QuidelOrtho™

For use with Sofia 2 only
CLIA Complexity: Moderate
For *in Vitro* diagnostic use

℞ ONLY

Sofia® 2 SARS Antigen+ FIA

A symbols glossary may be found at www.quidel.com/glossary.

Intended Use

The Sofia 2 SARS Antigen+ FIA is a lateral flow immunofluorescent sandwich assay that is used with the Sofia 2 instrument for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 6 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when tested at least twice over three days with at least 48 hours between tests.

The test does not differentiate between SARS-CoV and SARS-CoV-2.

A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient management decisions.

Positive results do not rule out co-infection with other respiratory pathogens.

Performance characteristics for SARS-CoV-2 were established during the 2021-2022 SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary.

This test is intended for prescription use only and can be used in Point-of-Care settings.

Summary and Explanation

SARS-CoV-2, also known as the COVID-19 virus, was first identified in Wuhan, Hubei Province, China December 2019. The WHO declared that COVID-19 was a pandemic on March 11, 2020, and human infection has spread globally, with millions of confirmed infections and hundreds of thousands deaths.[1]

The pooled incubation time is estimated to be approximately 6 days[2] with symptoms expected to be present within 12 days of infection.[3] The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough, and shortness of breath.[4]

Principle of the Test

The Sofia 2 SARS Antigen+ FIA employs immunofluorescence technology in a sandwich design and is used with Sofia 2 instrument to detect nucleocapsid protein from SARS-CoV-2.

An Anterior Nasal Swab from the patient 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 SARS-CoV-2 viral antigens are 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 Sofia2 to be scanned (READ NOW Mode).

Sofia 2 will scan the test strip and measure the fluorescent signal by processing the results using a method-specific algorithm. 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): containing monoclonal anti-SARS antibodies
- Pre-filled Reagent tubes (25): Buffer with detergents, reducing agents, and Proclin 300
- Dropper Tips (25)
- Sterile Nasal Swabs (25)
- SARS Positive Control Swab (1): Swab is coated with non-infectious recombinant SARS antigens
- Negative Control Swab (1): Swab is coated with heat-inactivated, non-infectious Streptococcus C antigen
- QC Card (located on the kit box)

Materials Not Supplied in Kit

- Timer or watch
- Sofia 2
- Calibration Cassette

Warnings and Precautions

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- 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.
- Do not use on anyone under 2 years of age.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Do not use if any of the test kit contents or packaging is damaged.
- 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, Reagent Tubes, Dropper Tip, 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 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.
- Do not open the Test Cassette until you are ready to perform the test. Once opened, the Test Cassette should be used within 60 minutes.
- Sample collection and handling procedures require specific training and guidance.
- The test is intended to be used with direct anterior nasal swabs and is not validated for use with swabs in viral transport media.
- When collecting a nasal swab sample, use the Nasal Swab supplied in the kit.
- Do not touch the swab tip.
- 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.
- 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.
- 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.
- 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.
- The control swabs are not for patient specimen collection. Risk of false results. Use only as a test quality control sample.
- Dispose of containers and unused contents in accordance with Federal, State, and Local regulatory requirements.
- For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID.

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.

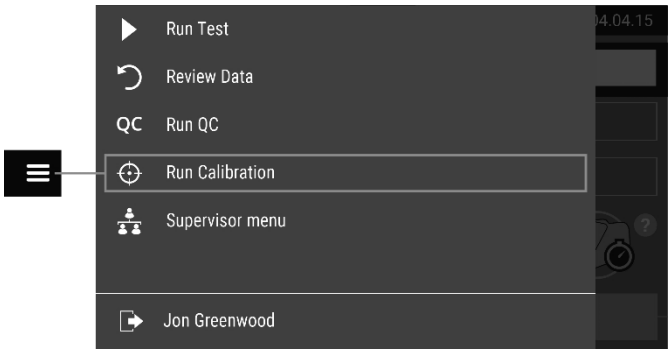
Sofia 2 Calibration Check Procedure

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.

