Sofia®
Influenza A+B FIA

For use with the Sofia Analyzer only
CLIA Complexity: MODERATE

INTENDED USE

The Sofia Influenza A+B FIA employs immunofluorescence to detect influenza A and influenza B viral nucleoprotein antigens in nasal swab, 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 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 virus 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 management decisions. The test is intended for professional and laboratory use.

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, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

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% to 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 the Sofia Analyzer to detect influenza virus nucleoproteins. This test allows for the differential detection of influenza A and influenza B antigens.

The Sofia Influenza A+B FIA involves the disruption of influenza A and B viral antigens. 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 influenza viral antigen is present, they will be trapped in a specific location.

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

The Sofia Analyzer will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. The Sofia Analyzer 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-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)
- 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 Analyzer instrument
- Micropipettor
- Specimen container
- Sterile saline
- Equipment used for collection of Nasopharyngeal Aspirate or Nasopharyngeal Wash
- Nylon flocked nasopharyngeal swab
- Calibration Cassette (supplied with the Sofia Analyzer)

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]
- 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 specimen collection, storage, and transport may yield false test results.
- Specimen collection and handling procedures require specific training and guidance.
- Use the Viral Transport Media recommended in this Package Insert.
- When collecting a nasal swab specimen, use the nasal swab supplied in the kit.
- When collecting a nasopharyngeal swab specimen, use a nylon flocked nasopharyngeal swab.
- Do not write on the barcode of the Cassette. This is used by the Sofia Analyzer 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 Analyzer.
- If infection with a novel influenza A virus is suspected, based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens 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 specimens.
- 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 specimens 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. The Sofia Analyzer must be used for result interpretation.

KIT STORAGE AND STABILITY

Store the kit at room temperature, 59–86°F (15–30°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 Analyzer and Cassette: Sofia Analyzer calibration procedure, built-in procedural control features, and External Controls.

Sofia Analyzer Calibration Check Procedure

Note: This is a "Calibration Check" procedure.

The Calibration Check Procedure should be performed every thirty (30) days. The Sofia Analyzer can be set to remind the user to complete the calibration check procedure.

The Calibration Check is a required function that checks the Sofia Analyzer optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is shipped with the Sofia Analyzer. Refer to the Sofia Analyzer 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 the Sofia Analyzer, select "Calibration" from the Main Menu.

2. Following the prompts, insert the Calibration Cassette into the Sofia Analyzer and close the drawer. The Analyzer performs the calibration check automatically with no user input required.

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

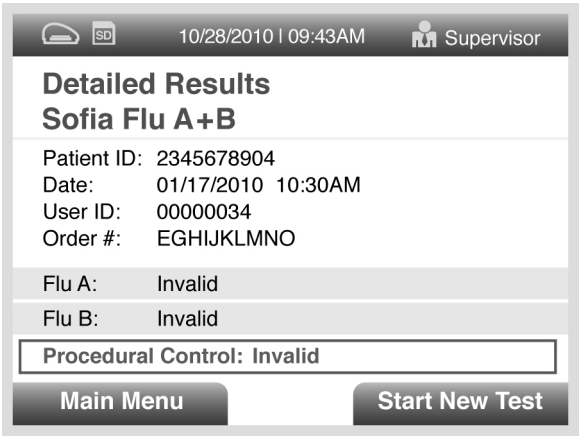
NOTE: If calibration cannot be completed successfully, notify the on-site Supervisor or contact Quidel Technical Support for assistance from 7:00 a.m.-5:00 p.m. PST at (800) 874-1517 (within the USA); (858) 552-1100 (outside the USA); Fax: (858) 455-4960; custserv@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support) or contact your local distributor.

Built-in Procedural Control

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

A valid result obtained from the procedural control demonstrates that the test flowed correctly and the functional integrity of the Cassette was maintained. **The procedural control is interpreted by the Sofia Analyzer after the Cassette has developed for fifteen (15) minutes. If the test does not flow correctly, the Sofia Analyzer 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 result shows that an invalid result had occurred.

