QUIDEL

Sofia 2 SARS-CoV-2 Antibody IgG FIA

FOR USE WITH SOFIA 2

For *in vitro* diagnostic use only.

A symbols glossary can be found at quidel.com/glossary

INTENDED USE

The Sofia 2 SARS-CoV-2 Antibody IgG FIA is a single-use rapid immunochromatographic test for the qualitative detection of Immunoglobulin G (IgG) antibodies to SARS-CoV-2 in fingerstick whole blood, venous whole blood, serum, or plasma (lithium or sodium heparin) samples. The Sofia 2 SARS-CoV-2 Antibody IgG FIA is intended for use either as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection or determine the presence of IgG antibodies post vaccination.

Results from the Sofia 2 SARS-CoV-2 Antibody IgG FIA test should not be used as the sole basis for diagnosis of prior SARS-CoV-2 infection, or immune status to the virus.

Results are for the detection of SARS-CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2 become detectable later following infection. Positive results for IgG antibodies could occur after infection and can be indicative of acute or recent infection. Laboratories are required to report all positive results from non-vaccinated individuals to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results must be combined with clinical observations, patient history, and epidemiological information. IgG antibodies may not be detected in the first few days of infection; the sensitivity of the Sofia 2 SARS-CoV-2 Antibody IgG FIA test early after infection is unknown.

IgG antibodies to SARS-CoV-2 are generally detectable in blood 10-days after the first vaccination shot. The presence of IgG antibodies after the initial vaccination shot of a two-shot protocol does not indicate immunity to the virus, and the second shot should be administered.

False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

At this time, it is unknown for how long IgG antibodies may persist following infection.
The Sofia 2 SARS-CoV-2 Antibody IgG FIA test is intended for use by trained clinical laboratory personnel and individuals proficient in performing the test in patient care settings.

PRINCIPLE OF THE TEST

The Sofia 2 SARS-CoV-2 Antibody IgG FIA is an immunofluorescence-based, lateral flow assay for detection of IgG antibodies to SARS-CoV-2 Nucleocapsid Protein (N), Spike Protein 1, and Spike Protein 2. Reagents for the assay are ready-to-use and provided in the kit.

The assay uses a bidirectional test format that consists of a sample port that distributes sample onto two assay paths, which include antibody detection test and process control areas in separate readout windows.

To perform the test, the patient specimen (25 µL fingerstick or venous whole blood or 10 µL serum/plasma) is added to a pre-filled Reagent Vial. 100 µL of the diluted sample is then dispensed into the round sample well located near the center of the Test Cassette. The sample flows onto both sides of the lateral flow test strip (enclosed in the test cassette housing) and hydrates the two dried antibody binding fluorescent conjugates located on either side of the sample pad in separate label pads. If the sample contains SARS-CoV-2 antibodies, the fluorescent conjugate containing anti-human IgG antibodies binds to the antibodies (IgG) from the sample. The sample continues to migrate through the strip in both directions to the test areas where SARS-CoV-2 antibodies (if present in the sample) bind to the immobilized Nucleocapsid Protein antigen on the Top test area and bind to the immobilized Spike S1 and S2 antigens on the bottom test area to form test lines. Finally, the sample migrates to the procedural control (C) areas where separate control lines form. The total development time of the assay is 15 minutes.

The Test Cassette is loaded into Sofia 2 for an automatically defined development time (WALK AWAY Mode) or pre-incubated on the bench top prior to loading into Sofia 2 (READ NOW Mode). Sofia 2 scans the test strip, analyzes the fluorescent signal, and then displays three (3) test results: N-protein (positive, negative), S1 (positive, negative) and S2 (positive, negative).

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Test Cassettes (25) containing non-infectious SARS and SARS-CoV-2 proteins, monoclonal rabbit anti-human IgG, and bovine proteins
- 25 µL Capillary Tubes (25)
- 100 µL Fixed Volume Pipettes (25)
- Reagent Vials, filled with 1.0 mL reagent solution (25) comprised of buffer with Microcide III as a preservative
- One (1) Bottle of Positive Control (2.8 mL) containing human anti-SARS IgG (to N protein, S1, and S2) in buffer with Sodium Azide and Microcide III as preservatives
- One (1) Bottle of Negative Control (2.8 mL) containing human serum that is negative for COVID-19 in buffer with sodium azide and Microcide III as preservatives
- Package Insert (1)
- Quick Reference Instructions (1)
- QC Card (located on kit box)

MATERIALS REQUIRED BUT NOT SUPPLIED IN KIT

- Sofia 2 Fluorescent Immunoassay Analyzer
- Calibration Cassette (supplied with the Sofia 2)
- Timer or watch for use in READ NOW Mode
- Calibrated micropipette – for measuring 10 µL and 25 µL volumes
- Blood collection tubes
- Lancets for fingerstick samples

WARNINGS AND PRECAUTIONS

- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.[4]
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.
- Do not reuse the used Test Cassette or Reagent Vial.
- The user should never open the foil pouch of the Test Cassette exposing it to the ambient environment until the Test Cassette is ready for immediate use.
- Discard and do not use any damaged Test Cassette or material.
- To obtain accurate results, the Package Insert instructions must be followed.
- The Calibration Cassette must be kept sealed in the provided foil storage pouch between uses.
- Inadequate or inappropriate specimen collection, storage, and transport may yield false test results.
- Specimen collection and handling procedures require specific training and guidance.
- 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. Sofia 2 must be used for result interpretation.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

KIT STORAGE AND STABILITY

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

QUALITY CONTROL

There are three types of Quality Control for Sofia 2 and the Test Cassette: Calibration Check Procedure, Built-in Procedural Control features, and External Controls.

