FDA U.S. FOOD & DRUG ADMINISTRATION

EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set
DECISION SUMMARY

I Background Information:

A De Novo Number
DEN220039

B Applicant
Quidel Corporation

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVF	Class II	21 CFR 866.3982 - Simple qualitative device to directly detect SARS-CoV-2 virus targets in human clinical specimens for settings operating under a certificate of waiver or at home use	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
De Novo request for evaluation of automatic class II designation for the Sofia 2 SARS Antigen + FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set

B Measurand:
Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

C Type of Test:
Qualitative lateral flow immunoassay

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

III Indications for Use:

A Intended Use(s):
See Indications for Use below.

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

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

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

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

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

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

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Sofia 2 Analyzer

IV Device/System Characteristics:

A Device Description:
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.

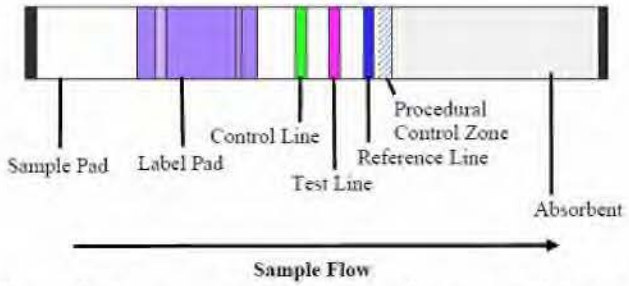

Figure 1: Schematic of the Sofia 2 Antigen + FIA Test Strip

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.

B Principle of Operation

The Sofia 2 SARS Antigen+ FIA employs immunofluorescence based lateral flow technology in a sandwich design to detect nucleocapsid protein from SARS-CoV-2. Following sample collection, the patient's anterior nasal swab sample is placed in the reagent tube, to lyse the sample and solubilize viral nucleoproteins of the analyte. After lysis, the sample is loaded onto the test cassette sample well. The sample migrates across the test strip first and then flows across 3 distinct areas: 1) the test line, 2) the procedural control line, and 3) the reference line. If SARS-CoV-2 viral antigens are present, they will be captured by antibodies and the fluorescence-tagged complex will be bound to the test line on the test strip.

The test cassette is placed inside of the Sofia 2 instrument for timed test reaction and signal development (WALK AWAY Mode). Thereafter, the Sofia 2 will automatically scan the test strip, expose the strip to UV light, and measure the fluorescent signal by processing the results using method-specific algorithms. A positive result for the analyte is determined by detection of fluorescent signals at the Test Line that are above a software specific cutoff. If SARS-CoV-2 viral antigens are not present, no fluorescent complexes are formed at the Test Line and no signal is detected by Sofia 2. In the absence of SARS-CoV-2 a signal is formed only at the Control Line. If the Control Line is not detected by the Sofia 2 the sample result is invalid. Test results are displayed on the screen as Positive, Negative, or Invalid.

C Instrument Description Information

1. Instrument Name:
   Sofia 2

2. Specimen Identification:
   SARS-CoV-2 nucleocapsid protein antigen

3. Specimen Sampling and Handling:
   Direct anterior nasal swab specimens

4. Calibration:
   Sofia 2 reminds the user to check the calibration status of the instrument every thirty days, utilizing a specially labeled Calibration Cassette. This cassette uses a fluorescent reagent embedded in plastic along with a unique barcode that prevents the analyzer from mistaking the Calibration Cassette for a standard assay cassette. If calibration is needed, the Sofia 2 analyzer will calibrate automatically after the cassette is inserted into the instrument. If the calibration has expired, the Sofia 2 analyzer will not allow tests to be run.

5. Quality Control:
   Quality Control is facilitated by scanning the lot-specific QC card that is provided on the unit carton of each kit. The QC card "informs" the Sofia 2 analyzer as to which test is being subjected to quality control evaluation, and provides the kit's lot number, test cassette's lot number, and the kit's expiration date. Following scanning of the QC card, the user is prompted to insert a cassette containing the extracted Positive Control test sample, followed by the extracted Negative Control test sample.