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 – and 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 the Sofia Analyzer 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 Analyzer will then prompt the user 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. **Note: the Influenza Positive Control Swab should give a positive result for both influenza A and influenza B.**

Do not perform patient tests or report patient test results if the control tests do not produce the expected results. Repeat the test or contact Quidel Technical Support before testing patient specimens.

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.A.) or (858) 552.1100.

SPECIMEN COLLECTION AND HANDLING

SPECIMEN 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 specimens. **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 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.

SPECIMEN TRANSPORT AND STORAGE

Specimens 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. One (1) milliliter or less is suggested for optimal rapid test performance. The following viral transport media listed in Table 1 are compatible with the Sofia Influenza A+B FIA:

Table 1
Recommended Viral Transport Media

Viral Transport Media	Recommended Storage Condition	
	2–8°C	25°C
Copan Universal Transport Media	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

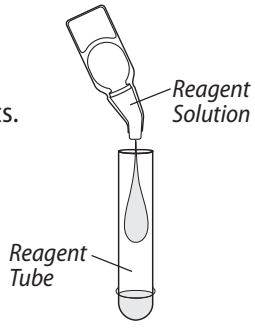
TEST PROCEDURES

All clinical specimens must be at room temperature before beginning the assay.

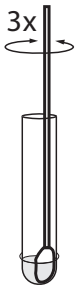
Expiration date: Check expiration on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

Swab Test Procedure (Nasal / Nasopharyngeal Swab)

1. Add the Reagent Solution to the Reagent Tube. Gently swirl the Reagent Tube to dissolve its contents.

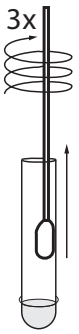


2. Immediately place the patient swab sample into the Reagent Tube. Roll the swab a minimum of three (3) times while pressing the head against the bottom and side of the Reagent Tube.

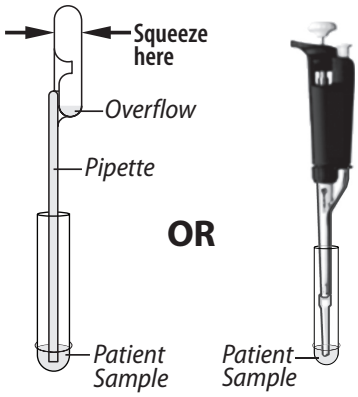


Leave the swab in the Reagent Tube for one (1) minute.

3. Express all liquid from the swab head by rolling it against the inside of the Reagent Tube as the swab is being removed. Discard the swab in accordance with your biohazard waste disposal protocol.



4. Fill the provided fixed volume pipette (120 µL) or a micropipettor with **120 µL** of the patient sample from the Reagent Tube.



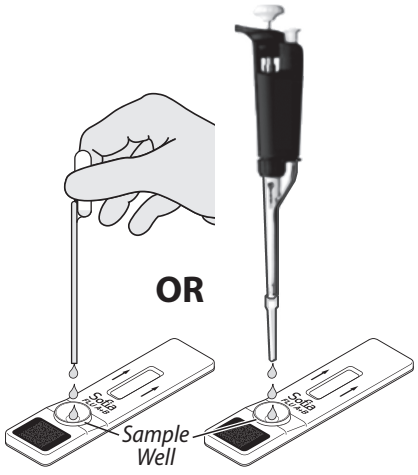
To fill the fixed volume pipette with the patient sample:

a. FIRMLY squeeze the top bulb.

b. Still squeezing, place the pipette tip into the liquid sample.

c. With the pipette tip still in the liquid sample, release pressure on bulb to fill the pipette.

5. Dispense the patient sample into the Cassette sample well. Firmly squeeze the top bulb to empty the contents of the fixed volume pipette into the Cassette sample well (extra liquid in the overflow bulb is OK).

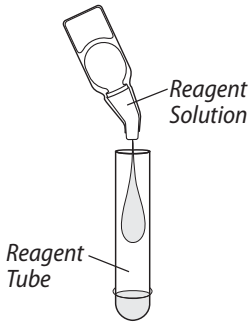


NOTE: The fixed volume pipette is designed to collect and dispense the correct amount of liquid sample. Discard the pipette in accordance with your biohazard waste disposal protocol.