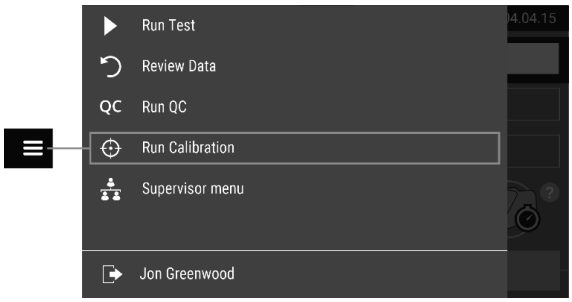
Sofia 2 Calibration Check Procedure

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.

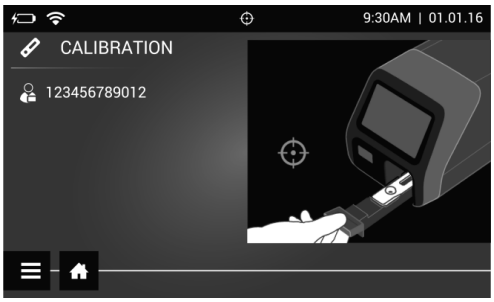
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

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.

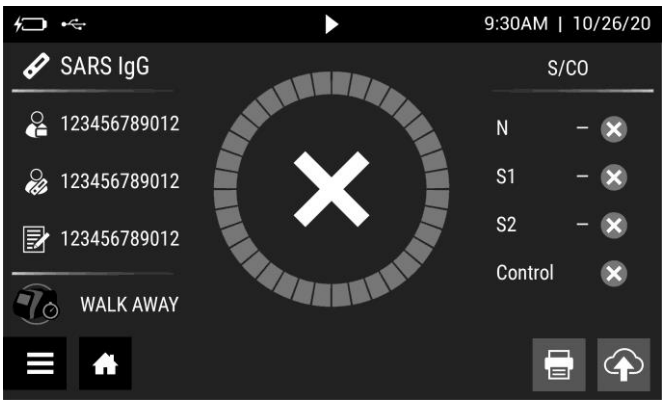
NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer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

Built-in Procedural Controls

The Sofia 2 SARS-CoV-2 Antibody IgG FIA contains built-in procedural control features. Each time a test is run, the procedural control areas are 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 in 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 are used to demonstrate that the reagents and assay procedure perform properly. Quidel recommends that Positive and Negative External Controls be run:

- Once for each new 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 Quality Control Test Procedure

1. From the main menu, select "Run QC".
2. Following the prompt on the screen, scan the QC Card (located on the assay kit box).
3. The user will select the desired mode (WALK AWAY or READ NOW) and then run the External Controls. A full description of each mode can be found in the "Using Sofia 2" section of this Package Insert.
4. Use the following procedure to test each of the Control solutions. **The Positive Control must be run first, followed by the Negative Control.**

    a. Prepare a Positive Control Cassette by adding **2 drops** of the Positive Control solution (red cap) to a Test Cassette sample well. Then follow the Sofia 2 screen instructions for developing and analyzing the Positive Control Cassette.

    NOTE: The round Test Cassette sample well is located between the rectangular windows and has a green-tinted sample pad. DO NOT place sample into the left or right rectangular windows. When adding drops, hold the bottle vertically so that a complete drop forms.

    b. Prepare a ***Negative Control Cassette*** by adding **2 drops** of the Negative Control solution (white cap) to a Test Cassette sample well. Then follow the Sofia 2 screen instructions for developing and analyzing the Negative Control Cassette.

5. After both the Positive and Negative Controls have been run, the results will be displayed as ✔ or ✖ on Sofia 2.

Do not perform patient tests or report patient test results if either of the QC test results fail. 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.

Repeat the test or contact Quidel Technical Support at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.).

SAMPLE COLLECTION AND STORAGE

Fingerstick

The pad (or the palmar surface of the distal segment) of the middle finger or ring finger of the non-dominant hand are the preferred sites for collecting a finger-stick whole blood sample. Avoid the side or tip of the finger.[5] Instruct the patient to rest the arm in a downward position for at least 30 seconds to allow blood to flow to fingertips. A warming pad, or holding the patient's hand under warm water, may be needed if patient has cold hands. Cleanse the puncture site with alcohol and let the skin air-dry before proceeding. Puncture the skin with the lancet. Discard the lancet in a sharps disposal container. Wipe away the first drop of blood with dry gauze.

NOTE: DO NOT massage or apply strong repetitive pressure ("milking") to the punctured finger. This may contaminate the sample with tissue-fluid and affect the test result. Collect the finger-stick whole blood using the Capillary Tube and immediately test the sample as described in the Test Procedure.

Venous Whole Blood

Collect venous whole blood specimens per standard procedures in lithium heparin or sodium heparin tubes. Samples may be used immediately without centrifugation or stored at 2-8 °C for up to 72 hours prior to use in the assay.

Serum or Plasma

Collect and process serum or plasma per standard procedures. Samples may be stored refrigerated (2°C to 8°C) up to 48 hours, at room temperature (15°C to 30°C) up to 8 hours, and frozen (–20°C) up to 2 freeze/thaw cycles. **Make sure sample is at room temperature before using in the test. For samples that have been previously refrigerated or frozen, mix well by fully thawing and inverting the tube 10 times**.

Performance of the Sofia 2 SARS-CoV-2 Antibody IgG FIA has been established with the following tubes:

- Serum Separator Tube (RED)
- Serum Separator Tube (TIGER)
- Lithium Heparin
- Sodium Heparin

Note: EDTA is NOT compatible with Sofia 2 SARS-CoV-2 Antibody IgG FIA.

TEST PROCEDURE (FINGERSTICK WHOLE BLOOD)

Precautions

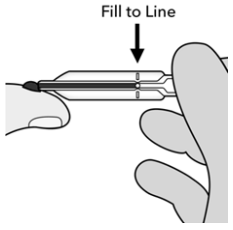
DO NOT open the foil pouch containing the Test Cassette until ready to test the sample. Place the Test Cassette on a clean and level surface.

