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 Influenza A+B FIA

For use with Sofia and Sofia 2
CLIA Complexity: Waived for direct nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens
CLIA Complexity: Moderate for nasopharyngeal swab and nasopharyngeal aspirate/wash specimens eluted in transport media

For *in vitro* use only, RX only

A Certificate of Waiver is required to perform this test in a CLIA waived setting. This test may also be used by laboratories that perform moderate and high complexity testing. 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 Influenza A+B FIA employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens in direct nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens and nasopharyngeal swab and nasopharyngeal aspirate/wash specimens in transport media from symptomatic patients. This qualitative test is intended for use as an aid in the rapid differential diagnosis of acute influenza A and influenza B viral infections. The test is not intended to detect influenza C antigens. A negative test is presumptive and it is recommended these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infections and should not be used as the sole basis for treatment or other patient management decisions. This test is intended for professional and laboratory use.

The Sofia Influenza A+B FIA may be used with Sofia or Sofia 2.

Performance characteristics for influenza A and B were established during February through March 2011 when influenza viruses A/California/7/2009 (2009 H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 (Victoria-Like) were the predominant influenza viruses in circulation according to the Morbidity and Mortality Weekly Report from the CDC entitled “Update: Influenza Activity--United States, 2010-2011 Season, and Composition of the 2011-2012 Influenza Vaccine.” Performance characteristics may vary against other emerging influenza viruses.

If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, samples should be collected with appropriate infection

control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture samples.

SUMMARY AND EXPLANATION

Influenza viruses are causative agents of highly contagious, acute, viral infections of the respiratory tract.

Influenza viruses are immunologically diverse, single-stranded RNA viruses. There are three types of influenza viruses: A, B, and C. Type A viruses are the most prevalent and are associated with most serious epidemics. Type B viruses produce a disease that is generally milder than that caused by type A. Type C viruses have never been associated with a large epidemic of human disease. Both Type A and B viruses can circulate simultaneously, but usually one type is dominant during a given season.[1]

Every year in the United States, on average 5%-20% of the population contract influenza; more than 200,000 people are hospitalized from influenza complications; and, about 36,000 people die from influenza-related causes. Some people, such as adults 65 years of age and older, young children, and people with certain health conditions, are at high risk for serious influenza complications.[2]

PRINCIPLE OF THE TEST

The Sofia Influenza A+B FIA employs immunofluorescence technology that is used with Sofia and Sofia 2 to detect influenza virus nucleoproteins. This test allows for the differential detection of influenza A and influenza B antigens.

The patient sample is placed in the Reagent Tube, during which time the virus particles in the sample are disrupted, exposing internal viral nucleoproteins. After disruption, the sample is dispensed into the Test Cassette sample well. From the sample well, the sample migrates through a test strip containing various unique chemical environments. If influenza viral antigen is present, they will be trapped in a specific location.

Note: Depending upon the user's choice, the Test Cassette is either placed inside of Sofia or Sofia 2 for automatically timed development (WALK AWAY Mode) or placed on the counter or bench top for a manually timed development and then placed into Sofia or Sofia 2 to be scanned (READ NOW Mode).

Sofia and Sofia 2 will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. Sofia and Sofia 2 will display the test results (Positive, Negative, or Invalid) on the screen.

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Test Cassettes (25): Mouse monoclonal anti-influenza A and anti-influenza B antibodies
- Reagent Tubes (25): Lyophilized buffer with detergents and reducing agents
- Reagent Solution (25): Ampoules with salt solution
- Sterile Nasal Swabs (25)
- Small, Clear 120 µL Fixed Volume Pipettes (25)
- Large, Pink 250 µL Fixed Volume Pipettes (25)
- Influenza A and Influenza B Positive Control Swab (1): Swab is coated with non-infectious recombinant influenza A and influenza B antigens
- 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
- Sofia or Sofia 2
- Sample/Specimen container
- Sterile saline for sample collection
- Equipment used for collection of nasopharyngeal aspirate or nasopharyngeal wash
- Nylon flocked nasopharyngeal swab
- Calibration Cassette (supplied with the Sofia Installation Pack or with Sofia 2)

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.[3]
- Use of Nitrile, Latex (or equivalent) gloves is recommended when handling patient samples.[3]
- Do not reuse the used Test 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 Test Cassette is ready for immediate use.
- Discard and do not use any damaged Test 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 Media (VTM) recommended in this Package Insert.
- When collecting a nasal swab sample, use the Nasal Swab supplied in the kit.
- When collecting a nasopharyngeal swab sample, use a nylon flocked nasopharyngeal swab.
- 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 for all sample types.
  - **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 sample 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.
- To obtain accurate results, do not use visually bloody or overly viscous samples.
- Do not write on the barcode of the Test Cassette. This is used by Sofia and Sofia 2 to identify the type of test being run and to identify the individual Test Cassette so as to prevent a second read of the Test Cassette by the same Sofia or Sofia 2.
- If infection with a novel influenza A virus is suspected, based on current clinical and epidemiological screening criteria recommended by public health authorities, samples should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture samples.

- Although this test has been shown to detect cultured avian influenza viruses, including avian Influenza A subtype H5N1 virus, the performance characteristics of this test with samples from humans infected with H5N1 or other avian influenza viruses are unknown.
- As the detection reagent is a fluorescent compound, no visible results will form on the test strip. Sofia or Sofia 2 must be used for result interpretation.
- To obtain accurate results, an opened and exposed Cassette should not be used inside a laminar flow hood or in a heavily ventilated area.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State, and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

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 Sofia or Sofia 2 and the Test 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 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 two 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. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support); or contact your local distributor.

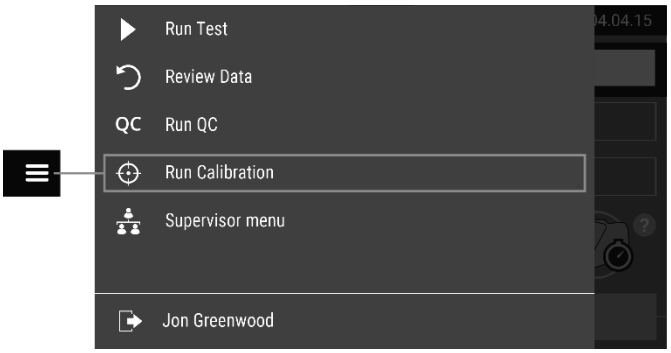
Sofia 2 Calibration Check Procedure

The Calibration Check Procedure should be performed every 30 days. Sofia 2 can be set to remind the user to complete the Calibration Check Procedure.

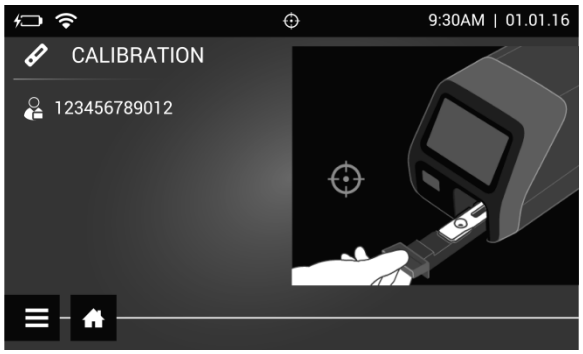
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

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 2, select “Run Calibration” from the Main Menu.



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



Sofia 2 indicates when the Calibration Check is completed. Select to return to the Run Test screen.

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. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support); or contact your local distributor.

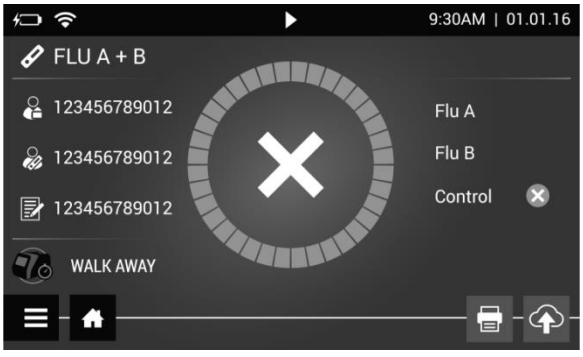
Built-in Procedural Controls

The Sofia Influenza A+B FIA contains a built-in procedural control feature. Each time a test is run in Sofia or Sofia 2, the procedural control zone is scanned by Sofia or Sofia 2 and the result is displayed on the Sofia or Sofia 2 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 or sofia 2 with each test result.

