CLIA Waiver Determination Decision Summary

A. Document Number:

CW170001

B. Parent Document Number:

K162911

C. Purpose of the Submission:

To obtain CLIA Waived categorization of the Sofia RSV FIA test when used with the Sofia 2 analyzer.

The Sofia RSV FIA test for use with the Sofia analyzer was cleared under K130398 and subsequently CLIA waived under K130398/A001. The data supporting equivalence of the assay performance when used with the new Sofia 2 and Sofia analyzers were submitted under K162911 and the device, Sofia RSV FIA test with Sofia 2, was cleared.

D. Measurand (analyte):

Respiratory Syncytial Virus (RSV) A and B nucleoprotein antigens

E. Sample Type:

Direct nasopharyngeal swabs and nasopharyngeal aspirate/wash specimens

F. Type of Test:

Qualitative antigen based immunoassay

G. Applicant:

Quidel Corporation

H. Proprietary and Established Names

Sofia RSV FIA
Sofia 2 Analyzer

I. Test System Description:

1. Overview

The Sofia RSV FIA is a lateral flow immunoassay that uses a sandwich design to qualitatively detect RSV A and RSV B nucleoprotein from nasopharyngeal (NP) swab and nasopharyngeal aspirate/wash (NA/W) specimens collected from pediatric patients with signs and symptoms of respiratory infection. The Sofia RSV FIA lateral flow device has four main components, which are identical to those previously described in submission K130398. Briefly, the four test components consist of: (1) a sample pad for receiving the specimen; (2) a label pad containing dried, fluorescently dyed microparticles coated with RSV-specific monoclonal antibodies; (3) a nitrocellulose test strip for the capture of RSV analyte; and (4) an absorbent pad to drive capillary flow.

To perform the Sofia RSV FIA test, a pre-measured volume of Reagent Solution is added to the Reagent Tube and the contents are swirled. The patient nasopharyngeal swab is then placed into the Reagent Tube, rolled at least three times against the wall and bottom of the tube, and left to stand for 1 minute prior to transferring the sample to the sample pad using the provided clear, fixed volume (120 µL) pipette. If the sample is a nasopharyngeal wash or aspirate, the sample is transferred to the Reagent Tube using the provided large pink, fixed volume (250 µL) pipette, swirled, and then similarly transferred to the sample pad using the provided clear, fixed volume (120 µL) pipette. As the specimen migrates through the test cassette, fluorescently dyed microparticles coated with anti-RSV antibodies are hydrated and bind to RSV A and RSV B nucleoprotein antigens present in the sample. The bead-coupled, antigen-antibody complexes become immobilized on the surface of the nitrocellulose test strip at a fixed location via capture antibodies, forming an analyte-specific test line. In addition to the RSV test line, the nitrocellulose test strip contains several built-in controls to ensure that the test is performing properly. Controls include a negative control line, procedural control zone and a reference line. Importantly, because of the fluorescent nature of the microparticles used in the immunoassay, the RSV test line and each of the control lines are invisible to the naked eye.

2. Results Interpretation

A positive result is determined by detection of a fluorescent signal at levels above a signal threshold set after the image capture of the Negative Control line and interpretation by a specific algorithm in the Sofia 2 analyzer.

Two operational modes are available with the Sofia 2 analyzer, Walk Away and Read Now.

- In Walk Away Mode, the user inserts the test cassette into the analyzer immediately following addition of the specimen to the Sofia RSV FIA sample port. The Sofia analyzer automatically times the test development and provides positive or negative test results at 15 minutes. The Sofia 2 analyzer

scans the test cassette periodically during the test development time and displays a positive test result between 3 and 15 minutes. If the test is negative, the result will be displayed at 15 minutes.

- In the Read Now mode, the user incubates the test cassette on the benchtop for 15 minutes before inserting the cassette into the Sofia 2 analyzer. Positive and negative test results are displayed within 1 minute.

The test results are reported as text on the display screen of Sofia 2 analyzer as one of three possible outputs: RSV positive, RSV negative, or invalid. If an invalid test result is reported, the Sofia RSV FIA should be repeated with a new patient sample and a new test cassette.

