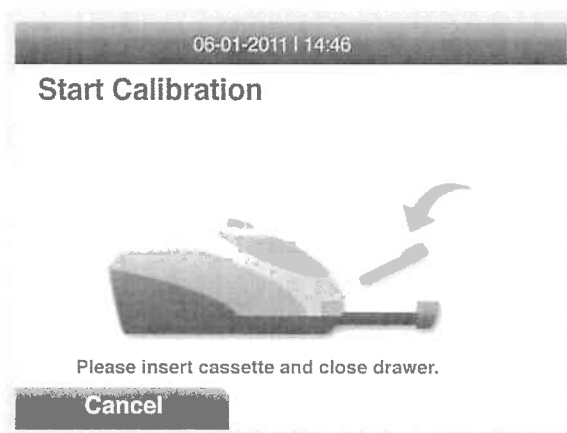
The Calibration Check is a required function that checks the Sofia optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with the Sofia Installation Pack. Refer to the Sofia User Manual for details regarding the Calibration Check Procedure.

Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses to protect from exposure to light.

1. To check the calibration of Sofia, select "Calibration" from the Main Menu.



2. Following the prompts, insert the Calibration Cassette into Sofia and close the drawer. Sofia performs the Calibration Check automatically within 2 minutes with no user input required.



Sofia indicates when the Calibration Check is completed. Select **OK** to return to the Main Menu.

NOTE:

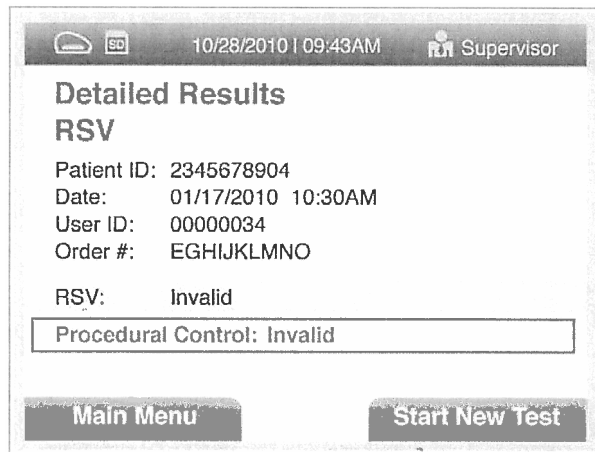
If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. PST at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; custserv@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support); or contact your local distributor.

Built-in Procedural Controls

The Sofia RSV FIA contains built-in procedural control features. Each time a test is run in Sofia, the procedural control zone is scanned by Sofia and the result is displayed on the Sofia screen.

The manufacturer's recommendation for daily control is to document the results of these built-in procedural controls for the first sample tested each day. This documentation is automatically logged into Sofia with each test result.

A valid result obtained from the procedural controls demonstrates that the test flowed correctly and the functional integrity of the Cassette was maintained. **The procedural controls are interpreted by Sofia after the Cassette has developed for 15 minutes. If the test does not flow correctly, Sofia will indicate that the result is invalid.** Should this occur, review the procedure and repeat the test with a new patient sample and a new Cassette.



For example: This display shows an invalid result.

External Quality Control

External Controls may also be used to demonstrate that the reagents and assay procedure perform properly.

Quidel recommends that Positive and Negative External Controls be run:

- once for each untrained operator
- once for each new shipment of kits – provided that each different lot received in the shipment is tested
- as deemed additionally necessary by your internal quality control procedures, and in accordance with Local, State and Federal regulations or accreditation requirements.

To test External Controls, the user must first select Run QC on the Sofia Main Menu. Then, when prompted, scan the QC Card (located on kit box). This card provides information specific to the kit lot, including lot number and expiration date. Sofia will prompt the user to select the desired mode (Walk Away or Read Now) and then to run the External Control swabs.

External Positive and Negative Control swabs are supplied in the kit and should be tested using the Swab Test Procedure provided in this Package Insert or in the Quick Reference Instructions. **The Positive Control test must be run prior to the Negative Control test.** When the QC run is complete, each result will be displayed as “Passed” or “Failed” for the Positive Control and the Negative Control.

Do not perform patient tests or report patient test results if either of the QC test results fail. Repeat the test or contact Quidel Technical Support before testing patient samples.

Additional External Control swabs may be obtained separately by contacting Quidel’s Customer Support Services at 800.874.1517 (toll-free in the U.S.) or 858.552.1100.

SAMPLE COLLECTION AND HANDLING

SAMPLE COLLECTION

Nasopharyngeal Swab Sample

Use the nasopharyngeal swab supplied in the kit.

To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril that presents the most secretion under visual inspection. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. Rotate the swab several times then remove it from the nasopharynx.

Nasopharyngeal Aspirate/Wash Sample

Follow your institution's protocol for obtaining nasopharyngeal aspirate/wash samples. **Use the minimal amount of saline that your procedure allows.** Alternatively, if your institution does not provide a protocol, then consider the following procedures that are used by clinicians.

