CLIA Waiver by Application Approval Determination
Decision Memorandum

A. Document Number
CW230010

B. Parent Document Number
DEN220039

C. CLIA Waiver Type:
CLIA Waiver by Application

D. Applicant
Quidel Corporation

E. Proprietary and Established Names
Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set

F. Measurand (analyte)
Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

G. Sample Type(s)
Direct anterior nasal swab specimens

H. Type of Test
Qualitative lateral flow immunoassay

I. Test System Description

1. Overview

The Sofia 2 SARS Antigen+FIA is based upon a lateral flow technology that employs immunofluorescence technology in a sandwich design that is used with Sofia 2 to detect nucleocapsid protein from the SARS-CoV-2 virus in human anterior nasal swab specimens.

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. The Test Strip is composed of the following biochemical components dried and immobilized onto the nitrocellulose membrane: 1) sample pad that receives the specimen; 2) a label pad that contains detection fluorescent micro-particles, coated with monoclonal antibodies that are specific for SARS-CoV-2 nucleocapsid antigen; 3) embedded monoclonal antibodies specific for SARS-CoV-2 nucleocapsid antigen to capture the antigen-microparticle complex at the test line location. The sample pad facilitates migration of the sample fluid across the nitrocellulose strip into the absorbent pad (See Figure 1 below). The test strip also contains a desiccant that does not participate in the assay but serves as a stabilizing agent during storage.

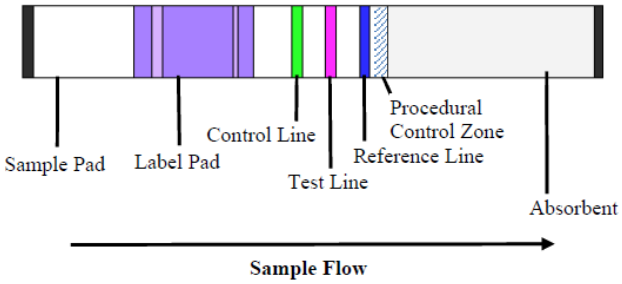

Figure 1: *Schematic of the Sofia 2 Antigen+ FIA Test Strip*

Sample is applied to in the sample well and migrates through a test strip, then passes through the test and control lines. If SARS-CoV-2 viral antigen is present, they will be bound by the fluorescent microparticles in the label pad region, forming an antigen-microparticle complex. The test line is coated with monoclonal antibodies that are specific to SARS-CoV-2 nucleocapsid antigen and is intended to capture the antigen-microparticle complex. If SARS-CoV-2 viral antigen is not present, the fluorescent microparticles will not be trapped by the capture antibodies nor detected by Sofia 2.

The Sofia 2 SARS Antigen+ FIA employs antibody-tagged microparticles dyed with a fluorescent compound, to be detected and read by the Sofia 2 reader instrument. The Sofia 2 analyzers automatically scan/image the test strip, collect and analyze the fluorescence data, and then calculate and report the result as either positive, negative, or invalid.

Additionally, the Sofia 2 Antigen+ FIA utilizes a reference line for the Sofia 2 reader (to locate the test line and negative control line) and a procedural control (to assess for sample presence and adequate sample flow). No colored lines will be visible in the test window of the fluorescent assay cassette, thereby preventing visual interpretation of the test results. The operator must use the Sofia 2 analyzer to obtain a test result.

The Sofia SARS Antigen FIA Control Swabs are intended to be used as quality control samples representative of positive and negative test samples, to demonstrate that the reagents are functional and that the assay procedure is correctly perform.

This specific test device was de novo granted in DEN220039. Additionally, the Sofia 2 instrument platform was previously cleared (in K211342 and K173496) and CLIA waived (in CW170015, CW170009, CW210001, and CW160016). This current submission is to obtain a CLIA waiver for the DEN220039 test device, the Sofia 2 Antigen+ FIA.

2. Test System Components

The assay kit contains all materials needed to run the test, including external controls. For a 25-test kit, this includes:

- Individually Packaged Test Cassettes (25): containing monoclonal anti-SARS antibodies

- Pre-filled Reagent tubes (25): Buffer with detergents, reducing agents, and Proclin 300
- Dropper Tips (25)
- Sterile Nasal Swabs (25)
- SARS Positive Control Swab (1): Swab is coated with non-infectious recombinant SARS antigens
- Negative Control Swab (1): Swab is coated with heat-inactivated, non-infectious Streptococcus C antigen
- QC Card (located on the kit box)

Table 1. Kit Components & Other Materials/Information

Kit Components
Test Cassette with test strip
Negative control swab
Positive control swab
Dropper Tips
Pre-filled Extract Reagent: • Extraction Reagent Solution • Extraction Reagent Vial

Sofia 2 analyzer instrument and the Calibration Cassette are not included with the assay kit but are required for operation of this test. They are available for purchase separately.