V Standards/Guidance Documents Referenced:

Table 1. Referenced Standards and Guidance Documents

Document Number	Title	Publishing Organization	Applicable Study
EN 13612:2002/ AC:2002	Performance evaluation of in vitro diagnostic medical devices	European Standard	All
EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition (Chapter 1	CLSI	Precision/ Repeatability and Reproducibility

	Introduction and 3 Single-Site Precision Evaluation Study)		
EP12-A2	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition (Sections 7.1 Controls, Section 8 Bias and Imprecision Studies (with focus on 8.3), and Appendix: Statistical Reasoning for Precision Experiment Conclusions)	CLSI	Precision/ Repeatability and Reproducibility
N/A	U.S. FDA's SARS-CoV-2 Emergency Use Authorization Antigen Template for Test Developers (dated 26 Oct 2020)	FDA	All
N/A	Guidance for Industry and FDA Staff, Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses (15 Jul 2011; Section 9.A.i)	FDA	Limit of Detection
N/A	Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices; Guidance for Industry and Food and Drug Administration Staff (dated 26 Feb 2020; Section IV.A. Tier 1:Risk Analysis and Flex Studies)	FDA	Cross-reactivity and Microbial Interference; Development Read Time and Test Result Stability
EN ISO 14971:2019	Application of Risk Management to Medical Devices	ISO	Risk Analysis
EN ISO 13485:2016	Medical devices - Quality management systems – Requirements for regulatory purposes	ISO	Software Validation
IEC 60601-1-2:2014	Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance Collateral standard: Electromagnetic Disturbances Requirements and tests.	IEC	Electromagnetic Compatibility and Electrical Safety
CISPR 11 Edition 6.0:2015	Industrial, scientific and medical equipment Radio-frequency disturbance characteristics Limits and methods of measurement.	CISPR	Electromagnetic Compatibility and Electrical Safety
EN 61326-1:2013 / EN 61326-2-6:2012	Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements. (Appendix 17-2)	IEC	Electromagnetic Compatibility and Electrical Safety
IEC 61010-1:2010 (third edition)	Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements. (Appendix 17-6)	IEC	Electromagnetic Compatibility and Electrical Safety
IEC 62133:2012	Secondary cells and batteries containing alkaline or other non-acid electrolytes – Safety requirements for portable sealed secondary cells, and for batteries made from them, for use in portable applications. (Appendix 17-7)	IEC	Electromagnetic Compatibility and Electrical Safety

VI Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

a) Precision and Repeatability

The precision/repeatability (P/R) studies herein evaluated four (4) levels of UV-inactivated SARS-CoV-2 in Negative Clinical Matrix (NCM): Negative (C0), High Negative (C5), Low Positive (C95), and Positive (3X LoD) — in two (2) events ('runs') per day, two (2) replicates per event, over a minimum of twenty (20) days on each of two Sofia 2 SARS Antigen+ FIA devices/reagent lots (see table below). The precision testing was conducted according to the Package Insert using two operators.

Table 2. Precision and Repeatability Study Design and Test Parameters

Precision and Reproducibility Study Conditions		
Parameter	**Per Lot**	**Overall**
Analyte Levels	4	4
Days	20	20
Events ('runs')	2 runs per day	40
Replicates	2 per run	160 per level
Operators	1 per level	at least 2
Overall	80 replicates per level. 320 data points	640 data points

Table 3. Precision and Repeatability Study – Test Sample Panel

Spiking Concentrations	
Level	**Concentration (TCID50/mL)**
Negative Sample (C_0)	N/A
High Negative (C_5)	0.04 x LoD
Low Positive (C_{95})	1 x LoD
Moderate Positive	3 x LoD

Qualitative results, Quantitative results, and Variance Component Analysis are summarized here:

- Out of 640 replicates tested, there were zero (0) invalid test results obtained.
- The negative samples produced an overall 99.4% expected negative agreement (159/160)
- The high negative samples produced 95.0% expected negative agreement (152/160).
- The low positive samples produced 98.1% expected positive agreement (157/160).
- The moderate positives sample produced 99.4% expected positive agreement (159/160).

Table 4. Precision and Repeatability Study – Summary Results

Kit Lot	Negative		High Negative		Low Positive		Moderate Positive	
	1	2	1	2	1	2	1	2
Run 1	40/40	40/40	39/40	40/40	40/40	40/40	40/40	40/40
Run 2	40/40	39/40	35/40	38/40	40/40	37/40	40/40	39/40
Total	80/80	79/80	74/80	78/80	80/80	77/80	80/80	79/80

% Agreement	100.0%	98.75%	92.5%	97.5%	100.0%	96.25%	100.0%	98.75%
(95% CI)	[95.4% - 100.0%]	[93.3% - 99.8%]	[84.6% - 96.5%]	[91.3% - 99.3%]	[95.4% - 100.0%]	[89.5% - 98.7%]	[95.4% - 100.0%]	[93.3% - 99.8%]
% Agreement	99.4%		95.0%		98.1%		99.4%	
95% CI)	[96.5% - 99.9%]		[90.4% - 97.4%]		[94.6% - 99.4%]		[96.5% - 99.9%]	

b) Reproducibility

The study was designed to evaluate site-to-site, operator-to-operator, and level-to-level variability and demonstrate that the Sofia 2 SARS Antigen+ FIA can be performed consistently and correctly. This study was conducted at (b)(4) distinct sites, each with two (2) different operators, testing two (2) test lots, using a coded panel of contrived samples (refer to Table 3 above). Four analyte levels were each tested as follows: 2 operators x 3 sites x 5 days x 2 device lots x 2 replicates = 120 replicates for each of the different analyte levels to a total of 480 replicates per site.