The kit contains the following test components:

- 25 individually Packaged Test Cassettes
- 25 Reagent Tubes with lyophilized buffer with detergents and reducing agents
- 25 Ampoules with salt solution (Reagent Solution)
- 25 Sterile Nasopharyngeal Swabs
- 25 Small (120 µL), clear fixed volume pipettes
- 25 Large (250 µL), pink fixed volume pipettes
- 1 Positive Control Swab (coated with non-infectious RSV antigen)
- 1 Negative Control Swab (coated with heat-inactivated, non-infectious Streptococcus C antigen)

A calibration cassette for the Sofia 2 analyzer is provided separately.

3. Description of Changes

The primary difference between the original Sofia and Sofia 2 analyzer is the design of the optical detection system. Sofia uses a motorized optics unit to collect fluorescent signal data as it performs a series of scans across the longitudinal axis of the Sofia RSV FIA test strip, whereas Sofia 2 captures a still image of the entire test strip window using a complimentary color-oxide semiconductor (CMOS) camera. To emulate Sofia, the Sofia 2 analyzer converts pixels captured by the CMOS camera to fluorescent signal data, after which the resulting data is analyzed in an equivalent manner to Sofia to yield qualitative test results. Other minor adjustments to the Sofia 2 analyzer affect mainly the user interface and include the addition of a touchscreen display and an integrated barcode scanner for sample identification. The test procedure for performing the assay remains unchanged, and the test cassettes used with both instruments are identical.

J. Demonstrating "Simple":

The Sofia RSV FIA with Sofia 2 was designed to be simple and easy to use by incorporating the following features:

- The test uses direct unprocessed nasopharyngeal swab and nasopharyngeal wash/aspirate specimens.
- The test requires basic, non-technique-dependent specimen and reagent handling to obtain accurate test results.
- The provided reagents are premeasured and provided in single-use vials.
- Color coded fixed volume pipettes are provided for sample addition.
- The test cartridges are unitized and contain all the reagents required for analysis.
- The test does not require any operator intervention during the analysis step.
- The test cartridges are keyed and can be inserted into the analyzer only in one direction.
- The Sofia 2 analyzer performs automated analysis of test results and eliminates subjectivity associated with visual reading of results by the end-user.
- The results are printed on a touchscreen as positive, negative or invalid and there is no interpretation required.
- The Sofia 2 touchscreen is designed for ease of use and features a color display that facilitates easy-to-read messages.
- Error messages are unambiguous and include easy-to-interpret solutions.
- No complex troubleshooting or interpretation of error codes are required to operate Sofia 2.
- There is no maintenance required other than wiping of the external surface of the analyzer.
- Calibration, which is required every 30 days, is easily performed with a provided calibration cassette.
- There are no serviceable parts and the instrument is to be returned to Quidel if maintenance is required.
- 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 Assessment

   A comprehensive risk analysis for the Sofia RSV FIA when used with Sofia 2 has been conducted according to ISO 14971 and Quidel's internal procedures. 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 potential failure modes and effect of the failure, potential causes, built in design controls and evaluation of severity, frequency of occurrence, and ability to detect the failure. The elements considered include the intended user, environment, 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 identified risks which could result in erroneous test

results were evaluated in flex studies that stressed the functional limits of the test system (see below).

Detailed software validation and verification documentation was provided, including requirements related to assay performance when using Sofia 2. The instrument software was reviewed under the 510(k) submission (K162911).

2. The Sofia RSV FIA with the Sofia 2 was designed to include numerous features and "lockouts" built into the hardware and software to prevent erroneous results.

   Fail-safe and Failure Alert Mechanisms

   - Cassette drawer and Presence Sensor prevent the test from proceeding when the drawer is not closed or when the test cassette is not present. If the cassette drawer is opened during a test, the analysis will not continue and an invalid result will be reported.
   - Internal sensors prohibit Sofia 2 from performing a test if the internal temperature of the device falls above or below the operating limits (15-35°C).
   - Calibration is required every 30 days to ensure that any signal drift of the optics is controlled. The analyzer reminds the user to check the calibration status of the instrument after 30 days from last calibration. The calibration process takes less than two minutes and is performed with a provided calibration cassette. The analyzer activates a lock-out function that prevents the user from initiating a test procedure if the 30-day calibration interval has passed. The user must perform a calibration check to proceed with testing.
   - If a calibration check fails, the analyzer activates a lock-out function that prevents the user from initiating a test procedure. The user must call for technical support before additional testing can be completed.
   - An internal barcode reader is designed to read the assay cassette barcode and will not allow the test to continue:
     - if the barcode cannot be read.
     - if the assay selected does not match the test type of the cartridge.
     - if the assay cartridge has previously been used.
     - if the assay cartridge is expired.
   - During power initialization, the analyzer performs a start-up self-test to check for the integrity of the optics, the ambient temperature, the clock functionality and the integrity of the memory and functionality of the electronic sensors. All measurements must be within predetermined specifications; otherwise an error message is displayed and the software prevents the use of the analyzer.