A valid result obtained from the procedural control demonstrates that the test flowed correctly and the functional integrity of the Test Cassette was maintained. **The procedural control is interpreted by Sofia or Sofia 2 after the Test Cassette has developed for 15 minutes. If the test does not flow correctly, Sofia or Sofia 2 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 Test Cassette.

For example: This display shows an invalid result on Sofia.



For example: This display shows an invalid result on Sofia 2.

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.

The user must first select Run QC on the Main Menu of Sofia or Sofia 2 and 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.

The user will select the desired mode (WALK AWAY or READ NOW) then 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 Influenza Positive Control Swab contains both influenza A and influenza B antigen. **The Positive Control Swab must be run first, followed by the Negative Control Swab.**

When the QC run is complete, each result will be displayed as "Passed" or "Failed" on Sofia or  or on Sofia 2, 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.

On Sofia, if both the Positive and Negative Controls fail, repeat testing with new Positive and Negative Controls a second time. If only a single Control fails, the user has the option of repeating both the Positive and Negative Controls OR to repeat only the Control that failed. The user may select "Skip" on the Sofia display in order to skip the Control test that previously passed. The QC Results will show a skipped Control test as "unknown."

On Sofia 2, if either or both of the Positive and Negative Controls fail, repeat testing with new Positive and Negative Controls a second time.

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

SAMPLE COLLECTION AND HANDLING

SAMPLE COLLECTION
Nasal Swab Sample
Use the nasal swab supplied in the kit.
To collect a nasal swab sample, carefully insert the swab (provided in the kit) 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 turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then remove it from the nostril.

Nasopharyngeal Swab Sample

Use a nylon flocked nasopharyngeal swab, not supplied.

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: a child could 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 sample 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, with viral transport medium (VTM), 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 were tested by Quidel and determined to be compatible with the Sofia Influenza A+B FIA. However, lot-to-lot variation of viral transport media may impact the performance.

Table 1
Recommended Viral Transport Media (VTM)

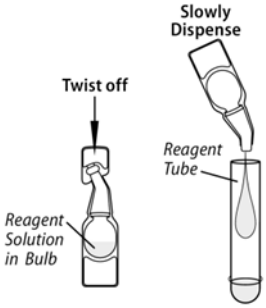
Viral Transport Medium (VTM)	Recommended Storage Condition	
	2°C to 8°C	25°C
Copan Universal Transport Medium	72 hours	72 hours
Hank's Balanced Salt Solution	24 hours	Not recommended
M4	72 hours	72 hours
M4-RT	72 hours	72 hours
M5	72 hours	72 hours
M6	72 hours	72 hours
Modified Liquid Stuarts	6 hours	Not recommended
Saline	24 hours	4 hours
Starplex Multitrans	72 hours	72 hours

Note: When using viral transport medium (VTM), it is important to ensure that the VTM containing the sample is warmed to room temperature. Cold samples will not flow correctly and can lead to erroneous or invalid results. Several minutes will be required to bring a cold sample to room temperature.

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.*

Swab Test Procedure (Nasal / Nasopharyngeal Swab)



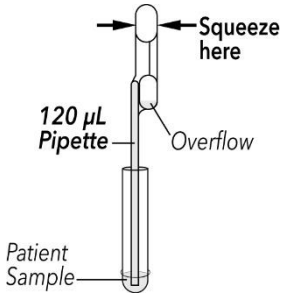
1. Verify that Sofia or Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**.
   See the "Using Sofia and Sofia 2" section for more information.

2. Dispense all of the Reagent Solution into the Reagent Tube. **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.

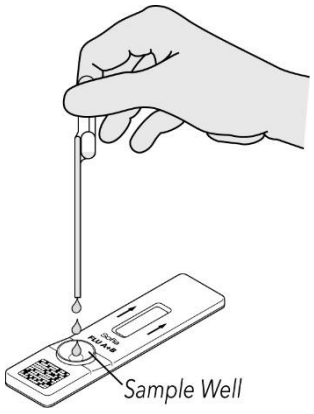


5. Fill the provided **Small, Clear 120 µL Fixed Volume Pipette** with the patient sample from the Reagent Tube.

   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.

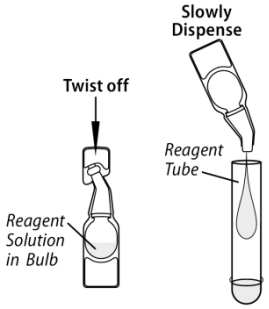
6. Firmly squeeze the top bulb to empty the contents of the **Small, Clear 120 µL Fixed Volume Pipette** into the Test Cassette sample well. Extra liquid left over in the overflow bulb should be left behind.

   NOTE: The Fixed Volume Pipettes are designed to collect and dispense the correct amount of liquid sample. Discard the pipette in your biohazard waste.



7. Promptly proceed to the next section, "Using Sofia and Sofia 2," to complete the test.

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



1. Verify that Sofia is set to the desired Mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia and Sofia 2" section for more information. Also ensure that the liquid sample is at **room temperature** before proceeding.

2. Dispense all of the Reagent Solution into the Reagent Tube. **Swirl the Reagent Tube to dissolve its contents.**

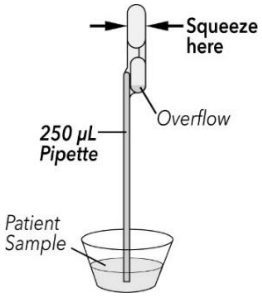
3. Fill the provided **Large, Pink 250 µL Fixed Volume Pipette** with patient sample from the collection cup or test tube.

   To fill the Fixed Volume Pipette with the sample:

   a) FIRMLY squeeze the top bulb.

   b) Still squeezing, place the Pipette tip into the patient sample.

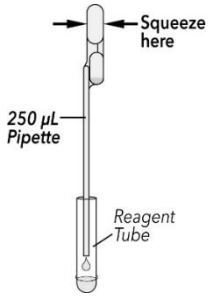
   c) With the Pipette tip still in the patient sample, slowly release pressure on bulb to fill the Pipette.



   NOTE: To obtain accurate results, avoid mucoid substances when filling the **Large, Pink 250 µL Fixed Volume Pipette** with patient sample from the collection cup.

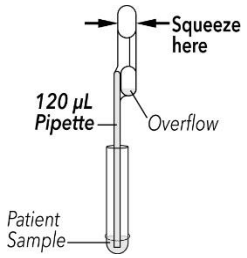
4. Firmly squeeze the top bulb to empty the contents of the **Large, Pink Fixed Volume Pipette** into the Reagent Tube. Extra liquid left over in the overflow bulb should be left behind.

   NOTE: Once the sample is added to the Reagent Tube, <u>vigorously mix</u> prior to adding the sample to the Test Cassette.



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

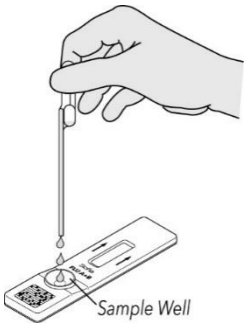
5. Fill the provided **Small, Clear 120 µL Fixed Volume Pipette** with patient sample from the Reagent Tube, by slowly releasing pressure on the bulb.



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

   NOTE: Do not pour sample from the Reagent Tube. Use the provided Small Clear 120 µL Fixed Volume Pipette.

7. Promptly proceed to the next section, "Using Sofia and Sofia 2," to complete the test.



USING SOFIA AND SOFIA 2

WALK AWAY/READ NOW Modes

Refer to the Sofia or Sofia 2 User Manual for operating instructions.

Sofia and Sofia 2 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 Test Cassette into Sofia or Sofia 2. The development time may differ between Sofia and Sofia 2.

- Sofia – Sofia will automatically time the test development, and the results will be displayed in 15 minutes.
- Sofia 2 – Sofia 2 scans the Test Cassette periodically during the test development time. Positive test results will be displayed between 3 and 15 minutes. Negative test results will be displayed at 15 minutes.