- Out of 480 samples tested, there were zero (0) invalid test results obtained.
- The negative samples produced an overall 100.0% expected negative agreement (120/120)
- The high negative samples produced 55.0% expected negative agreement (66/120).
- The low positive samples produced 99.2% expected positive agreement (119/120).
- The moderate positive samples produced 99.2% expected positive agreement (119/120).

Table 5. Reproducibility Study – Summary Results

Site	Negative	0.04x LoD	1x LoD	3x LoD
1	40/40	26/40	40/40	39/40
2	40/40	11/40	40/40	40/40
3	40/40	29/40	39/40	40/40
Total	120/120	66/120	119/120	119/120
% Agreement	100%	55%	99.2%	99.2%
(95% CI)	[96.9% - 100.0%]	[46.1% - 63.6%]	[95.4% - 99.9%]	[95.4% - 99.9%]

2. Linearity:

This study is not applicable as this test device is a qualitive assay.

3. Analytical Specificity/Interference:

a) Cross-reactivity and Microbial Interference Study

The cross-reactivity and potential interference were evaluated by testing various bacteria (7), viruses (19), fungus (1), and negative matrixes (2) with the Sofia 2 SARS Antigen+ FIA. Each organism and virus were tested in five (5) replicates in the absence or presence of 2xLoD of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020). None of the

organisms and viruses evaluated demonstrated cross-reactivity and interference in the assay at the concentrations tested.

Table 6. Cross-Reactivity and Microbial Interference Study – Summary Results

Virus/Microorganism	Strain	Conc.	Cross-Reactivity (Negative Agreement) (SCV-2 negative replicates/all replicates)	Interference (Positive Agreement) (SCV-2 positive replicates/all replicates)
Adenovirus Culture Fluid	Type 1 (Species C)	2.04E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Coronavirus Culture Fluid (Heat Inactivated)	229E	1.26E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Coronavirus Culture Fluid (Heat Inactivated)	OC43	1.00E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Coronavirus Culture Fluid (Heat Inactivated)	NL63	3.40E+04 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Enterovirus Type 68 Major Group Culture Fluid	2014 Isolate 1	2.29E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Human Metapneumovirus 9	A1	1.27E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Human Rhinovirus Type 1A	N/A	1.78E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Influenza A	A/Brisbane/10/07	1.00E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Influenza A H1N1	New Caledonia/20/99	1.78E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Influenza A	New Caledonia/20/99 Custom formalin inactivated	4.02E+05 $TCID_{50}$/mL	Not Tested	100.0% (5/5)
Influenza A	Brisbane/02/18	5.62E+03 $TCID_{50}$/mL	Not Tested	100.0% (5/5)
Influenza A	California/07/09	4.17E+04 $TCID_{50}$/mL	Not Tested	100.0% (5/5)
Influenza B Virus Culture Fluid	Brisbane/33/08	2.34E+04 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
MERS-CoV (Heat Inactivated)	Florida/USA-2_Saudi Arabia_2014	1.04E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)