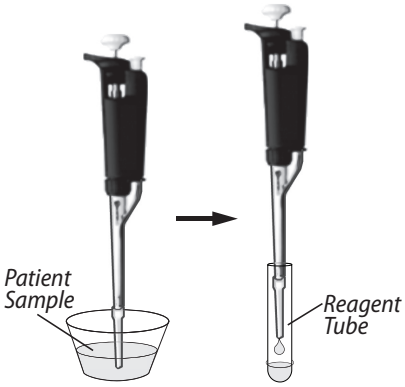
6. Proceed to the "Using the Sofia Analyzer" section of this Package Insert.

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

1. Add the Reagent Solution to the Reagent Tube. Gently swirl the Reagent Tube to dissolve its contents.



2. Fill a micropipettor with **260 µL** of the patient's liquid sample. Dispense the contents of the micropipettor into the Reagent Tube. Gently swirl the Reagent Tube to mix its contents.



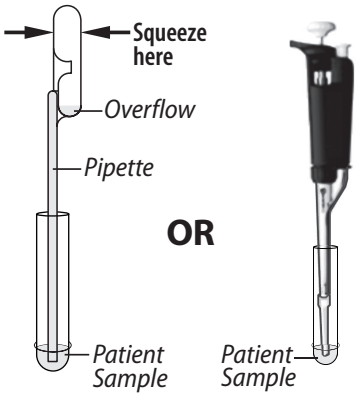
3. Fill the provided fixed volume pipette (120 µL) or a micropipettor with **120 µL** of the patient sample from the Reagent Tube.

To fill the fixed volume pipette with the patient sample:

a. FIRMLY squeeze the top bulb.

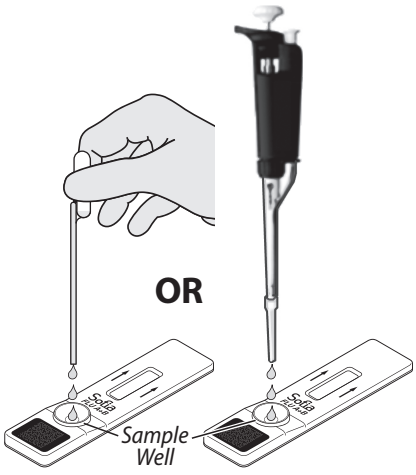
b. Still squeezing, place the pipette tip into the liquid sample.

c. With the pipette tip still in the liquid sample, release pressure on bulb to fill the pipette.



4. Dispense the patient sample into the Cassette sample well. Firmly squeeze the top bulb to empty the contents of the fixed volume pipette into the Cassette sample well (extra liquid in the overflow bulb is OK).

NOTE: The fixed volume pipette is designed to collect and dispense the correct amount of liquid sample. Discard the pipette in accordance with your biohazard waste disposal protocol.



5. Proceed to the "Using the Sofia Analyzer" section of this Package Insert.

USING THE SOFIA ANALYZER

Walk Away/Read Now Modes

Refer to the Sofia Analyzer User Manual for operating instructions.

The Sofia Analyzer may be set to two different development timed modes (Walk Away and Read Now). The procedures for each mode are described below.

Walk Away Mode

Using the barcode reader, scan in the user and patient IDs. After the user adds the patient sample and inserts the Cassette into the Sofia Analyzer, the Analyzer will automatically time the test development, scan, and display the test result in about fifteen (15) minutes.

Read Now Mode

The Read Now mode provides a convenient way for busy laboratories to conduct batch testing. The user adds the patient sample to the Cassette and places the Cassette on the counter or bench top for fifteen (15) minutes (outside of the Analyzer). The user must carefully and manually time the development step. When the development time is approaching completion, enter user ID and patient ID with the provided barcode reader. Once the development time is complete, the user immediately inserts the Cassette into the Sofia Analyzer. The Analyzer will scan and display the test result within one (1) minute. **Note:** Results will remain stable for an additional fifteen (15) minutes after the recommended development time of fifteen (15) minutes.