READ NOW Mode

Critically important: Allow the test to develop for the FULL 15 minutes BEFORE placing it into Sofia or Sofia 2.

The user must first place the Test Cassette onto the counter or bench top for 15 minutes (outside of Sofia or Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia or Sofia 2. In READ NOW Mode, Sofia and Sofia 2 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 (RT) for up to 12 hours without loss of activity before adding the sample(s).

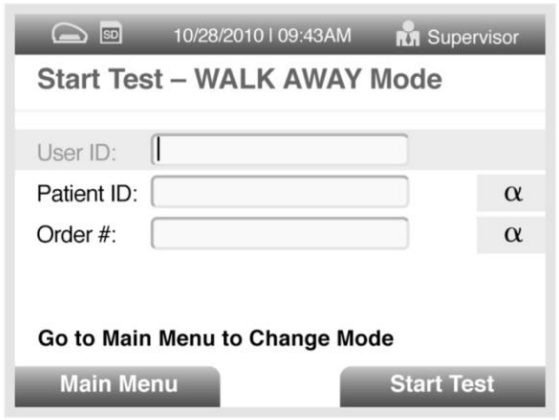
Alternatively, after addition of the Reagent Solution, the user can process swab or liquid samples in the Reagent Tube, then after removing the swab (if applicable), recap the tube and let them stand at RT for up to 12 hours without loss of activity before testing.

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

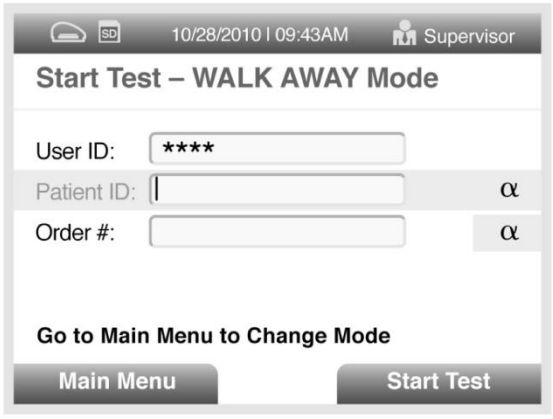
RUN TEST WITH SOFIA

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

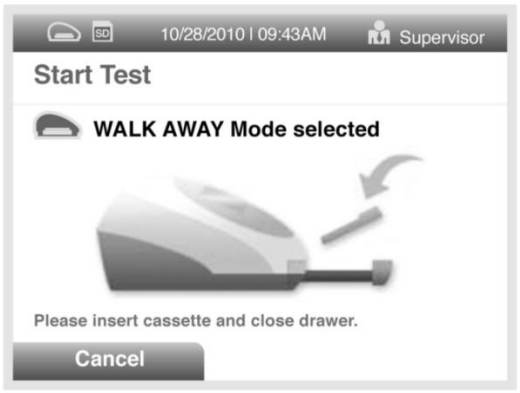
NOTE: *If you mistakenly scan the incorrect barcode, use the Arrow Buttons on the Sofia key pad to re-highlight the field. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*



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

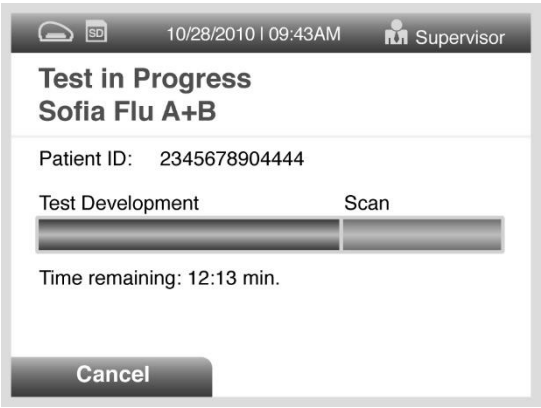


3. Press Start Test and the Sofia drawer will automatically open.



4. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Insert the prepared patient Test Cassette into the drawer of Sofia 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.



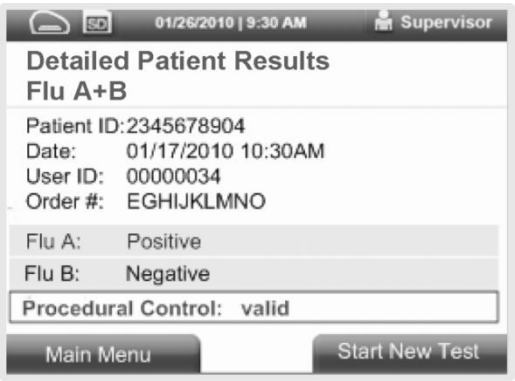
For example: This display shows that the test in WALK AWAY mode has 12 minutes, 13 seconds remaining. Sofia will read and display the results after 15 minutes.

INTERPRETATION OF RESULTS USING SOFIA

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.

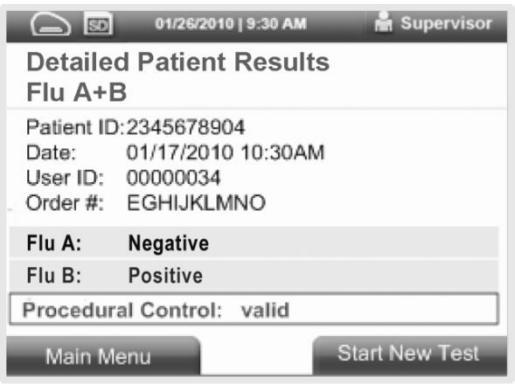
The Sofia screen will display results for the procedural control as being “valid or invalid,” and will individually provide a positive or negative result for both influenza A and influenza B. If the procedural control is “invalid,” retest with a new patient sample and a new Test Cassette.

Positive Results:



For example: This display shows a valid <u>positive result for Influenza A</u>.

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



For example: This display shows a valid <u>positive result for Influenza B</u>.

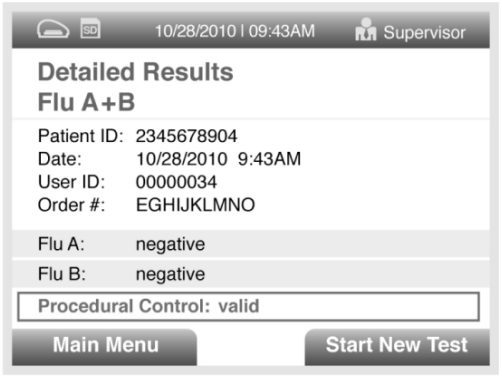
NOTE: A positive result does not rule out co-infections with other pathogens.

For example: This display shows a valid <u>positive result for both Influenza A and Influenza B</u>.

NOTE: A positive result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.

NOTE: Co-infection with influenza A and B is rare. Sofia Influenza A+B FIA "dual positive" clinical samples (influenza A and influenza B positive) should be re-tested. It is recommended that repeatable influenza A and B "dual positive" results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay before reporting results.

Negative Results:



For example: This display shows a valid <u>negative</u> result for Influenza A and Influenza B.

NOTE: A negative result does not exclude influenza viral infection. It is recommended that negative results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay.

Invalid Results:

For example: This display shows an invalid result.

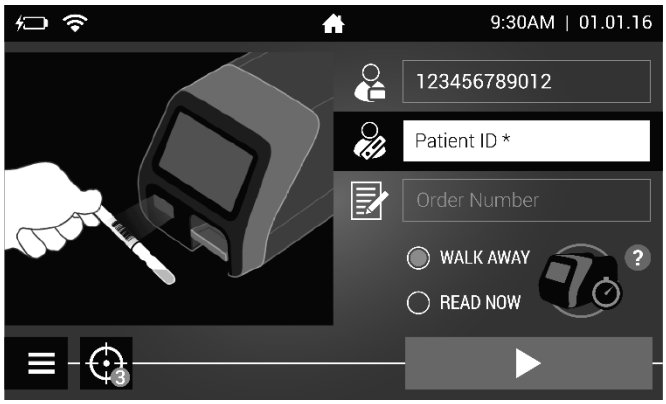
Invalid Result: If the test is invalid, a new test should be performed with a new patient sample and a new Test Cassette.