   The functionality of Fail-Safe mechanisms built into the software of the Sofia 2 analyzer was demonstrated in studies conducted using the Sofia RSV FIA cassettes and the Sofia 2 analyzer.

Table 1. Fail-Safe Mechanisms for the Sofia 2 Analyzer

	User Action	Expected Results
1	Analyzer fails the start-up self-test (POST) during power initialization	Error message: *POST Failure* User is instructed to discontinue testing and contact Quidel
2	Ambient temperature outside of the instrument specifications (below or above the range limits)	Error message: *Temperature Out of Range* Testing cannot be initiated
3	Attempt to start the test with the drawer open	Error message: *Drawer Open* Testing cannot be initiated
4	Open the drawer while the test cassette is inserted in the Read Now mode	If the image is already captured when the drawer is opened, test analysis continues and the result is reported
5	Open the drawer while the test cassette is inserted in the Walk Away mode (during incubation)	If the drawer is opened during the incubation period, the test is cancelled and the results are not reported
6	Light leak in the instrument during power-on self-test (POST)	Error message: *POST Error* Testing cannot be initiated
7	Image unable to focus during test run	Error message: *Unreadable Cassette* Results analysis will not initiate without scan data from an entire cassette
8	Inserting a cassette with an unreadable barcode	Error message: *Unreadable Cassette* Testing cannot proceed
9	Inserting a cassette for an incorrect assay	Error message: *Cassette not Valid for Current Test* Testing cannot proceed
10	Inserting a previously used cassette	Error message: *Cassette previously used* Testing cannot proceed
11	Inserting an expired cassette	Error message: *Expired Barcode* Testing cannot proceed
12	Assay failure due to procedural issues (QC results, insufficient sample volume etc.)	Error Message: *Invalid* Test must be repeated for results

	User Action	Expected Results
13	Pressing the power switch briefly (1 second) while the test is running in Walk Away mode (to simulate an inadvertent action)	The test continues
14	Pressing the power switch for 5 seconds while the test is running in Walk Away mode (to simulate an intentional power down)	The testing stops, the instrument powers down and no results are reported
15	Expired calibration	Error message: *Calibration Overdue* Testing cannot be initiated
16	Calibration failure	Error Message *Calibration Error* Testing cannot be initiated

Built-in procedural Controls

- The Negative Control Line is designed to control for non-specific binding. If the measured signal is outside of the predetermined specifications, the test will be reported as "invalid."
- 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."
- The Reference Line provides additional information used to verify adequate sample flow through the nitrocellulose test strip and is used by the Sofia 2 to accurately orient the position of the image with respect to the negative control line and procedural control zone.

Dark Image 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

One positive control swab (coated with non-infectious RSV culture lysate) and one negative control swab (coated with heat-inactivated, non-infectious Streptococcus C antigen) are included in each test kit. Each control is processed using a separate test cassette following the procedure outlined in the

instructions. If one or both of the external controls do not perform within specification, the instrument will not proceed.

3. Flex Studies

The operational limits of the Sofia RSV FIA performed on the Sofia 2 analyzer were evaluated in a series of experiments under conditions of "stress."

Samples used for flex study testing were prepared in clinical matrix derived from nasal swab specimens eluted in universal viral transport media (UTM) collected from individuals confirmed to be RSV negative with the Sofia RSV FIA on the original Sofia analyzer. All RSV negative samples were pooled and retested to confirm the absence of RSV antigen. The negative pool was then spiked with commercially available RSV A (Long) and/or RSV B (9318) strains at concentrations 2-3X the assay LoD unless stated otherwise. Negative samples consisted of pooled negative clinical matrix only.