To collect a nasopharyngeal aspirate sample: instill a few drops of sterile saline into the nostril to be suctioned. Insert the flexible plastic tubing along the nostril floor, parallel to the palate. After entering the nasopharynx, aspirate the secretions while removing the tubing. The procedure should be repeated for the other nostril if inadequate secretions were obtained from the first nostril.

To collect a nasopharyngeal wash sample: the child should sit in the parent's lap facing forward, with the child's head against the parent's chest. Fill the syringe or aspiration bulb with the minimal volume of saline required per the subject's size and age. Instill the saline into one nostril while the head is tilted back. Aspirate the wash specimen back into the syringe or bulb. The aspirated wash sample will likely be approximately 1 cc in volume.

Alternatively, following instillation of the saline, tilt the head forward and let the saline drain out into a clean collection cup.

SAMPLE TRANSPORT AND STORAGE

Samples should be tested as soon as possible after collection. However, if transport of samples is required, minimal dilution of the sample is recommended, as dilution may result in decreased test sensitivity. Whenever possible, 1 milliliter or less is best to avoid excessive dilution of the patient sample. The following viral transport media listed in Table 1 are compatible with the Sofia RSV FIA:

Table 1
Recommended Viral Transport Media

Viral Transport Medium (VTM)	Recommended Storage Condition	
	2°C to 8°C	25°C
Copan Universal Transport Medium	24 hours	24 hours
Hank's Balanced Salt Solution	24 hours	24 hours
Liquid Amies Media	24 hours	24 hours
M4	24 hours	24 hours
M4-RT	24 hours	24 hours
M6	24 hours	24 hours
Modified Liquid Stuarts Media	24 hours	24 hours

Saline	24 hours	24 hours
Starplex Multitrans	24 hours	24 hours
Phosphate Buffered Saline	24 hours	24 hours

TEST PROCEDURE

All clinical samples, including samples in VTM, must be at room temperature before beginning the assay.

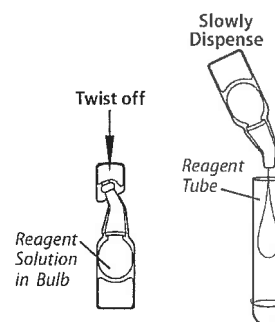
Expiration date: Check expiration date on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

Nasopharyngeal Swab Test Procedure

1. Verify that Sofia is set to the desired Mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia" section for more information.

2. **Prepare Reagent:**

- a. Flick or shake the Reagent Solution vial down so that all fluid is in the bulb.
- b. Twist off the tab.
- c. Slowly dispense all of the Reagent Solution into the Reagent Tube.
- d. Gently swirl the Reagent Tube to dissolve its contents.



3. Place the patient swab sample into the Reagent Tube. Roll the swab at least 3 times while pressing the head against the bottom and side of the Reagent Tube.

Leave the swab in the Reagent Tube for 1 minute.



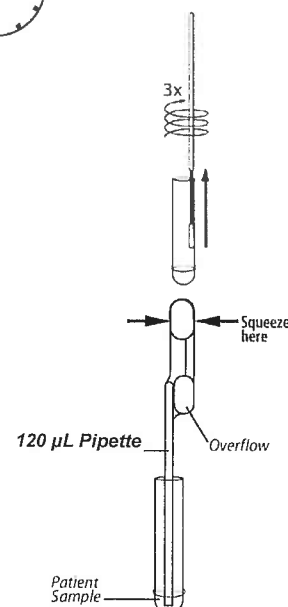
4. Roll the swab head against the inside of the Reagent Tube as you remove it. Dispose of the used swab in your biohazard waste.



5. Fill the provided **Small, Clear 120 µL Fixed Volume Pipette** with the patient sample from the Reagent Tube.

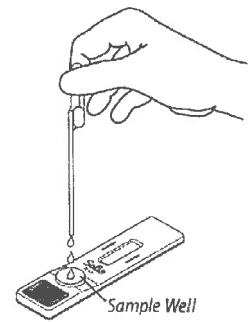
To fill the Fixed Volume Pipette with the patient sample:

- a. FIRMLY squeeze the top bulb.
- b. Still squeezing, place the Pipette tip into the patient sample.
- c. With the Pipette tip still in the patient sample, slowly release pressure on bulb to fill the Pipette.



- Firmly squeeze the top bulb to empty the contents of the **Small, Clear 120 μL Fixed Volume Pipette** into the Cassette sample well. Extra liquid left over in the overflow bulb should be left behind.

NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of patient sample. Discard the Pipette in your biohazard waste.



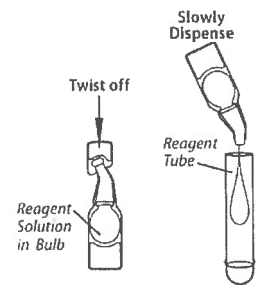
- Promptly proceed to the next section, "Using Sofia," to complete the test.

