For use under the Emergency Use Authorization (EUA) only

For in vitro diagnostic use

℞ ONLY

QUIDEL

Sofia 2 Flu+SARS Antigen FIA

INTENDED USE

The Sofia 2 Flu + SARS Antigen FIA is a lateral flow immunofluorescent sandwich assay that is used with Sofia 2. Sofia 2 Flu + SARS Antigen FIA is intended for the simultaneous qualitative detection and differentiation of nucleocapsid protein antigen from SARS-CoV-2, influenza A, and influenza B directly from nasopharyngeal (NP) and nasal (NS) swab specimens collected from individuals who are suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider within the first five (5) days of symptom onset when tested at least twice over three days with at least 48 hours between tests. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate, high or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

The test is intended for use in the simultaneous rapid in vitro detection and differentiation of SARS-CoV-2, influenza A virus, and influenza B virus nucleocapsid protein antigen, but does not differentiate, between SARS-CoV and SARS-CoV-2 viruses and is not intended to detect influenza C antigens. 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.

SARS-CoV-2, influenza A, and influenza B viral antigens are generally detectable in upper respiratory specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status.

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

All negative SARS-CoV-2 results are presumptive and should be confirmed with a molecular assay, if necessary,

for patient management. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control measures such as isolating from others and wearing masks. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

All negative influenza A and B test results are presumptive. It is recommended these results be confirmed by an FDA-cleared influenza A and B molecular assay. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.

The Sofia 2 Flu + SARS Antigen FIA is intended for use on the Sofia 2 only and by medical professionals or trained operators who are proficient in performing tests using the Sofia 2 Instrument. The Sofia 2 Flu + SARS Antigen FIA test is only for in vitro diagnostic use under the Food and Drug Administration's Emergency Use Authorization. This product has not been FDA cleared or approved.

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.[i]

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.[ii]

SARS-CoV-2, also known as the COVID-19 virus, was first identified in Wuhan, Hubei Province, China in December 2019. This virus, as with the novel coronavirus SARS-1 and MERS, is thought to have originated in bats, however SARS-CoV-2 may have had an intermediary host such as pangolins, pigs or civets.[iii] The WHO declared the COVID-19 pandemic on March 11, 2020, and human infection has spread globally, with hundreds of thousands of confirmed infections and deaths.[iv]

The median incubation time is estimated to be 5.1 days with symptoms expected to be present within 12 days of infection.[v] The symptoms of COVID-19 are similar to those of other viral respiratory diseases, and include fever, cough, and shortness of breath.[vi]

PRINCIPLE OF THE TEST

The Sofia 2 Flu + SARS Antigen FIA employs immunofluorescence technology in a sandwich design that is used with Sofia 2 to detect nucleocapsid protein from influenza A, influenza B, and SARS-CoV-2. This test allows for the detection of SARS-CoV and SARS-CoV-2. The test detects, but does not differentiate, between the two viruses.

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 A, influenza B, SARS-CoV or SARS-CoV-2 viral antigen is present, they will be trapped in a specific location.

Note: Depending upon the user's choice, the Test Cassette is placed inside 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 2 to be scanned (READ NOW Mode).

Sofia 2 will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. 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; Monoclonal anti-SARS antibodies
- Reagent Tubes (25): Lyophilized buffer with detergents and reducing agents
- Reagent Solution (25): Ampoules with salt solution
- Sterile Nasal (SKU # 20377) or Nasopharyngeal Swabs (SKU # 20390) (25)
- Small, Clear 120 µL Fixed Volume Pipettes (25)
- Flu + SARS Positive Control Swab (1): Swab is coated with non-infectious recombinant influenza A, influenza B, and SARS 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)

MATERIALS NOT SUPPLIED IN KIT

- Timer or watch
- Sofia 2
- Calibration Cassette (supplied with the Sofia 2)
- Dry transport tube (SKU # 20385) (25). Store at room temperature.
- Sofia 2 Flu + SARS Control Swab Set for additional Quality Control (SKU # 20391)

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- For prescription use only.
- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- This test is for use with the Sofia 2 instrument only.
- This test has been authorized only for the detection and differentiation of proteins from SARS-CoV-2, influenza A, and influenza B, not for any other viruses or pathogens.
- In the USA, this product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization. This product has been authorized only for the detection of proteins from SARS-CoV-2, influenza A, and influenza B, not for any other viruses or pathogens. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- The test has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories certified under the CLIA that meet the requirements to perform moderate, high or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

- **Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
- **Consistent with serial testing recommendations for SARS-CoV-2, for multi-analyte tests, symptomatic individuals who test positive for influenza A or B on the initial test but test negative for SARS-CoV-2 should be tested again in 48 hours to evaluate for co-infection with SARS-CoV-2 infection.**
- The Sofia 2 Flu + SARS FIA is intended to be used with direct nasal or nasopharyngeal swabs and is not validated for use with viral transport media.
- Do not use if any of the test kit contents or packaging is damaged.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Test components are single-use. Do not re-use.
- Once opened, the test kit components should be used immediately.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- Use of Nitrile, Latex (or equivalent) gloves is recommended when handling patient samples.
- 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 or dropped 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.
- When collecting a nasal swab sample, use the Nasal Swab supplied in the kit.
- When collecting a nasopharyngeal swab sample, use the nylon flocked nasopharyngeal swab supplied in the kit.
- Use the appropriate Fixed Volume Pipette in accordance with test procedures.
- **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 2 to identify the type of test being run and to identify the individual Test Cassette to prevent a second read of the Test Cassette by the same 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 2 must be used for result interpretation.
- **Do not read test results before 15 minutes or after 30 minutes. Results read before 15 minutes or after 30 minutes may lead to a false positive, false negative, or invalid result.**
- To obtain accurate results, an opened and exposed Test 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.
- Do not ingest any kit components. The reagent solution contains harmful chemicals (see table below). If the solution contacts your [e.g., *skin, eyes, nose, or mouth]*, flush with large amounts of water. **If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Chemical Name/CAS	Harms (GHS Code) for each ingredient	Concentration
TCEP / 51805-45-9	H314 - Causes severe skin burns and eye damage H318 - Causes serious eye damage	0.003%
Mouse IgG / N/A	-	0.006%
Empigen BB / 66455-29-6	-	0.058%
EDTA, Tetrasodium Salt / 10378-23-1	H302 - Harmful if swallowed H318 - Causes serious eye damage	0.639%
Tris Base / 77-86-1	H315 - Causes skin irritation H319 - Causes serious eye irritation H335 - May cause respiratory irritation	0.048%
Tris HCl / 1185-53-1	-	0.081%
Azide / 26628-22-8	H300 - Fatal if swallowed H310 – Fatal in contact with skin H400 – Very toxic to aquatic life H410 – Very toxic to aquatic life with long lasting effects	0.004%

- For more information on EUAs please visit: https://www.fda.gov/emergencypreparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergencyuse-authorization
- For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID19

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 2 and the Test Cassette: Sofia 2 Calibration Check procedure, built-in procedural control features, and External Controls.