The effect of the following conditions on the performance of the assay was evaluated:

Human Factors/Operator Errors

a. Non-level positioning of the Sofia 2 analyzer

Four different tilt positions of the Sofia 2 analyzer were evaluated by positioning the instrument at two longitudinal (fore and aft) and two latitudinal 15° angles (left and right). Five RSV positive and five RSV negative samples were tested in Walk Away mode on five separate analyzers for each work surface condition. No failures were observed as a result of non-leveled surfaces, and all samples generated expected results.

b. Movement of the cassette during analysis

Sample was added to the Sofia RSV FIA test cassettes and allowed to absorb fully into the sample pad before being tilted vertically at a 90° angle from the work surface. Cassettes remained in the vertical position for 1, 5, or 13 minutes and were then placed horizontally until the 15 minute incubation time was complete. Five RSV positive and five RSV negative replicates were tested in Read Now mode on five separate Sofia 2 analyzers. No failures were observed as a result of placing the cassette vertically, and all samples generated the expected results.

c. Inadvertent dropping of the test cassette

Performance of the Sofia RSV FIA on the Sofia 2 analyzer was evaluated using test cassettes that may have sustained physical damage after falling from either benchtop (3 ft) or storage shelf (8 ft) height. Five RSV positive and five RSV negative replicates were tested in Read Now mode for each of the following conditions:

i. Un-pouched cassettes were dropped from 3 feet (workbench height) onto a hard surface such as tile or linoleum prior to use in the assay. After dropping, each cassette was briefly examined for damage. No damage was observed.

ii. Pouched cassettes were dropped from a height of 8 feet (high storage shelf height) onto a hard surface such as tile or linoleum prior to use in assay. After dropping, each cassette was briefly examined for damage. No damage was observed.

No failures occurred as a result of cassettes being mishandled, and all samples generated the expected results.

d. Varying the sample volume applied to the test cassette

A flex study evaluating varied sample volume was previously conducted for the Sofia RSV FIA on the original Sofia analyzer under K130398/A001. An additional sample volume study was conducted for the Sofia RSV FIA on the Sofia 2 analyzer to evaluate the effect of adding a volume of sample that is greater than the volume specified in the test procedure (120 uL). Although the test specifies that the provided fixed-volume transfer pipette must be used, a larger volume could be delivered if the operator does not follow instructions and "floods" the sample well of the cassette. The study evaluated sample volumes ranging from 140 uL to 300 uL. The data showed that sample volumes 140 uL, 160 uL, 180 uL, 200 uL and 240 uL all generated expected results; at 300 uL false results were observed. The possibility of this error is minimized by the fixed volume pipette that is included with the kit. In addition, the test procedure includes a bolded caution directing the operator to use the provided transfer pipette and not to pour the sample onto the sample pad.

e. Varying development/read time in Read Now mode

The effect of varied incubation time (0 to 30 minutes) of the Sofia RSV FIA prior to inserting the test cassette into the analyzer was evaluated on nine different Sofia 2 instruments using the Read Now mode. A total of 27 replicates were tested per RSV strain in addition to negative sample matrix after 0, 2, 5, 8, 10, 15, 20, 25 and 30 minute incubation times. All positive and negative samples generated expected results when the Read Time was as early as 8 minutes and as late as 30 minutes. To mitigate the risk of obtaining invalid or false negative test results observed with short incubation times (<8 minutes), The assay procedure clearly states that the result must be interpreted at 15 minutes when the Sofia 2 analyzer is used in the Read Now mode.

f. Varying reagent and sample extraction volumes

Flex studies that examined sources of errors related to the steps of the test procedure were previously conducted in support of the CLIA Waiver application

for the Sofia RSV FIA for use with Sofia analyzer (K130398/A001). Those studies evaluated factors such as the effect of utilizing an incorrect volume of Reagent Solution to rehydrate the lyophilized extraction material, and adding varying amounts of nasopharyngeal aspirate/wash or other liquid patient sample to the rehydrated lyophilized reagents prior to testing. Those studies demonstrated that the test is robust and not vulnerable to the evaluated errors.