Nasopharyngeal Aspirate/Wash or Samples in Viral Transport Media Test Procedure

- Verify that Sofia is set to the desired Mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia" section for more information.

2. Prepare Reagent:

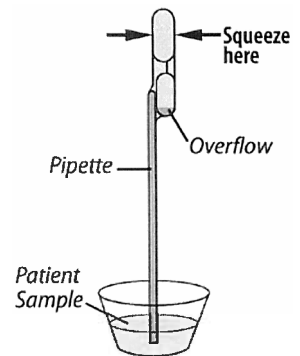
- Flick or shake the Reagent Solution vial down so that all fluid is in the bulb.
- Twist off the tab.
- Slowly dispense all of the Reagent Solution into the Reagent Tube.
- Gently swirl the Reagent Tube to dissolve its contents.



- Fill the provided **Large, Pink 250 μL Fixed Volume Pipette** with patient sample from the collection cup.

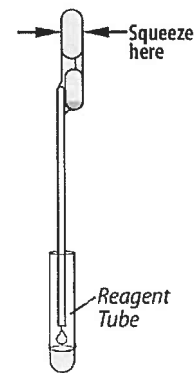
To fill the Fixed Volume Pipette with the sample:

- FIRMLY** squeeze the top bulb.
- Still squeezing, place the Pipette tip into the patient sample.
- With the Pipette tip still in the patient sample, slowly release pressure on bulb to fill the Pipette.

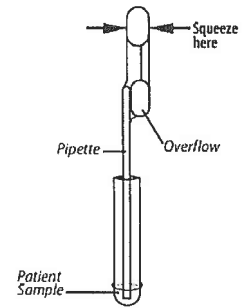


- Firmly squeeze the top bulb to empty the contents of the **Large, Pink 250 μL Fixed Volume Pipette** into the Reagent Tube. Extra liquid left over in the overflow bulb should be left behind. **Gently swirl the Reagent Tube to mix.**

NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of patient sample. Discard the Pipette in your biohazard waste.

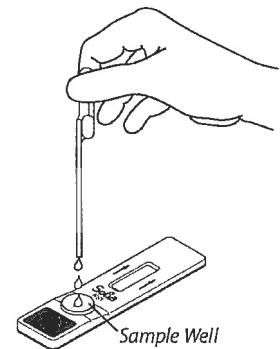


5. Fill the provided **Small, Clear 120 μ L Fixed Volume Pipette** with patient sample from the Reagent Tube, by slowly releasing pressure on the bulb.



6. Firmly squeeze the top bulb to empty the contents of the **Small, Clear 120 μ L Fixed Volume Pipette** into the Cassette sample well. Extra liquid left over in the overflow bulb should be left behind. Discard the Pipette in your biohazard waste.

NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of patient sample. Discard the Pipette in your biohazard waste.



7. Proceed to the next section, "Using Sofia," to complete the test.

Using Sofia

WALK AWAY/READ NOW Modes

Refer to the **Sofia User Manual** for operating instructions.

Sofia may be set to two different modes (WALK AWAY and READ NOW). The procedures for each mode are described below.

WALK AWAY Mode

In WALK AWAY Mode, the user **immediately** inserts the Cassette into Sofia. The user then returns after 15 minutes to get the test result. In this mode, Sofia will automatically time the test development before scanning and displaying the test result.

READ NOW Mode

Critically important: Allow the test to develop for the FULL 15 minutes BEFORE placing it into Sofia.

The user must first place the Cassette onto the counter or bench top for 15 minutes (outside of Sofia) and manually time this development step. The Cassette **MUST** stand 15 minutes to get an accurate result. Then, the user inserts the Cassette into Sofia. In READ NOW Mode, Sofia will scan and display the test result within 1 minute. **Note:** Results will remain stable for an additional 15 minutes after the recommended development time of 15 minutes.

Tips for Batch Testing

Depending on the workload, several options exist to make batch testing easier. The user can add the Reagent Solution to one or more Reagent Tubes, recap them, and store them on the bench at room temperature for up to 4 hours without loss of activity before adding the sample(s). Alternatively, after addition of the Reagent Solution, the user can process swab or liquid specimens in the Reagent Tube. Then after removing the swab (if applicable), recap the tube and let them stand at room temperature for

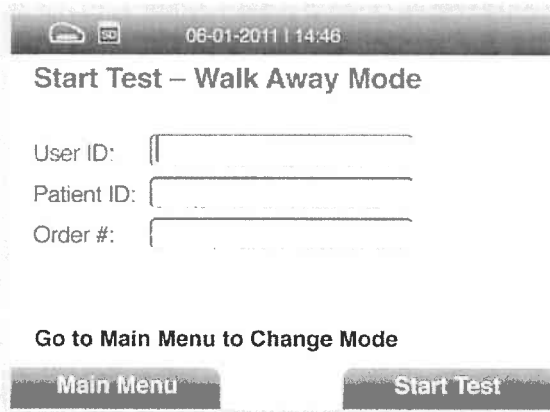
up to 4 hours without loss of activity before testing.