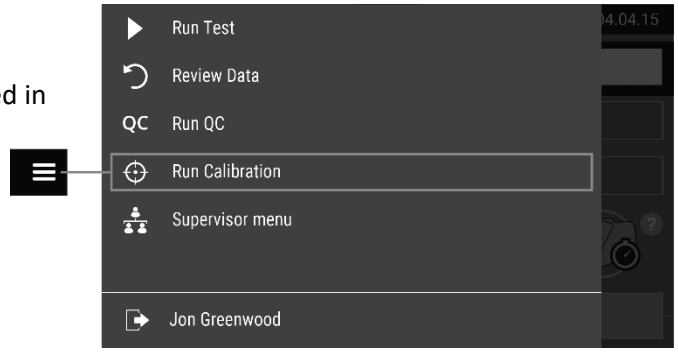
Sofia 2 Calibration Check Procedure

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.

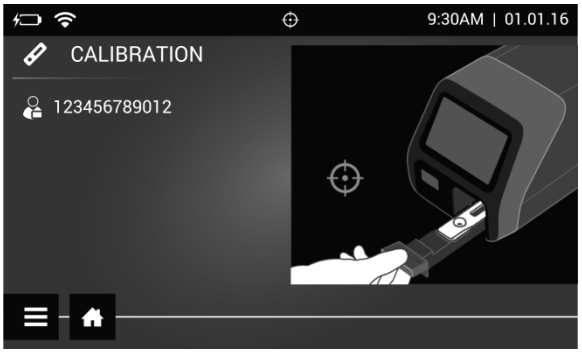
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for

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.

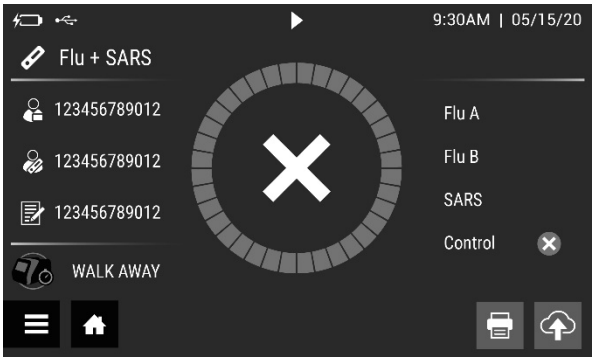
NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer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

Built-in Procedural Controls

The Sofia 2 Flu + SARS Antigen FIA contains a built-in procedural control feature. Each time a test is run in Sofia 2, the procedural control zone is scanned by Sofia 2 and the result is displayed on the 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 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 2 after the Test Cassette has developed for 15 minutes. If the test does not flow correctly, 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 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 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 Flu + SARS Positive Control Swab contains influenza A, influenza B, and SARS 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 ✓ 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.

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 » on the Sofia 2 display to skip the Control test that previously passed. The QC Results will show a skipped Control test as » on Sofia 2.

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.

Prior to collecting the nasal swab, the patient should be instructed to blow their nose. 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 the nasopharyngeal swab provided in the kit or an alternate nylon flocked nasopharyngeal swab.

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.

SAMPLE TRANSPORT AND STORAGE

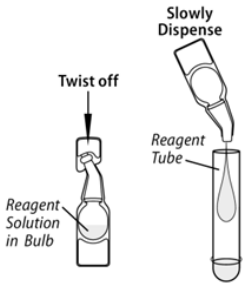
Samples should be tested as soon as possible after collection. Based on data generated with the SARS Antigen FIA, nasal or nasopharyngeal swabs are stable for up to 48-hours at room temperature or 2° to 8°C in a clean, dry transport tube.

TEST PROCEDURE

All clinical samples 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)



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

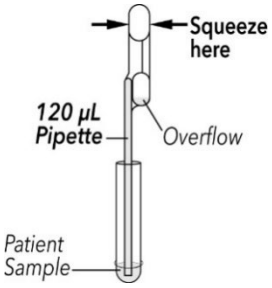
3. Place the patient swab sample into the Reagent Tube. Roll the swab at least 3 times while pressing the head against the bottom and side of the Reagent Tube.

   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.

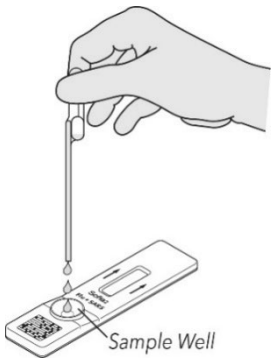
   To fill the Fixed Volume Pipette with the patient sample:
   - **a)** FIRMLY squeeze the top bulb.
   - **b)** Still squeezing, place the Pipette tip into the patient sample.
   - **c)** With the Pipette tip still in the patient sample, slowly release pressure on bulb to fill the Pipette.



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.

   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 2," to complete the test.

USING SOFIA 2

WALK AWAY/READ NOW Modes

Refer to the Sofia 2 User Manual for operating instructions.
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 2. Sofia 2 scans the Test Cassette periodically during the test development time. Positive and negative test results will be displayed in 15 minutes.

READ NOW Mode

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

The user must first place the Test Cassette onto the counter or bench top for 15 minutes (outside of Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia 2. In READ NOW Mode, Sofia 2 will scan and display the test result within 1 minute.

Warning: Results must not be interpreted past 30 minutes after inoculation. Using the Sofia 2 past this time may result in false results.

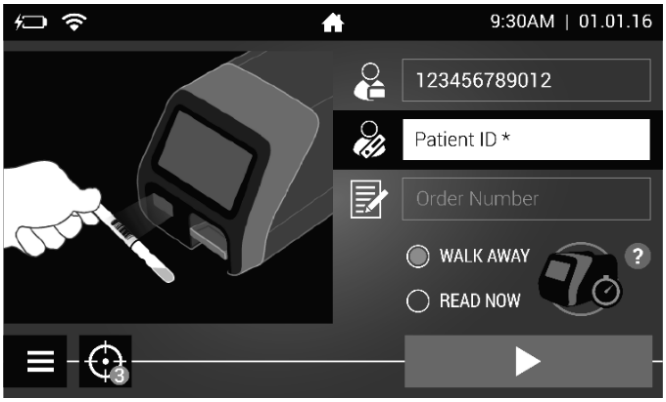
Critically important: The user should never open the foil pouch exposing the Test Cassette to ambient environment until ready for immediate use.

RUN TEST WITH SOFIA 2

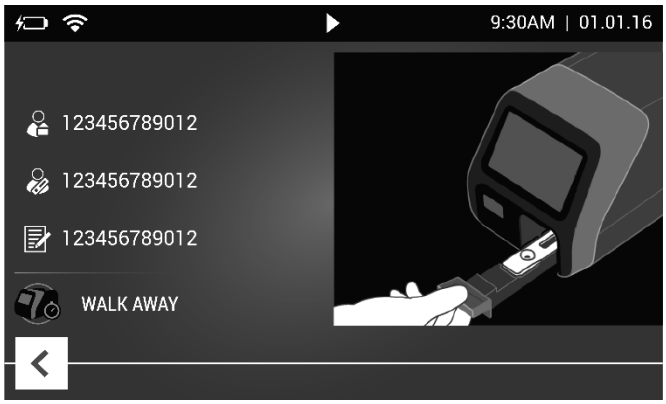
1. Input the User ID using the integrated barcode scanner or manually enter the data using the on-screen key pad.