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

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.*

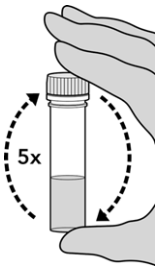
1. Verify that Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia 2" section for more information.

2. Hold the provided **25 µL** capillary tube **horizontally** and touch the tip to the patient blood sample. Blood will automatically draw into the capillary tube – **Do not squeeze the bulb**. Fill to the black line.

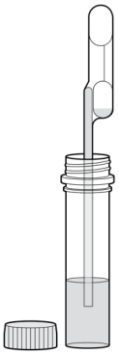


3. **Submerge** capillary in buffer and **squeeze** the bulb on the end of the capillary tube to add patient sample to the Reagent Vial. **While submerged**, quickly squeeze bulb 5 times to **mix**.

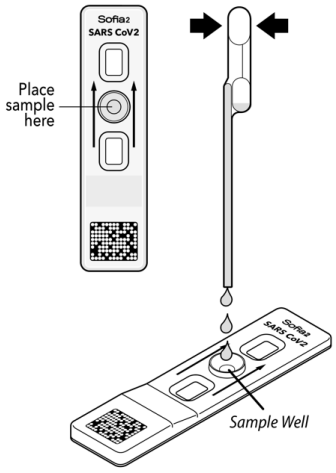
4. Screw cap on Reagent vial and **invert** 5 times.



5. Fill the **100 µL** pipette with diluted sample from the Reagent Vial. Sample may go into overflow bulb.



6. Align the tip of the pipette with the round Test Cassette sample well and empty its contents.



7. Proceed to the section “Using Sofia 2” to complete the test.

TEST PROCEDURE (VENOUS WHOLE BLOOD)

Precautions

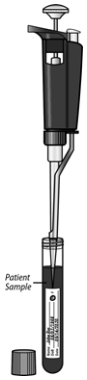
DO NOT open the foil pouch containing the Test Cassette until ready to test the sample. Place the Test Cassette on a clean and level surface.

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

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.*

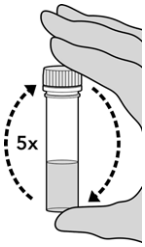
1. Verify that Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia 2" section for more information.

2. Fill a calibrated micropipette with **25 µL** of the patient venous whole blood sample.

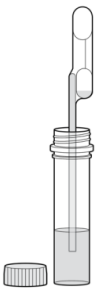


3. Add **25 µL of the** patient sample to Reagent Vial and **gently mix**.

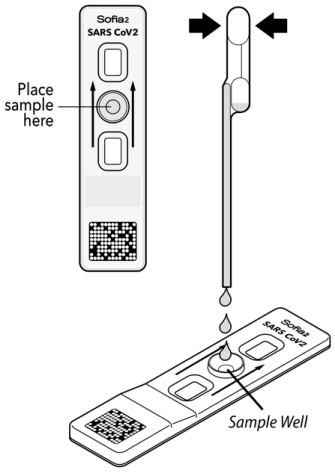
4. Screw cap on Reagent Vial and **invert** 5 times.



5. Fill the **100 µL** pipette with diluted sample from the Reagent Vial. Sample may go into overflow bulb.



6. Align the tip of the pipette with the round Test Cassette sample well and empty its contents.



7. Proceed to the section "Using Sofia 2" to complete the test.

TEST PROCEDURE (SERUM/PLASMA)

Precautions

DO NOT open the foil pouch containing the Test Cassette until ready to test the sample. Place the Test Cassette on a clean and level surface.

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

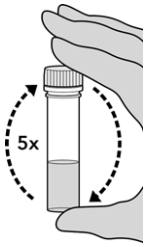
Check expiration date on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

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

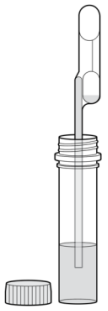
9. Fill a calibrated micropipette with **10 µL** of the patient serum/plasma sample.

10. Add patient sample to Reagent Vial and **gently mix**.

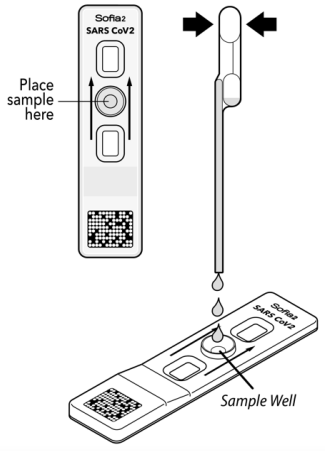
11. Screw cap on Reagent Vial and **invert** 5 times.



12. Fill a calibrated micropipette or the provided disposable pipette with **100 µL** of diluted sample from the Reagent Vial. Sample may go into overflow bulb of the transfer pipette.



13. Align the tip of the micropipette or transfer pipette with the round Test Cassette sample well and empty its contents (100 uL).



14. 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. Positive test results will be displayed at 15 minutes.

READ NOW Mode

Allow the test to develop for the full 15 minutes BEFORE placing it into Sofia 2.

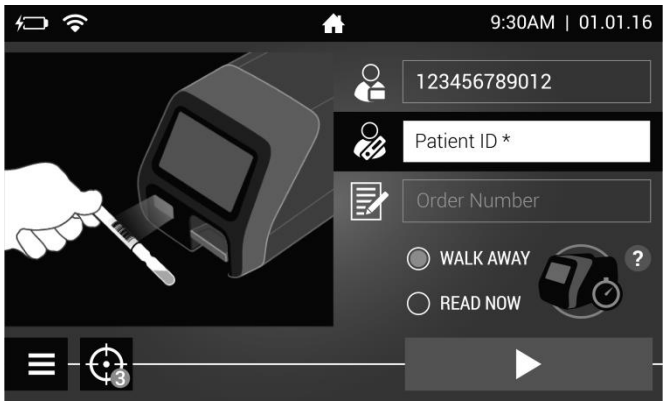
The user must first place the Test Cassette onto the counter or bench top for 15 minutes (outside of Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia 2. In READ NOW Mode, Sofia 2 will scan and display the test result in approximately 1 minute. **Note:** Results will remain stable for an additional 5 minutes after the recommended development time of 15 minutes.