Critically important, the user should never open the foil pouch exposing the Cassette to ambient environment until ready for immediate use.

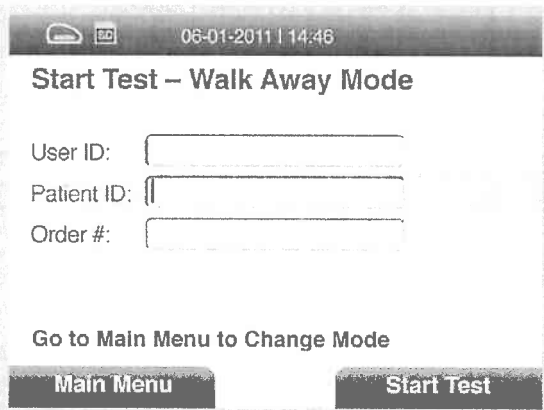
Run Test

1. Input the User ID using the handheld barcode scanner or manually enter the data using the key pad.

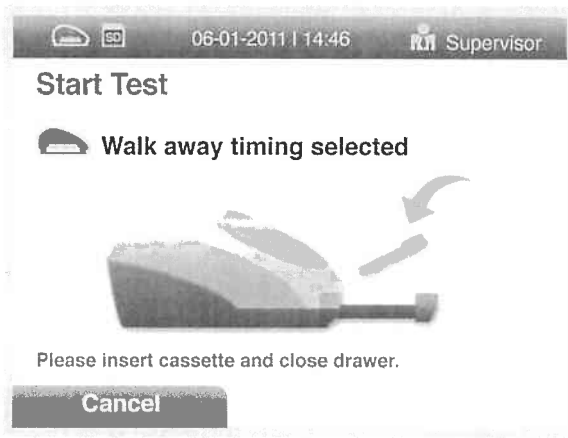
***NOTE:** If you mistakenly scan the wrong barcode, use the Arrow Buttons on the Sofia key pad to re-highlight the field. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*



2. Input Patient ID or Order # using the handheld barcode scanner or manually enter the data using the key pad.



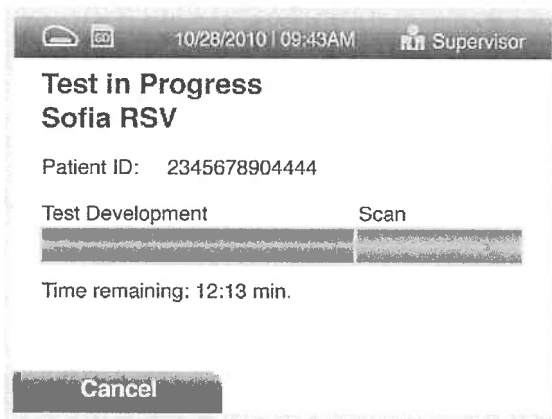
3. Press Start Test and the Sofia drawer will automatically open.



4. Verify that the correct development mode, Walk Away or Read Now, has been selected. Insert the prepared patient Cassette into the Sofia drawer and close the drawer.



5. Sofia will start automatically and display the progress, as shown in the example below. In Walk Away Mode, the test results will be displayed on the screen in approximately 15 minutes. In Read Now Mode, the test results will be displayed on the screen within 1 minute. See Interpretation of Results section.



For example: This display shows that the test in Walk Away mode has 12 minutes, 13 seconds remaining.

INTERPRETATION OF RESULTS

When the test is complete, the results will be displayed on the Sofia screen. The results can be automatically printed on the integrated printer if this option is selected. Test Lines, which are fluorescent, cannot be seen with the naked eye.

The Sofia screen will display results for the procedural control as being “valid or invalid,” and will provide a positive or negative result for RSV. If the procedural control is “invalid,” retest with a new patient sample and a new Cassette.

Positive Results:

10/28/2010 10:09:43AM Supervisor

Detailed Results
RSV

Patient ID: 2345678904
Date: 01/17/2010 10:30AM
User ID: 00000034
Order #: EGHJKLMNO

RSV: Positive

Procedural Control: valid

Main Menu Start New Test

For example: This display shows a valid positive result for RSV.

NOTE: A positive result does not rule out co-infections with other pathogens.

Negative Results:

10/28/2010 10:09:43AM Supervisor

Detailed Results
RSV

Patient ID: 2345678904
Date: 01/17/2010 10:30AM
User ID: 00000034
Order #: EGHJKLMNO

RSV: Negative

Procedural Control: valid

Main Menu Start New Test

For example: This display shows a valid negative result for RSV.

NOTE: A negative result does not exclude RSV viral infection. Negative results should be confirmed by viral culture.