Specimen Integrity/Handling and Assay Stability

a. Effect of mucin and accuracy of Early Read feature

A flex study was conducted to evaluate the effects of varying concentrations of mucin, which may be present in natural clinical sample matrix, on performance of the Sofia RSV FIA when tested on the Sofia 2 analyzer.
RSV negative samples used in this study were prepared in 45.5% M4-RT or M6 viral transport media in saline containing varying concentrations of porcine mucin (0.2%, 0.4%, 0.5%, 0.6% and 0.8%). For each medium and concentration of mucin tested, ten RSV negative replicates were prepared and analyzed at 3, 5, 8, 10, and 15 minutes. RSV positive samples were similarly prepared, spiked with RSV A strains over a series of low and high concentrations, and analyzed at early read and 15 minute time points. The study demonstrated that the test is robust with regard to the effect of mucin.

b. Specimen Handling and Assay Stability

Studies that examined additional factors related to specimen integrity and handling, and stability of reagents were previously conducted in support of the CLIA Waiver application for the Sofia RSV FIA for use with the original Sofia analyzer (K130398/A001) and were not repeated for this application. Those studies evaluated deviations from the swab specimen handling protocol (i.e., swab rotating, squeezing, omitting 1 minute incubation step), the stability period of the extracted specimens prior to adding to the test cassette, and the stability period for the rehydrated extraction reagent prior to mixing with patient sample. Study results showed that the system is robust, insensitive to errors related to deviations from the specimen handling protocol, and stable for at least 24 hours after rehydration of lyophilized material. Studies also showed that extraction time can be extended for up to 4 hours without the risk of obtaining false negative RSV test results.

c. Effect of Various Transport Media on Sample Stability

The performance of the assay using specimens that had been stored in various types of viral transport media at 2-8°C or 25°C for 0, 4, 8, 24, and 72 hours was previously assessed under K130398/A001 using the original Sofia analyzer and was not repeated for this application. In this study, ten different types of media were tested for compatibility by collecting 20 nasopharyngeal swabs from

asymptomatic, RSV negative patients per medium and testing each clinical matrix with or without the addition of RSV analyte at concentrations near the assay LoD under the conditions specified above. The studies demonstrated that the assay yields expected results with the media types and storage conditions listed in the product package insert.

d. Calibration Cycle Stability

This study assessed the effectiveness of the calibration procedure in preventing signal drift of the Sofia 2 and its effect on assay performance during the maximum 30-day calibration cycle. Using a method file specifically designed for the evaluation of the signal drift over time, the variability in RFU for each of the four control lines in the test strip window of the calibration cassette was measured to determine the percentage change in RFU over 3 reads per working day for 45 calendar days. The observed change in the fluorescence was less than 6% for all four lines across five Sofia 2 instruments.

Environmental Factors

a. Operational temperature and humidity outside of the expected conditions of use

The recommended operational parameters for the Sofia 2 analyzer are between 15°C and 35°C. In this study, the performance of the Sofia RSV FIA was examined under conditions in which either the reagents alone, or both the reagents and the Sofia 2 analyzer were exposed to temperatures and humidity outside of the normal operating range.

To test the effect of temperature and humidity on reagents alone, the test kits were equilibrated under normal laboratory conditions (20.7°C/ambient humidity) and at two temperatures outside of the instruments specifications (3.8°C/ambient humidity and 40°C/90% humidity) for 30 minutes prior to testing. Five RSV positive and five RSV negative replicates were tested in Read Now mode on five separate Sofia 2 analyzers kept at ambient temperature and humidity. No failures were observed and all samples generated the expected results.

In the second study, both the reagents and the Sofia 2 analyzers were equilibrated to either ambient temperature (20.7°C) and ambient humidity, 3.8°C and ambient humidity, or 40°C at 90% humidity and tested in Read Now mode. As expected, no data could be acquired for the extreme conditions at 3.8°C and 40°C due to the Sofia 2 analyzer internal temperatures being out of range. At ambient temperature and humidity, no failures were observed and all samples generated the expected results.

b. Comparing the effect of temperature on the two operational modes

All test materials including the Sofia RSV FIA test cassette and the Sofia 2 analyzer were equilibrated to 15°C, room temperature or 30°C for at least 30 minutes prior to testing. The 15 minute incubation period for samples tested in Read Now mode was also performed at the specified condition. Ten RSV positive and ten RSV negative sample replicates were tested under each condition using five separate Sofia 2 analyzers. No failures were observed regardless of the selected mode, and all samples generated the expected results.

c. Vibrations due to surrounding instrumentation

Five individual Sofia 2 analyzers were placed on a benchtop at various distances relative to a lab centrifuge (20, 39.5, 60, 84 and 104 centimeters). While the centrifuge was running at 10,000rpm, five RSV positive and five RSV negative sample replicates were prepared and placed into the Sofia 2 analyzers set in Walk Away mode. All tests gave expected results. No failures were observed as a result of proximity to vibrating machinery, and all samples generated the expected results.