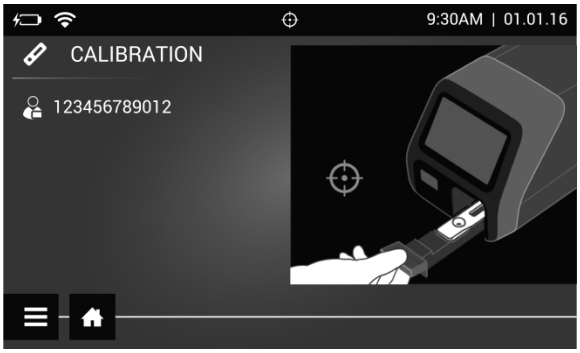
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

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

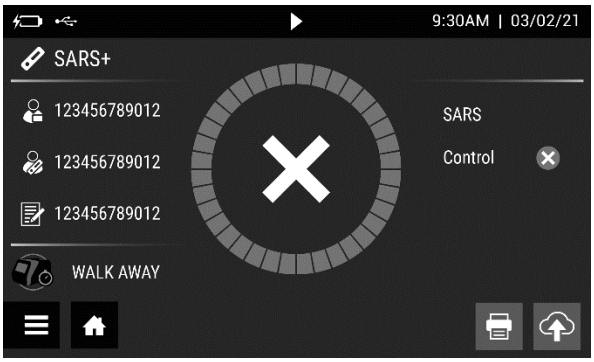
Built-in Procedural Controls

The Sofia 2 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 ✓ 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 10 minutes. If the test does not flow correctly, Sofia 2 will indicate that the result is ⊗.** 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.

QuidelOrtho 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.

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 SARS Positive Control Swab contains SARS antigen. **The Positive Control Swab must be run first, followed by the Negative Control Swab.**

The user must first select Run QC on the Main Menu of Sofia 2 and then, when prompted, scan the QC Card (located on the kit box). This card provides information specific to the kit lot, including the lot number and expiration date.

The user will select the desired mode (WALK AWAY or READ NOW) then run the External Control swabs.

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 proceed with testing if either the Positive Control and/or Negative Control fail the QC check** (result displayed as ⊗).

Do not perform patient tests or report patient test results if either of the QC test results fail. Repeat the test or contact QuidelOrtho 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. For repeat testing of either the Positive or Negative Controls, it may be necessary to obtain additional External Control Swabs.

Additional External Control swabs (Catalog # 20482) may be purchased separately as needed.

Sample Collection

Nasal Swab Specimen

Use the nasal swab supplied in the kit.

Prior to collecting the nasal swab, the patient should be instructed to blow their nose. The use of nitrile, latex (or equivalent) gloves, suitable protective clothing, and eye/face protection is recommended when handling patient samples. 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.

Sample Transport and Storage

Samples should be tested as soon as possible after collection. Based on data generated with the Sofia 2 SARS Antigen+ FIA, nasal swabs are stable for up to 12 hours at room temperature, 24 hours at 2° to 8°C or 4 days when frozen (≤-20°C).

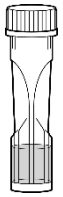
Test Procedure

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

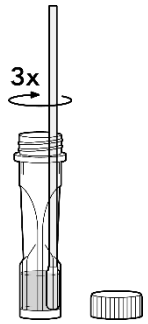
Expiration date: Check the expiration date on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

Swab Test Procedure

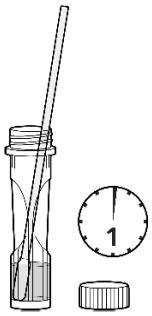
1. Verify that Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia 2" section for more information.



2. Open and remove the cap of the Reagent tube. Insert the swab into the Reagent tube. **Roll the swab at least 3 times** while pressing the head against the bottom and side of the Reagent tube.

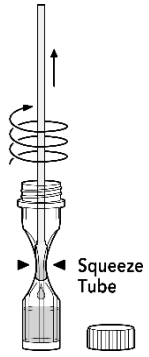


Leave the swab in the Reagent tube for 1 minute.

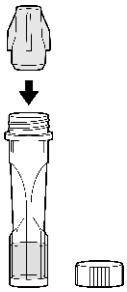


3. Express as much liquid as possible from the swab by squeezing the sides of the Tube as the swab is withdrawn in a complete twisting motion.

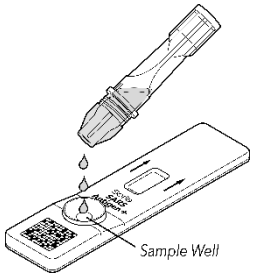
   Discard the Swab in accordance with your biohazard waster disposal protocol.