Invalid Results:

10/28/2010 | 09:43AM Supervisor

Detailed Results
RSV

Patient ID: 2345678904
Date: 01/17/2010 10:30AM
User ID: 00000034
Order #: EGHJKLMNO

RSV: Invalid

Procedural Control: Invalid

Main Menu Start New Test

For example: This display shows an invalid result.

Invalid Result: If the test is invalid, a new test should be performed with a new patient sample and a new Cassette.

LIMITATIONS

- This test is suitable for the pediatric population (less than 19 years of age) only. Performance characteristics have not been established for use with patients older than 19 years of age and for immunocompromised patients.
- The contents of this kit are to be used for the qualitative detection of RSV antigen from nasopharyngeal swab and nasopharyngeal aspirate/wash samples.
- This test detects both viable (live) and non-viable RSV. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the Test Procedure 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.
- Positive test results do not rule out co-infections with other pathogens.
- Negative test results are not intended to rule in other non-RSV viral or bacterial infections.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low RSV activity when prevalence is moderate to low.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, RSV viruses that have undergone minor amino acid changes in the target epitope region.
- Samples contaminated with whole blood >1% may interfere in the interpretation of the test. Visually bloody samples should not be used.
- Mycoplasma pneumoniae at levels greater than 1×10^5 cfu/mL may cross-react or interfere with the performance of the test.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.

EXPECTED VALUES

The rate of positivity observed in RSV testing will vary depending on the method of specimen collection, handling/transport system employed, detection method utilized, time of year, age of the patient, and disease prevalence. The prevalence observed with culture during the clinical study was 12% (211/1755).

PERFORMANCE CHARACTERISTICS

Sofia RSV FIA Performance vs. Cell Culture

The performance of the Sofia RSV FIA was compared to viral cell culture methods followed by DFA in a multi-center clinical field study during February through April of 2012 and October through December of 2012 in the United States. This study was conducted by health care personnel at 17 distinct sites in various geographical regions within the United States. In this multi-center, point-of-care (POC) field trial, two (2) nasopharyngeal swabs or nasopharyngeal aspirate/wash samples were collected from each of 1,736 patients. A pair of nasopharyngeal swab samples was provided by 972 patients and a nasopharyngeal aspirate/wash sample was provided by 764 patients. All clinical samples were collected from symptomatic patients (less than 19 years of age): 55% were male and 45% were female.

On-site testing of one nasopharyngeal swab sample or a portion of nasopharyngeal aspirate/wash sample was performed by medical personnel in the physician’s office or hospital facility with the Sofia RSV FIA. The samples were freshly collected and tested. The remaining sample was placed in viral transport media for culturing. The paired swab samples were randomized with respect to the order of testing in the Sofia RSV FIA versus culture. Viral cell culture was performed either at a local clinical laboratory at the test site, or the samples were transported cold on ice packs, not frozen, overnight to a central laboratory for culture within 48 hours. Results are presented in Tables 2 and 3.

Table 2
Sofia RSV FIA Nasopharyngeal Swab Results Versus Culture
(Ages 0-<19 Years)

	Culture		
	Pos	Neg	
Sofia Pos	126	25	Sens. = 126/146 = 86% (95% C.I. 80-91%)
Sofia Neg	20	801	Spec. = 801/826 = 97% (95% C.I. 96-98%)
Total	146	826	

Table 3
Sofia RSV FIA Nasopharyngeal Aspirate/Wash Results Versus Culture
(Ages 0-<19 Years)

	Culture		
	Pos	Neg	
Sofia Pos	57	12	Sens. = 57/64 = 89% (95% C.I. 79-95%)
Sofia Neg	7	688	Spec. = 688/700 = 98% (95% C.I. 97-99%)
Total	64	700	

Sofia RSV FIA Performance vs. Cell Culture When Testing Specimens Placed into Viral Transport Media

The performance of the Sofia RSV FIA when testing specimens placed into VTM was compared to viral cell culture methods followed by DFA in the same multi-center clinical field study during February through April of 2012 and October through December of 2012 in the United States. This portion of the study was conducted by laboratory personnel at two (2) distinct laboratory sites within the United States. A nasopharyngeal swab or nasopharyngeal aspirate/wash specimen collected from each of 1,749 patients

was placed in viral transport media and then transported cold on ice packs, not frozen, overnight to the laboratory. The Sofia RSV FIA test was performed on a portion of each specimen, and the culture was performed using the remainder of the same specimen in VTM. Nasopharyngeal swab specimens were provided by 968 patients and nasopharyngeal aspirate/wash specimens were provided by 781 patients. Results are presented in Tables 4 and 5.