RUN TEST WITH SOFIA 2

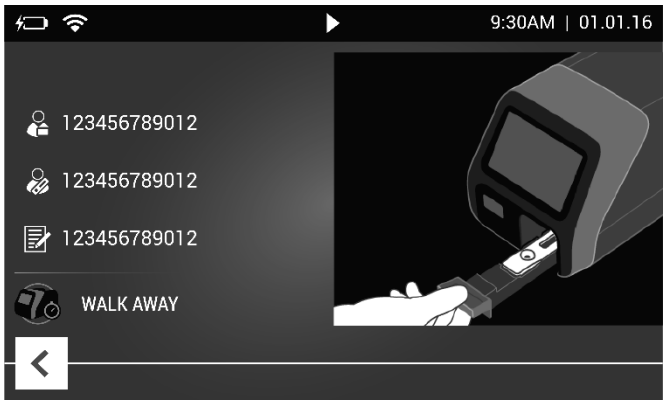
1. Input the User ID using the integrated barcode scanner or manually enter the data using the on-screen key pad.

NOTE: *If you mistakenly scan the incorrect barcode, select the field again to re-highlight it. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*

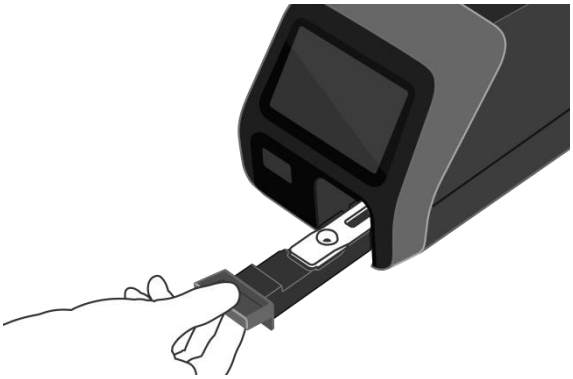
2. Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the on-screen key pad.



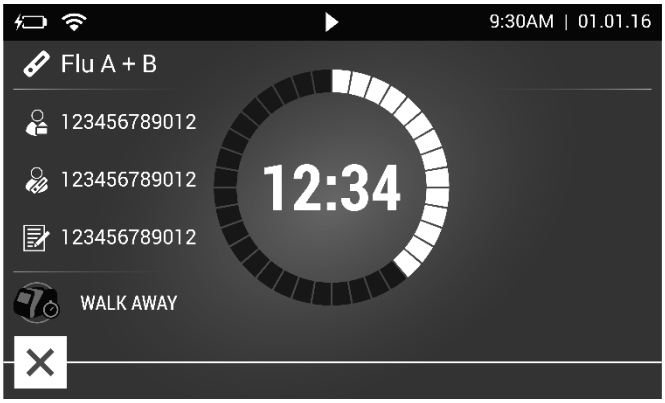
3. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



4. Insert the prepared Test Cassette into the drawer of Sofia 2 and close the drawer.



5. Sofia 2 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 between 3 and 15 minutes. In READ NOW Mode, the test results will be displayed on the screen within 1 minute. See Sofia 2 Interpretation of Results section.



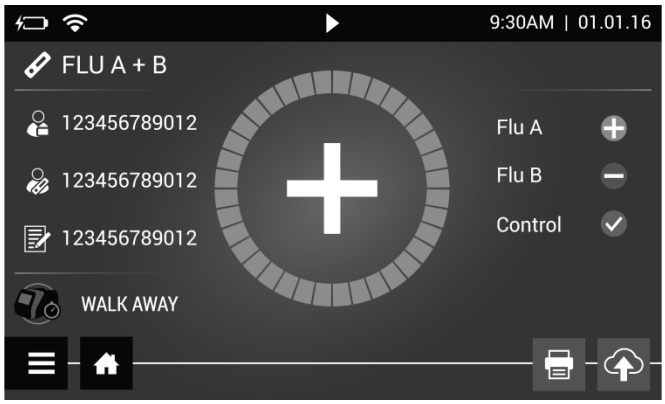
For example: This display shows that the test in WALK AWAY Mode has 12 minutes, 34 seconds remaining. Sofia 2 will read and display the results between 3 and 15 minutes.

INTERPRETATION OF RESULTS USING SOFIA 2

When the test is complete, the results will be displayed on the Sofia 2 screen. Test Lines, which are fluorescent, cannot be seen with the naked eye.

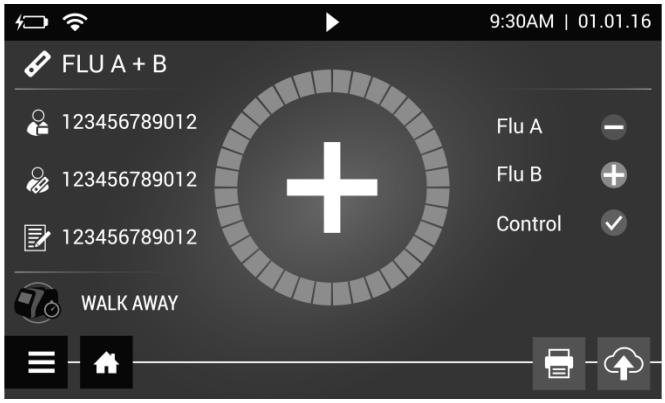
The Sofia 2 screen will display results for the procedural control as being ✓ or ✗, and will individually provide a positive or negative result for both influenza A and influenza B. If the procedural control is ✗ retest with a new patient sample and a new Test Cassette. If a printer is connected, the results can be printed manually by selecting the print icon while the test results are displayed on the screen.

Positive Results:



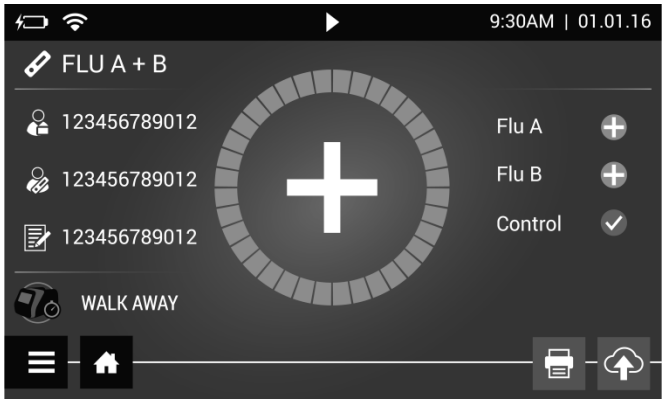
For example: This display shows a valid <u>positive result for Influenza A</u>.

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



For example: This display shows a valid <u>positive result for Influenza B</u>.

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

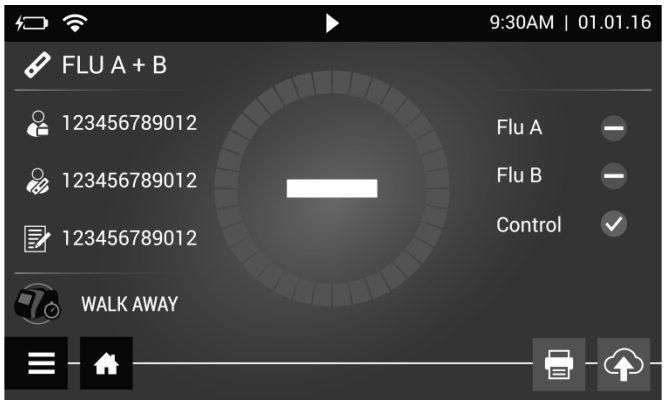


For example: This display shows a valid <u>positive result for both Influenza A and Influenza B</u>.

NOTE: A positive result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.

NOTE: Co-infection with influenza A and B is rare. Sofia Influenza A+B FIA "dual positive" clinical samples (influenza A and influenza B positive) should be re-tested. It is recommended that repeatable influenza A and B "dual positive" results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay before reporting results.