4. Press the dropper tip firmly into the Reagent Tube to seal the sample.



5. Add three (3) drops into the Test Cassette sample well.



6. 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 10 minutes.

READ NOW Mode

Critically important: Using a timer, allow the test to develop for the FULL 10 minutes BEFORE placing it into Sofia 2.

The user must first place the Test Cassette onto the counter or bench top for 10 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 20 minutes after inoculation. Using the Sofia 2 past this time may result in false results.

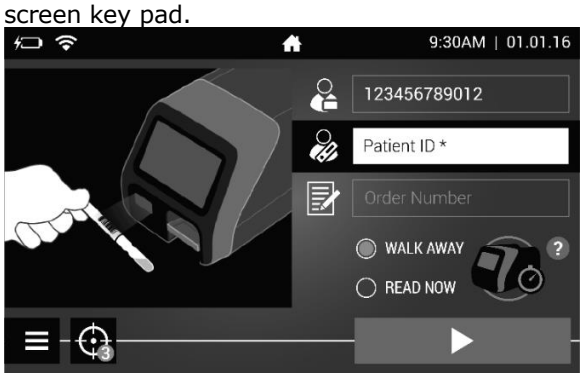
Critically important: The user should never open the foil pouch exposing the Test Cassette to ambient environment until ready for immediate use.

Run Test With Sofia 2

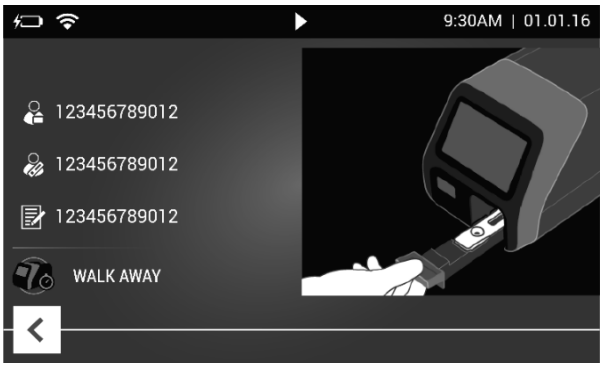
1. Input the User ID using the integrated barcode scanner or manually enter the data using the on-screen key pad.

   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.*

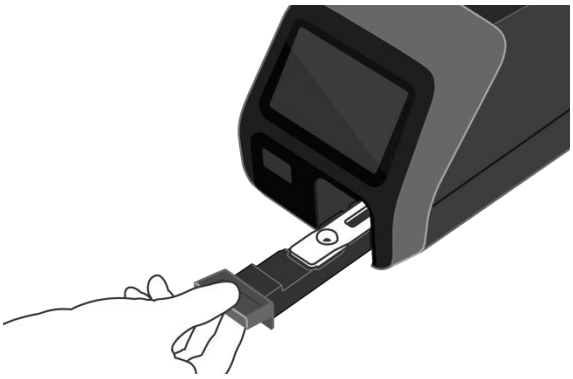
2. Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the on-screen key pad.



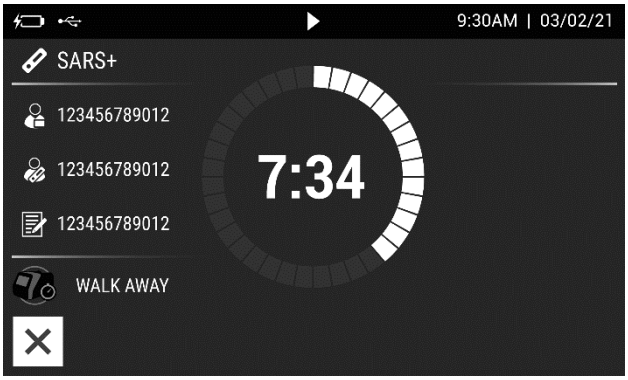
3. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



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 10 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 7 minutes, 34 seconds remaining.

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.