Table 4
Sofia RSV FIA Nasopharyngeal Swab in VTM Results Versus Culture
(Ages 0-<19 Years)

	Culture		Sens. = 125/143 = 87% (95% C.I. 81-92%)
	Pos	Neg	
Sofia Pos	125	26	Spec. = 799/825 = 97% (95% C.I. 95-98%)
Sofia Neg	18	799	
Total	143	825	

Table 5
Sofia RSV FIA Nasopharyngeal Aspirate/Wash in VTM Results Versus Culture
(Ages 0-<19 Years)

	Culture		Sens. = 59/67 = 88% (95% C.I. 78-94%)
	Pos	Neg	
Sofia Pos	59	12	Spec. = 702/714 = 98% (95% C.I. 97-99%)
Sofia Neg	8	702	
Total	67	714	

Reproducibility Studies

The reproducibility of the Sofia RSV FIA was evaluated at three (3) different laboratories. Two (2) different operators at each site tested a series of coded, contrived samples, prepared in negative clinical matrix, ranging from low negative to moderate positive RSV. The inter-laboratory agreement (Table 6) for negative samples was 98%-100% and 98%-100% for positive samples. The intra-laboratory agreement (Table 7) for all samples ranged from 98%-100%.

Table 6
Sofia RSV FIA Reproducibility Study Inter-laboratory Agreement

Site	Low Neg (no virus)	High Negative (C ₅)	Low Positive (C ₉₅)	Mod. Positive (C _{3x LOD})
1	30/30	28/30	30/30	30/30
2	30/30	30/30	28/30	30/30
3	30/30	30/30	30/30	30/30
Total	90/90	88/90	88/90	90/90
% Overall Agreement (95% CI)	100% (95%-100%)	98% (92%-100%)	98% (92%-100%)	100% (95%-100%)

Table 7
Sofia RSV FIA Reproducibility Study Intra-laboratory Agreement

Site	Low Neg (no virus)	High Negative (C ₅)	Low Positive (C ₉₅)	Mod. Positive (C _{3X LOD})	% Overall Agreement (95% CI)
1	30/30	28/30	30/30	30/30	98% (118/120) (94%-100%)
2	30/30	30/30	28/30	30/30	98% (118/120) (94%-100%)
3	30/30	30/30	30/30	30/30	100% (120/120) (96%-100%)

Limit of Detection and Analytical Reactivity

The limit of detection (LOD) for the Sofia RSV FIA was determined using a total of four (4) strains of RSV, two (2) isolates of RSV A and two (2) isolates of RSV B (Table 8).

Table 8
Limit of Detection with Human Isolates of RSV A and B

Viral Strain	Minimum Detectable Level (TCID ₅₀ /mL)
RSV A-2	3153
RSV A Long	372
RSV B CH93-18(18)	476
RSV B Washington/18537/62	32.3

TCID₅₀/mL=50% tissue culture infectious dose. TCID₅₀ levels were determined by the Reed-Muench method.

Analytical reactivity was demonstrated using two (2) additional strains of RSV B: West Virginia Strain/14617/85 at 163 TCID₅₀/mL and RSV 9320 at 8.7 TCID₅₀/mL.

Analytical Specificity

Cross Reactivity

The cross reactivity of the Sofia RSV FIA was evaluated with a total of 32 bacterial and fungal microorganisms and 42 non-RSV viral isolates. None of the organisms or viruses listed below in Table 9 showed any sign of cross reactivity in the assay. When the same organisms in Table 9 were pre-mixed with RSV and tested in the Sofia RSV FIA, all results were positive indicating that the potential cross-reactants did not interfere with the detection of RSV.

Table 9
Analytical Specificity and Cross Reactivity

Organism/Non-RSV Virus	Concentration*
<i>Acinetobacter baumannii</i>	2.32x10 ⁶ cfu/mL
<i>Bacteroides fragilis</i>	2.32x10 ⁶ cfu/mL
<i>Bordetella pertussis</i>	2.32x10 ⁶ cfu/mL
<i>Candida albicans (yeast)</i>	2.32x10 ⁶ cfu/mL
<i>Corynebacterium diphtheriae</i>	2.32x10 ⁶ cfu/mL
<i>Escherichia coli</i>	2.32x10 ⁶ cfu/mL
<i>Haemophilus influenzae</i>	2.32x10 ⁶ cfu/mL
<i>Klebsiella pneumoniae</i>	2.32x10 ⁶ cfu/mL
<i>Lactobacillus plantarum</i>	2.32x10 ⁶ cfu/mL
<i>Legionella pneumophila</i>	2.32x10 ⁶ cfu/mL
<i>Moraxella catarrhalis</i>	2.32x10 ⁶ cfu/mL
<i>Mycobacterium avium</i>	2.32x10 ⁶ cfu/mL
<i>Mycobacterium intracellulare</i>	2.32x10 ⁶ cfu/mL
<i>Mycobacterium tuberculosis</i>	2.32x10 ⁶ cfu/mL
<i>Mycoplasma pneumoniae</i>	1x10 ⁵ cfu/mL
<i>Neisseria meningitidis</i>	2.32x10 ⁶ cfu/mL
<i>Neisseria mucosa</i>	2.32x10 ⁶ cfu/mL
<i>Neisseria sicca</i>	2.32x10 ⁶ cfu/mL
<i>Neisseria subflava</i>	2.32x10 ⁶ cfu/mL
<i>Pseudomonas aeruginosa</i>	2.32x10 ⁶ cfu/mL
<i>Serratia marcescens</i>	2.32x10 ⁶ cfu/mL
<i>Staphylococcus aureus</i>	2.32x10 ⁶ cfu/mL
<i>Staphylococcus aureus</i> (Cowen 1)	2.32x10 ⁶ cfu/mL
<i>Staphylococcus epidermidis</i>	2.32x10 ⁶ cfu/mL
<i>Streptococcus mutans</i>	2.32x10 ⁶ cfu/mL
<i>Streptococcus pneumoniae</i>	2.32x10 ⁶ cfu/mL
<i>Streptococcus pyogenes</i> Group A	2.32x10 ⁶ cfu/mL
<i>Streptococcus sanguis</i>	2.32x10 ⁶ cfu/mL
Streptococcus sp. Group B	2.32x10 ⁶ cfu/mL
Streptococcus sp. Group C	2.32x10 ⁶ cfu/mL
Streptococcus sp. Group F	2.32x10 ⁶ cfu/mL
Streptococcus sp. Group G	2.32x10 ⁶ cfu/mL
Adenovirus 3	2.32x10 ⁵ TCID ₅₀ /mL
Adenovirus 4	2.64x10 ⁴ TCID ₅₀ /mL