d. Exposure to sunlight during processing and incubation (environmental lighting)

Sample-loaded cassettes were placed on the workbench in direct environmental light, just 17 cm from the laboratory window, for the full 15 minute incubation period prior to being inserted into the Sofia 2 analyzer. Five RSV positive and five RSV negative replicates were tested on three separate Sofia 2 analyzers in Read Now mode. No failures were observed as a result of the incubation of the RSV FIA test cassette in direct environmental sunlight, and all samples generated the expected results.

The flex studies that were conducted demonstrated that the system is robust when operating under conditions of stress. The combination of built in fail-safe mechanism and explicit cautions in the labeling provide adequate controls to ensure that improper use of the device is not likely to yield false results.

L. Demonstrating "Accuracy":

The accuracy of the Sofia RSV FIA assay with Sofia 2 when used by untrained operators in CLIA Waived settings was determined based on the clinical performance data generated on the Sofia analyzer (reviewed under K130398/A001) and on a method comparison study conducted with trained operators to demonstrate comparable performance between the Sofia and Sofia 2 analyzers. An additional study was conducted at CLIA Waived sites to demonstrate that untrained operators can obtain accurate results when testing weakly reactive samples using the Sofia RSV FIA test with the Sofia 2 analyzer.

1. Clinical Performance of the Sofia RSV FIA with the Sofia analyzer

   The sensitivity and specificity of the Sofia RSV FIA used with Sofia analyzer was demonstrated in a prospective CLIA Waiver clinical study at CLIA waived sites with untrained operators, with specimens from pediatric patients ages 0-<7 years. The test results obtained with the Sofia RSV FIA were compared to the results obtained by viral cell culture. This study was conducted at 16 CLIA-waived sites during the months of spring (Feb-April) and fall (Oct-Dec) of 2012 with 37 untrained operators representative of CLIA-waived settings. The study included 2193 subjects: one thousand fifty-seven (1,057) subjects provided a pair of nasopharyngeal swabs and 1,136 provided a nasopharyngeal aspirate/wash specimen. The sensitivity of the assay was demonstrated to be 87% [(134/154), 95% CI: 81%-92%] with NP specimens and 92% [(141/154), 95% CI: 86%-95%] with NA/W specimens when combining the results for 0-<7 years of age. The combined specificity of the assay was demonstrated to be 96% [(869/903), 95% CI: 95%-97%] and 98% [(960/982), 95% CI: 97%-99%] with NP and NA/W specimens, respectively.

   Data from the clinical study supported the CLIA Waiver approval of the Sofia RSV FIA with Sofia, and was reviewed under K130398/A001. The decision to grant CLIA Waiver for the Sofia RSV FIA on the Sofia analyzer was based on the information provided in the CLIA Waiver application, as well as a benefit/risk assessment. Conclusions from the benefit/risk assessment supported a public health need for improved tests that could offer a more timely diagnosis of RSV infection in pediatric patients in order to provide optimal patient management, particularly to aid in the establishment of quick infection control measures. This device offered a fast time to result and had a number of fail-safe mechanisms including the requirement for a cassette reader to obtain test results, which eliminates test subjectivity and enhances overall assay consistency.

2. Comparison of the Sofia and Sofia 2 analyzers

   A study was conducted to demonstrate comparable performance of the Sofia RSV FIA when tested on Sofia vs. Sofia 2 in the hands of trained operators. This study (reviewed under K162911) was conducted at three clinical testing using identical panels of known positive and negative clinical and contrived samples prepared in viral transport media (VTM). The viral concentrations of the panel members were distributed across the range of the assay based on the signal-to-cutoff ratio (S/CO). For each sample, three Sofia and three Sofia 2 results were obtained from all three sites combined. All samples were coded and used to prepare the randomized panels. A total of 200 samples per site were tested resulting in a total of 600 results.

   The PPA and NPA between the Sofia 2 and Sofia analyzers was 97.2% ((314/323); 95% CI: (94.7%-98.6%)) and 96.4% ((267/277); 95% CI: (93.4%-98.1%)), respectively, demonstrating that the performance of the assay on both analyzers was similar. Additionally, an appropriate regression analysis of the numeric values of Sofia RSV FIA of Sofia 2 analyzer vs Sofia analyzer for the samples close to the

cutoff was performed and showed a minimal bias between the results from the two instruments. Please refer to K162911 for further details.