Run Test with Sofia 2

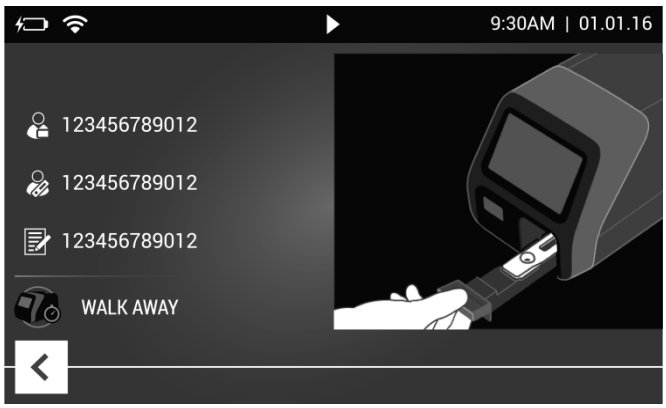
1. Input the User ID using the barcode scanner or manually enter the data using the on-screen key pad.

   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.*

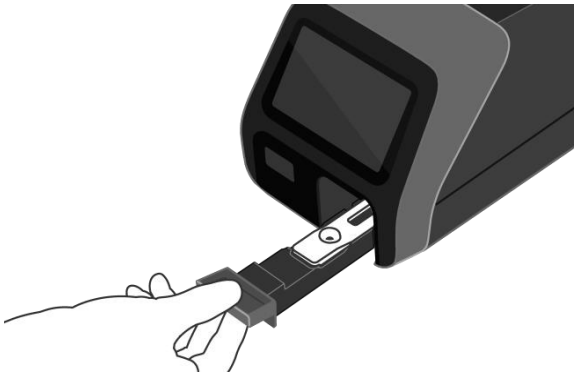
2. Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the on-screen key pad.



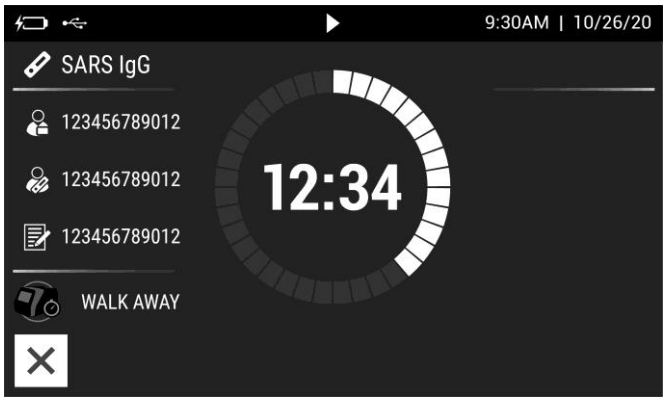
Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



3. Insert the prepared patient Test Cassette into the drawer of Sofia 2 and gently close the drawer.



4. 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 at 15 minutes. In READ NOW Mode, the test results will be displayed on the screen within 1 minute. See Interpretation of Results section.



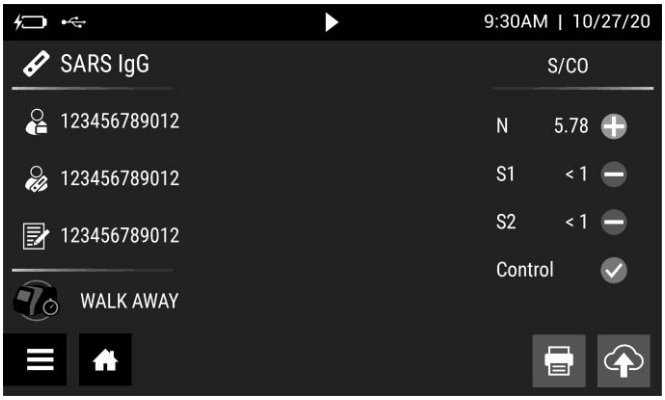
For example: This display shows that the test in WALK AWAY Mode has 12 minutes, 34 seconds remaining. Sofia 2 will read and display the results at 15 minutes.

INTERPRETATION OF RESULTS ON SOFIA 2

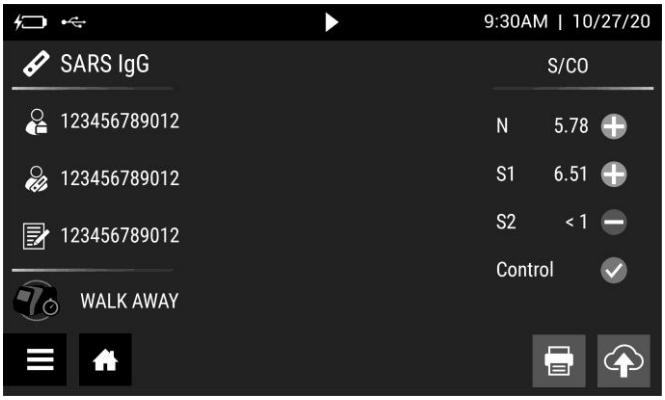
When the test is complete, the results will be displayed on the Sofia 2 screen. Test Lines, which are fluorescent, cannot be seen with the naked eye.

The Sofia 2 screen will display results for the procedural controls as being ✓ or ✗ and will provide a ⊕ or ⊖ result for the detection of IgG antibodies to SARS-CoV-2 N, S1, and S2. Signal to cutoff (S/CO) values will also be reported for each protein. If the procedural controls are ✗, retest the patient's sample with a new Test Cassette.