J. Demonstrating "Simple"

Table 2. Demonstration of Simplicity for the Sofia 2 Antigen+ FIA

"Simple" Criteria	**Device Characteristics**
Is a fully automated instrument or a unitized or self-contained test.	The device is fully automated and only requires sample incubation in the extraction reagent prior to sample application.
Uses direct unprocessed specimens, such as capillary blood (fingerstick), venous whole blood, nasal swabs, throat swabs, or urine.	The test uses direct anterior nasal swab specimens.
Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination.	• An untrained operator can conduct the test by performing 6 simple steps without sample manipulation: 1) collect the anterior nasal swab, 2) incubate the sample swab in the extraction reagent, 3) express excess liquid from the swab by pinching the sides of the tube, 4) attach the dropper cap to the tube, 5) apply 4 drops of the sample to the cartridge, and then 6) load the sample cartridge into the Sofia 2 analyzer.

	• No specialized equipment is needed for sample processing.
Needs only basic, non-technique-dependent reagent manipulation, such as "mix reagent A and reagent B."	• The test requires only basic reagent handling to obtain accurate test results. No processing of reagents is needed prior to combining test reagent and sample. • The provided reagent is premeasured and provided in single-use vials. • The test cartridges are unitized and contain all the reagents required for analysis. • The test cartridges are keyed and can be inserted into the analyzer only in one direction.
Needs no operator intervention during the analysis steps.	• The test does not require any operator intervention during the analysis step. • The Sofia 2 analyzer performs automated analysis of test results and eliminates subjectivity associated with visual reading of results by the end-user. • The Sofia 2 touchscreen is designed for ease of use and features a color display that facilitates easy-to-read messages. • After insertion of the sample cartridge into the Sofia 2 analyzer and the test run initiated, the test does not require any operator intervention during the analysis step.
Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.	• Error messages are unambiguous and include easy-to-interpret solutions. • No complex trouble-shooting or interpretation of error codes are required to operate Sofia 2.
Needs no electronic or mechanical maintenance beyond simple tasks, e.g., changing a battery or power cord.	• There is no maintenance required other than wiping of the external surface of the analyzer. • System Control Checks for temperature are built-in to ensure the instrument is operating within validated heating and cooling specifications. • There are no serviceable parts and the instrument is to be returned to Quidel if maintenance is required.
Produces results that require no operator calibration, interpretation, or calculation.	Calibration, which is required every 30 days, is easily performed with a provided calibration cassette.
Produces results that are easy to determine, such as 'positive' or 'negative,' a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations.	Interpretation of results is automated. Results are displayed on the instrument screen as positive, negative or invalid and no additional interpretation or calculations are required.
Contains a quick reference instruction sheet that is written at no higher than a 7th grade reading level.	The test procedure is written at a 7th grade comprehension level.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

   Risk Management of the Sofia 2 SARS Antigen+ FIA and the Sofia 2 analyzer has been conducted in accordance with ISO 14971 and the FDA guidance, "*Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices*".

   The sponsor utilized the Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods to assess the risks of failure that may occur during use or misuse of the device. The FMEA includes use-related (i.e., influenced by the user) failure modes associated with incorrect diagnosis, invalid results, unable to run test, and no result. The use-related FMEA evaluates potential failures that may be caused by the user during use or misuse of the product. Elements considered include the intended user, environment (e.g., Physician Office, Lab, Hospital), human factors/potential human errors, and historical field data from similar devices.

   Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. The following safety risk control methods were used to eliminate or reduce the probability of occurrence of identified safety hazards:

   - Design – Incorporate design decisions to ensure a fail-safe architecture, including providing redundant hardware or selection of high reliability components to minimize potential safety risks
   - Manufacturing procedures – Procedures that are used in the manufacturing process are compliant with applicable quality system regulations to ensure product safety and efficacy
   - Testing and verifications – Specific device test or verification activities to verify product performance of in-process and final released product consistent with the cleared performance described in the labeling.
   - Labeling / Instructions for use – Develop external labels for the device and instructions for use that define intended use and required operational use procedures and contra-indicate improper device use.

   The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (Section K.3).

2. Fail-Safe and Failure Alert Mechanisms

   The Sofia 2 was designed to include safety features based on design control measures and risk analysis. Sofia 2 includes an audio alert system for informing the user of warnings and errors. A summary of failures, alerts, and mitigations incorporated into the design of Sofia 2 are in this document (Table 3).

Table 3. Sofia 2 Fail-Safe Features

#	Feature	Function

1	Temperature Sensor	Monitors instrument ambient temperature: • If the internal temperature sensor is lower than 15°C or higher than • 33.5 on power up, the Reader shall display a temperature error • Locks operation if ambient outside specified instrument range • Locks specific assays if temperature outside range defined in assay method.

2	Assay Cassette 2-D Barcode	• Determine assay method automatically
		• A test cassette with no associated test type file shall not be scanned and user shall be notified.
		• Determines expiry date; Sofia 2 will not run a test if:
		  ○ If the cassette was used before
		  ○ If the test cassette is expired
		•