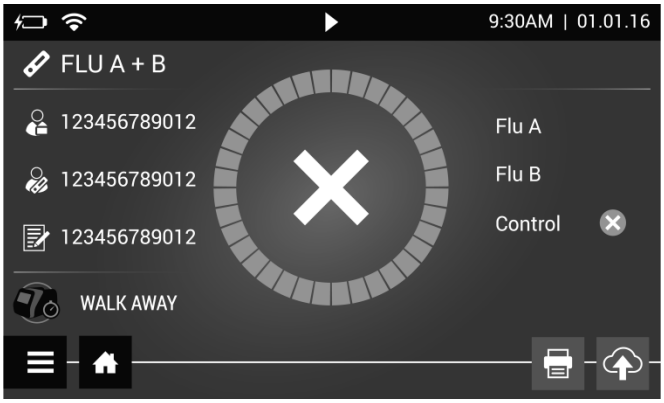
Negative Results:



For example: This display shows a valid <u>negative</u> result for Influenza A and Influenza B.

NOTE: A negative result does not exclude influenza viral infection. It is recommended that negative results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay.

Invalid Results:



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 Test Cassette.

LIMITATIONS

- The contents of this kit are to be used for the qualitative detection of influenza type A and B antigens from nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash samples.
- This test detects both viable (live) and non-viable influenza A and B. 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.
- The clinical performance of the Sofia Influenza A+B FIA for nasopharyngeal aspirate/wash samples has not been established in patients 22 years of age and older and may not be consistent with the clinical performance obtained with younger patients.
- 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.
- Positive test results do not identify specific influenza A virus subtypes.
- Negative test results are not intended to rule in other non-influenza viral or bacterial infections.
- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing samples from adults will often yield lower sensitivity than testing samples from children.

- 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 influenza activity when prevalence is moderate to low.
- Individuals who received nasally administered influenza A vaccine may have positive test results for up to 3 days after vaccination.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.
- If differentiation of specific influenza A subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Samples contaminated with whole blood >4% v/v or mucin >0.5% v/v may interfere in the interpretation of the test. Visually bloody or overly viscous samples should not be used.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.

EXPECTED VALUES

Seasonal outbreaks of influenza occur worldwide in both the northern and southern hemispheres causing widespread illness each winter. The average attack rate of influenza is 26-33 cases per 100 people per year. The risk of hospitalization is roughly 1/300 of those infected among the very young and elderly. Over a period of 30 years, between 1976 and 2006, estimates of flu-associated deaths in the United States ranged from a low of about 3,000 to a high of about 49,000 people.[2] Ninety percent (90%) of deaths occur in those 65 years of age and older.[4] Influenza pandemics occurred in 1918, 1957, 1968 and 2009. The 1918 pandemic resulted in an estimated 40-50 million deaths worldwide. The prevalence observed with the reference test (viral culture) during the 2011 clinical study for Sofia Influenza A+B FIA was 15% for influenza A and 13% for influenza B.

PERFORMANCE CHARACTERISTICS

The following studies were performed with Sofia Influenza A+B FIA and Sofia

Sofia Influenza A+B FIA Performance vs. Cell Culture

The performance of the Sofia Influenza A+B FIA with Sofia was compared to viral cell culture methods followed by Direct Fluorescent Assay (DFA) in a multi-center clinical field study during February through March 2011 in the United States. This study was conducted by health care personnel at seventeen (17) distinct professional and CLIA waived sites (combined) in various geographical regions within the United States. In this multi-center, point-of-care (POC) field trial, two (2) nasal or two (2) nasopharyngeal swabs or nasopharyngeal aspirate/wash samples were collected from each of two thousand sixty-six (2066) patients. Six hundred seventy-one (671) provided a pair of nasal swab samples, seven hundred thirty-four (734) provided a pair of nasopharyngeal swab samples, and six hundred sixty-one (661) proved a nasopharyngeal aspirate/wash sample. All clinical samples were collected from symptomatic patients: 74% were <6 years of age, 22% 6-21 years of age, 4% 22-59 years of age, and 1% ≥60 years of age. Fifty-three percent (53%) were male and forty-seven percent (47%) were female.

A total of two thousand forty-seven (2047) prospective clinical samples were tested using the Sofia Influenza A+B FIA and gave valid results during this clinical study. These results were included in Tables 2-6. The invalid rate was 0.9% (19/2066) with 95% CI: 0.6% to 1.4%. The invalid results were excluded from Tables 2-6 because new patient samples were not collected for re-testing.

On-site testing of one nasal swab or nasopharyngeal swab, or a portion of nasopharyngeal aspirate/wash sample, was performed by medical personnel in the physician's office or hospital facility with the Sofia Influenza A+B FIA. All samples were freshly collected and tested. The remaining sample was placed in viral transport media for culturing. The paired swab samples or paired aspirate/wash samples were randomized with respect to the order of testing in the Sofia Influenza A+B 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 2-6.

Table 2
Sofia Influenza A+B FIA Nasal Swab Results Versus Culture
(All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	124	27
Sofia Neg	14	500

Sens = 124/138 = 90% (95% C.I. 84%-94%)

Spec = 500/527 = 95% (95% C.I. 93%-96%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	100	23
Sofia Neg	12	530

Sens = 100/112 = 89% (95% C.I. 82%-94%)

Spec = 530/553 = 96% (95% C.I. 94%-97%)

Table 3
Sofia Influenza A+B FIA Nasopharyngeal Swab Results Versus Culture
(All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	100	34
Sofia Neg	3	596

Sens = 100/103 = 97% (95% C.I. 91%-99%)

Spec = 596/630 = 95% (95% C.I. 93%-96%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	101	19
Sofia Neg	11	602

Sens = 101/112 = 90% (95% C.I. 83%-95%)

Spec = 602/621 = 97% (95% C.I. 95%-98%)

Table 4
Sofia Influenza A+B FIA Nasopharyngeal Aspirate/Wash Results Versus Culture
(All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	68	26
Sofia Neg	1	554

Sens = 68/69 = 99% (95% C.I. 91%-100%)

Spec = 554/580 = 96% (95% C.I. 93%-97%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	46	22
Sofia Neg	6	575

Sens = 46/52 = 88% (95% C.I. 77%-95%)

Spec = 575/597 = 96% (95% C.I. 94%-98%)

Table 5
Performance Compared to Culture for Each Sample Type by Age Group for Influenza A

	Nasal Swabs		Nasopharyngeal Swabs		Nasopharyngeal Aspirate/Wash	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
All Ages	**90%** (124/138) (95%CI=84%-94%)	**95%** (500/527) (95%CI=93%-96%)	**97%** (100/103) (95%CI=91%-99%)	**95%** (596/630) (95%CI=93%-96%)	**99%** (68/69) (95%CI=91%-100%)	**96%** (554/580) (95%CI=93%-97%)
<6 years	**95%** (62/65) (95%CI=87%-99%)	**95%** (210/221) (95%CI=91%-97%)	**97%** (61/63) (95%CI=89%-100%)	**94%** (444/470) (95%CI=92%-96%)	**99%** (68/69) (95%CI=91%-100%)	**95%** (544/570) (95%CI=93%-97%)
6 to 21 years	**87%** (46/53) (95%CI=75%-94%)	**95%** (193/204) (95%CI=91%-97%)	**97%** (35/36) (95%CI=85%-100%)	**94%** (136/144) (95%CI=89%-97%)	**N/A** (0/0)	**100%** (10/10) (95%CI=68%-100%)
22 to 59 years	**78%** (14/18) (95%CI=54%-92%)	**96%** (82/85) (95%CI=90%-99%)	**100%** (4/4) (95%CI=45%-100%)	**100%** (15/15) (95%CI=76%-100%)	**N/A** (0/0)	**N/A** (0/0)
60 Years and up	**100%** (2/2) (95%CI=29%-100%)	**88%** (15/17) (95%CI=64%-98%)	**N/A** (0/0)	**100%** (1/1) (95%CI=17%-100%)	**N/A** (0/0)	**N/A** (0/0)

Table 6
Performance Compared to Culture for Each Sample Type by Age Group for Influenza B

