INTENDED USE
The Sofia 2 SARS Antigen FIA is a lateral flow immunofluorescent sandwich assay that is used with the Sofia 2 instrument intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal (NP) and nasal (NS) swab specimens directly or after the swabs have been added to viral transport media from individuals who are suspected of COVID-19 by their healthcare provider. 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 SARS Antigen FIA does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is 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 positive results to the appropriate public health authorities.

Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary for patient management. Negative results do not rule out COVID-19 and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. 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.

The SARS Antigen FIA is intended for use by trained clinical laboratory personnel and individuals trained in point of care settings. The SARS Antigen FIA is only for use under the Food and Drug Administration’s Emergency Use Authorization.

The Sofia 2 SARS Antigen FIA is intended for use by medical professionals or trained operators who are proficient in performing tests using the Sofia 2 Instrument. The Sofia 2 SARS Antigen FIA test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

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

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

PRINCIPLE OF THE TEST
The Sofia 2 SARS Antigen FIA employs immunofluorescence technology in a sandwich design that is used with Sofia 2 to detect nucleocapsid protein from SARS-CoV 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 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): Monoclonal anti-SARS antibodies
- Reagent Tubes (25): Lyophilized buffer with detergents and reducing agents
- Reagent Solution (25): Ampoules with salt solution
- Sterile Nasal Swabs (25)
- Small, Clear 120 µL Fixed Volume Pipettes (25)
- SARS Positive Control Swab (1): Swab is coated with non-infectious recombinant SARA 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
Vortex
1.0-mL Calibrated Micropipette with pipette tips
Nylon flocked nasopharyngeal swab
Polyester wound swab
Viral Transport Media
Calibration Cassette (supplied with the Sofia 2)

WARNINGS AND PRECAUTIONS
- For in vitro diagnostic use.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- 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.
- To obtain accurate results, use the Viral Transport Media (VTM) recommended in this Package Insert.
- When collecting a nasal swab sample, use the Nasal Swab supplied in the kit.
- When collecting a nasopharyngeal swab sample, use a nylon flocked nasopharyngeal swab.
- Use the appropriate Fixed Volume Pipette in accordance with test procedures.
- 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 so as 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.
- 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: 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); technicalessupport@quidel.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 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.

1. 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 SARS Positive Control Swab contains 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.

To collect a nasal swab sample, carefully insert the swab (provided in the kit) into the nostril that presents the most secretion under visual inspection. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then remove it from the nostril.

Nasopharyngeal Swab Sample

Use a nylon flocked nasopharyngeal swab, not supplied.

To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril that presents the most secretion under visual inspection. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx. Rotate the swab several times then remove it from the nasopharynx.

SAMPLE TRANSPORT AND STORAGE

Samples should be tested as soon as possible after collection. Based on data generated with influenza virus, nasal or nasopharyngeal swabs are stable for up to 24-hours at room temperature or 2° to 8°C.

If transport of samples with viral transport medium (VTM) is required, minimal dilution of the sample is recommended, as dilution may result in decreased test sensitivity. Whenever possible, 1 milliliter or less is best to avoid excessive dilution of the patient sample. While holding the swab, remove the cap from the tube. Insert the swab into the tube until the breakpoint is level with the tube opening. Bend the swab shaft at a 180 degrees angle to break it off at the breaking point. You may need to gently rotate the swab shaft to complete the breakage. Based on data generated with influenza virus, nasal or nasopharyngeal swabs in VTM are stable for up to 72-hours at 2° to 8°C.

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

**CDC Media/ Viral Transport Media 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. Also ensure that the liquid sample is at **room temperature** before proceeding.

2. Mix the specimen received in viral transport media by vortexing the tubes for 5 seconds.

3. Fill a calibrated micropipette with **250 µL** of patient sample from the viral transport media (VTM).

   **NOTE:** There is no need to dispense any reagent solution into the reagent tube. The patient sample in VTM will be used to rehydrate the reagent tube.

   **NOTE:** To obtain accurate results, avoid mucoid substances when filling the micropipette with patient sample in VTM.

4. Empty the contents of the micropipette into the Reagent Tube.

   **NOTE:** Once the sample is added to the Reagent Tube, **vortex the tubes for 5 seconds** prior to adding the sample to the Test Cassette.