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.*

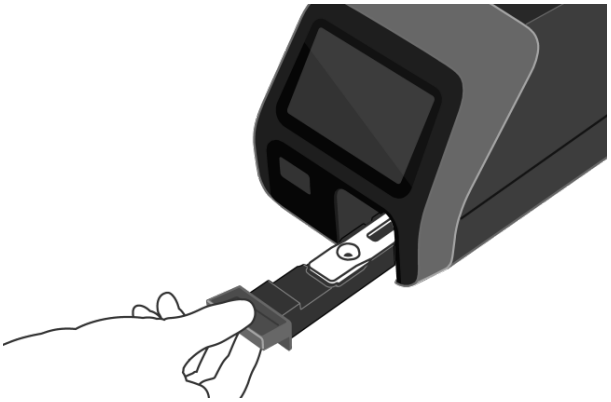
2. Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the on-screen key pad.



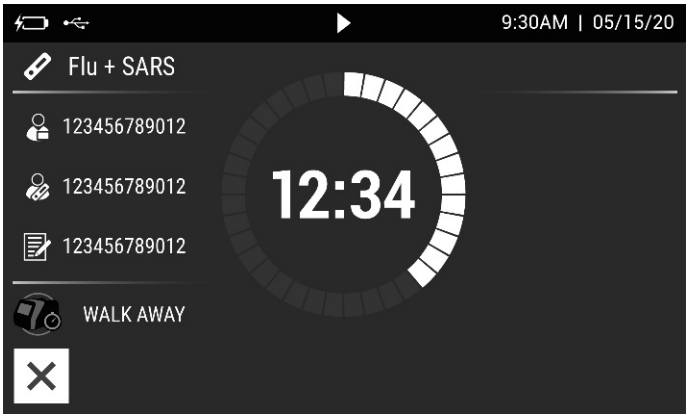
3. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



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 in 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.



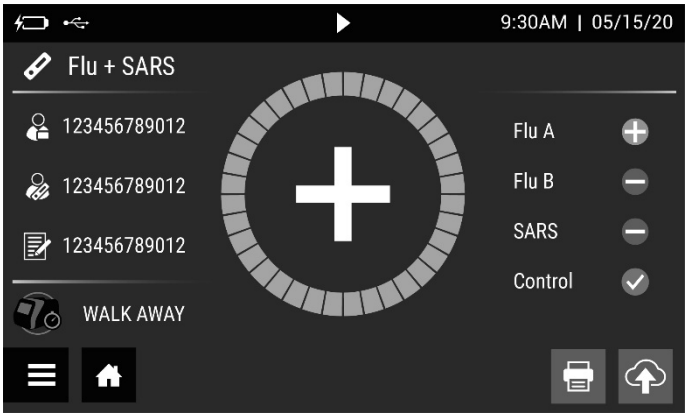
For example: This display shows that the test in WALK AWAY Mode has 12 minutes, 34 seconds remaining. Sofia 2 will read and display the results in 15 minutes.

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.

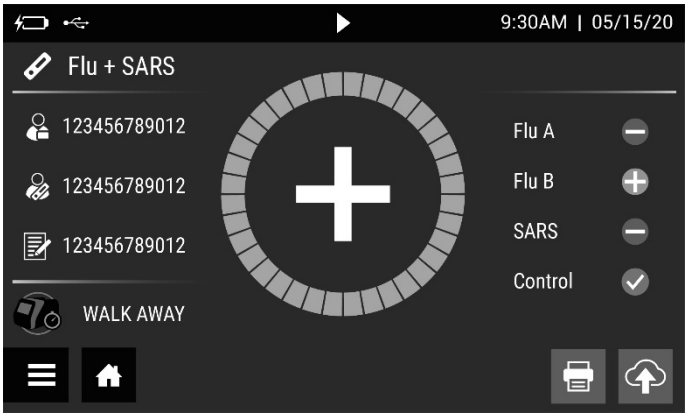
The Sofia 2 screen will display results for the procedural control as being ✓ or ✗, and will individually provide a ⊕ or ⊖ result for influenza A, influenza B, and SARS. If the procedural control is ✗ retest with a new patient sample and a new Test Cassette. If a printer is connected, the results can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

Positive Results:



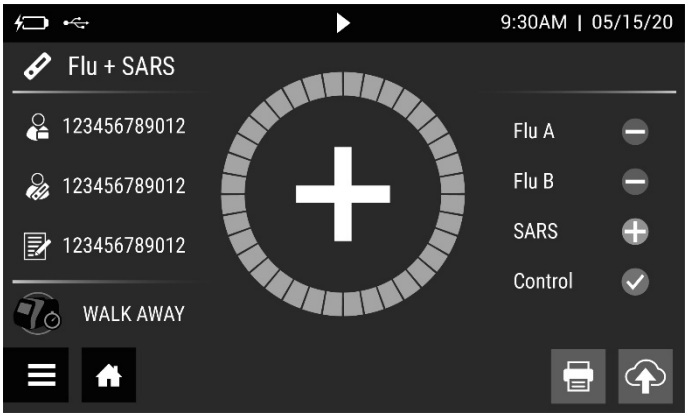
For example: This display shows a valid <u>positive result for influenza A</u>.

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



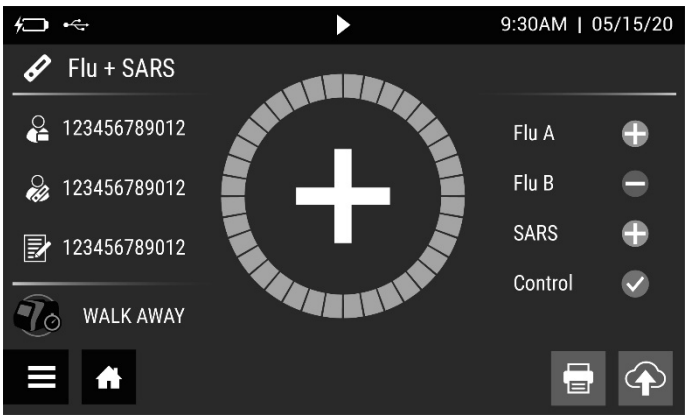
For example: This display shows a valid <u>positive result for influenza B</u>.

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



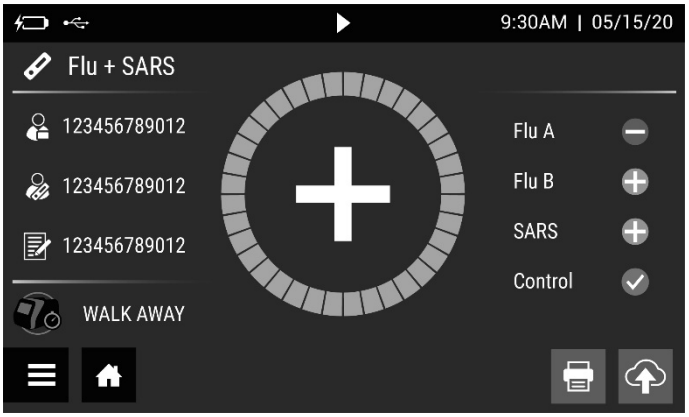
For example: This display shows a valid <u>positive result for SARS</u>.

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 A and SARS</u>.