3	Calibration Cassette 2-D Barcode	• When a calibration fails, the Reader shall set to a lockout mode which disables patient tests and QC tests • Prior to running a calibration, the reader shall check to be sure the calibration cassette is not expired. • Determines expiry date & will not run calibration if expired. • When a CM-4 cassette is verified, the firmware shall determine the position of line 1 • When a CM-4 cassette is verified, the firmware shall determine from the cassette barcode the expected values of the fluorescent line • The Analyzer shall read the x-offset from the calibration cassette barcode • The Analyzer shall change the x-offset according to what was read from the calibration cassette barcode
4	User Access Control	3 levels of user (Operator, Supervisor, Quidel Only Service): • Entry to the Service and Supervisor modes shall require separately designated ID codes. Service mode is accessible by Quidel only.
5	Calibration	• Displays “Calibration Due” warning when < 24 hrs. • Prevents running patient tests and QC if calibration interval exceeded. • Locks instrument if certain adjustment limits are exceeded or if calibration cassette expired.
7	Software Error Detection - Power on self-test (POST)	• If power on self-test (POST) fails, the reader shall display the error code and display that the instrument is unable to be used. • If there is a POST failure, the reader shall log the failure item in the error log. • If POST detects that no measurements may be performed, all scanning testing functions shall be disabled.
8	“Soft” Power Switch	During a test run, attempting to power off with the power switch shall cause a message to appear asking the user to cancel the test first, then power off.
9	Accidental loss of AC power	Sofia 2 includes a rechargeable battery. The battery will automatically charge while plugged into AC power. Sofia 2 will automatically switch to battery power when AC power is not available.

10	Cassette Orientation	When the drawer is closed and no readable cassette is present, the Reader displays "Cassette Error."
11	Quality Control	• While running the QC protocol, if the user inserts a control cassette that is expired, the Reader shall not run the test and warn the use the cassette has expired • While running the QC protocol, if the user inserts a control cassette that is a different test type than the QC data card, the Reader shall warn the user that the cassette is the wrong test type • The expected value of a control shall be determined from a 1-D barcode on the control container • The tolerance of expected control value to measured control value shall be determined from a 1-D barcode on the control container • The System shall print measured QC value and Pass / Fail Status • The Analyzer shall display measured QC value and pass / fail status

Built-in Procedural Control

- The Control Line is the first line that the extracted specimen encounters and acts as a filter to prevent non-specific binding downstream in the test line and reference line formation areas of the test strip.
- The Reference Line is used for determination of the orientation of the test strip and the locations of Test Line and Procedural Control Zone in the test strip. The Reference Line is the last line that the extracted specimen encounters before it enters the absorbent pad at the end of the test strip. The analyzer images the strip and uses specific algorithms to validate the peaks and locate the exact position of the Reference Line within the range.
- The Procedural Control Zone is designed to control for the flow of reagents and must produce a signal within the predetermined specifications, otherwise the test will be reported as "invalid." For a valid test result the reagents must flow to the end of the test strip and produce a minimum fluorescent signal in the Procedural Control zone.

Excessive Lighting Check

- Prior to each test run, a dark image is captured by Sofia 2 and analyzed for excessive ambient lighting. If the number of pixels obtained from the dark image exceeds the instrument specifications, Sofia 2 will generate an internal error and the test cannot continue.

External Controls

Positive and negative controls will be provided in each Sofia 2 SARS Antigen+ FIA kit, but can also be obtained as a separately distributed kit. The control set will consist of:

- One SARS positive control swab: the SARS positive control swab is coated with non-infectious recombinant SARS antigen.
- One negative control swab: the negative control swab is coated with heat-inactivated, non-infectious Streptococcus C antigen.

The amount of antigen in the positive control is set to give a high positive signal on the Sofia 2 SARS Antigen+ FIA device. The positive control swab must be run first, followed by the negative control swab. When the Quality Control run is complete, each

result will be displayed as "Passed" or "Failed" on Sofia 2, for the Positive Control and the Negative Control.

3. Flex Studies
   The operational limits of the Sofia 2 SARS Antigen+ FIA performed on the Sofia 2 analyzer were evaluated in a series of experiments under conditions of "stress." The studies to support the CLIA Waiver Application for Sofia 2 SARS Antigen+ FIA are listed in Table 4.

Table 4. Summary of Flex Studies Performed

Procedural Step	**Potential Use Error**	**Potential Hazard**	**Flex Study and Verification Results**
NA	Testing materials and Sofia analyzer operating outside of recommended temperature and humidity levels.	False Negative, Invalid.	Operating Environment Flex Study demonstrates that operation of the test kit at a wide range of temperatures (15°C to 30°C) and humidity conditions (5% to 85%) does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA.
Do not open the Test Cassette until you are ready to perform the test.	Cassette removed from pouch prematurely exposing strip to moisture / environmental elements, which caused incomplete flow.	False Negative, False Positive, Invalid.	Open Pouch Flex Study demonstrates that open pouch time ranging from 0 minutes up to 2.5 hours does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA.
Insert the swab into the Reagent tube.	User runs samples not at room temperature.	False Negative; Invalid.	Sample Temperature Flex Study demonstrates that potential use error related to sample temperature does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA.
Leave the swab in the Reagent tube for 1 minute. Remove the Swab.	User does not leave the swab in the Reagent Tube containing Reagent Solution for 1 minute	False Negative.	Extraction Time Flex Study demonstrates that variations in the extraction time ranging from < 30 seconds to 60 minutes (<0.5X to 60X the required extraction time) does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA. The study also demonstrates that up to 1 hour delay in applying the extracted sample to the test cassette does not impact the performance of Sofia 2 SARS Antigen+ FIA. The failure mode observed with too much delay before adding the extracted sample to the test cassette for analysis resulted in decreased detection of positive samples.