Virus/Microorganism	Strain	Conc.	Cross-Reactivity (Negative Agreement) (SCV-2 negative replicates/all replicates)	Interference (Positive Agreement) (SCV-2 positive replicates/all replicates)
Parainfluenza Type 1 Culture Fluid	N/A	1.00E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Parainfluenza Type 2 Culture Fluid	N/A	9.97E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Parainfluenza Type 3 Culture Fluid	N/A	2.29E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Parainfluenza Type 4B Culture Fluid	N/A	1.00E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Respiratory Syncytial Virus	Type A	1.51E+05 $TCID_{50}$/mL	100.0% (5/5)	100.0% (5/5)
Bordetella pertussis, ATCC 18323 (NCTC 10739)	Type b; Eagan	3.05E+07 CFU/mL	100.0% (5/5)	100.0% (5/5)
Chlamydophila pneumoniae	Z500[IOL207]	1.06E+07 IFU/mL	100.0% (5/5)	100.0% (5/5)
Haemophilus influenzae	Type B, NCTC 8468	2.60E+07 CFU/mL	100.0% (5/5)	100.0% (5/5)
Legionella pneumophila	ATCC 33152 (Philadelphia-1)	2.00E+07 CFU/mL	100.0% (5/5)	100.0% (5/5)
Pneumocystis jirovecii-S. cerevisiae Recombinant	W303-Pji	5.29E+05 CFU/mL	100.0% (5/5)	100.0% (5/5)
Mycoplasma pneumoniae	M129	1.35E+07 CCU/mL	100.0% (5/5)	100.0% (5/5)
Streptococcus pneumoniae, Type 19F	ATCC 49619 (262 [CIP 104340])	1.90E+07 CFU/mL	100.0% (5/5)	100.0% (5/5)
Streptococcus pyogenes	ATCC 19615 (Bruno [CIP 104226])	1.60E+07 CFU/mL	100.0% (5/5)	100.0% (5/5)
S. aureus MRSA	N/A	1.03E+06 CFU/mL	100.0% (5/5)	100.0% (5/5)
S. aureus MSSA	N/A	1.15E+06 CFU/mL	100.0% (5/5)	100.0% (5/5)
S. epidermidis	N/A	1.29E+06 CFU/mL	100.0% (5/5)	100.0% (5/5)
Negative Nasal Matrix (normal flora)	UTM	N/A	100.0% (5/5)	100.0% (5/5)

Virus/Microorganism	Strain	Conc.	Cross-Reactivity (Negative Agreement) (SCV-2 negative replicates/all replicates)	Interference (Positive Agreement) (SCV-2 positive replicates/all replicates)
Negative Nasal Matrix (normal flora)	CDC Viral Transport	N/A	100.0% (5/5)	100.0% (5/5)

b) Endogenous and Exogenous Interfering Substances Study

This study evaluated endogenous or exogenous substances that may potentially interfere with the Sofia 2 SARS Antigen+ FIA. Each potentially interfering substance (see table below) was tested in negative clinical matrix (NCM) at the targeted initial concentrations in the absence (negative) and presence (positive) of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020) at the 2X LoD level. The effects of the substances were evaluated by the agreement with the expected negative or positive results. Five (5) replicates were tested for each sample prepared.

Table 7. Interfering Substances Study – Summary Results

Substance	Active Ingredient of Substance	Interferent Conc.	Positive Agreement (SCV-2 positive replicates/all replicates)	Negative Agreement (SCV-2 negative replicates/all replicates)
Afrin-nasal spray	Oxymetazoline	5% v/v	100.0% (5/5)	100.0% (5/5)
Blood (human)	Blood	5% v/v	100.0% (5/5)	100.0% (5/5)
Chloraseptic, Cepacol	Benzocaine, Menthol	0.7 g/mL	100.0% (5/5)	100.0% (5/5)
Flonase	Fluticasone	5% v/v	100.0% (5/5)	100.0% (5/5)
Halls Relief Cherry Flavor	Menthol	0.8 g/mL	100.0% (5/5)	100.0% (5/5)
Nasocort Allergy 24 Hour	Triamcinolone	5% v/v	100.0% (5/5)	100.0% (5/5)
Neo-Synephrine	Phenylephrine hydrochloride	5% v/v	100.0% (5/5)	100.0% (5/5)
Oseltamivir	Oseltamivir	2.2 µg/mL	100.0% (5/5)	100.0% (5/5)
Purified mucin protein	Mucin protein	2.5 mg/mL	100.0% (5/5)	100.0% (5/5)
Rhinocort	Budesonide (Glucocorticoid)	5% v/v	100.0% (5/5)	100.0% (5/5)
Saline nasal spray	Saline	15% v/v	100.0% (5/5)	100.0% (5/5)

Substance	Active Ingredient of Substance	Interferent Conc.	Positive Agreement (SCV-2 positive replicates/all replicates)	Negative Agreement (SCV-2 negative replicates/all replicates)
Tobramycin	Tobramycin	1.25 mg/mL	100.0% (5/5)	100.0% (5/5)
Zanamivir	Zanamivir	282 ng/mL	100.0% (5/5)	100.0% (5/5)
Zicam cold remedy	Galphimia glauca, Luffa operculata, Sabadilla	5% v/v	100.0% (5/5)	100.0% (5/5)

4. Assay Reportable Range:

   This section is not applicable as this test device is a qualitative assay.

5. Traceability, Stability and Expected Values (Controls, Calibrators, or Methods):

   a) Internal Controls

   The test strip has several built-in control features to ensure that each test is performed properly. These include the: 1) Control Line, 2) Reference Line and 3) the Procedural Control Zone.