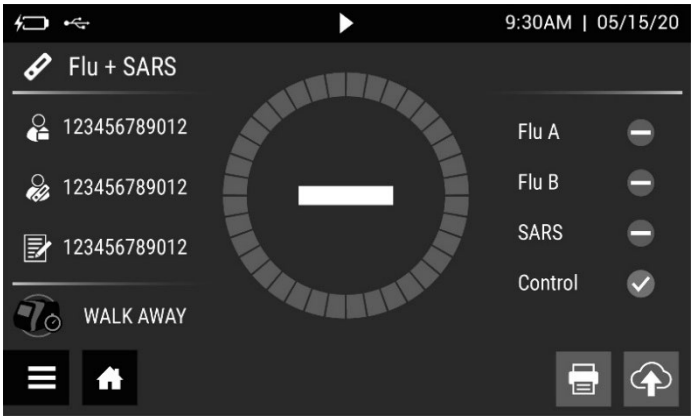
NOTE: A positive result does not rule out co-infections with other pathogens.



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

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

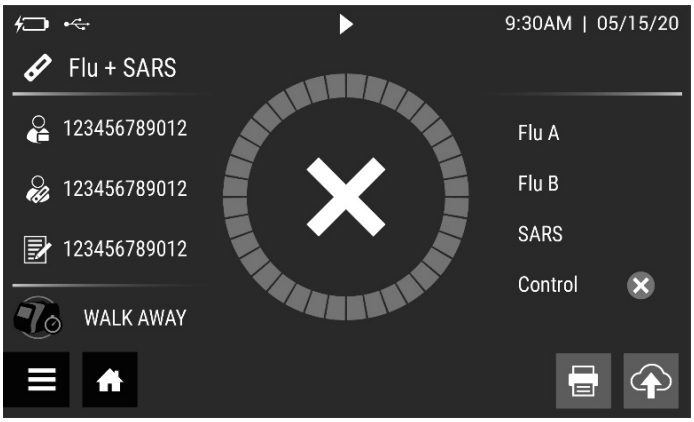
Negative Results:



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

NOTE: A negative result does not exclude infection.

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.

COVID-19 Test Interpretation

Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results. Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

Status on First Day of Testing	Day 0 (Test 1)	Day 2 (Test 2)
With Symptoms	COVID-19 (-) Serial testing recommended for COVID Flu A or B (-) Flu A or B result is Negative	COVID-19 (-) COVID result is Negative COVID-19 (+) COVID-19 result is Positive Flu A or B (-) Flu result is Negative Flu A or B (+) Flu result is Positive
	COVID-19 (-) Serial testing recommended for COVID Flu (+) Flu A or B result is Positive	COVID-19 (-) COVID-19 result is Negative COVID-19 (+) COVID-19 result is Positive Flu A or B (-) **Maintain Flu Positive interpretation** Flu A or B (+) Flu A or B result is Positive
	COVID-19 (+) COVID Positive Flu A or B (-) Flu A or B Negative	No serial testing recommended
	COVID-19 (+) COVID Positive Flu A or B (+) Flu A or B Positive	No serial testing recommended

COVID-19 Positive (+)

Repeat testing does not need to be performed if patients have a positive SARS result at any time.

A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self- isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive).

Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Individuals who test positive with the [Test Name] should self-isolate and seek follow up care with their physician or healthcare provider as additional confirmatory testing with a

molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

COVID-19 Negative (-)

To increase the chance that the negative result for COVID-19 is accurate, you should:

- **Test again in 48 hours if the individual has symptoms on the first day of testing.**
- **Test 2 more times at least 48 hours apart if the individual does not have symptoms on the first day of testing.**

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow up testing for SARS-CoV-2 with a molecular test or testing for other respiratory disease should be considered. If applicable, seek follow up care with the primary health care provider.

All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

LIMITATIONS

- The contents of this kit are to be used for the qualitative detection of influenza A, influenza B, and SARS antigens directly from nasal swab and nasopharyngeal swab.
- Viral Transport Media (VTM) should not be used with this test as it may cause false results.
- This test detects both viable (live) and non-viable, influenza A, influenza B, SARS-CoV, and SARS-CoV-2. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- Positive test results do not identify specific influenza A virus subtypes.
- Negative test results are not intended to rule in other non-influenza or SARS viral or bacterial infections.
- Negative results, from patients with COVID-19 symptom onset beyond five days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.
- Negative influenza A or influenza B results should be treated as presumptive and confirmed with an FDA authorized molecular assay, if necessary, for clinical management, including infection control.
- Children tend to shed influenza 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 SARS or influenza A subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- The performance of this test was established based on the evaluation of a limited number of SARS clinical specimens collected between June through August 2020. The clinical performance has not been established for all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARSCoV-2 and/or influenza and their prevalence, which change over time.
- There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 and/or influenza as compared to a molecular test, especially in samples with low viral load.
- All antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- If the patient continues to have symptoms of COVID-19 and/or influenza, and both the patient's first and second tests are negative, the patient may not have COVID-19 and/or influenza, however additional follow-up may be needed.
- If the test is positive, then proteins from the virus that causes COVID-19 and/or influenza have been found in the sample and the individual likely has COVID-19 or influenza.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- This test detects both viable (live) and nonviable SARS-CoV-2 and influenza A/B. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY AND PATIENT CARE SETTINGS

The Sofia 2 Flu + SARS Antigen FIA Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist in using the Sofia 2 Flu + SARS Antigen FIA ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using your product will use your product as outlined in the "Sofia 2 Flu + SARS Antigen FIA" Instructions for Use and Quick Reference Instructions. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quidel (via email:

QDL.COV2.test.event.report@quidel.com, or via phone by contacting Quidel Customer Support Services at 800.874.1517 (in the U.S.) or 858.552.1100) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

- All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- Quidel Corporation, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high, moderate, or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation." as "authorized laboratories."

CLINICAL PERFORMANCE

The Sofia 2 Flu + SARS Antigen FIA is a lateral flow fluorescent immunoassay (FIA). It is a modification of the test cassette used in the FDA-cleared assay (Sofia Influenza A+B FIA (k112177, k131606, k153012, k162438)) to include monoclonal antibodies for the detection of SARS-CoV-2. Data for the detection of influenza A + B by the Sofia Influenza A+B FIA is presented below.

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.

Sofia Influenza A+B FIA Nasal Swab Results Versus Culture (All Age Groups) – Influenza A								
		Viral Culture					95% CI	
		POS	NEG	Total	Sensitivity	90%	84%	94%
Sofia Influenza A+B FIA	POS	124	27	151	Specificity	95%	93%	96%
	NEG	14	500	514				
	Total	138	527	665				

Sofia Influenza A+B FIA Nasal Swab Results Versus Culture (All Age Groups) – Influenza B								
		Viral Culture					95% CI	
		POS	NEG	Total	Sensitivity	89%	82%	94%
Sofia Influenza A+B FIA	POS	100	23	123	Specificity	96%	94%	97%
	NEG	12	530	542				
	Total	112	553	665				

Sofia Influenza A+B FIA Nasopharyngeal Swab Results Versus Culture (All Age Groups) – Influenza A								
		Viral Culture					95% CI	
		POS	NEG	Total	Sensitivity	97.1%	91.8%	99.0%
Sofia Influenza A+B FIA	POS	100	34	134	Specificity	94.6%	92.6%	96.1%
	NEG	3	596	599				
	Total	103	630	733				

Sofia Influenza A+B FIA Nasopharyngeal Swab Results Versus Culture (All Age Groups) – Influenza B								
		Viral Culture					95% CI	
		POS	NEG	Total	Sensitivity	90%	83%	94%
Sofia Influenza A+B FIA	POS	101	19	120	Specificity	97%	95%	98 %
	NEG	11	602	613				
	Total	112	621	733				

Retrospective Comparison of the Sofia Influenza A+B FIA and Sofia 2 Flu + SARS Antigen FIA
To demonstrate the addition of the SARS-CoV-2 to the Sofia Influenza A+B FIA had no impact to the detection of influenza A or influenza B a study was performed using remnant clinical samples (72 influenza A positive, 15 influenza B positive, 56 negative). The specimens were tested with a FDA-cleared molecular device (Solana Influenza Assay, k161814) to confirm the presence or absence of influenza A or influenza B.