	Nasal Swabs		Nasopharyngeal Swabs		Nasopharyngeal Aspirate/Wash	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
All Ages	**89%** (100/112) (95%CI=82%-94%)	**96%** (530/553) (95%CI=94%-97%)	**90%** (101/112) (95%CI=83%-95%)	**97%** (602/621) (95%CI=95%-98%)	**88%** (46/52) (95%CI=77%-95%)	**96%** (575/597) (95%CI=94%-98%)
<6 years	**90%** (35/39) (95%CI=76%-97%)	**96%** (238/247) (95%CI=93%-98%)	**87%** (54/62) (95%CI=76%-94%)	**97%** (455/471) (95%CI=95%-98%)	**87%** (39/45) (95%CI=73%-94%)	**96%** (572/594) (95%CI=94%-98%)
6 to 21 years	**92%** (56/61) (95%CI=82%-97%)	**95%** (187/196) (95%CI=91%-98%)	**94%** (45/48) (95%CI=83%-98%)	**98%** (130/132) (95%CI=94%-100%)	**100%** (7/7) (95%CI=60%-100%)	**100%** (3/3) (95%CI=38%-100%)
22 to 59 years	**73%** (8/11) (95%CI=43%-91%)	**97%** (89/92) (95%CI=90%-99%)	**100%** (2/2) (95%CI=29%-100%)	**94%** (16/17) (95%CI=71%-100%)	**N/A** (0/0)	**N/A** (0/0)
60 Years and up	**100%** (1/1) (95%CI=17%-100%)	**89%** (16/18) (95%CI=66%-98%)	**N/A** (0/0)	**100%** (1/1) (95%CI=17%-100%)	**N/A** (0/0)	**N/A** (0/0)

Sofia Influenza A+B FIA Performance vs. Cell Culture When Testing Specimens Placed into Viral Transport Media

The performance of the Sofia Influenza A+B FIA with Sofia when testing specimens placed into VTM was compared to viral cell culture methods in the same multi-center clinical field study during February through March 2011 in the United States. This portion of the study was conducted by laboratory personnel at three (3) distinct reference laboratories within the United States. Nasopharyngeal swab and nasopharyngeal aspirate/wash specimens were evaluated in this study. Both specimen types were tested after the sample had been suspended into viral transport media (VTM) and transported to the corresponding reference laboratory. The Sofia Influenza A+B 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 swabs were provided by six hundred ninety (690) patients and nasopharyngeal aspirate/wash specimens were provided by six hundred sixty-four (664) patients. All clinical samples were collected from symptomatic patients: 84% were <6 years of age, 14% 6–21 years of age, 1% 22–59 years of age, and .1% ≥ 60 years of age.

A total of one thousand three hundred fifty-four (1354) clinical samples were used in the analysis. Data from certain subjects were excluded. Results are presented in Tables 7-8. The invalid rate was 2.1% (28/1354) with 95% CI: 1.4% to 3.0%. The invalid results were excluded from Tables 7-8 because new patient samples were not collected for re-testing.

Table 7
Sofia Influenza A+B FIA Nasopharyngeal Swab Results in VTM Versus Culture
(All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	94	12
Sofia Neg	9	573

Sens = 94/103 = 91% (95% C.I. 84-96%)

Spec = 573/585 = 98% (95% C.I. 96-99%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	88	6
Sofia Neg	19	575

Sens = 88/107 = 82% (95% C.I. 74-88%)

Spec = 575/581 = 99% (95% C.I. 98->99%)

Table 8
Sofia Influenza A+B FIA Nasopharyngeal Aspirate/Wash Results in VTM Versus Culture
(All Age Groups)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	66	17
Sofia Neg	2	553

Sens = 66/68 = 97% (95% C.I. 89->99%)

Spec = 553/570 = 97% (95% C.I. 95-98%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	43	20
Sofia Neg	8	567

Sens = 43/51 = 84% (95% C.I. 72-92%)

Spec = 567/587 = 97% (95% C.I. 95-98%)

Reproducibility Studies

The reproducibility of the Sofia Influenza A+B FIA with Sofia was evaluated at three different laboratories, one of which was Quidel. Two different operators at each site tested a series of coded, contrived samples, prepared in negative clinical matrix, ranging from low negative to moderate positive influenza A and influenza B. Testing occurred on 5 different days spanning over approximately a 2-week period. The inter-laboratory agreement (Table 9) for negative samples was 94%-100% and 98%-100% for positive samples.

Table 9
Sofia Influenza A+B Reproducibility Study Inter-Laboratory Agreement

Laboratory Site	Neg (no virus)	Flu A High Neg (C_5)	Flu A Low Pos (C_{95})	Flu A Mod Pos (C_{3X})	Flu B High Neg (C_5)	Flu B Low Pos (C_{95})	Flu B Mod Pos (C_{3X})
1	30/30	29/30	30/30	30/30	28/30	29/30	30/30
2	30/30	29/30	30/30	30/30	30/30	29/30	30/30
3	30/30	30/30	30/30	30/30	27/30	30/30	30/30
Total	90/90	88/90	90/90	90/90	85/90	88/90	90/90
% Overall Agreement with Expected Result (95% CI)	**100% (95%-100%)**	**98% (92%-100%)**	**100% (95%-100%)**	**100% (95%-100%)**	**94% (87%-98%)**	**98% (92%-100%)**	**100% (95%-100%)**

Limit of Detection

The limit of detection (LOD) for the Sofia Influenza A+B FIA with Sofia was determined using a total of seven (7) strains of human influenza viruses, two (2) influenza A and two (2) influenza B viruses for direct specimens, and two (2) influenza A and two (2) influenza B viruses for specimens eluted in VTM (Table 10).

Table 10
Limit of Detection with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)	
A/California/07/2009	A	2009 H1N1	202	
A/Hong Kong/8/68	A	H3N2	105	
B/Allen/45	B		40	
B/Malaysia/2506/04	B		24	
Specimens in Transport Media				
Viral Strain	**Viral Type**	**Sub-Type**	**Minimum Detectable Level ($TCID_{50}$/mL)**	
A/New Jersey/8/76	A	H1N1	M5	2460
			UTM	1645
A/Port Chalmers/1/73	A	H3N2	M5	1482
			UTM	1446
B/Allen/45	B		M5	5
			UTM	4.6
B/Florida/07/2004	B		M5	40
			UTM	35

$TCID_{50}$ levels were determined by either the Reed-Muench method or Rowe ELISA

Analytical Reactivity

Analytical reactivity was demonstrated with Sofia Influenza A+B FIA and Sofia using a total of 30 strains of human influenza viruses comprised of 21 Influenza A and 9 influenza B viruses (Table 11).

Table 11
Analytical Reactivity with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)	Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)
A/Fort Monmouth/1/47	A	H1N1	50	A/Wisconsin/67/05	A	H3N2	20
A/New Caledonia/20/1999	A	H1N1	200	A2/Aichi/2/68	A	H3N2	1.25
A/New Jersey/8/76	A	H1N1	500	A/Anhui/01/2005	A	H5N1	5
A/NWS/33	A	H1N1	0.63	A/GWT/LA/169GW/88	A	H10N7	20
A/Puerto Rico/8/34	A	H1N1	100	A/Shearwater/ Australia2576/79	A	H15N9	10
A/Solomon Islands/3/06	A	H1N1	0.31				
A/Taiwan/42/06	A	H1N1	200	B/Brisbane/60/2008	B		10
A/WI/629-9/2008	A	H1N1	200	B/Florida/04/2006	B		250
A1/Denver/1/57	A	H1N1	20	B/Florida/07/2004	B		500
Influenza/Mexico/4108/2009	A	2009 H1N1	200	B/GL/1739/54	B		2000
A/WI/629(D02312)/2009	A	2009 H1N1	50	B/Hong Kong/5/72	B		20
A/WI/629(D02473)/2009	A	2009 H1N1	25	B/Lee/40	B		5
A/Port Chalmers/1/73	A	H3N2	1000	B/Maryland/1/59	B		50
A/Victoria/3/75	A	H3N2	200	B/Ohio/1/2005	B		50
A/WI/629-2/2008	A	H3N2	20	B/Taiwan/2/62	B		50
A/Anhui/1/2013*	A	H7N9	3.95×10^6				

$TCID_{50}$/mL = 50% tissue culture infectious dose. EID_{50}/mL = 50% egg infective dose. $TCID_{50}$ and EID_{50} levels were determined by the Reed-Muench method.

*Although this test has been shown to detect H7N9 virus cultured from a positive human respiratory sample, the performance characteristics of this device with clinical samples that are positive for H7N9 influenza virus have not been established. The Sofia Influenza A+B FIA can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

Analytical reactivity was further evaluated using a total of 12 influenza A viruses isolated from birds. The Sofia Influenza A+B FIA detected all of the strains examined (Table 12).