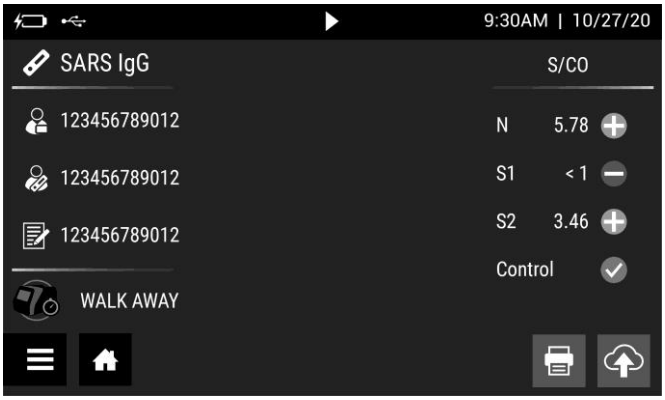
Positive Results:



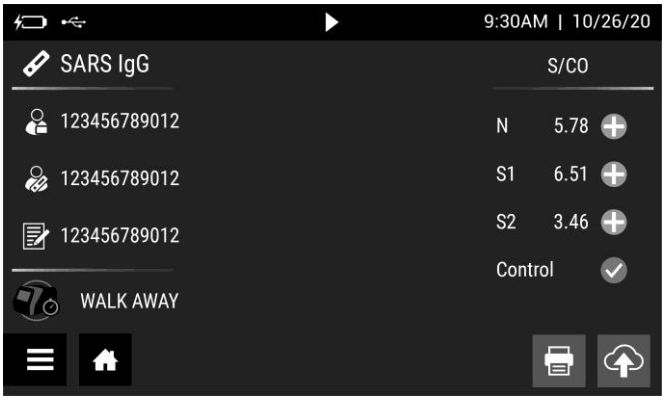
This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 N Protein. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2.



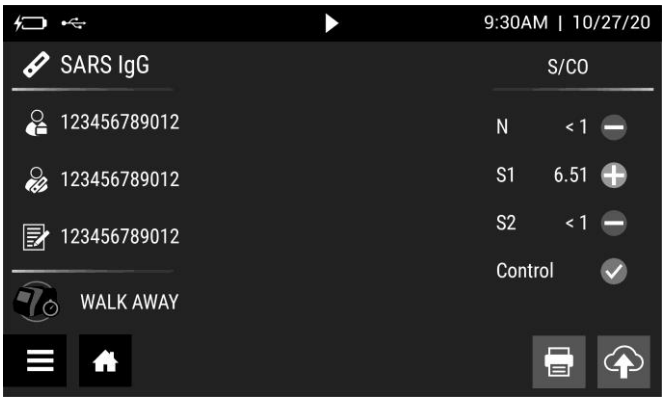
This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 N Protein and S1. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2



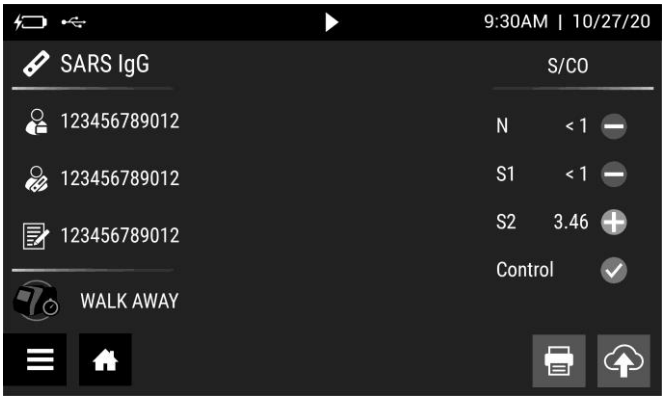
This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 N Protein and S2. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2.



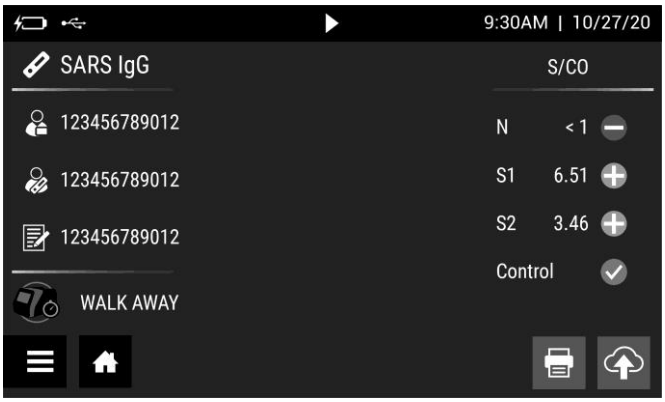
This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 N Protein, S1, and S2. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2



This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 S1. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2.

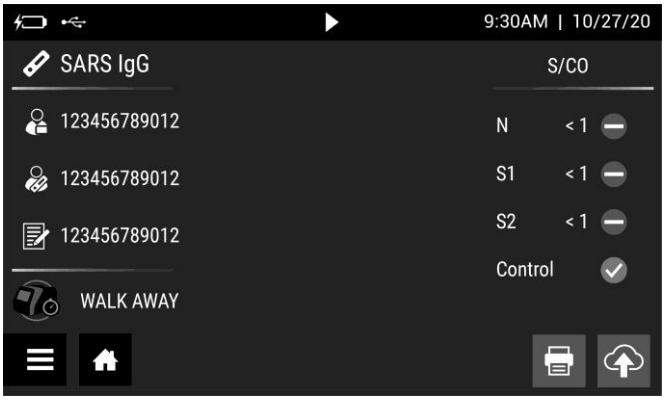


This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 S2. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2.