   - The Control Line is the first line that the extracted specimen encounters as it begins its migration across the length of the nitrocellulose test strip. The Control Line is comprised of mouse immunoglobulin (Ig). The Control Line 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 by the instrument to determine the orientation of the test strip and the locations of Test Line and Procedural Control Zone on 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 reagents must flow through the test strip and the fluorescent signals at the Reference Line location must meet the specifications by the fluorescent response, width, orientation and location for a valid Reference Line peak. If there is no valid Reference Line peak, the analyzer will report the result as "invalid". After the Reference Line peak is found, the software calculates the location of the Test Line and Procedural Control zone fixed distances from the Reference Line.
   - The Procedural Control Zone (PCZ) is used by the Sofia 2 analyzer to assess for adequate sample flow and sample volume. This PCZ of the nitrocellulose test strip is located between the Absorbent Pad and the Reference Control Line. As the assay is run, the fluid carries the europium-dyed microparticles through the test strip. For a valid assay the reagents must flow to the end of the test strip and produce a minimum fluorescent signal in the PCZ. If the fluorescent signal obtained in the zone is below the specification, the analyzer will report the result as "invalid".

b) External Controls

Ten (10) control swabs were tested per cassette lot – control lot combination and a minimum of ten (10) Sofia 2 Analyzers were used All testing was conducted in the "Read Now" mode following the package insert instructions. For each control lot – cassette lot combination, the percent agreement to expected result was calculated as follows:

- % Agreement to Expected Positive Result = 100 X [# Positive / (# Negative + # Positive)]
- % Agreement to Expected Negative Result = 100 X [# Negative / (# Negative + # Positive)]

For each control lot – cassette lot combination, all the positive and all the negative controls produced 100% agreement with the expected results.

Table 8. Validation of External Control Materials – Summary Results

SARS External Controls Performance							
Control	**Control Lot#**	**Cassette Lot#**	**n**	**# of invalid**	**# of Neg**	**# of Pos**	**% Expected Agreement**
Control Swab, Neg, SARS Antigen, Pouched	146067	210144	10	0	10	0	100
		210325	10	0	10	0	100
	147223	210144	10	0	10	0	100
		210325	10	0	10	0	100
Control Swab, Pos, SARS Antigen, Pouched	146072	210144	10	0	0	10	100
		210325	10	0	0	10	100
	147227	210144	10	0	0	10	100
		210325	10	0	0	10	100
	147367	210144	10	0	0	10	100
		210325	10	0	0	10	100

For three positive and two negative control lots tested across two test cassette lots, the positive and negative controls produced 100% agreement with the expected results.

c) Specimen Stability

Two (2) test samples were prepared for testing in the specimen stability study: a negative sample (NCM with no analyte) and a low positive sample (NCM spiked with heat inactive SARS-CoV-2 (isolate USA-WA1/2020) at 2x LoD. A total of [redacted] negative swabs and [redacted] low positive swabs were prepared by submerged/dipping a foam nasal swab into a liquid test sample for a minimum of five (5) minutes prior to placing into a dry transport tube. The tubes were then stored at 15°C±2°C, 30°C±2°C, in the cold room (2-8°C), or in the freezer (-20°C) for the duration of the study and were tested at the time points indicated in the table below:

Table 9. Specimen Stability Study Design and Test Parameters

Specimen Storage Temperature/Time					
Specimen Storage Temperature	**Specimen Storage Time**				
Ambient Temperature (23.7°C)	0 hrs.	N/A	N/A	N/A	N/A
Room Temperature (15±2°C)	2 hrs.	6 hrs.	12 hrs.	24 hrs.	N/A

Room Temperature (30±2°C)	2 hrs.	6 hrs.	12 hrs.	24 hrs.	N/A
Refrigerated (2-8°C)	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.
Frozen (-20°C)	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.

A minimum of ten (10) Sofia 2 Analyzers were used. (b)(4) negative swabs and (b)(4) positive swabs were tested at each time point and storage temperature. Swab samples were processed using the *Swab Test Procedure* according to the Package Insert. All samples were tested on the Sofia 2 SARS Antigen+ FIA using the "Read Now" mode.

At time 0 hours, all the negative and positive test swabs produced 100% agreement with the expected results. No false positives occurred with the negative samples at any of the timepoints tested for any of the temperature conditions evaluated. Across the timepoints and temperature conditions evaluated, positive samples were positive, except for some individual replicates at 30°C storage and one at refrigerated storage.