The samples were tested according to the respective Package Inserts for both devices.

Influenza A Performance

Influenza A results for both the Sofia 2 Flu + SARS and Sofia Influenza A+B assays were combined in the following Table:

Influenza A Performance								
		Sofia Influenza A+B					95% CI	
		POS	NEG	Total	PPA	100.0%	94.8%	100.0%
Sofia 2 Flu + SARS Antigen FIA	POS	70	2*	72	NPA	96.6%	88.3%	99.0%
	NEG	0	56	56				
	Total	70	58	128				

*2 Discrepant samples were confirmed Positive for Influenza A on Solana

Influenza A results for both the Sofia 2 Flu + SARS and Solana Influenza A+B assays were combined in the following Table:

Influenza A Performance								
		Solana Influenza Assay					95% CI	
		POS	NEG	Total	PPA	100.0%	94.9%	100.0%
Sofia 2 Flu + SARS Antigen FIA	POS	72	0	72	NPA	100.0%	93.6%	100%
	NEG	0	56	56				
	Total	72	56	128				

Influenza B Performance

Influenza B results for both the Sofia 2 Flu + SARS and Sofia Influenza A+B assays were combined in the following Table:

Influenza B Performance								
		Sofia Influenza A+B					95% CI	
		POS	NEG	Total	PPA	100.0%	78.5%	100.0%
Sofia 2 Flu + SARS Antigen FIA	POS	14	1*	15	NPA	98.2%	90.7%	99.7%
	NEG	0	56	56				
	Total	14	57	71				

* Discrepant sample was confirmed Positive for Influenza B on Solana

Influenza B results for both the Sofia 2 Flu + SARS and Solana Influenza A+B assays were combined in the following Table:

Influenza B Performance								
		Solana Influenza A+B					95% CI	
		POS	NEG	Total	PPA	100.0%	79.6%	100.0%
Sofia 2 Flu + SARS Antigen FIA	POS	15	0	15	NPA	100.0%	93.6%	100.0%
	NEG	0	56	56				
	Total	15	57	71				

Prospective Study of the Sofia 2 Flu + SARS Antigen FIA

A study of one hundred sixty-five (165) direct nasal swabs was performed. The samples were enrolled from symptomatic patients suspected of COVID-19 at six (6) locations and tested fresh with the Sofia assay at either a single central laboratory (113-specimens) or at the collection site (52-specimens). All patients had a matched nasal swab collected for RT-PCR at the central location. The order of swab collection was randomized between assays. The Sofia 2 Flu + SARS Antigen FIA was compared to the Reference Extracted RT-PCR assay.

Patient Demographics

Patient demographics (age, elapsed time from date of on-set) are available for the one hundred sixty-five (165) samples used in the study. Demographics are shown in the table below.

Patient Demographics for Nasal Swabs (Sofia Positive = 40)			
	Sofia SARS Antigen FIA		
Age	Total #	Total Positive	Prevalence
≤ 5 years	0	0	N/A
6 to 21 years	15	4	26.7%
22 to 59 years	123	33	26.8%
≥ 60 years*	26	3	11.5%

* One specimen was Invalid in the Sofia Flu + SARS Antigen Assay and removed from further analysis

The specimen positivity breakdown based on days post onset:

Days Post Symptom Onset for Nasal Swabs (Sofia Positive = 33)			
Days Post Symptom Onset	# Specimens Tested	# Positive Specimens	% Positive
0	1	0	0
1	32	14	43.8%
2*	39	10	25.6%
3***	36	4	11.1%
4	32	10	31.3%
5**	25	2	8.0%

* One specimen was Sofia 2 Flu + SARS Antigen FIA Negative and Positive by Reference Extracted RT-PCR

** One specimen was Sofia 2 Flu + SARS Antigen FIA Negative and Positive by Reference Extracted RT-PCR

*** One specimen was Invalid in the Sofia Flu + SARS Antigen Assay and removed from further analysis

Sofia 2 Flu + SARS Antigen FIA Performance Compared to Reference Extracted RT-PCR Assays for Influenza A and Influenza B and for SARS-CoV-2								
	Reference Extracted Influenza A + B RT-PCR assay – Influenza A						95% CI	
Influenza A		POS	NEG	Total	PPA	N/A	N/A	N/A
Sofia 2 Flu + SARS Antigen FIA Assay	POS	0	0	0	NPA	100.0%	97.7%	100.0%
	NEG	0	164	164	PPV	N/A	N/A	N/A
	Total	0	164	164**	NPV	100.0%	97.7%	100.0%
					Prevalence	0.0%	0.0%	3.0%

	Reference Extracted Influenza A + B RT-PCR assay – Influenza B						95% CI	
Influenza B		POS	NEG	Total	PPA	N/A	N/A	N/A
Sofia 2 Flu + SARS Antigen FIA Assay	POS	0	0	0	NPA	100.0%	97.7%	100.0%
	NEG	0	164	164	PPV	N/A	N/A	N/A
	Total	0	164	164**	NPV	100.0%	97.7%	100.0%
					Prevalence	0.0%	0.0%	3.0%
	Reference Extracted SARS-CoV-2 RT-PCR assay						95% CI	
SARS-CoV-2		POS	NEG	Total	PPA	95.2%	84.2%	98.7%
Sofia 2 Flu + SARS Antigen FIA Assay	POS	40	0	40	NPA	100.0%	96.9%	100.0%
	NEG	2*	122	124	PPV	100.0%	91.2%	100.0%
	Total	42	122	164**	NPV	98.4%	94.3%	99.6%
					Prevalence	25.6%	19.5%	32.8%

***** The two discordant samples (Sofia 2 Negative/Reference Extracted RT-PCR assay Positive) had Ct Values of 31.95 and 38.72.

****** One specimen was Invalid in the Sofia Flu + SARS Antigen Assay and removed from further analysis.

COVID-19 Serial Screening

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA authorized SARSCoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result is considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If results of the first two molecular test were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RTPCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection.

Performance of the antigen test with serial testing in individuals is described in the table below.
Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single day testing throughout the course of infection with serial testing. Data is from all antigen tests in study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	SYMPTOMATIC ON FIRST DAY OF TESTING Ag Positive / PCR Positive (Antigen Test Performance % PPA)		
	1 Test	2 Tests	3 Tests
0	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	4/9 (44.4%)	3/7 (42.9%)	--

1 Test = one (1) test performed on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.
2 Tests = two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.
3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

NIH/RADx Omicron Testing

The performance of the Sofia 2 SARS Antigen FIA (which includes the same chemistry and antibodies for the detection of SARS-CoV-2 as the Sofia 2 Flu + SARS Antigen FIA) in the detection of the Omicron variant of SARS-CoV-2 was evaluated in a dilution series of clinical specimens which were positive for the Omicron variant. This testing was conducted by the National Institutes of Health (NIH) as a component of the Rapid Acceleration of Diagnostics (RADx®) initiative. Specimen pools were prepared by the RADx® team using clinical pooled samples from currently circulating Omicron strains and tested by RADx® to assess performance with the Omicron variant. Results from this dilution series cannot be compared to other specimen pools and do not indicate that a test will have different clinical performance compared to other EUA authorized tests. Compared to an EUA authorized RT-PCR method, the Sofia 2 SARS Antigen FIA detected 100% of live virus Omicron samples at a Ct-value of 24 (n=25, 5 lots tested at 5 replicates each). Testing was also compared to two additional EUA-authorized OTC antigen tests (Assay #1 and Assay #2). Omicron dilutions at lower viral concentrations (Ct-values greater than 24) were not detected by the Sofia 2 SARS Antigen FIA in this study.