Add Three (3) drops into the Test Cassette sample well.	Excessive volume of diluted sample is added to cassette.	False Negative.	Sample Volume Delivery Flex Study demonstrates that sample volumes ranging from 2 drops to 4 drops which could reasonably occur with miscounted number of drops does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA.
	Insufficient volume of diluted sample is added to cassette.	False Negative, False Positive, Invalid.	
Run test on Sofia 2.	Cassette developed on bench top before placing into analyzer – Read Now Mode. User runs the test on the Sofia 2 sooner or later than 10 mins.	False Negative, False Positive.	Development/Read Time and Test Result Stability Flex Study* demonstrates the test results for the Sofia 2 SARS Antigen+ FIA are not impacted by variation in cassette development time between 8 minutes to 30 minutes.

** The study summary and line data were submitted in Sofia 2 SARS Antigen+ FIA De Novo, DEN220039, granted March 8, 2023. Please refer to DEN220039, Section 018 Performance Bench, 18.7. Development/Read Time and Test Result Stability Flex Study.*

Samples used for flex study testing were prepared in negative clinical matrix (NCM), which was prepared by pooling negative nasal swabs collected from healthy individuals in saline. Nasal swabs were collected from a minimum of twenty healthy donors per batch. Each nasal swab was eluted in one mL of saline solution in a 15 mL polypropylene tube for a minimum of five minutes. The eluted respiratory samples in saline were pooled into a polypropylene container to formulate the stock NCM. The pooled sample was confirmed to be negative on the Sofia 2 SARS Antigen+ FIA. Contrived positive samples were prepared at 2X LoD by spiking NCM with heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020) to obtain a concentration of 2.88E+04 $TCID_{50}$/mL.

Except where indicated otherwise, the flex studies were all conducted utilizing ten Sofia 2 Analyzers were used. The negative and positive test samples (under the applicable flex conditions) were evaluated in replicates of five. Samples were tested according to the IFU protocol, except for the noted deviations dictated by the flex parameter under evaluation. All samples were tested on the Sofia 2 SARS Antigen+ FIA using the "Read Now" mode.

a) Operating Environment Flex Study

The purpose of this study was to evaluate the impact of operating the Sofia 2 SARS Antigen+ FIA at the extremes of the recommended temperature and humidity. The Sofia 2 analyzer and associated assays were intended to be operated at room temperature between 15°C and 30°C. The Sofia 2 analyzer includes temperature sensors and will prevent interpretation of assay results if the detected temperature is lower than 15°C or higher than 34.5°C. This study simulated the variable operating conditions of temperature and humidity reasonably expected to occur indoors near-patient and point-of-care facilities, including the extremes of recommended temperature.

Table 5. Operating Environment Conditions

Condition #	Operating Temperature	Operating Humidity
1	15±2°C	15 ± 10% RH
2	25±5°C	5 ± 10% RH
3	25±5°C	45 ± 10% RH
4	30±2°C	85 ± 10% RH

A minimum of five Sofia 2 Analyzers were used. All materials and components used in the test, including external control swabs, test sample aliquots, nasal swabs, reagent solution, reagent tubes, reagent tips, cassettes, and Sofia 2 analyzers were allowed to equilibrate to the pre-determined operating environment temperature and humidity levels for at least one hour prior to testing. 100% agreement to the expected negative and positive results were obtained at all environmental test conditions.

b) Open Pouch Flex Study

The purpose of this study was to evaluate the impact of delays in using the test cassette of Sofia 2 Antigen+ FIA after the pouch is opened. This study simulated the use error of leaving a cassette un-pouched for up to 2.5 hours before performing tests on Sofia 2 SARS Antigen+ FIA.

Table 7. Open Pouch Conditions

Condition #	Open Pouch Time points
1	**0 minutes (Control)**
2	1 hour
3	2 hours
4	2.5 hours

The general procedure was to delay using the test cassette of Sofia 2 Antigen+ FIA after the pouch is opened in 0 minutes, 1 hour, 2 hours, and 2.5 hours. Ten (10) Sofia 2 Analyzers were used. 100% agreement to expected results was obtained for all test conditions with cassettes un-pouched from 0-2.5 hours.

c) Sample Temperature Flex Study

The purpose of this study was to evaluate the impact of running samples on Sofia 2 SARS Antigen+ FIA that were not at room temperature before beginning the assay. The Sofia 2 SARS Antigen+ FIA Instructions for Use (IFU) state that all samples must be at room temperature before beginning the assay. This study simulated user error in testing a swab sample from refrigerated conditions without equilibrate to room temperature prior to testing.

Table 6. Sample Temperature Conditions

Condition #	Sample Temperature Condition
1 (control)	Cold sample equilibrated to ambient temperature
2	Cold sample removed directly from 2-8°C

The test samples were kept at 2-8 °C for at least 1 hour prior to testing. The test samples for Condition 1 were tested after being equilibrated at room temperature for at least 15 minutes. The test samples for Condition 2 were tested cold directly from refrigerated conditions 2-8°C. 100% agreement to expected results was obtained with cold samples either equilibrated to ambient temperature before testing (control) or used immediately from 2-8°C in testing.

d) Extraction Time Flex Study

The purpose of this study was to evaluate the impact of incorrect extraction time of the swab sample on the Sofia 2 SARS Antigen+ FIA. The IFU state to leave the swab in the Reagent Tube containing Reagent Solution for 1 minute. This study simulated use error by flexing the duration of time the swab was left in the tube (also called extraction time) below and above this control condition until failure was observed, where practicable.