Table 10. Specimen Stability Study – Positive Agreement Summary Results

Specimen Storage Parameters	0 hrs.	2 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
Control Ambient Temp. (23.7°C)	100.0% (15/15)								
Room Temp. (15±2°C)		100.0% (15/15)	100.0% (15/15)	100.0% (15/15)	100.0% (15/15)				
Room Temp. (30±2°C)		93.3% (14/15)	100.0% (15/15)	100.0% (15/15)	86.7% (13/15)				
Refrigerated (2-8°C)			100.0% (15/15)	100.0% (15/15)	100.0% (15/15)	93.3% (14/15)	100.0% (15/15)		
Frozen (-20°C)					100.0% (15/15)	100.0% (15/15)	100.0% (15/15)	100.0% (15/15)	100.0% (15/15)

Based upon this study design and the results thereof, the specimen stability data support, storage of nasal swab samples at: 2-8°C for up to 24 hours, 15°C to 30°C for up to 12 hours, -20°C or below for up to 4 days (96 hours) including two freeze-thaw cycles.

6. Detection Limit:

The Limit of Detection (LoD) of the Sofia 2 SARS Antigen+ FIA was determined by evaluating different dilutions of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020) in negative clinical matrix. The SARS-CoV-2 USA-WA1/2020 stock was diluted into negative clinical matrix (NCM) in order to target the LoD level. The negative clinical matrix was pooled human nasal swab samples eluted into saline. Six serial dilutions were made from SARS-CoV-2 USA-WA1/2020 stock into a negative clinical matrix. Five (5) replicates were tested for each of the six dilutions to determine the preliminary LoD concentration on two lots of the Sofia 2 SARS Antigen+ FIA cassettes. Thereafter, the LoD was confirmed by

testing 20 replicates at the preliminary LoD concentration. The Sofia 2 SARS Antigen+ FIA LOD was confirmed to be 1.44 x10^4 $TCID_{50}$/mL.

Table 11. Limit of Detection Study – Summary Results

Conc. (TCID50/mL)	Range Finding				Confirmation			
	n	Inv	Neg	Pos (% Pos)	n	Inv	Neg	Pos (% Pos)
(b)(4)								
2.88E+04	5	0	0	5 (100%)				
1.44E+04	**5**	**0**	**0**	**5 (100%)**	**20**	**0**	**0**	**20 (100%)**
(b)(4)								
NCM (Negative Clinical Matrix)	5	0	5	0 (0.0%)				
(b)(4)								
2.88E+04	5	0	0	5 (100%)				
1.44E+04	**5**	**0**	**1**	**4 (80.0%)**	**20**	**0**	**0**	**20 (100%)**
(b)(4)								
NCM (Negative Clinical Matrix)	5	0	5	0 (0.0%)				

7. High-dose Hook Effect Study

A High Dose Hook Effect study was conducted to determine if a hook effect would be observed at high concentrations of the analyte (i.e., a false negative at high concentrations of SARS-CoV-2). A series of four concentrations were prepared in NCM and tested in five (5) replicates on the Sofia 2 SARS Antigen+ FIA, between and including (b)(4) LoD (the maximum virus concentration possible) and (b)(4) LoD (high positive). The starting virus stock was (b)(4) (isolate USA-WA1/2020). All spiked samples were 100% positive, as expected, at all tested concentrations. The Sofia 2 SARS Antigen+ FIA did not display a Hook Effect for high concentrations of SARS-CoV-2 tested herein.

Table 12. High-dose Hook Effect Study – Summary Results

Sample Level	Concentration ($TCID_{50}$/mL)	%Expected Agreement
(b)(4) **LoD**	2.30E+06	100.0% (5/5)

40X LoD		(b)(4)	100.0% (5/5)
20X LoD			100.0% (5/5)
10X LoD			100.0% (5/5)

8. Inclusivity

This study was performed to demonstrate that the Sofia 2 SARS Antigen+ FIA assay can detect the viral strain/isolate SARS-CoV-2 (isolate Italy-INMI1). The heat-inactivated SARS-CoV-2 (isolate Italy-INMI1) stock ((b)(4)) was diluted into (b)(4) at different concentrations. Each concentration was tested with (b)(4) replicates until two consecutive dilutions produced 1 or more negative replicates out of (b)(4)