Because the Sofia and Sofia 2 devices are similar in both design and function, with limited changes to the user interface and QRI, an additional CLIA Waiver clinical study for the Sofia 2 analyzer was not needed.

3. Performance with Analyte Concentrations Near the Assay Cutoff

A study was conducted to demonstrate the ability of untrained users in CLIA Waived settings to obtain accurate results with weakly reactive samples when performing tests using the Sofia RSV FIA on the Sofia 2 analyzer.

The near the cutoff CLIA Waiver study was conducted by three operators at each of three geographically diverse CLIA-Waived sites located throughout the U.S. The operators that participated in the study were non-laboratory personnel and included medical assistants, nurses, clinical research assistants, and office staff. The work experience of the operators ranged from < 1 year to 12 years and their education level ranged from high school to college. The operators performed the testing using the Quick Reference Instructions; no additional training was provided to the operators.

For this study, each of the three CLIA Waived sites received 48 coded, low positive or negative samples prepared in clinical swab matrix. Low positive samples contained inactivated RSV at concentrations near the assay LoD (C95). The 48 coded samples at each testing site were distributed evenly among operators such that each individual tested at a minimum five positive and five negative samples. Testing was performed on ten different days over a period of approximately two weeks, and was incorporated into the daily workflow.

One sample required repeat testing due to an initial invalid test result. Repeat testing of the sample resulted in a valid result, which was included in calculations of agreement with expected results shown in Table 2 below.

Table 2: Performance of the Sofia RSV FIA with Sofia 2 with Samples Containing Virus Concentrations Near the Assay Cutoff

Percent Agreement with Expected Results					
Sample Type	**Site 1**	**Site 2**	**Site 3**	**Overall**	**Overall 95% CI**
RSV Low Positive	95.8% (23/24)	95.8% (23/24)	100% (24/24)	97.2% (70/72)	90.4 - 99.2%
Negative	100% (24/24)	100% (24/24)	100% (24/24)	100% (72/72)	94.9 - 100%

Testing of the samples was divided between the two operational modes available on the Sofia 2 analyzer, the Read Now and the Walk Away modes. The results are presented below.

Table 3: Performance of the Sofia 2 Analyzer in Read Now and Walk Away Modes

Percent Agreement with Expected Results						
Sample Type	**Operational Mode**	**Site 1**	**Site 2**	**Site 3**	**% Overall Agreement**	**95% CI**
RSV Low Positive	Read Now	11/12	12/12	12/12	97.2% (35/36)	85.8 – 99.5%
	Walk Away	12/12	11/12	12/12	97.2% (35/36)	85.8 – 99.5%
Negative	Read Now	12/12	12/12	12/12	100% (36/36)	90.4 - 100%
	Walk Away	12/12	12/12	12/12	100% (36/36)	90.4 – 100%

There were no significant differences in the observed reactivity of the device weakly reactive samples between operators, between sites, and between the two operational modes. All negative samples yielded expected results at all three sites for all operators.

The study results demonstrated that untrained users were able to perform the test correctly and the test provided the expected results for samples with virus concentrations near the assay cutoff.

M. Proposed Labeling

Quick Reference Instructions (QRI)

The QRI was reviewed in detail to ensure that the directions are clear and easy to understand and that all precautions are included as appropriate. The QRI for the use of the test with either one of the instruments is written in simple language (at 7th grade reading level) and contains pictorial descriptions of the individual steps. The test instructions for performing the assay using Sofia 2 remain unchanged from the original instructions for use of the assay with the Sofia. The QRI has separate sections for Sofia and Sofia 2 for easy reference by the user. The only difference between the two sections is the Early Read feature when using the Sofia 2 in the Walk Away mode and the pictorial images of the two instruments. The interpretation of results is identical on both instruments; however, the interpretation of results section for Sofia 2 shows the specific graphics on the Sofia 2 screen designed to make the display more user-friendly.

N. Operator Questionnaire Results

Each operator that participated in the study completed the Operator Questionnaire designed to assess the operator impressions from using the test. The users found the test easy to perform and the written instructions easy to follow.

O. Conclusion

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