Table 8. Extraction Time Test Conditions

	Condition #	**Time**
Extraction Time Flex (swab left in Reagent Tube)	1	Dip and Remove (1 to 3 seconds)
	2	<30 seconds
	3	30 seconds
	4	**1 minute (control)**
	5	5 minutes
	6	10 minutes
	7	20 minutes
	8	1 hour

The general procedure was to leave the testing swab in the extraction solution for different extraction time prior to loading the extracted sample into the sample well of the Sofia 2 SARS Antigen+ FIA cassette. All positive and negative samples tested under Condition #1 ("dip and remove") produced negative result. 100% agreement to expected results was obtained for all other test conditions (<30 seconds to 1 hour) in the extraction time flex.

e) Delay in Sample Testing (following extraction)

The purpose of this study was to determine the stability of the extracted sample *prior* to application on the Sofia 2 SARS Antigen+ FIA. The IFU state to add extracted sample into the Test Cassette sample well after pressing the dropper tip firmly into the Reagent Tube. This study simulated use error by flexing the delays in adding the extracted sample to the test cassette sample well after the swab has been removed and the dropper tip has been firmly pressed into the Reagent Tube.

Table 9. Delay in Sample Testing/ Extracted Sample Stability Conditions

	Condition #	**Time**
Delay in Sample Testing /	9	0 minute (control)
	10	10 minutes

Extracted Sample Stability (swab removed and dropper tip inserted)	11	30 minutes
	12	1 hour
	13	2 hours
	14	3 hours
	15	4 hours
	16	8 hours
	17	24 hours

The general procedure was to delay the time when adding the extracted sample to the test cassette sample well after the swab has been removed and the dropper tip has been firmly pressed into the Reagent Tube. 100% agreement to expected results was obtained for test conditions up to a 1-hour delay in adding the extracted sample to the test cassette. At 2 hours, variable results begin to appear, with 1 false negative results (out of 5 total positive samples). At 3 hours and later, false negative results were observed with the moderate positive sample, indicating instability of the extracted sample. The study results demonstrate that up to 1 hour delay in applying the extracted sample to the test cassette does not impact the performance of Sofia 2 SARS Antigen+ FIA.

f) Sample Volume Delivery Flex Study

The purpose of this study was to evaluate the impact of incorrect drops of sample delivered to the test cassette of the Sofia 2 SARS Antigen+ FIA. The IFU state to add 3 drops into the Test Cassette sample well. This study simulated use error by flexing the number of drops of sample added onto the test cassette sample well.

Table 10. Sample Volume Delivery Conditions

Condition	Sample Delivery Conditions
1	1 drop
2	2 drops
3	3 drops (control)
4	4 drops
5	5 drops

The general procedure was to add different volumes of the sample from the Reagent Tube on the Sofia 2 SARS Antigen+ FIA cassette sample well. 100% agreement to expected results was obtained for 2-4 drops of extracted sample.

- Invalid results occurred under Condition 1 (1 drop) with both the negative and positive samples. This appears to be the near or below minimum volume needed to flow through the test cassette.
- Invalid results (5/10) and false negative results (5/10) occurred with 5 drops of extracted sample added to the test cassette.

The study results demonstrate that sample volumes ranging from 2 drops to 4 drops does not adversely impact the performance of Sofia 2 SARS Antigen+ FIA.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparison Study

a. Study Design

i. Study Sites and Duration

The CLIA Waiver Clinical Performance Study was conducted to demonstrate the clinical performance of Quidel's Sofia 2 SARS Antigen+ FIA to detect SARS-CoV-2 viral nucleoprotein antigen in specimens obtained from nasal swabs. In this study, nasal swabs were prospectively collected from symptomatic subjects and tested on the Sofia 2 SARS Antigen+ FIA test and the comparator device. This was a multi-center, prospective study performed at six clinical sites; comparator samples were tested at one reference laboratory. Symptomatic subjects were enrolled and two nasal swabs were collected from each subject, within one minute or each other:

- One swab, was tested on the Sofia 2 SARS Antigen+ FIA by an untrained operator at the CLIA-waived site
- The second swab was shipped to a central reference laboratory and evaluated with an EUA authorized SARS-CoV-2 RT-PCR assay as the comparator method.

ii. Operators

There were a total of 12 operators representative of intended CLIA waived users across the six clinical testing sites, consisting of administrative personnel, medical assistants, nurses, research/study coordinators, administrative managers, and other patient care providers. All operators were employed in subject enrollment and sample collection, testing, and shipping for the CLIA Waiver clinical performance study. All operators employed in this CLIA Waiver clinical performance study, except two, had prior experience in CLIA waived settings. However, no operators herein had any prior experience in high-moderate complexity laboratory settings. Furthermore, no operators were previously trained in the use of the Sofia 2 SARS Antigen+ FIA test or operation of the Sofia 2 instrument and relied solely on the Package Insert, Quick Reference Instructions, User Manual, and QuickStart Guide. Upon completion of the study, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure (Section L.2).

iii. Instructions for Use

The Sofia 2 SARS Antigen+ FIA assay was performed in accordance with the assay procedure in the draft Package Insert, Sofia 2 User Manual and Quick Reference Instructions. Each site was provided with copies of these materials.

iv. Subjects (Patients)

Performance characteristics of the Sofia 2 SARS Antigen+ FIA were established during a prospective study conducted from August 2021 to April 2023. A total of 832 subjects were enrolled in the study.