Table 13. Inclusivity Study – Summary Results

Strain / Isolate / Variant (Lineage)	Concentration ($TCID_{50}$/mL)	n	# of Inv	# of Neg	# of Pos	% Positivity	# of Valid Results Mean ± SD (%CV) of S/CO
Heat Inactivated SARS-CoV-2: Isolate Italy (INMI1)	(b)(4)	(b)(4)					(b)(4)
	2.43E+05	5	0	0	5	100.0	5 2.2±0.66 (30.5%)
	(b)(4)						(b)(4)
Heat Inactivated SARS-CoV-2: Delta (B.1.617.2)	(b)(4)						
	1.00E+04	5	0	0	5	100.0	5 2.1±0.48 (22.5%)
	(b)(4)						(b)(4)
Heat Inactivated SARS-CoV-2: Omicron BA.1 (BA.1.18)	(b)(4)						
	2.36E+04	5	0	0	5	100.0	5 1.9±0.70 (37.7%)
	(b)(4)						(b)(4)

Heat Inactivated SARS-CoV-2: Omicron BA.2 (B.1.1.529)	(b)(4)						
	8.22E+03	5	0	0	5	100.0	5 2.3±0.75 (32.8%)
	(b)(4)						

9. Assay Cut-Off:

The Sofia 2 analyzer workflow calculates the cutoff values (COs) and the signal over CO ratio value (S/CO) prior to reporting the result. Values from reference lots during development were used to establish the reference values used in these calculation algorithms. Sofia 2 determines a positive or negative result based on predetermined, specific fluorescence based cutoff values, programmed into the software and established on a lot-to-lot basis. Final cut-off values were further validated as part of the analytical and clinical studies.

10. Accuracy (Instrument):

Please refer to Section VI.C (Clinical Studies) for the clinical evaluation study and data that establish clinical performance and accuracy of the test device.

11. Carry-Over:

Carry-over contamination is not applicable to this test device as each sample uses an independent, new, single-use test cassette that is discarded after each run. No fluidic handling occurs in the instrument therefore the risk of carry-over was determined to be low.

B Comparison Studies:

1. Method Comparison:

Please refer to Section VI.C (Clinical Studies) below for the clinical validation, regarding the method comparison studies.

2. Matrix Comparison:

The Sofia 2 SARS Antigen+ FIA is only intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal swab specimens. As no other specimen or sample type is claimed herein, a Matrix Comparison study is not applicable to this test device.

C Clinical Studies:

The performance of the Sofia 2 SARS Antigen+ FIA in detecting SARS-CoV-2 viral nucleoprotein antigen was evaluated in a multi-center, prospective study conducted from August 2021 to November 2022, using 12 operators who conducted enrollment and testing of the subjects. An additional 7 operators conducted enrollment and/or sample shipments only. The study only enrolled subjects with symptoms of respiratory infection consistent with a SARS-CoV-2 infection. A total of five hundred thirty (581) subjects were consecutively enrolled and tested across six different CLIA-waived sites.

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), pre-labeled with the subject ID, refrigerated (2-8°C) in the biohazard bag within one hour from the collection, and shipped to the reference laboratory on ice packs on the same day. If same-day shipping was not possible, the swabs used for the comparator method were kept refrigerated (2-8°C) until shipment to the reference laboratory. Upon receipt by the reference laboratory, the second nasal swab was tested with a highly sensitive Emergency Use Authorization (EUA) authorized RT-PCR comparator assay, within six days of receipt. Demographics, symptoms, and health history were also collected from each subject.

There were 581 evaluable subjects with 37.5% (218/581) male and 62.5% (363/581) female, with a mean age of 41.6 years; one subject was excluded. Results obtained with the Quidel Sofia 2 SARS Antigen+ FIA test were compared to the results obtained with the RT-PCR comparator test to determine clinical sensitivity and specificity. The study cohort included 36.7% low positive samples.

The Sofia 2 SARS Antigen+ FIA demonstrated a clinical sensitivity of 89.0% (97/109; 95% CI: 81.7% - 93.6%) and a specificity of 99.6% (470/472; 95% CI: 98.5% - 99.9%) versus the comparator.

Table 14. Clinical Performance of the Sofia 2 SARS Antigen+ FIA Compared to an EUA authorized highly sensitive RT-PCR comparator

	EUA authorized RT-PCR Comparator		
	Pos	**Neg**	**Total**
Sofia Pos	97	2	99
Sofia Neg	12	470	482
Total	109	472	581

- Positive Percent Agreement (PPA) = 89.0% (97/109; 95% CI: 81.7% - 93.6%)
- Negative Percent Agreement (NPA) = 99.6% (470/472; 95% CI: 98.5% - 99.9%)
- Positivity in Study Cohort = 18.761% (109/581; 95% CI: 15.8% - 22.1%)

1. Clinical Sensitivity:

   Please refer to Section VI.C (Clinical Studies) above for the clinical validation.
   The PPA for the test is 89.0% (97/109; 95% CI: 81.7% - 93.6%).