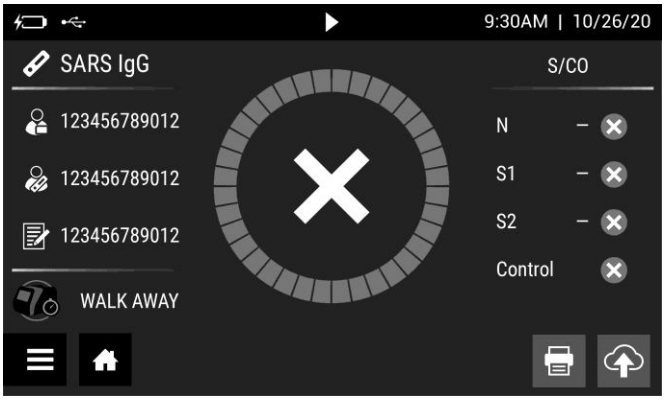
This display shows a valid positive result for IgG antibodies to SARS-CoV or SARS-CoV-2 S1 and S2 Protein. Interpret to be presumptive of exposure to SARS-CoV or SARS-CoV-2.

Negative Results:



This display shows a valid negative result for IgG antibodies to SARS-CoV-2. Interpret to be presumptive of no exposure to SARS-CoV-2.

Invalid Results:



This display shows an invalid result.

If the test is invalid, a new test should be performed starting with Step 1 and a new Test Cassette.

LIMITATIONS

- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- False positive results may occur if samples are read before the minimum 15-minute development time.
- False negative results may occur if samples are read more than 5-minutes after the required 15-minute development time.
- Antibody detection methods do not provide definitive results for establishing or ruling out exposure to SARS-CoV-2
- A negative result in the Sofia 2 SARS-CoV-2 Antibody IgG FIA does not rule out the possibility of SARS-CoV-2 exposure in a patient.
- Positive results in the Sofia 2 SARS-CoV-2 Antibody IgG FIA must be interpreted with caution. Cross-reactivity may be observed with certain diseases.
- Levels of bilirubin above 10 mg/dL may cause false negative results in the assay.
- Levels of hemoglobin above 500 mg/dL may cause false negative results in the assay.

PERFORMANCE CHARACTERISTICS

Clinical Specificity of Sofia 2 SARS-CoV-2 Antibody IgG FIA

A total of eight-hundred and sixteen (816) serum/plasma samples known to be negative for COVID-19, from the pre-COVID-19 pandemic, were obtained from external vendors and the Quidel biorepository. Each specimen was tested on the Sofia 2 SARS-CoV2 Antibody IgG FIA at Quidel. The results are summarized below in Table 1. The overall clinical specificity was 97.4% for all three targeted analytes.

Table 1. Clinical Specificity Results

Pre-COVID19 Serum/Plasma Samples	# Specimens Tested	# Negative	# Positive	Specificity	95% CI	
Sofia Overall	**816**	**795**	**21**	**97.4%**	**96.1%**	**98.3%**
Sofia N Results	816	803	13	98.4%	97.3%	99.1%
Sofia S1 Results	816	815	1	99.9%	99.3%	100%
Sofia S2 Results	816	808	8	99.0%	98.1%	99.5%

Clinical Agreement of Sofia 2 SARS-CoV-2 Antibody IgG FIA to Comparator Methods

A multi-center prospective study was performed at three (3) sites. One-hundred and nine (109) patients were enrolled and had one fingerstick sample and one serum separator tube sample collected at a single visit. The fingerstick sample was processed immediately on the Sofia 2 SARS-CoV-2 Antibody IgG FIA according to the Instructions for Use. The serum samples were processed sent to a 3rd party for comparator testing: one aliquot was tested with Abbott Architect SARS-CoV-2 IgG for detection of IgG antibody against the N-protein and the other aliquot was tested with Diasorin LIAISON® SARS-CoV-2 S1/S2 IgG for detection of IgG antibody against the S-protein (undifferentiated S1 and S2 results). The sample was considered positive on comparator methods if either or both comparator methods tested positive and is negative if both comparator methods tested negative. The specimen was considered positive on Sofia 2 SARS-CoV-2 Antibody IgG FIA if any of the three (3) analytes (N or S1 or S2) tested positive and was negative if all of the analytes tested negative. The results are presented in Tables 2 through 4 below.

Table 2. Sofia 2 SARS-CoV-2 Antibody IgG FIA Compared to Abbott Architect and Diasorin (N, S1, S2)

Overall Results		Abbott Architect SARS-CoV-2 IgG + Diasorin LIAISON® SARS-CoV-2 S1/S2 IgG (Serum)				95% CI	
		POS	**NEG**	**Total**	**PPA**	96.4%	90.0%	98.8%
Sofia 2 SARS-CoV-2 Antibody IgG FIA (Fingerstick Whole Blood)	**POS**	81	5	86	**NPA**	80.0%	60.9%	91.1%
	NEG	3	20	23	**PPV**	94.2%	87.1%	97.5%
	Total	84	25	109	**NPV**	87.0%	67.9%	95.5%
					% Agr.	92.7%	86.2%	96.2%

Table 3. Sofia 2 SARS-CoV-2 Antibody IgG FIA Compared to Abbott Architect Only (N)

N Results		Abbott Architect SARS-CoV-2 IgG (Serum)				95% CI	
		POS	**NEG**	**Total**	**PPA**	87.8%	79.0%	93.2%
Sofia 2 SARS-CoV-2 Antibody IgG FIA (Finger Stick Whole Blood)	**POS**	72	2	74	**NPA**	92.6%	76.6%	97.9%
	NEG	10	25	35	**PPV**	97.3%	90.7%	99.3%
	Total	82	27	109	**NPV**	71.4%	54.9%	83.7%
					% Agr.	89.0%	81.7%	93.6%