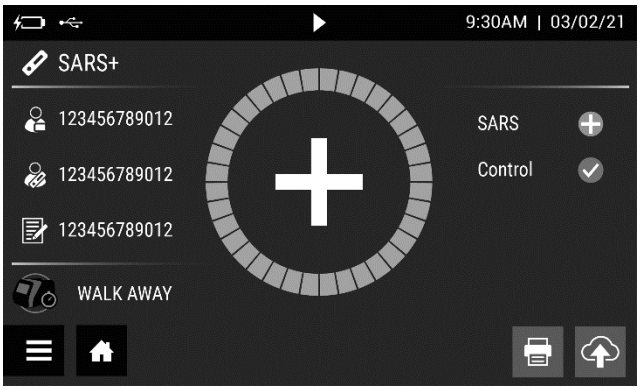
The Sofia 2 screen will display results for the procedural control as being ✓ or ✗, and will individually provide a ⊕ or ⊖ result for SARS. If the procedural control is ✗ retest with a new patient sample and a new Test Cassette. If a printer is connected, the results can be printed manually by selecting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

Repeat testing is needed for negative results 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.

Test results should be reported in accordance with local, state, and federal regulations.

Status on first day of Testing	First Result Day 1	Second Result Day 3	Interpretation
With Symptoms	Positive	N/A	Positive for COVID-19
	Negative	Positive	Positive for COVID-19
	Negative	Negative	Negative for COVID-19

Positive Results:



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

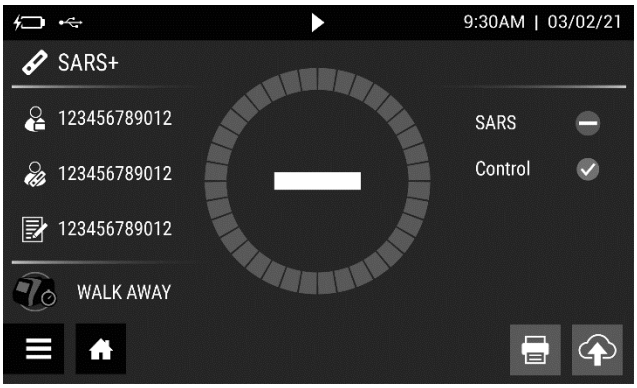
NOTE: A positive result does not rule out co-infections with other pathogens.

Repeat testing does not need to be performed if patients have a positive 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 Sofia 2 SARS Antigen+ FIA 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.

Negative Results:



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

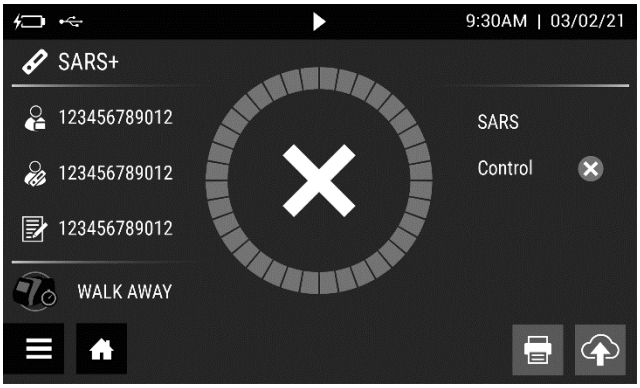
NOTE: Negative results, from patients with symptom onset beyond five days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.

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.

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. A negative test result does not preclude the possibility of infection with other bacteria or viruses. 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 are 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.

Invalid Results:



For example: This display shows an invalid result.

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

Limitations

- 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 SARS-CoV-2 infection as compared to a molecular test, especially in samples with low viral load.
- This test detects both viable (live) and non-viable SARS-CoV-2 virus.
- This test does not differentiate between SARS-CoV and SARS-CoV-2.
- Test results should be interpreted in conjunction with other clinical and laboratory information available to the healthcare provider.
- Test performance depends on the amount of virus (antigen) in the 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 lead to incorrect test results. Accurate results are dependent on adequate specimen collection, transport, storage, and processing (as applicable).
- Negative results are presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed.
- Positive test results do not rule out co-infections with other pathogens.
- This test should not be used beyond the expiration date listed on the packaging. Use of expired tests can lead to incorrect results.
- There is a risk of erroneous results (i.e., false negatives) due to the presence of novel, emerging respiratory viral variants (e.g., specific strains or isolates). The performance of this test was established based on the evaluation of a limited number of SARS clinical specimens collected between August 2021 through November 2022. 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. Due to the propensity of the virus to mutate, new strains emerge over time which may potentially affect the performance of this device and have serious public health implications. Additional testing with a molecular test and/or sequencing should be considered in situations where a new virus strain or variant is suspected.
- If infection with a novel SARS-CoV-2 virus variant is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health department for testing.
- The performance of this test has not yet been clinically validated for use in patients without signs and symptoms of respiratory infection or for serial screening applications, and performance may differ in these populations.