Depending upon 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 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 the swab or liquid specimens in the Reagent Tube, then after removing the swab, recap the tube and let them stand at RT for up to 12 hours without loss of activity before testing.

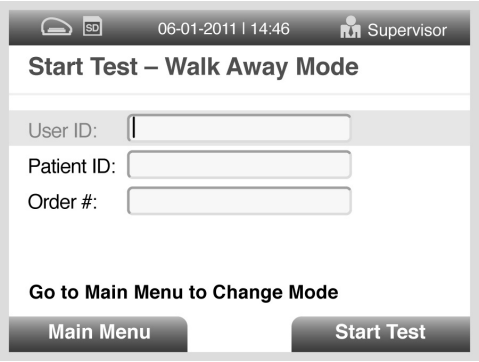
Critically important, the user should never open the foil pouch thus exposing the test Cassette to the ambient environment, until they are ready for immediate use.

Run Test

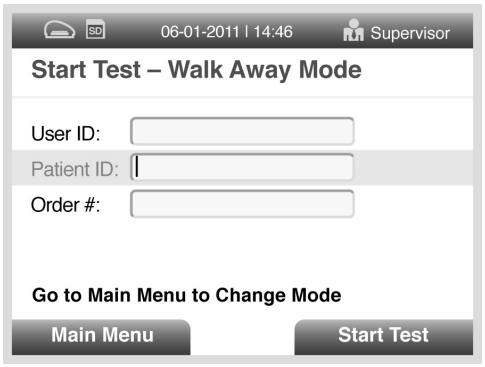
When placing the Cassette into the Sofia Analyzer, the user ID, patient ID, and order # can be entered via a handheld barcode scanner or by manually entering the information onto the keypad of the Sofia Analyzer. The test ID (test type, lot #, Cassette serial #, and test expiration date) on the Cassette is scanned automatically into the Sofia Analyzer with an internal barcode reader. The test is automatically interpreted at fifteen (15) minutes when the Analyzer is set to the Walk Away Mode and will give the result within one (1) minute after inserting the Cassette when the Analyzer is set to the Read Now Mode.

NOTE: If you mistakenly scan the incorrect barcode, simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.

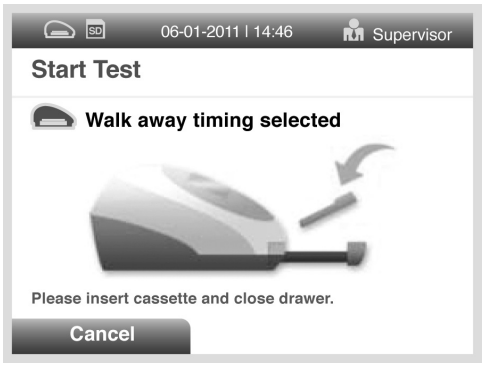
1. Highlight User ID field using the Arrow Buttons on the Sofia Analyzer key pad. Input the user ID using the barcode scanner or manually enter the data using the key pad.



2. Press the Down Arrow on the Sofia Analyzer keypad to go to the Patient ID or Order # field. Input patient ID or Order # using the barcode scanner or manually enter the data using the key pad.

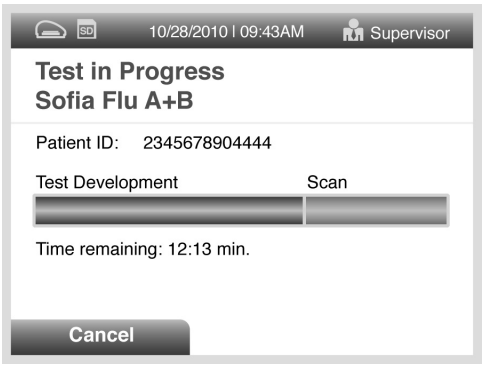


3. Press Start Test and the Sofia Analyzer drawer will automatically open.



4. Verify that the correct development mode, Walk Away or Read Now, has been selected. **Immediately** insert the prepared patient test Cassette into the drawer of the Sofia Analyzer and close the drawer.