Table 4. Sofia 2 SARS-CoV-2 Antibody IgG FIA Compared to Diasorin Only (S1, S2)

S1 + S2 Results		Diasorin LIAISON® SARS-CoV-2 S1/S2 IgG (Serum)				95% CI	
		POS	NEG	Total	PPA	87.8%	78.5% 93.5%
Sofia 2 SARS-CoV-2 Antibody IgG FIA (Finger Stick Whole Blood)	POS	65	9	74	NPA	74.3%	57.9% 85.8%
	NEG	9	26	35	PPV	87.8%	78.5% 93.5%
	Total	74	35	109	NPV	74.3%	57.9% 85.8%
					% Agr.	83.5%	75.4% 89.3%

The performance of the Sofia 2 SARS-CoV2 Antibody IgG FIA was further analyzed by days post symptom onset (Table 5). The eighty-four (84) finger stick whole blood samples that were confirmed positive via their matched positive serum samples per the comparator methods, Abbott Architect SARS-CoV-2 IgG or Diasorin LIAISON® SARS-CoV-2 S1/S2 IgG, was used in the analysis.

Table 5. Sofia 2 SARS-CoV-2 Antibody IgG FIA Compared to Diasorin Only (S1, S2)

Days Post Symptom Onset	# Positive by Comparator Methods	*Sofia 2 SARS-CoV-2 Antibody IgG FIA*			
		# Positive by Sofia	% Agreement	95% CI	
≤ 7 Days	**4**	4	100%	51%	100%
8 - 14 Days	**3**	3	100%	43.9%	100%
≥ 15 Days	**75**	72	96.0%	88.9%	98.6%
No days*	**2**	2	100%	34.2%	100%
All Days	**84**	**81**	**96.4%**	**90%%**	**98.8%**

*Two (2) consensus positive samples have missing data for the days post symptom onset

Clinical Agreement and Longitudinal Monitoring of Antibody Response for Vaccinated Subjects

A multi-center, IRB approved study was designed to collect samples prior to and after the first and second Moderna and Pfizer-BioNTech vaccine doses. Subjects with prior infection or no known infection prior to the first vaccination were included in this study. Subjects included in the study were male or female, age 18 years or older, and must have had the first blood draw within 7 days of receiving the first COVID-19 mRNA vaccine. Subjects were also required to obtain the second vaccination according to the manufacturer's requirement. Following consent, demographics, symptoms, and health history were collected from each subject. Matched fingerstick, venous whole blood, plasma, and serum samples were collected from each subject on a weekly basis. Samples were collected for 3 weeks after the second vaccination dose. Demographics of study participants is shown in Table 6.

Table 6. Demographics of the Study Participants

Vaccine manufacturer:	Moderna	Pfizer-BioNTech	Total
Subjects received 1st and 2nd dose	50	32	82
Subjects 3 weeks past 2nd dose	50	28	78
Average Days Between Doses	33	21	NA
Male	26	14	40
Female	24	18	42
Age Range (Years)	19-79	19-66	19-83
Median Age (Years)	40	40	40
Subjects with known Prior Infection	3	1	4
Subjects with no known Prior Infection	47	31	78

Each of the seventy-eight (78) subjects with no known prior infection were negative by the Sofia 2 SARS-CoV-2 Antibody IgG FIA at the time of enrollment. The four (4) subjects with known prior infection had detectable antibody with Sofia 2 SARS-CoV-2 Antibody IgG FIA at the time of enrollment.

All seventy-eight (78) subjects with no known prior infection developed detectable S1 antibody after the first vaccination. It was observed that most subjects receiving the Moderna vaccine had an immune response to S1 approximately 2 weeks after the first vaccine dose. Similarly, subjects that received the Pfizer-BioNTech vaccine had an immune response to S1 approximately 3 weeks after the first vaccine dose. The average antibody response to S1 was similar for both vaccines approximately 2 weeks after the second dose.

A total of twenty-seven (27) of the seventy-eight (78) subjects with no prior infection (nineteen (19) for Moderna and eight (8) for Pfizer-BioNTech) showed an immune response indicating likely previous exposure to SARS-CoV-2. After the first vaccination dose, the S1 IgG response in these subjects was much higher (S/CO ~ 45) as compared to other subjects in the same group (S/CO ~ 15). No IgG response was observed against S2 and N protein. Although, S2 is part of the vaccine S protein construct, S2 has been reported to have low immunogenicity. As a result of this analysis, a new category was created for the 27 subjects as "no recorded infection - possible asymptomatic infection". The remaining 51 subjects then comprised the "no prior infection" group.

The immune response for subjects previously exposed to SARS-CoV-2 (known to have prior infection) and vaccinated with Moderna (n=3) and Pfizer-BioNTech (n=1) were higher than the IgG immune response from subjects with no prior exposure to the virus (referred to as "no prior infection" group). Following the first vaccination, S1 IgG response of the prior infection group was rapid and visibly higher (S/CO > 100) than the non-exposed subject response (S/CO ~15). The increased S1 IgG levels in the prior infection group was observed 4-5 days after the first vaccination compared to the non-exposed subjects at 13 days. Following the second dose, the prior infection subjects' S1 response increased to a similar high response seen after the first vaccination dose. The prior infection subjects had detectable antibodies against S2 and N antigens as well.

The data generated by the three studies demonstrate the following:

- Sofia 2 SARS-CoV-2 Antibody IgG FIA has excellent specificity (97.4%) when testing a large (816 specimens) pre-pandemic specimen bank.
- Sofia 2 SARS-CoV-2 Antibody IgG FIA has excellent % agreement (92.7%) to consensus result of two current EUA devices (Abbott Architect SARS-CoV-2 IgG for detection of IgG antibody against the N-protein and Diasorin LIAISON® SARS-CoV-2 S1/S2 IgG).
- Sofia 2 SARS-CoV-2 Antibody IgG FIA can be used to detect immune response to the Moderna and Pfizer-BioNTech vaccines.