5. Fill the provided **Small, Clear 120 µL Fixed Volume Pipette** with patient sample from the Reagent Tube, by slowly releasing pressure on the bulb.
6. Firmly squeeze the top bulb to empty the contents of the Small, Clear 120 µL Fixed Volume Pipette into the Test Cassette sample well. Extra liquid left over in the overflow bulb should be left behind. Discard the Pipette in your biohazard waste.  

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

7. Promptly proceed to the next section, “Using Sofia 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 past this time may result in false results.

**Tips for Batch Testing**

Depending on the workload, several options exist to make batch testing easier. The user can add the Reagent Solution to one or more Reagent Tubes, recap them, and store them on the bench at room temperature (RT) for up to 12 hours without loss of activity before adding the sample(s).

**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 rescans 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 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 positive result for SARS.

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

- The contents of this kit are to be used for the qualitative detection of SARS antigens from nasal swab and nasopharyngeal swab.
- This test detects both viable (live) and non-viable, 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.
- Negative test results are not intended to rule in other non-SARS viral or bacterial infections.
- Negative results should be treated as presumptive and confirmed with an FDA authorized molecular assay, if necessary, for clinical management, including infection control.
Clinical performance was evaluated with frozen samples, and performance may be different with fresh samples.

Specimen stability recommendations are based upon stability data from influenza testing and performance may be different with SARS-CoV-2. Users should test specimens as quickly as possible after specimen collection.

If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The Sofia 2 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the Sofia 2 SARS Antigen FIA (“your product” in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories1 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 SARS Antigen FIA” Instructions for Use. 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: ODL_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 and patient care settings 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

Study 1: Due to the limited availability of direct swabs, the clinical performance of the SOFIA 2 SARS Antigen FIA was established with a study using one hundred forty-three (143) previously characterized frozen NP swabs originally collected in 3-mL viral transport media.

<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2 Molecular</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>Sofia 2 SARS Antigen FIA Assay</td>
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<td>0</td>
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<tr>
<td>NEG</td>
<td>12</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>84</td>
</tr>
</tbody>
</table>

The assay demonstrated acceptable clinical sensitivity (80%) when compared to an EUA molecular device. The assay demonstrated excellent clinical specificity (100%). There was no demonstrable cross-reactivity with seventy-nine (79) specimens containing seasonal CoVs detected by the BioFire® FilmArray® Respiratory Panel (20-229E, 19-HKU1, 20-NL63, 20-OC43).

Study 2: A limited study using forty-eight (48) direct nasal swabs was performed. The samples were sequentially enrolled from four locations and tested fresh. The SOFIA 2 SARS Antigen FIA was compared to the Lyra SARS-CoV-2 Assay (EUA200016/A002), an extracted RT-PCR assay.

<table>
<thead>
<tr>
<th></th>
<th>Lyra SARS-CoV-2 Assay EUA200016/A002</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>Sofia 2 SARS Antigen FIA Assay</td>
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<td>0</td>
</tr>
<tr>
<td>NEG</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>43</td>
</tr>
</tbody>
</table>

Supportive Contrived Performance

Study 3: In this study, thirty NP swabs were spiked with approximately 50-µL of a virus dilution. Twenty specimens were spiked with 1x LoD (TCID50 1.13 x10² mL) of virus. Ten additional specimens were spiked with 5x LoD (TCID50 5.65 x10² mL) of virus. The spiked swabs were added to the Sofia 2 SARS Antigen FIA extractant concurrently to thirty NP swabs from thirty different individuals. Forty-seven negative NP swabs were also tested. The swabs were processed concurrently according to the Sofia 2 SARS Antigen FIA package insert. The table below presents the data for Study 2:
Clinical evaluation in spiked nasopharyngeal swab specimens

<table>
<thead>
<tr>
<th>Sample SARS-CoV-2 Concentration</th>
<th># Positives/# Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>unspiked</td>
<td>0/47</td>
</tr>
<tr>
<td>1.0x LoD</td>
<td>20/20</td>
</tr>
<tr>
<td>5x LoD</td>
<td>10/10</td>
</tr>
</tbody>
</table>

Performance against the expected results are:
Positive Percent Agreement 30/30 = 100% (95% CI: 88.6%-99.4%)
Negative Percent Agreement 47/47 = 100% (95% CI: 92.4%-100%)

ANALYTICAL PERFORMANCE

LIMIT OF DETECTION

a) *Limit of Detection (LoD):*

The Limit of Detection (LoD) of the Sofia 2 SARS Antigen FIA was determined using limiting dilutions of heat-inactivated SARS-CoV-2 (bei Resources NR-52286) in two separate studies. The NR-52286 is a preparation of SARS-Related Coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, that has been inactivated by heating at 65°C for 30-minutes. The material was supplied frozen at a concentration of TCID\textsubscript{50} of 3.40 x 10\textsuperscript{5} per mL.