Inclusion Criteria

1. Male or female subjects 2 years and older.
2. Individual is suspected of having a SARS-CoV-2 infection.
3. Individual is exhibiting symptoms characteristic of a possible COVID-19 viral respiratory illness, such as fever, cough, shortness of breath or difficulty breathing, fatigue, chills, muscle or body aches, headache, sore throat, new loss of taste or smell, congestion or runny nose, nausea or vomiting, or diarrhea.

Exclusion Criteria

1. Individual or parent/ guardian (if subject under 18 years old) is unable to understand and consent to participation.

v. Samples

Two nasal swabs were collected from each study subject during the same visit in a randomized manner. One swab was tested on the Sofia 2 SARS Antigen+ FIA by a CLIA-waived test operator at the site. The second swab was collected for testing with the comparator test, placed into a transport tube containing 3mL of Quidel Transport Medium (QTM), refrigerated (2-8°C), and shipped to the reference laboratory on ice packs on the same day. Upon receipt by the reference laboratory, the second nasal swab was tested on the chosen comparator method, within six days of receipt.

A total of 832 paired nasal swab specimens were enrolled in the study. Of those, nine specimens were excluded from the final analysis:

- One sample was excluded because the site personnel accidentally forgot to test Sofia2 SARS+.
- One samples was exlcuded because the site personnel was unable to perform Sofia2 Sars + Antigen Test due to running out of cassettes
- One sample was excluded as it was a duplicate of a previous smaple, logged in error.
- Six samples were excluded because of receiving an invalid result for Sofia 2. These samples were not retested.

Accordingly, a total of 823 nasal swab specimens were considered evaluable for the purpose of data analysis in the accuracy study.

vi. Comparative Method (CM)

The Sponsor utilized an EUA authorized SARS-CoV-2 RT-PCR as the comparator method. The chosen comparator is a RT-PCR test with high sensitivity, employs an RNA extraction and purification steps, was validated with clinical samples that contained an acceptable number of low positive samples and demonstrated acceptable performance.

b. Results and Analysis

i. Statistical Analysis of Comparison Study Results

The results from this CLIA Waiver Clinical Evaluation study demonstrate acceptable performance in a total of 823 evaluable subjects, comprising 133 positives and 690 negatives, with an overall sensitivity of 88.72% and a specificity of 99.28% (Table 11).

Table 11. Sofia 2 SARS Antigen+ FIA Performance Compared to an EUA authorized SARS-CoV-2 RT-PCR Assay

	EUA authorized SARS-CoV-2 RT-PCR Positive	EUA authorized SARS-CoV-2 RT-PCR Negative	Total
Sofia Positive	118	5	123
Sofia Negative	15	685	700
Total	133	690	823

- Positive Percent Agreement (PPA): 88.7% (118/133); 95% CI: 82.2% - 93.0%
- Negative Percent Agreement (NPA): 99.3% (685/690); 95% CI: 98.3% - 99.7%

Clinical performance was also stratified by each Operator (Table 12) and by each Site (Table 13).

Table 12. Clinical Study Operators – Performance per Operator

Site	Operator	n	TP	FP	FN	TN	PPA	NPA
1	1	27	5	0	1	21	83.3%	100.0%
	2	68	3	1	4	60	42.9%	98.4%
2	1	98	18	1	2	77	90.0%	98.7%
3	2	113	9	0	0	104	100.0%	100.0%
	3	18	2	0	1	15	66.7%	100.0%
4	1	45	5	0	0	40	100.0%	100.0%
	2	40	5	0	0	35	100.0%	100.0%
	3	46	4	1	0	41	100.0%	97.6%
	4	68	2	1	0	65	100.0%	98.5%
5	1	126	27	1	3	95	90.0%	99.0%
	2	24	5	0	1	18	83.3%	100.0%
6	1	150	33	0	3	114	91.7%	100.0%

Table 13. Clinical Study Sites – Performance per Site

Site	n	TP	FP	FN	TN	PPA	NPA
1	95	8	1	5	81	61.5%	98.8%
2	98	18	1	2	77	90.0%	98.7%
3	131	11	0	1	119	91.7%	100.0%
4	199	16	2	0	181	100.0%	98.9%
5	150	32	1	4	113	88.9%	99.1%
6	150	33	0	3	114	91.7%	100.0%

ii. Device Performance with Analyte Concentrations Near the Cutoff

The Sponsor conducted a Reproducibility study to evaluate the inter-site reproducibility of the Sofia 2 SARS Antigen+ FIA with a panel of test samples at three distinct laboratory sites in the hands of the intended user. This study was conducted at three distinct CLIA waiver sites using a coded panel contrived samples to demonstrate that personnel at these sites could perform the Sofia 2 SARS Antigen+ FIA consistently and correctly. This reproducibility study evaluated a panel of contrived positive samples, prepared at four levels of UV-inactivated SARS-CoV-2 in Negative Clinical Matrix (NCM): Negative, High Negative, Low Positive, and Moderate Positive (Table 14). Each site received ten panels, corresponding to five panels for each of two operators at each site. Each panel included four blinded samples, tested in duplicate on each of two cassette lots. Operators were untrained and reflective of the intended users of the Sofia 2 SARS Antigen+ FIA. The Sofia 2 SARS+ FIA test was performed according to the IUO Package Insert and IUO Quick Reference Instructions. Sofia 2 instruments were used in accordance with the User Manual.