Matrix Comparison

The Sofia 2 SARS-CoV-2 Antibody IgG FIA is designed to be used with fingerstick whole blood, venous whole blood, serum, or plasma samples. In this study we compared the IgG response against S1, S2 and N antigens from each of the four sample types. Sample matrix equivalency was demonstrated by qualitative percent agreement of the results from fingerstick, plasma, serum, and venous whole blood samples. The qualitative agreement of the fingerstick whole blood results was greater than 95% with all combinations of sample matrices for all analytes as shown in Table 7 below. Based on the robust sample equivalency results, the fingerstick sample data was used for analysis of the longitudinal study.

Table 7. Matrix Equivalency Study Results			
Sample Type	**% Agreement**		
	S1 – Fingerstick	**S2 – Fingerstick**	**N – Fingerstick**
Plasma	98.7% (546/553)	97.6% (540/553)	96.2% (532/553)
Serum	98.4% (544/553)	96.7% (535/553)	95.5% (528/553)
Venous Whole blood	99.5% (550/553)	97.8% (541/553)	97.5% (539/553)

Cross-Reactivity

A study was performed to evaluate the effects of potentially cross-reacting disease state serum or plasma samples when tested on the Sofia 2 SARS-CoV-2 Antibody IgG FIA test. Each sample was tested once in the assay and summarized below in Table 8. The Percent Positivity (% Positivity) is calculated and reported. For all of the disease state samples tested (n=109) and evaluated by Sofia 2 SARS-CoV-2 Antibody IgG FIA, the percent positivity for N was 0.9% (1/109), the percent positivity for S1 was 0% (0/109), and the percent positivity for S2 was 0.9% (1/109) indicating a high level of specificity for each antigen with all disease state samples that were tested.

Table 8. Cross Reactivity Study Results									
Disease State		**# of samples**	**N**		**S1**		**S2**		**% Positivity**
			Neg	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	
Seasonal Coronavirus	anti-229E (alpha Coronavirus)	23	23	0*	23	0	23	0	0%
	anti-NL63 (alpha Coronavirus)	16	16	0	16	0	16	0	0%
	anti-OC43 (beta Coronavirus)	22	22	0*	22	0	22	0	0%
	anti-HKU1 (beta Coronavirus)	17	17	0*	17	0	17	0	0%
Antinuclear Antibodies (ANA)		5	5	0	5	0	5	0	0%
Chronic Fatigue Syndrome (CFS)		5	5	0	5	0	5	0	0%
CMV IgG/IgM		10	9	1*	10	0	10	0	10%
EBV IgG/IgM		10	10	0	10	0	10	0	0%
Influenza A/B		15	15	0*	15	0	14	1*	6.7%
Haemophilus Influenzae type B		6	6	0	6	0	6	0	0%
Fibromyalgia		5	5	0	5	0	5	0	0%
HIV		5	5	0	5	0	5	0	0%
Lupus		5	5	0	5	0	5	0	0%
Multiple Sclerosis		5	5	0	5	0	5	0	0%
Parvo B19 IgM		5	5	0	5	0	5	0	0%
Rheumatoid Factor		5	5	0	5	0	5	0	0%
Syphilis		5	5	0	5	0	5	0	0%

*There were samples that initially tested positive for Seasonal Coronavirus (N), CMV (N), and Influenza A/B (N and S2). All of the discrepant samples were re-tested with 10 more replicates to confirm the results. The Seasonal Coronavirus sample (containing antibodies to 229E, OC43, and HKU1) was confirmed as negative for N during retest in the Sofia 2 assay (10/10 replicates negative). The CMV sample was confirmed as positive for N during retest in the assay (1/10 replicates positive). The Influenza A/B sample that initially tested positive for N was confirmed negative for N during retest in the assay (10/10 replicates negative). The Influenza A/B sample that initially tested positive for S2 was confirmed as positive for S2 during retest in the assay (5/10 replicates were positive). For each sample, it is counted as positive or negative in the summary table above based on the outcome of this retesting.

Interfering Substances:

The potential interference of endogenous and exogenous substances that may be found in serum, plasma, or whole blood specimens were tested with the Sofia 2 SARS-CoV-2 Antibody IgG FIA. The potentially interfering substances were diluted to the final test concentrations in venous whole blood (negative condition) or plasma (low positive samples) listed below and tested in replicates of 10. The substances tested at their initial concentrations did not interfere in the assay (either negatively or positively) with the exception of bilirubin and hemoglobin. Bilirubin interfered at concentrations above 10 mg/dL. Hemoglobin interfered at a concentration above 500 mg/dL. The substances and concentrations listed below in Table 9 did not interfere with the performance of the SARS-CoV-2 Antibody IgG FIA.

Table 9. Non-interfering Substances

Substance	Concentration
Acetaminophen	156 µg/mL
Bilirubin	20 mg/dL
Cholesterol	400 mg/dL
Hemoglobin	500 mg/dL
Ibuprofen	219 µg/mL
Triglycerides	1500 mg/dL

ASSISTANCE

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

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Switzerland	0 800 554864	
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REF 20375 – Sofia 2 SARS-CoV-2 Antibody IgG FIA – 25 Test
20379 – Sofia 2 SARS-CoV-2 Antibody IgG FIA – 25 Test

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1443800EN00 (08/21)

REF Catalogue number	CE CE mark of conformity
EC REP Authorized representative in the European Community	LOT Batch code
 Use-by date	 Manufacturer
 Temperature limitation	 Consult instructions for use
IVD *In vitro* diagnostic medical device	 Keep away from sunlight
 Contains sufficient for <n> tests	CONTROL + Positive control
CONTROL − Negative control	