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

The second study to determine the Sofia 2 SARS Antigen FIA LoD was designed to reflect the assay when using NP swabs inoculated into UTM. In this study a NP swab was spiked with approximately 50-µL of the virus dilution in saline. The spiked swab was added to 3.0-UTM containing NP matrix. The inoculated UTM was tested according to the Sofia 2 SARS Antigen FIA PI.

The LoD was determined in three steps:

1. **LoD Screening**
   10-fold dilutions of the heat inactivated virus were made in saline and processed for each study as described above. These dilutions were tested in triplicate. The concentration demonstrating 3 of 3 positives was chosen for LoD range finding.

   Based on this testing, the concentration chosen for Study 1 (the swab) was TCID\textsubscript{50} of 3.40 x 10\textsuperscript{2} per mL, the concentration chosen for Study 2 (the swab in UTM) was TCID\textsubscript{50} of 8.50 x 10\textsuperscript{2} per mL.

2. **LoD Range Finding**
Study 1 - Five (5) doubling dilutions were made of the TCID₅₀ of 3.40 x10² per mL concentration in saline processed for the study as described above. These dilutions were tested in triplicate. The concentration demonstrating 3 of 3 positives was chosen for LoD confirmation.

Based on this testing the concentration chosen was TCID₅₀ of 1.13 x10² per mL.

Study 2 - Five (5) doubling dilutions were made of the TCID₅₀ of 8.50 x10² per mL concentration in saline processed for the study as described above. These dilutions were tested in triplicate. The concentration demonstrating 3 of 3 positives was chosen for LoD confirmation.

Based on this testing the concentration chosen was 8.50 x10² per mL.

3. LoD Confirmation

Study 1 - The concentration TCID₅₀ of 1.13 x10² per mL dilution was tested an additional seventeen (17) times, for a total of twenty (20) results. Twenty (20) of twenty (20) results were positive.

Study 2 - The concentration TCID₅₀ of 8.50 x10² per mL dilution was tested an additional seventeen (17) times, for a total of twenty (20) results. Twenty (20) of twenty (20) results were positive.

Based on this testing the concentration was confirmed as:
Swab LoD: TCID₅₀ 1.13 x10² per mL
Swab in 3.0-mL LoD: TCID₅₀ 8.50 x10² per mL

b) Cross-Reactivity:

Cross-reactivity of the Sofia 2 SARS Antigen FIA was evaluated by testing various microorganisms (8), viruses (16) and negative matrixes (3) that may potentially cross-react with the Sofia 2 SARS Antigen FIA. Each organism and virus were tested in triplicate. The final concentration of the organisms and viruses are documented in the Table below.

<table>
<thead>
<tr>
<th>Virus/Bacteria/Parasite*</th>
<th>Strain</th>
<th>Source/ Sample type</th>
<th>Concentration</th>
<th>Results**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Type 1</td>
<td>Isolate</td>
<td>1 x 10⁵.⁵³ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>229e</td>
<td>Isolate</td>
<td>1 x 10⁵.¹⁰ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>OC43</td>
<td>Isolate</td>
<td>9.55 x 10⁵ TCID₅₀/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>NL63</td>
<td>Isolate</td>
<td>5 x 10¹.⁶⁷ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>MERS-CoV (heat-inactivated)</td>
<td>Florida/USA-2_Saudia Arabia_2014</td>
<td>Isolate</td>
<td>1.17 x 10⁵ TCID₅₀/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>*Mycoplasma pneumoniae</td>
<td>M129</td>
<td>Isolate</td>
<td>3 x 10⁶ CCU/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Z018</td>
<td>Isolate</td>
<td>3.8 x 10⁶ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Influenza A H3N2</td>
<td>Brisbane/10/07</td>
<td>Isolate</td>
<td>1 x 10⁴.⁶⁷ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Influenza A H1N1</td>
<td>New Caledonia/20/99</td>
<td>Isolate</td>
<td>1 x 10⁵.⁶⁶ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Brisbane/33/08</td>
<td>Isolate</td>
<td>1 x 10⁵.¹⁵ U/mL</td>
<td>Negative</td>
</tr>
</tbody>
</table>
**Cross-Reactivity: Sofia 2 SARS Antigen FIA - Wet Testing**