Organism/Non-RSV Virus	Concentration*
Adenovirus 5	8.98x10 ⁵ TCID ₅₀ /mL
Adenovirus 7A	2.32x10 ⁵ TCID ₅₀ /mL
Adenovirus 11	2.32x10 ⁵ TCID ₅₀ /mL
Coronavirus OC43	2.32x10 ⁵ TCID ₅₀ /mL
Coronavirus 229E	2.32x10 ⁵ TCID ₅₀ /mL
Coxsackievirus B5 (Faulkner)	2.32x10 ⁵ TCID ₅₀ /mL
Cytomegalovirus AD-169	2.32x10 ⁵ TCID ₅₀ /mL
Cytomegalovirus Towne	2.32x10 ⁵ TCID ₅₀ /mL
Echovirus Type 3	2.32x10 ⁵ TCID ₅₀ /mL
Herpes Simplex virus 1	2.32x10 ⁵ TCID ₅₀ /mL
Herpes Simplex virus 2	2.32x10 ⁵ TCID ₅₀ /mL
Human Metapneumovirus A1	2.32x10 ⁵ TCID ₅₀ /mL
Human Metapneumovirus A2	2.32x10 ⁵ TCID ₅₀ /mL
Human Metapneumovirus B1	2.32x10 ⁵ TCID ₅₀ /mL
Human Metapneumovirus B2	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H1N1 (Mexico/4108/2009)	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H1N1 (Denver/1/57)	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H1N1 (FM/1/47)	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H1N1 (New Jersey/8/76)	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H1N1 (PR/8/34)	2.32x10 ⁵ TCID ₅₀ /mL
Influenza A H3N2	2.32x10 ⁵ TCID ₅₀ /mL
Influenza B Hong Kong	2.32x10 ⁵ TCID ₅₀ /mL
Influenza B Panama	2.32x10 ⁷ TCID ₅₀ /mL
Influenza C/Taylor/1233/47	2.32x10 ⁵ TCID ₅₀ /mL
Measles (Edmonston)	2.32x10 ⁵ TCID ₅₀ /mL
Metapneumovirus VR-03-00181 UIHC	2.32x10 ⁵ TCID ₅₀ /mL
Mumps (Enders)	2.32x10 ⁵ TCID ₅₀ /mL
Parainfluenza virus 1	2.32x10 ⁵ TCID ₅₀ /mL
Parainfluenza virus 2	2.32x10 ⁵ TCID ₅₀ /mL
Parainfluenza virus 3	2.32x10 ⁵ TCID ₅₀ /mL
Parainfluenza virus 4A	2.32x10 ⁵ TCID ₅₀ /mL
Parainfluenza virus 4B	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 1B	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 2	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 3	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 7	2.32x10 ⁵ TCID ₅₀ /mL

Organism/Non-RSV Virus	Concentration*
Rhinovirus Type 15	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 18	2.32x10 ⁵ TCID ₅₀ /mL
Rhinovirus Type 37	2.32x10 ⁵ TCID ₅₀ /mL
Varicella Zoster Virus	3.55x10 ⁴ TCID ₅₀ /mL

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL (cfu=colony forming unit). Virus concentrations were determined by standard virology methods, Reed-Muench.

Interfering Substances

Whole blood, mucin, and several over-the-counter (OTC) products and common chemicals were evaluated and did not interfere with the Sofia RSV FIA at the levels indicated below (Table 10).