Performance Characteristics

The following studies were performed with Sofia 2 instrument.

Limit of Detection

The Limit of Detection (LoD) of the Sofia 2 SARS Antigen+ FIA was determined using limiting dilutions of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020). The LoD was confirmed to be a concentration of 1.44E+04 $TCID_{50}$/mL (14,400 $TCID_{50}$/mL), equivalent to 7.2E+02 $TCID_{50}$/swab (720 $TCID_{50}$/swab).

Analytical Reactivity

Analytical reactivity for Sofia 2 SARS Antigen+ FIA was demonstrated using 4 additional strain/isolate of SARS CoV-2 virus. The minimum detectable concentration is listed in Table 1.

Table 1. Inclusivity Study Result

Strain/Variant	Minimum Detectable Concentration
Heat Inactivated SARS-CoV-2 (Omicron BA.1, Lineage BA.1.18)	2.36E+04 $TCID_{50}$/mL
Heat Inactivated SARS-CoV-2 (Omicron BA.2, Lineage B.1.1.529)	8.22E+03 $TCID_{50}$/mL
Heat Inactivated SARS-CoV-2 (Delta, Lineage B.1.617.2)	1.00E+04 $TCID_{50}$/mL
Heat Inactivated SARS-CoV-2 (isolate Italy-INMI1, Beta, Lineage B.1)	2.43E+05 $TCID_{50}$/mL

Cross-Reactivity

The cross-reactivity and potential interference with the Sofia 2 SARS Antigen+ FIA were evaluated by testing various bacteria (10), viruses (19), fungus (1), and negative matrices (2). Each organism and virus was tested in five (5) replicates in the absence or presence of inactivated SARS-CoV-2 at 2X LOD. None of the organisms and viruses in Table 2 below showed cross-reactivity and interference with the assay at the concentrations listed.

Table 2. Cross-Reactivity/Microbial Interference Study Results

Virus/Microorganism	Strain	Conc.	Cross-Reactivity (Yes/No)	Interference (Yes/No)
Adenovirus Culture Fluid	Type 1 (Species C)	2.04E+05 $TCID_{50}$/mL	No	No
Coronavirus Culture Fluid (Heat Inactivated)	229E	1.26E+05$TCID_{50}$/mL	No	No
Coronavirus Culture Fluid (Heat Inactivated)	OC43	1.00E+05 $TCID_{50}$/mL	No	No
Coronavirus Culture Fluid (Heat Inactivated)	NL63	3.40E+04 $TCID_{50}$/mL	No	No
Enterovirus Type 68 Major Group Culture Fluid	2014 Isolate 1	2.29E+05 $TCID_{50}$/mL	No	No
Human Metapneumovirus (hMPV) 9	A1	1.27E+05 $TCID_{50}$/mL	No	No
Human Rhinovirus Type 1A Culture Fluid	N/A	1.78E+05$TCID_{50}$/mL	No	No
Influenza A	A/Brisbane/10/07	1.00E+05 $TCID_{50}$/mL	No	No
Influenza A	A/New Caledonia/20/99 H1	1.78E+05$TCID_{50}$/mL	No	No
Influenza A	New Caledonia/20/99 Custom formalin inactivated	4.02E+05 $TCID_{50}$/mL	Not Tested	No
Influenza A	Brisbane/02/18	5.62E+03 $TCID_{50}$/mL	Not Tested	No
Influenza A	California/07/09	4.17E+04 $TCID_{50}$/mL	Not Tested	No
Influenza B Virus Culture Fluid	Brisbane/33/08	2.34E+04 $TCID_{50}$/mL	No	No