<table>
<thead>
<tr>
<th>Virus/Bacteria/Parasite*</th>
<th>Strain</th>
<th>Source/ Sample type</th>
<th>Concentration</th>
<th>Results**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parainfluenza</td>
<td>Type 1</td>
<td>Isolate</td>
<td>$1 \times 10^{5.01}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 2</td>
<td>Isolate</td>
<td>$1 \times 10^{5.34}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 3</td>
<td>Isolate</td>
<td>$8.5 \times 10^{5}$ TCID&lt;sub&gt;50&lt;/sub&gt;/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 4b</td>
<td>Isolate</td>
<td>$1 \times 10^{5.53}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Type 68</td>
<td>Isolate</td>
<td>$1 \times 10^{5.5}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>A1 (IA10-s003)</td>
<td>Isolate</td>
<td>$1 \times 10^{5.55}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>Type A (3/2015 Isolate #3)</td>
<td>Isolate</td>
<td>$1 \times 10^{5.62}$ U/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Human Rhinovirus</td>
<td>N/A</td>
<td>Inactivated virus</td>
<td>Not available</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Chlamydia phila pneumoniae</em></td>
<td>AR-39</td>
<td>Isolate</td>
<td>$2.9 \times 10^{6}$ IFU/mL</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Type b; Eagan</td>
<td>Isolate</td>
<td>$7.87 \times 10^{6}$ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Philadelphia</td>
<td>Isolate</td>
<td>$6.82 \times 10^{6}$ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Z022; 19f</td>
<td>Isolate</td>
<td>$2.26 \times 10^{6}$ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>A639</td>
<td>Isolate</td>
<td>$6.37 \times 10^{6}$ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Pneumocystis jiuroveci-S. cerevisiae Recombinant</em></td>
<td>W303-Pji</td>
<td>Isolate</td>
<td>$1.56 \times 10^{6}$ cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative Nasal Matrix</td>
<td>UTM</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative Nasal Matrix</td>
<td>CDC Viral Transport</td>
<td>N/A</td>
<td>N/A</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Coronavirus HKU1 was not tested for cross-reactivity due to lack of availability. Given that the 19 specimens in the clinical evaluation that were positive for this strain all resulted as negative, cross-reactivity wet testing was not required.

** Testing was performed in triplicate.

Based on the data generated by this study, the Sofia 2 SARS Antigen FIA does not cross-react with the organisms or viruses tested.

c) **Hook Effect:**

As part of the LoD study the highest concentration of heat-inactivated SARS-CoV-2 stock available (TCID<sub>50</sub> of $3.40 \times 10^5$ per mL) was tested. There was no Hook effect detected.

**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).

**REF** 20374 – Sofia 2 SARS Antigen FIA – 25 Test
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>CE</td>
<td>CE mark of conformity</td>
</tr>
<tr>
<td>EC REP</td>
<td>Authorized Representative in the European Community</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>?</td>
<td>Use by</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td>?</td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Intended use</td>
</tr>
<tr>
<td>Rx ONLY</td>
<td>Prescription use only</td>
</tr>
<tr>
<td>i</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>IVD</td>
<td>For In Vitro diagnostic use</td>
</tr>
<tr>
<td>Σ 25</td>
<td>Contains sufficient for 25 determinations</td>
</tr>
<tr>
<td>CONT</td>
<td>Contents/Contains</td>
</tr>
<tr>
<td>CONTROL +</td>
<td>Positive control</td>
</tr>
<tr>
<td>CONTROL -</td>
<td>Negative control</td>
</tr>
</tbody>
</table>
REFERENCES
i Baker, S., Frias, L., and Bendix, A. Coronavirus live updates: More than 92,000 people have been infected and at least 3,100 have died. The US has reported 6 deaths. Here's everything we know. Business Insider. March 03, 2020.
**Test Procedure**

All specimens must be at room temperature before testing.

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 / Oropharyngeal Swab)**

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.

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 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 sample.
   
   c) With the Pipette tip still in the sample, 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 in the overflow bulb is OK.

   NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of patient 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. Proceed to the “Using Sofia 2” section to complete the test.
**Liquid Specimens/ VTM Test Procedure**

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

2. Mix the specimen received in viral transport media by vortexing the tubes for 5 seconds.

3. Fill a calibrated micropipette with 250 µL of patient sample from the Viral Transport Media (VTM).

   **NOTE:** Note: There is no need to dispense any reagent solution into the reagent tube. The patient sample in VTM will be used to rehydrate the reagent tube.