5. Upon closing the drawer, the Sofia Analyzer will start automatically and display the progress as shown in example below. In the Walk Away Mode, the test results will be displayed on the screen in approximately fifteen (15) minutes after the Cassette is inserted into the Sofia Analyzer. In the Read Now Mode, the test results will be displayed on the screen within one (1) minute after the Cassette is inserted into the Analyzer. See Interpretation of Results section.



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

INTERPRETATION OF RESULTS

When the test is complete, the results will be displayed on the Sofia Analyzer screen. The results can be automatically printed on the integrated printer if this option is selected.

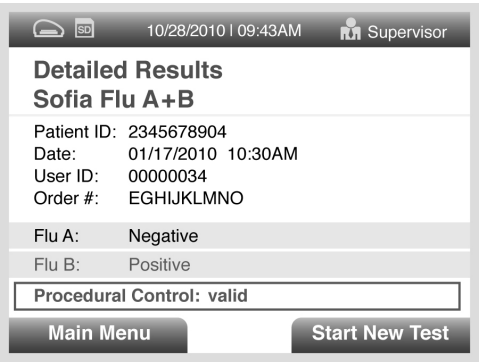
The Sofia Analyzer detects the test line. The test line will not be visible to the user.

The Sofia Analyzer 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 Cassette.

Positive Results:

For example: This result shows that a valid result had occurred and that the specimen was positive for Influenza A.

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



For example: This result shows that a valid result had occurred and that the specimen was positive for Influenza B.

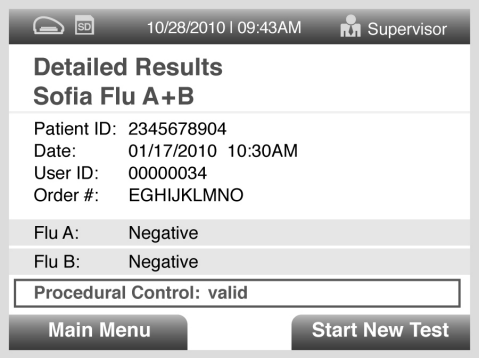
NOTE: A positive result does not rule out co-infections with other pathogens.

For example: This result shows that a valid result had occurred and that the specimen was positive for both Influenza A and Influenza B.

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

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

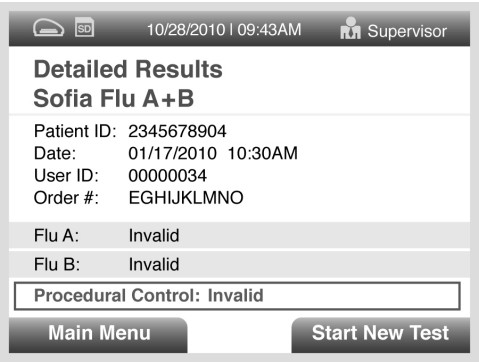
Negative Results:



For example: This result shows that a valid result had occurred and that the specimen was negative for Influenza A and Influenza B.

NOTE: A negative result does not exclude influenza viral infection. Negative results should be confirmed by virus culture or an FDA-cleared influenza A and B molecular assay.

Invalid Results:



For example: This result shows that an invalid result had occurred.

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 a nasal swab, nasopharyngeal swab, and nasopharyngeal aspirate/wash specimens.
- This test detects both viable (live) and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in the specimen and may or may not correlate with virus culture results performed on the same specimen.
- The clinical performance of the Sofia Influenza A+B FIA for nasopharyngeal aspirate/wash samples has not been established in patients 60 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 specimens from adults will often yield lower sensitivity than testing specimens 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 or activity when prevalence is moderate to low.
- Individuals who received nasally administered influenza A vaccine may have positive test results for up to three 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. In the 1918 pandemic, an estimated 40–50 million deaths resulted worldwide. The prevalence observed with the reference test (virus culture) during the 2011 clinical study for Sofia Influenza A+B FIA was 15% for influenza A and 13% for influenza B.