- At Site 1, Operator 1 conducted testing for both the Reproducibility study and the CLIA waiver study. Operator 2 conducted testing in Reproducibility study and non-testing activities in CLIA waiver study.
- At Site 2, Operator 2 conducted testing for both the Reproducibility study and the CLIA waiver study. Operator 1 conducted testing for the Reproducibility study, and non-testing activities in CLIA waiver study.

Table 14. Reproducibility Study – Test Sample Panel

Spiking Concentrations	
Level	**Concentration**
Negative Sample	N/A
High Negative	6.46×10^2 $TCID_{50}$/mL; *(0.277x LOD)*
Low Positive	2.33×10^3 $TCID_{50}$/mL; *(1x LOD)*
Moderate Positive	6.99×10^3 $TCID_{50}$/mL; *(3x LOD)*

Overall, this reproducibility study comprised a total of 720 results, with 240 results tested per site (4 levels x 2 operators x 5 days x 2 replicates x 3 lots = 240 samples). The overall qualitative and quantitative results are summarized in are shown below in Table 15-20. In summary:

- Out of 720 samples tested, no invalid test results obtained.
- True Negative specimens were called negative 100% (180/180) of the time.
- High Negative samples (0.277x LoD) were called negative 48.3% (87/180) of the time.
- Low Positive samples (1x LoD) were called positive 99.4% (179/180) of the time
- Moderate Positive samples (3x LoD) were positive 99.4% (179/180) of the time.

Table 15. Reproducibility Performance by Operator and Site.

Site	Sample	Op	Total	Inv	Neg	Pos	Agreement	Wilson Score 95% CI	Mean ± SD (%CV) of S/CO
1	Negative	1	30	0	30	0	100.0%	(88.6% - 100%)	0.22 ± 0.10 (46.2%)
		2	30	0	30	0	100.0%	(88.6% - 100%)	0.21 ± 0.11 (55.13%)
	High Negative	1	30	0	18	12	60.0%	(42.3% - 75.4%)	0.99 ± 0.40 (41.12%)
		2	30	0	17	13	56.7%	(39.1% - 72.6%)	1.01 ± 0.49 (48.94%)
	Low Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	4.11 ± 1.59 (38.8%)
		2	30	0	0	30	100.0%	(88.6% - 100%)	3.81 ± 2.34 (61.52%)
	Moderate Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	11.7 ± 5.55 (47.37%)
		2	30	0	1	29	96.7%	(83.3% - 99.4%)	8.09 ± 4.23 (52.36%)
2	Negative	1	30	0	30	0	100.0%	(88.6% - 100%)	0.30 ± 0.11 (38.13%)
		2	30	0	30	0	100.0%	(88.6% - 100%)	0.25 ± 0.10 (41.65%)
	High Negative	1	30	0	9	21	30.0%	(16.6% - 47.8%)	1.20 ± 0.41 (34.5%)
		2	30	0	7	23	23.3%	(11.7% - 40.9%)	1.39 ± 0.42 (30.56%)
	Low Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	5.04 ± 2.18 (43.26%)
		2	30	0	0	30	100.0%	(88.6% - 100%)	5.04 ± 2.12 (42.22%)
	Moderate Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	14.0 ± 6.00 (42.59%)
		2	30	0	0	30	100.0%	(88.6% - 100%)	13.1 ± 6.43 (48.86%)
3	Negative	1	30	0	30	0	100.0%	(88.6% - 100%)	0.21 ± 0.09 (42.32%)
		2	30	0	30	0	100.0%	(88.6% - 100%)	0.21 ± 0.07 (35.42%)
	High Negative	1	30	0	16	14	53.3%	(36.1% - 69.7%)	0.95 ± 0.24 (25.94%)
		2	30	0	20	10	66.7%	(48.7% - 80.7%)	0.95 ± 0.33 (34.85%)
	Low Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	3.55 ± 1.11 (31.34%)
		2	30	0	1	29	96.7%	(83.3% - 99.4%)	3.71 ± 1.84 (49.64%)

Site	Sample	Op	Total	Inv	Neg	Pos	Agreement	Wilson Score 95% CI	Mean ± SD (%CV) of S/CO
	Moderate Positive	1	30	0	0	30	100.0%	(88.6% - 100%)	9.63 ± 3.01 (31.32%)
		2	30	0	0	30	100.0%	(88.6% - 100%)	9.76 ± 4.11 (42.14%)