   **NOTE:** To obtain accurate results, avoid mucoid substances when filling the micropipette with patient sample in VTM.

4. Empty the contents of the micropipette into the Reagent Tube.

   **NOTE:** Once the sample is added to the Reagent Tube, vortex the tubes for 5 seconds prior to adding the sample to the Test Cassette.

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

   **NOTE:** Do not pour sample from the Reagent Tube. Use the provided Small, Clear 120 µL Fixed Volume 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 in the overflow bulb is OK.

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

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

WALK AWAY/READ NOW Modes

Refer to the Sofia 2 User Manual.

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 and negative test results will be displayed at 15 minutes.

### READ NOW Mode

The user first places the Test Cassette onto the counter or bench top for 15 minutes (outside of Sofia 2). The user manually times this development step. The Test Cassette MUST remain on the bench for 15 minutes to get an accurate result. 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 past this time may result in false results.

### RUN TEST

1. Input the User ID with the integrated barcode scanner or manually enter the data using the key pad.  
   **NOTE:** If you scan the wrong barcode, re-highlight the field using the touchscreen on Sofia 2. The previous one will be overwritten with the right barcode.

2. Input the Patient ID and Order # (if applicable) with the integrated barcode scanner or manually enter the data using the key pad.

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

4. Insert the Test Cassette into the drawer. Then gently close the drawer.

5. Sofia 2 will start automatically and display the progress. In WALK AWAY Mode, the test results will be displayed in approximately 15 minutes. In READ NOW Mode, the test results will be displayed within 1 minute. See Sofia 2 Interpretation of Results section.
**Sofia 2 Interpretation of Results**

When the test is complete, the results will be displayed on the Sofia 2 screen. Test Lines will not be visible to the naked eye.

**Results:** The Sofia 2 screen will display results for the procedural control as being ☑️ or ☐️. If the control is ☐️, retest with a new patient sample and new Test Cassette.

<table>
<thead>
<tr>
<th>Reader Display</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS: ☑️ Procedural Control: ☑️</td>
<td>Positive Test for SARS (SARS antigen present)</td>
</tr>
<tr>
<td>SARS: ☐️ Procedural Control: ☑️</td>
<td>Presumptive Negative Test for SARS (no SARS antigen detected)</td>
</tr>
<tr>
<td>SARS: ☐️ Procedural Control: ☐️</td>
<td>Result Invalid</td>
</tr>
</tbody>
</table>

**Sofia 2 External Quality Control** *(External Positive and Negative Swabs are supplied in the kit)*

1. From the main menu, select Run QC.

2. Follow the prompts on the screen. Scan the QC Card (located on the kit box).

3. Sofia 2 prompts the user to select the desired mode (WALK AWAY or READ NOW).

4. Run the External Control Swabs.

5. Follow the Swab Test procedure of this Quick Reference Instructions. First test the Positive Swab, followed by the Negative Swab.

6. After both the Positive and Negative Swabs have been run, the results will be displayed as ☑️ or ☐️.

**INTENDED USE**

The Sofia 2 SARS Antigen FIA is a lateral flow immunofluorescent sandwich assay that is used with the Sofia 2 instrument intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal (NP) and nasal (NS) swab specimens directly or after the swabs have been added to viral transport media from individuals who are suspected of COVID-19 by their healthcare provider. 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 SARS Antigen FIA does not differentiate between SARS-CoV and SARS-CoV-2. Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is 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 positive results to the appropriate public health authorities. Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary for patient management. Negative results do not rule out COVID-19 and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. 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.

The SARS Antigen FIA is intended for use by trained clinical laboratory personnel and individuals trained in point of care settings. The SARS Antigen FIA is only for use under the Food and Drug Administration’s Emergency Use Authorization. The Sofia 2 SARS Antigen FIA is intended for use by medical professionals or trained operators who are proficient in performing tests using the Sofia 2 Instrument. The Sofia 2 SARS Antigen FIA test is only for use under the Food and Drug Administration’s Emergency Use Authorization.

**Reference the Package Insert for Warnings and Precautions, Limitations, Specimen Collection and Handling, and Study the Package Insert and User Manual thoroughly before using Quick Reference Instructions. This is not a complete Package Insert.**