Table 12
Analytical Reactivity with Different Isolates of Avian Influenza A

Viral Strain*	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)
A/Mallard/NY6750/78	A	H2N2	100
A/Mallard/OH/338/86	A	H4N8	50
A/Mallard/WI/34/75	A	H5N2	100
A/Chicken/CA/431/00	A	H6N2	50
A/Chicken/NJ/15086-3/94	A	H7N3	5
A/Blue Winged Teal/LA/B174/86	A	H8N4	10

Viral Strain*	Viral Type	Sub-Type	Minimum Detectable Level ($TCID_{50}$/mL)
A/Chicken/NJ/122210/97	A	H9N2	10
A/Chicken/NJ/15906-9/96	A	H11N9	50
A/Duck/LA/188D/87	A	H12N5	50
A/Gull/MD/704/77	A	H13N6	0.625
A/Mallard/GurjevRussia/262/82	A	H14N5	20
A/Shorebird/DE/172/2006	A	H16N3	2

*The performance characteristics for influenza A virus subtypes emerging as human pathogens have not been established.

Analytical Specificity

Cross Reactivity

The Sofia Influenza A+B FIA with Sofia was evaluated with a total of 18 bacterial and fungal microorganisms and 16 non-influenza viral isolates. Bacterial and fungal isolates were evaluated at a concentration of $2x10^6$ cfu/mL. Viral isolates were evaluated at a concentration of $2x10^5$ $TCID_{50}$/mL. None of the organisms or non-influenza viruses listed below in Table 13 showed any sign of cross reactivity in the assay. Flow of the sample and appearance of the Control Line were also not affected.

Table 13
Analytical Specificity and Cross Reactivity

Organism/Non-Influenza Virus	Concentration*	Flu A Result	Flu B Result
Bordetella pertussis	$2x10^6$ cfu/mL	Negative	Negative
Canidida albicans	$2x10^6$ cfu/mL	Negative	Negative
Chlamydia trachomatis	$2x10^6$ cfu/mL	Negative	Negative
Corynebacterium diphtheriae	$2x10^6$ cfu/mL	Negative	Negative
Escherichia coli	$2x10^6$ cfu/mL	Negative	Negative
Haemophilus influenzae	$2x10^6$ cfu/mL	Negative	Negative
Lactobacillus plantarum	$2x10^6$ cfu/mL	Negative	Negative
Legionella pneumophila	$2x10^6$ cfu/mL	Negative	Negative
Moraxella catarrhalis	$2x10^6$ cfu/mL	Negative	Negative
Mycobacterium tuberculosis (avirulent)	$2x10^6$ cfu/mL	Negative	Negative
Mycoplasma pneumoniae	$2x10^6$ cfu/mL	Negative	Negative
Neisseria meningitidis	$2x10^6$ cfu/mL	Negative	Negative
Neisseria subflava	$2x10^6$ cfu/mL	Negative	Negative
Pseudomonas aeruginosa	$2x10^6$ cfu/mL	Negative	Negative
Staphylococcus epidermidis	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus pneumoniae	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus pyogenes	$2x10^6$ cfu/mL	Negative	Negative
Streptococcus salivarius	$2x10^6$ cfu/mL	Negative	Negative
Adenovirus type 1	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Adenovirus type 7	$2x10^5$ $TCID_{50}$/mL	Negative	Negative

Organism/Non-Influenza Virus	Concentration*	Flu A Result	Flu B Result
Human coronavirus (OC43)	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human coronavirus (229E)	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human coxsackievirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Cytomegalovirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Epstein Barr Virus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 1	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 2	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human parainfluenza type 3	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Measles	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Human metapneumovirus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Mumps virus	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus type A	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Respiratory syncytial virus type B	$2x10^5$ $TCID_{50}$/mL	Negative	Negative
Rhinovirus type 1B	$2x10^5$ $TCID_{50}$/mL	Negative	Negative

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL. 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 with Sofia Influenza A+B FIA and Sofia. No interference was observed at the levels tested (Table 14).

Table 14
Non-interfering Substances

Substance	Concentration
Whole Blood	4%
Mucin	0.5%
Ricola (Menthol)	1.5 mg/mL
Sucrets (Dyclonin/Menthol)	1.5 mg/mL
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
CVS Nasal Drops (Phenylephrine)	15% v/v
Afrin (Oxymetazoline)	15% v/v
CVS Nasal Spray (Cromolyn)	15% v/v
Nasal Gel (Oxymetazoline)	10% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Fisherman's Friend	1.5 mg/mL
Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL

Substance	Concentration
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL

CLIA Waiver Studies

As part of a larger prospective study described in the Performance Characteristics section above, the accuracy of the Sofia Influenza A+B FIA with Sofia, when used by untrained operators, was compared to the results obtained by viral cell culture. This study was conducted at eleven (11) CLIA waived sites with thirty-four (34) untrained operators representative of CLIA waived settings. The study included 1,973 subjects: five hundred eighty-five (585) subjects provided a pair of nasal swabs, seven hundred twenty-seven (727) provided a pair of nasopharyngeal swabs, and six hundred sixty-one (661) provided a nasopharyngeal aspirate/wash sample. Seventeen (17) subjects were excluded due to Sofia Influenza A+B FIA invalid results. The invalid rate was 0.9% (17/1973) with 95% CI: 0.5% to 1.4%.

A total of 1,956 clinical samples gave valid results when tested in the Sofia Influenza A+B FIA. These results are included in Tables 15 and 16.

The clinical sensitivity and specificity of the Sofia Influenza A+B FIA, as compared to viral culture (the comparator method), are presented below in Tables 15 and 16.

Table 15
Sofia Influenza A+B FIA Versus Culture (Nasal/Nasopharyngeal Swabs)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	219	58
Sofia Neg	16	1014

Sens = 219/235 = 93% (95% C.I. 89%-96%)

Spec = 1014/1072 = 95% (95% C.I. 93%-96%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	188	40
Sofia Neg	21	1058

Sens = 188/209 = 90% (95% C.I. 85%-93%)

Spec = 1058/1098 = 96% (95% C.I. 95%-97%)

Table 16
Sofia Influenza A+B FIA Versus Culture (Nasopharyngeal Aspirate/Wash)

TYPE A

	Culture	
	Pos	Neg
Sofia Pos	68	26
Sofia Neg	1	554

Sens = 68/36 = 99% (95% C.I. 91%-100%)

Spec = 554/580 = 96% (95% C.I. 93%-97%)

TYPE B

	Culture	
	Pos	Neg
Sofia Pos	46	22
Sofia Neg	6	575

Sens = 46/52 = 88% (95% C.I. 77%-95%)

Spec = 575/597 = 96% (95% C.I. 94%-98%)

Two studies were conducted to demonstrate that untrained intended users could perform the test consistently and accurately using weakly reactive samples. Each study consisted of three (3) distinct CLIA-waived sites where the Sofia Influenza A+B FIA with Sofia was evaluated using coded randomized panels of simulated samples, including one (1) weak positive (C_{95}—a concentration at the assay cutoff) and one (1) weak negative (C_5—a concentration just below the assay cutoff) for influenza A and influenza B. Two (2) or more operators at each site (15 operators total) tested the panel on each of 10 days, spanning a period of approximately 2 weeks.

Study A evaluated swab samples and the performance is shown in Table 19. Study B evaluated liquid samples. Study B was designed to provide contrived samples to untrained operators, which were a mix of liquid and

swab samples. The purpose of Study B was to demonstrate the ability of the operator to choose the correct procedure per the sample type. Table 17 shows the results obtained with liquid samples in Study B.