Virus/Microorganism	Strain	Conc.	Cross-Reactivity (Yes/No)	Interference (Yes/No)
MERS-CoV Culture Fluid (Heat Inactivated)	Florida/USA-2_Saudi Arabia_2014	1.04E+05$TCID_{50}$/mL	No	No
Parainfluenza Type 1 Culture Fluid	N/A	1.00E+05 $TCID_{50}$/mL	No	No
Parainfluenza Type 2 Culture Fluid	N/A	9.97E+05 $TCID_{50}$/mL	No	No
Parainfluenza Type 3 Culture Fluid	N/A	2.29E+05 $TCID_{50}$/mL	No	No
Parainfluenza Type 4B Culture Fluid	N/A	1.00E+05 $TCID_{50}$/mL	No	No
Respiratory Syncytial Virus Type A (RSV-A) Culture Fluid	Type A	1.51E+05$TCID_{50}$/mL	No	No
Bordetella pertussis, ATCC 18323 (NCTC 10739)	Type b; Eagan	3.05E+07 CFU/mL	No	No
Chlamydophila pneumoniae	Z500[IOL207]	1.06E+07 IFU/mL	No	No
Haemophilus influenzae	Type B, NCTC 8468	2.60E+07 CFU/mL	No	No
Legionella pneumophila	ATCC 33152 (Philadelphia-1)	2.00E+07 CFU/mL	No	No
Pneumocystis jirovecii-S. cerevisiae Recombinant	W303-Pji	5.29E+05 CFU/mL	No	No
Mycoplasma pneumoniae	M129	1.35E+07 CCU/mL	No	No
Streptococcus pneumoniae, Type 19F	ATCC 49619 (262 [CIP 104340])	1.90E+07 CFU/mL	No	No
Streptococcus pyogene	ATCC 19615 (Bruno [CIP 104226])	1.60E+07 CFU/mL	No	No
Staphylococcus methicillin resistant aureus (MRSA)	N/A	1.03E+06 CFU/mL	No	No
Staphylococcus methicillin susceptible aureus (MSSA)	N/A	1.15E+06 CFU/mL	No	No
Staphylococcus epidermidis	N/A	1.29E+06 CFU/mL	No	No
Negative Nasal Matrix (normal flora)	UTM	N/A	No	No
Negative Nasal Matrix (normal flora)	CDC Viral Transport	N/A	No	No

Interference Substances Study

Fourteen (14) potentially interfering substances were evaluated with the Sofia 2 SARS Antigen + FIA Assay. Each substance was tested in five (5) replicates in the absence or presence of 2.88E+04 $TCID_{50}$/mL of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020). None of the substances listed in Table 3 interfered with the assay at the levels tested.

Table 3. Interfering Substances Study Results

Interfering Substance	Active Ingredient	Concentration	Replicates tested/ Replicates detected	Interference (Yes/No)
Afrin – nasal spray	Oxymetazoline	5%	5/5	No
Blood (human)	Blood	5%	5/5	No
Chloraseptic, Cepacol	Benzocaine, Menthol	0.7 g/mL	5/5	No
Flonase	Fluticasone	5%	5/5	No
Halls Relief Cherry Flavor	Menthol	0.8 g/mL	5/5	No
Nasocort Allergy 24 hour	Triamcinolone	5%	5/5	No

Interfering Substance	Active Ingredient	Concentration	Replicates tested/ Replicates detected	Interference (Yes/No)
Neo-Synephrine	Phenylephrine hydrochloride	5%	5/5	No
Oseltamivir	Oseltamivir	2.2 µg/mL	5/5	No
Purified mucin protein	Mucin protein	2.5 mg/mL	5/5	No
Rhinocort	Budesonide (Glucocorticoid)	5%	5/5	No
Saline nasal spray	Saline	15%	5/5	No
Tobramycin	Tobramycin	1.25 mg/mL	5/5	No
Zanamivir	Zanamivir	282.0 ng/mL	5/5	No
Zicam Cold Remedy	Galphimia glauca, Luffa operculata, Sabadilla	5%	5/5	No

Hook Effect

As part of the LoD study the highest concentration of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020) stock available (2.30E+06 $TCID_{50}$/mL) was tested. There was no Hook effect detected.

Precision

The within-laboratory precision of the Sofia 2 SARS Antigen+ FIA was evaluated by using two (2) product lots. A series of coded, contrived samples were prepared as negative, high negative (0.277x LoD), low positive (1x LoD), and positive (3x LoD) using UV-inactivated SARS-CoV-2 (isolate USA-WA1/2020). Each sample was tested in duplicate in two (2) events per day over twenty (20) days. The negative sample produced results with a lower 95% confidence interval limit ≥95% negative agreement. The low positive sample at 1x LoD percent agreement to the expected positive result encompassed ≥95% agreement. The positive sample at 3X LoD produced results with a lower 95% confidence interval limit ≥95% positive agreement.