Omicron Pool 1 - Live	Average N2 Ct (n=9)	Assay #1 Percent Positive (n=5)	Assay #2 Percent Positive (n=5)	Quidel Sofia 2 SARS Antigen FIA Percent Positive (n=25)
Dilution 1	20.6	100	100	100
Dilution 2	21.5	100	100	100
Dilution 3	22.7	100	100	100
Dilution 4	24.0	100	100	100
Dilution 5	25.3	100	100	64
Dilution 6	26.0	100	100	0
Dilution 7	27.3	0	60	0
Dilution 8	28.8	0	0	0
Dilution 9	29.2	0	0	0
Dilution 10	30.6	0	0	0
Dilution 11	31.7	0	0	0
Dilution 11	32.6	0	0	0

ANALYTICAL PERFORMANCE

Limit of Detection

The LoD for the Sofia 2 Flu + SARS Antigen FIA was determined using limiting dilutions of the following virus strains of influenza A, influenza B, and SARS-CoV-2 in negative nasal matrix in UTM:

LoD Virus Strains		
Influenza A H3N2 Hong Kong/8/68	Zeptometrix 0810250CF	4.57 x 10e6 TCID50/mL
Influenza B Florida/05/06	Zeptometrix 0810037CF	4.17 x 10e5 TCID50/mL
SARS-CoV-2 USA-WA1/2020	Zeptometrix 0810587CHFI	4.17 x 10e5 TCID50/mL

The study to determine the Sofia 2 Flu + SARS Antigen FIA LoD was designed to reflect the assay when using direct swabs. In this study nasal swabs were spiked with approximately 50-µL of the virus dilution in saline. The spiked swab was added to the Sofia 2 Flu + SARS Antigen FIA extractant concurrently to a nasal swab containing NP matrix. The swabs were processed concurrently according to the Package Insert.

The table below provides the LoD of the Sofia 2 Flu + SARS Antigen FIA for influenza A, influenza B, and SARS-CoV-2.

Virus	Concentration ($TCID_{50}$/mL)	N	Negative	Positive	% Positive	LL 95% CI	UL 95% CI
Influenza A H3N2 Hong Kong/8/68	**50**	20	0	20	100%	83.9%	100%
Influenza B Florida/05/06	**1.8**	20	0	20	100%	83.9%	100%
SARS-CoV-2 USA-WA1/2020	**91.7**	20	1	19	95.0%	74.6%	99.1%

Based on 50-µL volume spiked on the swab, the $TCID_{50}$/mL equates to the following $TCID_{50}$/swab for influenza A, influenza B, and SARS-CoV-2.

Virus	Concentration ($TCID_{50}$/mL)	Concentration ($TCID_{50}$/swab)
Influenza A H3N2 Hong Kong/8/68	**50**	**2.5**
Influenza B Florida/05/06	**1.8**	**0.09**
SARS CoV-2 USA-WA1/2020	**91.7**	**4.59**

The 2020 CDC Human Influenza Panel was tested concurrently with the Sofia Influenza A + B FIA and Sofia 2 Flu + SARS FIA assays. The panel was tested per the **swab** protocol recommended by the CDC. Briefly, a series of 5-fild dilutions were prepared with each panel member. These dilutions were tested with five replicates until two consecutive dilution were negative. Test results generated for each influenza strain are listed below:

Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{9.3}$ EID50/mL	**2 x $10^{8.3}$**	**4 x $10^{7.3}$**	**8 x $10^{6.3}$**	**1.6 x $10^{6.3}$**	**3.2 x $10^{5.3}$**	**6.4 x $10^{4.3}$**	**1.28 x $10^{4.3}$**	**2.56 x $10^{3.3}$**
A(H3N2)	A/Perth/16/2009	Sofia Influenza A+B FIA	# Detected	5	5	5	5	5	1	0	NA
			% Detection	100%	100%	100%	100%	100%	20%	0%	NA
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	5	5	5	0
			% Detection	100%	100%	100%	100%	100%	100%	100%	0%
Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{7.5}$ EID50/mL	**2 x $10^{6.5}$**	**4 x $10^{5.5}$**	**8 x $10^{4.5}$**	**1.6 x $10^{4.5}$**	**3.2 x $10^{3.5}$**			
A(H3N2)	A/Hong Kong/2671/2019	Sofia Influenza A+B FIA	# Detected	5	5	2	0	0			
			% Detection	100%	100%	40%	0%	0%			
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	0	0			
			% Detection	100%	100%	100%	0%	0%			
Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{10.2}$ EID50/mL	**2 x $10^{9.2}$**	**4 x $10^{8.2}$**	**8 x $10^{7.2}$**	**1.6 x $10^{7.2}$**	**3.2 x $10^{6.2}$**	**6.4 x $10^{5.2}$**	**1.28 x $10^{4.2}$**	
A9H1N1pdm09	A/Christ Church/16/2010	Sofia Influenza A+B FIA	# Detected	5	5	5	5	5	0	0	
			% Detection	100%	100%	100%	100%	100%	0%	0%	
			# Detected	5	5	5	5	5	0	0	

Influenza Virus (Type/Subtype)	Virus Strain Name		Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)								
		Sofia 2 Flu + SARS Antigen FIA	% Detection	100%	100%	100%	100%	100%	0%	0%	
Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{9.1}$ EID50/mL	**2 x $10^{8.1}$**	**4 x $10^{7.1}$**	**8 x $10^{6.1}$**	**1.6 x $10^{6.1}$**	**3.2 x $10^{5.1}$**	**6.4 x $10^{4.1}$**		
A9H1N1pdm09	A/GuangDong-Maonan/1536/2019	Sofia Influenza A+B FIA	# Detected	5	5	5	5	0	0		
			% Detection	100%	100%	100%	100%	0%	0%		
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	0	0		
			% Detection	100%	100%	100%	100%	0%	0%		
			$10^{6.9}$ EID50/mL	**2 x $10^{5.9}$**	**4 x $10^{4.9}$**	**8 x $10^{3.9}$**	**1.6 x $10^{3.9}$**	**3.2 x $10^{2.9}$**	**6.4 x $10^{1.9}$**		
B (Victoria Lineage)	B/Michigan/09/2011	Sofia Influenza A+B FIA	# Detected	5	5	5	5	0	0		
			% Detection	100%	100%	100%	100%	0%	0%		
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	0	0		
			% Detection	100%	100%	100%	100%	0%	0%		
Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{8.3}$ EID50/mL	**2 x $10^{7.3}$**	**4 x $10^{6.3}$**	**8 x $10^{5.3}$**	**1.6 x $10^{5.3}$**	**3.2 x $10^{4.3}$**	**6.4 x $10^{3.3}$**	**1.28 x $10^{3.3}$**	**2.56 x $10^{2.3}$**
B (Yamagata Lineage)	B/Texas/81/2016	Sofia Influenza A+B FIA	# Detected	5	5	5	5	5	5	0	0
			% Detection	100%	100%	100%	100%	100%	100%	0%	0%
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	5	5	0	0
			% Detection	100%	100%	100%	100%	100%	100%	0%	0%
Influenza Virus (Type/Subtype)	**Virus Strain Name**		**Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)**								
			$10^{9.2}$ EID50/mL	**2 x $10^{8.2}$**	**4 x $10^{7.2}$**	**8 x $10^{6.2}$**	**1.6 x $10^{6.2}$**	**3.2 x $10^{5.2}$**	**6.4 x $10^{4.2}$**	**1.28 x $10^{4.2}$**	
B (Victoria Lineage)	B/Washington/02/2019	Sofia Influenza A+B FIA	# Detected	5	5	5	5	5	0	0	
			% Detection	100%	100%	100%	100%	100%	0%	0%	
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	5	0	0%	
			% Detection	100%	100%	100%	100%	100%	0%	0%	