Table 10
Non-interfering Substances

Substance	Concentration
Acetamidophenol	23 mg/mL
Acetylsalicylic acid	23 mg/mL
Albuterol	26 mg/mL
Chlorpheniramine	4 mg/mL
Dextromethorphan	4 mg/mL
Diphenhydramine	3 mg/mL
Guaiacol	46 mg/mL
Mucin	9 mg/mL
Nasal Spray #1 (Vick's)	23%
Nasal Spray #2 (4-Way)	23%
Nasal Spray #3 (Equate)	23%
OTC Mouthwash #1 (Listerine)	58%
OTC Mouthwash #2 (Crest Pro-Health)	58%
OTC Mouthwash #3 (Scope)	58%
OTC Cough Drop #1 (CVS)	19%
OTC Cough Drop #2 (Ricola)	15%
OTC Cough Drop #3 (Halls)	34%
Phenylephrine	11 mg/mL
Rimantadine	116 µg/mL
Whole Blood	1%

CLIA Waiver Studies

As part of the prospective study described in the Performance Characteristics section above, the accuracy of the Sofia RSV FIA, when used at CLIA waived sites by untrained operators, was evaluated with specimens from pediatric patients ages 0-<7 years. The test results obtained with the Sofia RSV FIA test were compared to the results obtained by viral cell culture. This study was conducted at sixteen (16) CLIA-waived sites with thirty-seven (37) untrained operators representative of CLIA-waived settings. The study included 2193 subjects: one thousand fifty-five (1,057) subjects provided a pair of nasopharyngeal swabs and one thousand one hundred thirty-six (1,136) provided a nasopharyngeal aspirate/wash specimen.

The clinical sensitivity and specificity of the Sofia RSV FIA with ages 0-<7 years, as compared to viral culture (the comparator method), are presented below in Tables 11 and 12.

Table 11
Sofia RSV FIA Versus Culture (Nasopharyngeal Swabs)
(Ages 0-<7 Years)

	Culture		
	Pos	Neg	
Sofia Pos	134	34	Sens. = 134/154= 87% (95% C.I. 81-92%)
Sofia Neg	20	869	Spec. = 869/903= 96% (95% C.I. 95-97%)
Total	154	903	

Table 12
Sofia RSV FIA Versus Culture (Nasopharyngeal Aspirate/Wash)
(Ages 0-<7 Years)

	Culture		
	Pos	Neg	
Sofia Pos	141	22	Sens. = 141/154= 92% (95% C.I. 86-95%)
Sofia Neg	13	960	Spec. = 960/982= 98% (95% C.I. 97-99%)
Total	154	982	

Another study was conducted to demonstrate that untrained intended users could perform the test consistently and accurately using weakly reactive samples. The study consisted of three (3) distinct CLIA-waived sites where the Sofia RSV FIA was evaluated using coded, randomized panels of simulated samples, including one (1) weak positive (C₉₅ - a concentration at the assay cutoff) and one (1) weak negative (C₅ - a concentration just below the assay cutoff). Two (2) or more operators at each site (8 operators total) tested the panel on each of ten (10) days, spanning a period of approximately two (2) weeks. The performance of the Sofia RSV FIA with samples near the assay cutoff was acceptable when used by untrained intended users. The percent agreement with expected results for each sample is shown in Table 13.

Table 13
Sofia RSV FIA Performance Near the Cutoff (All Sites)

Sample Level	Untrained Intended Users	
	Percent Agreement with Expected Results*	95% Confidence Interval
Weak RSV Positive (C ₉₅)	85% (51/60)	74-92%
Weak RSV Negative (C ₅)	93% (56/60)	84-98%

*The expected results for "Weak Positive" samples are "Positive," while the expected results for "Weak Negative" samples are "Negative."

Using the risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated that the test is insensitive to stresses of environmental conditions and potential user errors.

ASSISTANCE

If you have any questions regarding the use of this product or if you want to report a test system problem, please call Quidel’s Technical Support Number 800.874.1517 (in the U.S) or 858.552.1100, Monday through Friday, from 7:00 a.m. to 5:00 p.m., Pacific Time. If outside the United States contact your local distributor or technicalsupport@quidel.com. Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 1-800-FDA-1088; fax: 1-800-FDA-0178; <http://www.fda.gov/medwatch>).

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LOG SHEET

Sofia[®]

RSV FIA

Record Built-in Procedural Controls on the first patient tested each day.

	Date	Patient ID	Valid Procedural Control	Test Results At 15 minutes	Lot Number and Expiration Date	Technician Initials
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						

QC LOG SHEET



Facility Name: _____

Quidel recommends that positive and negative controls be run once for each untrained operator, once for each new shipment of kits — provided that each different lot received in the shipment is tested — and as deemed additionally necessary by your internal quality control procedures, and in accordance with local, state, and federal regulations or accreditation requirements. If you have any questions or concerns, please contact Quidel Technical Support at 800.874.1517 or at technicalsupport@quidel.com.

	Date MM/DD/YY	Kit Lot #	RSV Positive Control OK?	RSV Negative Control OK?	Comments	Technician Initials
1						
2						
3						
4						
5						
6						
7						
8						
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