Table 4. Precision Study Results

Lot	Negative*	High Negative* (0.277x LoD)	Low Positive** (1x LoD)	Positive** (3x LoD)
1	80/80	74/80	80/80	80/80
2	79/79	78/79	77/79	79/79
Total	159/159	152/159	157/159	159/159
% Agreement (95% CI)	**100% [97.6% - 100.0%]**	**95.6% [91.2% - 97.9%]**	**98.7% [95.5% - 99.7%]**	**100% [97.6% - 100.0%]**

*Virus not detected/total; **Virus detected/total

Reproducibility

The reproducibility of the Sofia 2 SARS Antigen+ FIA was evaluated at three (3) different laboratories using two (2) product lots (Table 5). Two (2) different operators at each site tested a series of coded, contrived samples, prepared as negative, high negative (0.277x LoD), low positive (1x LoD), and positive (3x LoD) using UV-inactivated SARS-CoV-2 (isolate USA-WA1/2020). Testing was conducted over 5 separate days. The operators obtained accurate qualitative results 100% (180/180) of the time for the negative samples and 99.2% (119/120) of the time for the positive samples at 1x and 3x LoD.

Table 5. Reproducibility Study Inter-Laboratory Agreement

Site	Negative* (C_0)	High Negative* (0.277x LoD)	Low Positive** (1x LoD)	Positive** (3x LoD)
1	40/40	26/40	40/40	39/40
2	40/40	11/40	40/40	40/40
3	40/40	29/40	39/40	40/40
Total	120/120	66/120	119/120	119/120

Site	Negative* (C_0)	High Negative* (0.277x LoD)	Low Positive** (1x LoD)	Positive** (3x LoD)
% Agreement (95% CI)	100% [96.9% - 100.0%]	55% [46.1% - 63.6%]	99.2% [95.4% - 99.9%]	99.2% [95.4% - 99.9%]

*Virus not detected/total; **Virus detected/total

Clinical Performance

The performance of the Sofia 2 SARS Antigen+ FIA with anterior nasal swab specimens was compared to a highly sensitive EUA authorized SARS-CoV-2 RT-PCR Comparator assay performed with anterior nasal swab specimens collected in VTM. The study was designed as a prospective multi-center clinical study. A total of five hundred eighty-one (581) subjects with symptoms of respiratory infection were evaluated in the study. Sixty-two percent (62%) of the subjects were female and thirty-eight percent (38%) were male. Subjects ranged in age from 3 years to over 92 years and with a mean age of 41.6 years. The results of the method comparison are shown in Table 6.

Table 5. Sofia 2 SARS Antigen+ FIA Performance Compared to EUA Extracted SARS-CoV-2 RT-PCR Assay

	EUA Extracted SARS-CoV-2 RT-PCR Assay Result		
	Pos	**Neg**	**Total**
Sofia Pos	97	2	99
Sofia Neg	12	470	482
Total	109	472	581

PPA = 89.0% (97/109) (95%CI=81.7% - 93.6%)

NPA = 99.6% (470/472) (95%CI=98.5% - 99.9%)

Positivity Rate = 18.8% (109/581) (95%CI=15.8% - 22.1%)

PPA= Positive Percentage Agreement; NPA= Negative Percentage Agreement

Table 7. Sofia 2 SARS Antigen+ FIA Performance Based EUA Extracted SARS-CoV-2 RT-PCR Assay Ct Values

Ct Value Range*	# Sofia SARS+ Positive	# RT-PCR Positive	% Detection
15 – 19	26	26	100%
20 – 24	47	47	100%
25 – 29	21	21	100%
30 – 34	0	4	0%
35 – 40	0	6	0%
Total	94	104	90%

*Ct values vary depending on several assay-dependent factors and therefore are specific to the RT-PCR comparator method used in a clinical study. Ct values of different molecular tests cannot be directly compared.

Serial Testing

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. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing.

Performance of the antigen test with serial testing in individuals is described in Table 8

Table 8. 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

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.

Assistance

If you have any questions regarding the use of this product, please contact QuidelOrtho Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidelortho.com. If outside the U.S., further information can be obtained from your distributor, or directly from QuidelOrtho at one of the numbers listed below. Reference **quidelortho.com** to see more options for Support.

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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).

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