Influenza Virus (Type/Subtype)	Virus Strain Name		Virus Serial Dilution Concentration (EID50/mL) and Number of Positive Results at Each Dilution (N=5)								
			$10^{9.9}$ EID50/mL	**2 x $10^{8.9}$**	**4 x $10^{7.9}$**	**8 x $10^{6.9}$**	**1.6 x $10^{6.9}$**	**3.2 x $10^{5.9}$**	**6.4 x $10^{4.9}$**	**1.28 x $10^{4.9}$**	
B (Yamagata Lineage)	B/Phuket/3073/2013	Sofia Influenza A+B FIA	# Detected	5	5	5	5	0	0	NA	
			% Detection	100%	100%	100%	100%	0%	0%	NA	
		Sofia 2 Flu + SARS Antigen FIA	# Detected	5	5	5	5	3	0	0	
			% Detection	100%	100%	100%	100%	60%	0%	0%	

Analytical Reactivity/Inclusivity

The analytical reactivity of the monoclonal antibodies targeting SARS-CoV-2 in the Sofia 2 Flu + SARS Antigen FIA were evaluated with the currently available SAR-CoV-2 strains (see table below).

2019-nCoV Strain/Isolate	Source/Sample Type	Concentration
USA-WA1/2020	BEI NR-52286	3.40 x10^5 $TCID_{50}$/mL
USA CA3/2020-P2	BEI NR-52385	1x10^7 $TCID_{50}$/mL

The analytical reactivity of the monoclonal antibodies targeting influenza A and influenza B 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. Additional information detailing this testing can be found in Table 11 of the Sofia Influenza A + B FIA Package Insert.

To further demonstrate analytical sensitivity with contemporary influenza strains, the Sofia 2 Flu + SARS Antigen FIA tested the 2020 CDC Human Influenza Panel. The panel was tested per the swab protocol recommended by the CDC. Test results generated for each influenza strain are listed below:

Influenza Virus (Type/Subtype)	Virus Strain Name	Virus Serial Dilution Concentration (EID_{50}/mL) and Number of Positive Results at Highest Detectable Dilution (N=5)
A(H3N2)	A/Perth/16/2009	1.28 x $10^{4.3}$
		5/5
A(H3N2)	A/Hong Kong/2671/2019	8 x $10^{4.5}$
		5/5
A(H1N1)pdm09	A/Christ Church/16/ 2010	3.2 x $10^{6.2}$
		5/5
A(H1N1)pdm09	A/GuangDong-Maonan/1536/2019	1.6 x $10^{6.1}$
		5/5
B (Victoria Lineage)	B/Michigan/09/2011	1.6 x $10^{3.9}$
		5/5
B (Victoria Lineage)	B/Texas/81/2016	6.4 x $10^{3.3}$
		5/5
B (Yamagata Lineage)	B/Washington/02/ 2019	3.2 x $10^{5.2}$
		5/5
B (Yamagata Lineage)	B/Phuket/3073/2013	3.2 x $10^{5.9}$
		3/5

Cross-Reactivity

The cross reactivity of the monoclonal antibodies used for the detection of influenza A and influenza B was determined as part of the Sofia Influenza A+B FIA (K112177) 510k submission. Additional information detailing this testing can be found in Table 13 of the Sofia Influenza A + B FIA Package Insert.

Cross-reactivity of the monoclonal antibodies used for the detection of SARS-CoV-2 was evaluated by testing various microorganisms (9), viruses (16) and negative matrixes (3) that may potentially cross-react with the Sofia 2 SARS FIA. Each organism and virus were tested in triplicate. The final concentration of the organisms and viruses are documented in the table below:

Cross-Reactivity/Interference of SARS-CoV-2					
Virus/Bacteria/Parasite*	**Strain**	**Source/ Sample Type**	**Concentration**	**Cross-Reactivity Results***	**Interference Results***
Adenovirus	Type 1	Isolate	$1 \times 10^{5.53}$ U/mL	No Cross-Reactivity	No Interference
Coronavirus	229e	Isolate	$1 \times 10^{5.10}$ U/mL	No Cross-Reactivity	No Interference
Coronavirus	OC43	Isolate	9.55×10^{5} $TCID_{50}$/mL	No Cross-Reactivity	No Interference
Coronavirus	NL63	Isolate	$5 \times 10^{3.67}$ U/mL	No Cross-Reactivity	No Interference
MERS-CoV (heat-inactivated)	Florida/USA-2_Saudia Arabia_2014	Isolate	1.17×10^{5} $TCID_{50}$/mL	No Cross-Reactivity	No Interference
Mycoplasma pneumoniae	M129	Isolate	3×10^{6} CCU/mL	No Cross-Reactivity	No Interference
Streptococcus pyogenes	Z018	Isolate	3.8×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Influenza A H3N2	Brisbane/10/07	Isolate	$1 \times 10^{5.07}$ U/mL	No Cross-Reactivity	No Interference
Influenza A H1N1	New Caledonia/20/99	Isolate	$1 \times 10^{5.66}$ U/mL	No Cross-Reactivity	No Interference
Influenza B	Brisbane/33/08	Isolate	$1 \times 10^{5.15}$ U/mL	No Cross-Reactivity	No Interference
Parainfluenza	Type 1	Isolate	$1 \times 10^{5.01}$ U/mL	No Cross-Reactivity	No Interference
Parainfluenza	Type 2	Isolate	$1 \times 10^{5.34}$ U/mL	No Cross-Reactivity	No Interference
Parainfluenza	Type 3	Isolate	8.5×10^{5} $TCID_{50}$/mL	No Cross-Reactivity	No Interference
Parainfluenza	Type 4b	Isolate	$1 \times 10^{5.53}$ U/mL	No Cross-Reactivity	No Interference
Enterovirus	Type 68	Isolate	$1 \times 10^{5.5}$ U/mL	No Cross-Reactivity	No Interference
Human Metapneumovirus	A1 (IA10-s003)	Isolate	$1 \times 10^{5.55}$ U/mL	No Cross-Reactivity	No Interference
Respiratory Syncytial Virus	Type A (3/2015 Isolate #3)	Isolate	$1 \times 10^{5.62}$ U/mL	No Cross-Reactivity	No Interference
Human Rhinovirus	N/A	Inactivated virus	Not available	No Cross-Reactivity	No Interference
Chlamydophila pneumoniae	AR-39	Isolate	2.9×10^{6} IFU/mL	No Cross-Reactivity	No Interference
Haemophilus influenzae	Type b; Eagan	Isolate	7.87×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Legionella pneumophila	Philadelphia	Isolate	6.82×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Streptococcus pneumoniae	Z022; 19f	Isolate	2.26×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Bordetella pertussis	A639	Isolate	6.37×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Pneumocystis jirovecii-S. cerevisiae Recombinant	W303-Pji	Isolate	1.56×10^{6} cfu/mL	No Cross-Reactivity	No Interference
Mycobacterium tuberculosis	H37Ra-1	Isolate	6.86×10^{7} cfu/mL	No Cross-Reactivity	No Interference