PERFORMANCE CHARACTERISTICS

Sofia Influenza A+B FIA Performance vs. Cell Culture

The performance of the Sofia Influenza A+B FIA was compared to viral cell culture methods followed by 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 sites 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 specimens were collected from each of two thousand forty-seven (2047) patients. Six hundred sixty-five (665) provided a nasal swab specimen, seven hundred thirty-three (733) provided a nasopharyngeal swab specimen and six hundred forty-nine (649) provided a nasopharyngeal aspirate/wash specimen. All clinical samples were collected from symptomatic patients. Seventy-one percent (71%) of the population tested were <6 years of age, 22% 6–21 years of age, 6% 22–59 years of age, and 1% ≥60 years of age. Fifty-three percent (53%) were male and forty-seven percent (47%) were female.

On-site testing of one nasal swab or nasopharyngeal swab or a portion of nasopharyngeal aspirate/wash specimen in the Sofia Influenza A+B FIA test was performed on the fresh specimen by medical personnel in the physician's office or hospital facility. The remaining sample was placed in viral transport media. The paired swab samples were randomized with respect to the order of testing in the Sofia Influenza A+B FIA versus culture. Cell culture was performed either at a local virus laboratory of the test site or 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	Culture 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	Culture 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	Culture 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	Culture 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	Culture 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	Culture 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 Specimen 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=86-100%)	**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 Specimen 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)

A total of 2047 prospective clinical specimens were tested and gave valid results during this clinical study. These results were included in Tables 2–6. There were nineteen (19) additional specimens (less than 1% of the total collected) that gave invalid results. The invalid results were excluded from Tables 2–6 because new patient specimens were not collected for re-testing.

Reproducibility Studies
The reproducibility of the Sofia Influenza A+B FIA 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 five (5) different days spanning over approximately a two-week period. The inter-laboratory agreement (Table 7) for negative samples was 94–100% and 98–100% for positive samples. The intra-laboratory agreement (Table 8) for all samples ranged from 98–99%.

Table 7
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%)**

Table 8
Sofia Influenza A+B Reproducibility Study Intra-Laboratory Agreement

Lab. 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})	% Overall Agreement with Expected Result (95% CI)
1	30/30	29/30	30/30	30/30	28/30	29/30	30/30	**98%** (206/210) **(95-100%)**
2	30/30	29/30	30/30	30/30	30/30	29/30	30/30	**99%** (208/210) **(96-100%)**
3	30/30	30/30	30/30	30/30	27/30	30/30	30/30	**99%** (207/210) **(96-100%)**

Limit of Detection
The limit of detection (LoD) for the Sofia Influenza A+B FIA was determined using a total of four (4) strains of human influenza viruses, two (2) influenza A and two (2) influenza B viruses (Table 9).

Table 9
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	H1N1	105
B/Allen/45	B		40
B/Malaysia/2506/04	B		24

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

Analytical Reactivity

Analytical reactivity was demonstrated using a total of twenty-nine (29) strains of human influenza viruses comprised of twenty (20) Influenza A and nine (9) influenza B viruses (Table 10).

Table 10
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/Australia 2576/79	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		1000
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	500	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

Viral Strain	Viral Type	Sub-Type	Minimum Detectable Level (EID_{50}/mL)
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 specimen, the performance characteristics of this device with clinical specimens 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.

Table 11
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
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 was evaluated with a total of eighteen (18) bacterial and fungal microorganisms and sixteen (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 12 showed any sign of cross reactivity in the assay. Flow of the sample and appearance of the Control Line were also not affected.

Table 12
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
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 and did not interfere with the Sofia Influenza A+B FIA at the levels tested (Table 13).

Table 13
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
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL

ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel's Technical Support Number (800) 874-1517 (toll-free in the U.S.A.) or (858) 552-1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time, U.S.A. If outside the United States contact your local distributor or technicalsupport@quidel.com.

For additional Quick Reference Instructions in your language, please see our website **quidel.com,** or contact Quidel Technical Support (858) 552-1100 or your local distributor for a copy delivered free of charge.

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