2. Clinical Specificity:
Please refer to Section VI.C (Clinical Studies) above for the clinical validation.
The NPA for the test is 99.6% (470/472; 95% CI: 98.5% - 99.9%).

3. Serial Testing:
As a mitigation for low performance of the device at Day 0 of symptom onset, as indicated by these clinical study data and in other studies for test devices of a similar principle and design, the Intended Use for this test device (and associated Instructions for Use) include recommendations for repeat testing (i.e., test at least twice over three days with at least 48 hours between tests.). This mitigation is supported by data generated by the National Institutes for Health (NIH) and the University of Massachusetts Chan Medical School (in collaboration with the FDA) demonstrating that repeat testing over multiple days improves test performance and increases the likelihood that a COVID-19 antigen test will accurately detect an infection. These results have informed the FDA's general understanding that repeat testing after a negative result from a COVID-19 antigen test reduces the risk of a false negative result. Please refer to the following studies for additional details:
   - Finding a Needle in the Haystack: Design and Implementation of a Digital Site-less Clinical Study of Serial Rapid Antigen Testing to Identify Asymptomatic SARS-CoV-2 Infection - https://www.medrxiv.org/content/10.1101/2022.08.04.22278274v1.
   - Performance of Screening for SARS-CoV-2 using Rapid Antigen Tests to Detect Incidence of Symptomatic and Asymptomatic SARS-CoV-2 Infection: findings from the Test Us at Home prospective cohort study - https://www.medrxiv.org/content/10.1101/2022.08.05.22278466v1

D Clinical Cut-Off:
There is no clinical cutoff related to the presence of SARS-CoV-2 in patient samples. This section is therefore not applicable.

E Expected Values/Reference Range:
A patient sample is expected to be negative for SARS-CoV-2. This section is therefore not applicable.

F Other Supportive Performance Characteristics Data:
This section is not applicable.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Identified Risks to Health	Mitigation Measures
Risk of false results	Certain labeling information including limitations, device descriptions, explanations of procedures and performance information identified in special controls (1) and (4). Use of certain specimen collection devices identified in special control (3). Certain design verification and validation including documentation of device descriptions, certain analytical studies and clinical studies, risk analysis strategies identified in special control (5). Testing of characterized viral samples and labeling information identified in special control (6).
Failure to correctly interpret test results	Certain labeling information including limitations, device descriptions, explanations of procedures and performance information identified in special controls (1) and (4). Use of certain specimen collection devices identified in special control (3). Certain design verification and validation including documentation of device descriptions, certain analytical studies and clinical studies, risk analysis strategies identified in special control (5).
Failure to correctly operate the device	Certain labeling information including limitations, device descriptions, explanations of procedures and performance information identified in special controls (1), (2), and (4). Use of certain specimen collection devices identified in special control (3).

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The evidence provided 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. An added benefit is the ability to use the Sofia 2 device, a device which has previous 510(k) clearance and is used in CLIA waived settings, for use in reading the antigen result.

B Summary of the Assessment of Risk:

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. False positive SARS-CoV-2 results may lead to include improper patient management, including treatment for SARS-CoV-2 with antiviral medication, monoclonal antibody treatment, or convalescent plasma. False positive SARS-CoV-2 results may also lead to unnecessary isolation or quarantine and additional health monitoring, mis-allocation of resources used for surveillance and prevention, and delayed diagnosis and treatment of other infections or health conditions. False negative SARS-CoV-2 results may lead to missing and not appropriately treating or monitoring a patient who has SARS-CoV-2 infection. False negative SARS-CoV-2 results may also lead to unnecessary additional diagnostic evaluation or treatment and delay in correct diagnosis or further spread of disease, which may lead to novel cases of infection and concomitant increase in patient morbidity and mortality.

C Summary of the Assessment of Benefit-Risk:

The clinical benefits outweigh the probable risk of false negative 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 validation data suggests that errors will be uncommon and will facilitate accurate assay implementation and interpretation of results. The device's performance observed in the clinical study, including with the Omicron variant of SARS-CoV-2, suggests that errors will be uncommon and are mitigated by the device on-screen instructions which instruct the user on initial data entry and correct placement of the cartridge. Given that the assay cartridge cannot be visually interpreted, the user interface reports test result therefore reducing the possibility of incorrect visual interpretation of a lateral flow device. 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.

X Conclusion:

The De Novo request is granted and the device is classified under the following Regulation and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s):	QVF
Device Type:	Simple point-of-care device to directly detect SARS-CoV-2 viral targets from clinical specimens in near-patient settings
Class:	Class II
Regulation:	21 CFR 866.3982