Cross-Reactivity/Interference of SARS-CoV-2					
Virus/Bacteria/Parasite*	**Strain**	**Source/ Sample Type**	**Concentration**	**Cross-Reactivity Results***	**Interference Results***
Staphylococcus epidermidis	MRSE; RP62A	Isolate	1.21×10^{10} cfu/mL	No Cross-Reactivity	No Interference
Staphylococcus aureus MSSA	NCTC 8325	Isolate	5.5×10^{9} cfu/mL	No Cross-Reactivity	No Interference
Staphylococcus aureus MRSA	0801638	Isolate	1.38×10^{10} cfu/mL	No Cross-Reactivity	No Interference
Coronavirus HKU1 was not tested for cross-reactivity due to lack of availability. 19 specimens containing Coronavirus HKU1 were tested and all resulted as negative, additional cross-reactivity wet testing was not required.					

* Testing was performed in triplicate

Hook Effect

The effects of high concentrations of the different viruses (high dose hook effect) were tested on the Sofia 2 Flu + SARS Antigen FIA. The general procedure was to test contrived samples prepared with virus at the maximum concentration possible.

When testing high concentrations of influenza A, influenza B, or SARS CoV-2 virus levels, the Sofia 2 Flu + SARS Antigen FIA demonstrated 100 % positive results for all tested for each analyte. The concentrations tested represent the maximum available concentrations for the viral strains evaluated. There was no hook evident for this assay.

The results generated in this study support the conclusion that in cases of SARS and Influenza coinfections, when the specimen has a high influenza A viral load, the test will generate 100% positive results for SARS. It also indicates that when the specimen has a high influenza B viral load, the test will generate 100% positive results for SARS.

Endogenous Interference Substances Studies

The potential interference or cross-reactivity of the monoclonal antibodies used for the detection of influenza A and influenza B by endogenous substances was determined as part of the Sofia Influenza A+B FIA (K112177) 510k submission. Additional information detailing this testing can be found in Table 14 of the Sofia Influenza A + B FIA Package Insert.

The potential interference or cross-reactivity of the monoclonal antibodies used for the detection of SARS-CoV-2 by endogenous substances was determined by testing fourteen substances in negative clinical matrix at target concentrations in the absence (negative) and presence (positive) SARS-CoV-2. Each condition (negative or positive) was tested with three replicates per substance.

Positive virus samples were prepared at approximately 4x LoD concentration in clinical negative matrix. Interfering substance samples were prepared at 2 times the final test concentration. Final samples were prepared by mixing 100-µL of the virus sample with 100-µL of the interfering substance sample. The target concentration for each virus was approximately 2 to 3 times the Limit of Detection (LoD).

None of the substances demonstrated interference or cross-reactivity with the SARS-CoV-2 antibodies. All samples prepared in the clinical negative matrix produced the expected negative Sofia 2 SARS result (cross-reactivity results), and all samples prepared at 4x LoD produced the expected positive Sofia 2 SARS result (interference results). The final concentrations of the non-interfering substances are summarized in the table below.

Interfering Substances for SARS-CoV-2				
Interfering Substance	**Active Ingredient**	**Concentration**	**Cross-Reactivity Results***	**Interference Results***
Afrin – nasal spray	Oxymetazoline	5%	No Cross-Reactivity	No Interference
Blood (human)	Blood	5%	No Cross-Reactivity	No Interference
Chloraseptic, Cepacol	Benzocaine, Menthol	0.7 g/mL	No Cross-Reactivity	No Interference
Flonase	Fluticasone	5%	No Cross-Reactivity	No Interference
Halls Relief Cherry Flavor	Menthol	0.8 g/mL	No Cross-Reactivity	No Interference
Nasocort Allergy 24 hour	Triamcinolone	5.00%	No Cross-Reactivity	No Interference
Neo-Synephrine	Phenylephrine hydrochloride	5%	No Cross-Reactivity	No Interference
Oseltamivir	Oseltamivir	2.2 µg/mL	No Cross-Reactivity	No Interference
Purified mucin protein	Mucin protein	2.5 mg/mL	No Cross-Reactivity	No Interference
Rhinocort	Budesonide (Glucocorticoid)	5%	No Cross-Reactivity	No Interference
Saline nasal spray	Saline	15%	No Cross-Reactivity	No Interference
Tobramycin	Tobramycin	1.25 mg/mL	No Cross-Reactivity	No Interference
Zanamivir	Zanamivir	282.0 ng/mL	No Cross-Reactivity	No Interference
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%	No Cross-Reactivity	No Interference

* Testing was performed in triplicate

Competitive Inhibition

For Competitive Interference, SARS-CoV-2 at levels near LoD was tested in the presence of high levels of influenza A or influenza B and near LoD influenza A and influenza B in the presence of high levels of SARS-CoV-2.

Competitive Virus	**Strain**	**Concentration**	**Competitive Target Virus**	**Concentration**	**Competitive Target Percent Positivity**
Influenza A H3N2	Brisbane/10/07	$1 \times 10^{5.07}$ U/mL	SARS-CoV-2	2.26×10^{2} U/mL	100%
Influenza A H1N1	New Caledonia/20/99	$1 \times 10^{5.66}$ U/mL	SARS-CoV-2	2.26×10^{2} U/mL	100%
Influenza B	Brisbane/33/08	$1 \times 10^{5.15}$ U/mL	SARS-CoV-2	2.26×10^{2} U/mL	100%
SARS-CoV-2	USA-WA1/2020	7.55×10^{5} U/mL	Flu A Hong Kong 6/68 H3N2	2.34×10^{1} U/mL	100%
SARS-CoV-2	USA-WA1/2020	7.55×10^{5} U/mL	Flu A Brisbane 10/07 H3N2	1.41×10^{2} U/mL	100%
SARS-CoV-2	USA-WA1/2020	7.55×10^{5} U/mL	Flu B Massachusetts 2/12	5.6×10^{0} U/mL	100%

In this testing there does not appear to be any competitive interference.

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).

INTELLECTUAL PROPERTY

REF 20377 – Sofia 2 Flu + SARS Antigen FIA – 25 Test (Nasal Swab)
20390 – Sofia 2 Flu + SARS Antigen FIA – 25 Test (Nasopharyngeal Swab)

IVD

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1449202EN00 (07/23)

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult instructions for use
IVD For *In Vitro* diagnostic use	 Contains sufficient for 25 determinations
CONT Contents/Contains	CONTROL + Positive control
CONTROL – Negative control	

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