Table 16. Reproducibility Performance by Overall Site

Site	Sample	Total	Inv	Neg	Pos	Agreement	Wilson Score 95% CI	Mean ± SD (%CV) of S/CO
1	Negative	60	0	60	0	100.0%	(93.9% - 100%)	0.21 ± 0.10 (50.28%)
	High Negative	60	0	35	25	58.3%	(45.7% - 69.9%)	1.00 ± 0.45 (44.96%)
	Low Positive	60	0	0	60	100.0%	(93.9% - 100%)	3.96 ± 1.99 (50.33%)
	Moderate Positive	60	0	1	59	98.3%	(91.1% - 99.7%)	9.90 ± 5.22 (52.76%)
2	Negative	60	0	60	0	100.0%	(93.9% - 100%)	0.28 ± 0.11 (40.24%)
	High Negative	60	0	16	44	26.7%	(17.1% - 39%)	1.30 ± 0.42 (32.97%)
	Low Positive	60	0	0	60	100.0%	(93.9% - 100%)	5.04 ± 2.13 (42.38%)
	Moderate Positive	60	0	0	60	100.0%	(93.9% - 100%)	13.6 ± 6.18 (45.39%)
3	Negative	60	0	60	0	100.0%	(93.9% - 100%)	0.21 ± 0.08 (38.72%)
	High Negative	60	0	36	24	60.0%	(47.3% - 71.4%)	0.95 ± 0.29 (30.47%)
	Low Positive	60	0	1	59	98.3%	(91.1% - 99.7%)	3.63 ± 1.51 (41.61%)
	Moderate Positive	60	0	0	60	100.0%	(93.9% - 100%)	9.69 ± 3.57 (36.89%)

Table 17. Reproducibility Performance by Overall Sample Type and Site

Site	1	2	3	Total	% Agreement (95% CI)
Negative	60/60	60/60	60/60	180/180	100.0% (97.9% - 100.0%)
High Negative	35/60	16/60	36/60	87/180	48.3% (41.1% - 55.6%)
Low Positive	60/60	60/60	59/60	179/180	99.4% (96.9% - 99.9%)
Moderate Positive	59/60	60/60	60/60	179/180	99.4% (96.9% - 99.9%)

2. Operator Questionnaire

At the end of the study, each operator in the CLIA Clinical Evaluation and Reproducibility studies was given a questionnaire to provide feedback on the ease of use

of the Sofia 2 instrument and the Sofia 2 SARS+ FIA. The questionnaire had 35 questions and was divided into the following sections:

1) Assay Procedure and IFU (7 questions)
2) Sofia 2 Operation (9 questions)
3) External Controls (9 questions)
4) Calibration Check (9 questions)
5) Overall Test (2 questions)

The operators performing the testing at each site also filled out a questionnaire about their professional training and background. Based on the 15 operators feedback, the overall Sofia 2 SARS+ FIA test was found to be easy to set up and operate, and the software interface for the Sofia 2 instrument was easy to navigate and use. The Sofia 2 error messages were easy to understand, and the result screen was clear and easy to interpret. Operators also found the Instructions for Use for the Sofia 2 SARS+ FIA easy to understand and follow.

M. Labeling for Waived Devices

1. The labeling submitted for the Sofia 2 SARS+ FIA test consists of:
    1) Instructions for Use: 5.1 Sofia 2 SARS Antigen+ FIA Package Insert.docx.doc
    2) Quick Reference Instructions: 5.2 Sofia 2 SARS Antigen+ FIA Quick Reference Instructions.docx.doc
    3) External Controls – Instructions for Use: 5.3 Sofia 2 SARS Antigen FIA Control Swab Set Package Insert.docx.doc
    4) Instrument Instructions for Use: 5.4 Sofia 2 User Manual.pdf
    5) Instrument Quick Reference Instructions: 5.5 Sofia 2 Quick Start Guide.pdf

2. <u>The following elements are appropriately present:</u>
   The Quick Reference Guide and Operator's Instrument Manual are written at no higher than a 7th grade reading level.
    - The User's Manual and Quick Reference Guide identify the test as CLIA waived.
    - The User's Manual and test cartridge package insert contain a statement that a Certificate of Waiver is required to perform the test in a waived setting.
    - The User's Manual and Quick Reference Guide contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1).
    - The User's Manual and Quick Reference Guide provide instructions for conducting quality control procedures.
    - The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Benefit-Risk Considerations

The evidence provided herein indicates that this assay will appropriately diagnose SARS-CoV-2. This assay was validated more vigorously as compared to an EUA device to support a full authorization and classification as a Class II device. The CLIA Waiver Clinical Evaluation study demonstrated an acceptable sensitivity of 88.72% (118/133) and an acceptable specificity of 99.28% (685/690). The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. The clinical benefits outweigh the probable risk of erroneous results for the proposed assay, considering the product labeling, special controls, as well as general controls. The clinical benefits of the assay include ease of use for the healthcare provider and instrument read results. Additionally of clinical benefit, the results of the CLIA Waiver Clinical Evaluation study, Reproducibility study, and Operator Questionnaire suggests that errors will be uncommon, are mitigated by the device on-screen instructions, and will facilitate accurate assay implementation and interpretation of results. This assay will provide substantial benefits to patients and healthcare providers as an aid in the diagnosis of SARS-CoV-2 when used in conjunction with other laboratory results and clinical information.

O. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.