Table 17
Sofia Influenza A+B FIA Performance Near the Cutoff—Study A

Sample Level	Untrained Intended Users	
	Percent Agreement with Expected Results*	**95% Confidence Interval**
Flu A Weak Negative (C_5)	87% (52/60)	76%-93%
Flu A Weak Positive (C_{95})	92% (55/60)	82%-97%
Flu B Weak Negative (C_5)	87% (52/60)	76%-93%
Flu B Weak Positive (C_{95})	92% (55/60)	82%-97%

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

Table 18
Sofia Influenza A+B FIA Performance Near the Cutoff—Study B

Sample Level	Untrained Intended Users	
	Percent Agreement with Expected Results*	**95% Confidence Interval**
Flu A Weak Negative (C_5)	95% (57/60)	86%-99%
Flu A Weak Positive (C_{95})	97% (58/60)	88%->99%
Flu B Weak Negative (C_5)	80% (48/60)	68%-88%
Flu B Weak Positive (C_{95})	97% (58/60)	88%->99%

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

In support of the CLIA waiver, an additional reactivity study was performed at an independent laboratory to demonstrate reactivity of the Sofia Influenza A+B FIA with a broad range of contemporary strains of influenza A and influenza B viruses. The Sofia Influenza A+B FIA yielded positive results with all 18 influenza A viruses and all 7 influenza B viruses included in the test panel at acceptable viral load levels.

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.

Sofia Influenza A+B FIA Performance with Sofia 2

The following studies were performed to demonstrate equivalency between Sofia and Sofia 2 when testing clinical samples with the Sofia Influenza A+B FIA.

Method Comparison

The performance of Sofia Influenza A+B FIA when used with Sofia vs. Sofia 2 was compared using a panel of 250 clinical samples. This field study was performed at 3 intended user laboratory sites using identical panels of known positive and negative clinical and contrived samples prepared in viral transport media (VTM). A total of 15 Sofia and 14 Sofia 2s were utilized in this study. One hundred (100) influenza A positive, 50 influenza B positive, and 100 influenza negative samples were incorporated into the panels. Panel members were prepared so that a broad range of negative and positive samples were evenly distributed across the dynamic range of the assay. All samples were coded and used to prepare the randomized panels. A total of 250 samples per site were tested resulting in a total of 749 results across the three testing sites (one specimen test result was not recoverable).

The Sofia vs. Sofia 2 comparison results are shown below in Table 19. Influenza A positive agreement was 98% and negative agreement was 97%. Influenza B positive agreement was 98% and negative agreement was 99%.

Table 19

Sofia Influenza A+B FIA – Sofia vs. Sofia 2 Method Comparison

TYPE A					TYPE B				
	Sofia					Sofia			
	Pos	Neg				Pos	Neg		
Sofia 2 Pos	304	15**	**Positive % Agreement/ (95% C.I.)**	304/311 = 98% (95.4%-98.9%)	**Sofia 2 Pos**	154	5**	**Positive % Agreement/ (95% C.I.)**	154/157 = 98% (94.5%-99.4%)
Sofia 2 Neg	7*	423	**Negative % Agreement/ (95% C.I.)**	423/438 = 97% (94.4%-97.9%)	**Sofia 2 Neg**	3*	587	**Negative % Agreement/ (95% C.I.)**	587/592 = 99% (98.1%-99.6%)
Total:	311	438			**Total:**	157	592		

*There were 7 discordant Sofia 2 negative/Sofia positive results for Influenza A which included 1 low positive (C_{95}) specimen and the rest high negative (C^5) specimens.

**There were 15 discordant Sofia 2 positive/Sofia negative results for Influenza A which included 3 low positive (C_{95}) specimens and the rest high negative (C_5) specimens.

‘There were 3 discordant Sofia 2 negative/Sofia positive results for nfluenza B which included all high negative (C_5) specimens.

‘*There were 5 discordant Sofia 2 positive/Sofia negative results for nfluenza B which included all high negative (C_5) specimens.

Reproducibility

A reproducibility study was performed with the Sofia Influenza A+B FIA and Sofia 2 at three different laboratories, one of which was Quidel. Two to three different operators at each site tested a series of coded, contrived samples, prepared in negative clinical matrix, ranging from negative to moderate positive influenza A and influenza B. Testing occurred on 5 different days spanning over approximately 1 week. A total of 10 Sofia 2s were utilized in this study. The inter-laboratory agreement (Table 20) for the Sofia Influenza A+B FIA with Sofia 2 for all samples ranged from 98.9% to 100%.

Table 20
Sofia Influenza A+B FIA Reproducibility Study (with Sofia 2)
Inter-laboratory Agreement

Site	Influenza A+B Negative (C_0)	Influenza A Weak Positive (C_{95})	Influenza A Moderate Positive (2-3X LOD)	Influenza B Weak Positive (C_{95})	Influenza B Moderate Positive (2-3X LOD)
1	30/30	30/30	30/30	30/30	30/30
2	30/30	30/30	30/30	30/30	30/30
3	29*/30	30/30	30/30	30/30	29*/30
Total	**89/90**	**90/90**	**90/90**	**90/90**	**89/90**
% Overall Agreement (95% CI)	**98.9% (93.9-99.8%)**	**100% (95.9-100%)**	**100% (95.9-100%)**	**100% (95.9-100%)**	**98.9% (93.9-99.8%)**

*The discordant result for the C_0 and the 2-3X LOD samples appeared to be a mix-up at the site as the samples were tested on the same day and by the same operator.
*The discordant result for the C_0 and the 2-3X LOD samples appeared to be a mix-up at the site as the samples were tested on the same day and by the same operator.

Limit of Detection

A limit of detection (LOD) study was performed with the Sofia Influenza A+B FIA on Sofia and Sofia 2 in parallel using a total of four (4) strains of human influenza viruses, two (2) influenza A and two (2) influenza B viruses for direct specimens (Table 21).

Table 21
Limit of Detection with Human Isolates of Influenza A and B

Viral Strain	Viral Type	Sub-Type	Platform	Minimum Detectable Level ($TCID_{50}$/mL)**
A/California/07/2009*	A	2009 H1N1	Sofia	5.48
			Sofia 2	9.79
A/Hong Kong/8/68*	A	H3N2	Sofia	1.80
			Sofia 2	2.19
B/Allen/45*	B		Sofia	113
			Sofia 2	82.3
B/Malaysia/2506/04*	B		Sofia	8.37
			Sofia 2	8.37

* $TCID_{50}$ levels were determined by either the Reed-Muench method or Rowe ELISA.
** New preparations of the viral strains were utilized accounting for the difference in $TCID_{50}$ results from the original Sofia LOD study.

CLIA Waiver Study

A study was conducted to demonstrate that untrained intended users could perform the test consistently and accurately using weakly reactive samples with the Sofia Influenza A+B FIA and Sofia 2. The study consisted of three (3) distinct CLIA-waived sites where the Sofia Influenza A+B FIA was evaluated using coded randomized panels of simulated samples, including one (1) weak positive (C_{95}—a concentration at the assay cutoff) for influenza A, one (1) weak positive (C_{95}—a concentration at the assay cutoff) influenza B, and one negative for influenza A and B. Three (3) or more operators at each site (10 operators total) tested the panel on each of 10 days, spanning a period of approximately 2 weeks (Table 22).

Table 22
Sofia Influenza A+B FIA Performance Near the Cutoff—with Sofia 2

Sample Level	Untrained Intended Users	
	Percent Agreement with Expected Results*	**95% Confidence Interval**
Flu A Weak Positive (C_{95})	98.6% (71/72)	92.5 – 99.8%
Flu B Weak Positive (C_{95})	98.6% (71/72)	92.5 – 99.8%
Negative (C_0)	100% (72/72)	94.9 – 100%

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 Technical Support at 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 U.S. 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: 800.FDA.1088; fax: 800.FDA.0178; http://www.fda.gov/medwatch).

REFERENCES

1. Murphy B.R. and Webster R.G., 1996, Orthomyxoviruses, pp. 1397-1445. In: Fields Virology, 3rd edition, B.N. Fields, D.M. Knipe, P.M. Howley, et al. (eds.), Lippincott-Raven, Philadelphia.
2. CDC, Key Facts About Seasonal Influenza. www.cdc.gov/flu/keyfacts.htm accessed 7/2011.
3. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. U.S. Department of Health and Human Services, CDC, NIH, Washington, DC (2007).
4. Bartlett J. Management of Respiratory Tract Infections. 2nd Ed, 1999: 149-169.

LOG SHEET

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 #	Influenza Positive Control passed?	Influenza Negative Control passed?	Comments	Technician Initials
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

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