**CLIA Waiver by Application**

**Approval Determination Decision Summary**

**A. Document Number**

CW160016

**B. Parent Document Number**

K162438

**C. Purpose of the Submission**

To obtain CLIA Waiver of the Sofia Influenza A+B FIA test when used with the Sofia 2 analyzer.

The Sofia Influenza A+B FIA test for use with the Sofia analyzer has been cleared under the K112177 and subsequently CLIA waived under K112177/A003 (for swab specimens) and K112177/A004 (for liquid specimens). The data supporting equivalence of the assay performance when used with the new Sofia 2 and Sofia analyzers were submitted under the K162438 and the device, Sofia Influenza A+B FIA with Sofia 2, was cleared on April 14, 2017.

**D. Measurand (analyte)**

Influenza A and influenza B viral nucleoprotein antigens

**E. Sample Type**

Direct nasal and nasopharyngeal swabs,
Nasopharyngeal aspirate/wash specimens

**F. Type of Test**

Qualitative Immunoassay

**G. Applicant**

Quidel Corporation

**H. Proprietary and Established Names**

Sofia Influenza A+B FIA
Sofia 2 Analyzer

## I. Test System Description

1. Overview

The Sofia Influenza A+B FIA is a lateral flow immunoassay that uses a sandwich design to detect and differentiate influenza A and influenza B viral antigens in patient specimens. The Sofia Influenza A+B FIA test components have not been modified; detailed description of the lateral flow device is available in submissions K153012 and K112177, describing the Sofia Influenza A+B FIA use with the original Sofia analyzer. A pre-measured volume of Reagent Solution is added to the Reagent Tube and the contents are swirled. The patient nasal or nasopharyngeal swab is then placed in the Reagent Tube and rolled against the wall and bottom of the tube, then left to stand for 1 minute. 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 and swirled. In either case, a sample of that mixture is then transferred to the sample well on the test cartridge using the provided clear, fixed volume (120 µL) pipette. The mixture flows through the test strip and, if influenza A or B antigens are present in the sample, they will be captured on the surface of the nitrocellulose by analyte specific antibodies to form immunofluorescent lines (invisible by a naked eye) in specific locations on the test strip.

2. Results Interpretation

A positive result for either analyte 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. There are five possible results: (1) positive for influenza A and negative for influenza B; (2) positive for influenza B and negative for influenza A; (3) positive for both influenza A and B; (4) negative for both influenza A and B; and (5) invalid. Influenza A and B dual positive results should be re-tested and repeated; a dual positive result should be confirmed by virus culture or an FDA-cleared influenza A and B molecular assay. If an invalid test result is reported, the Sofia Influenza FIA should be repeated with a new patient sample and a new test cassette.

Note: The Sofia and Sofia 2 analyzers may be set to one of two operating modes: Walk Away or Read Now. Time to results for the Sofia and Sofia 2 analyzer are described below.

- In Walk Away Mode, the user inserts the test cassette into the analyzer immediately following addition of the specimen to the Sofia Influenza A+B 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 or Sofia 2 analyzer. Positive and negative test results are displayed within 1 minute.

The results are displayed on the instrument screen. The results can also be printed on an integrated printer if this option is selected.

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 Nasal 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 recombinant influenza A and influenza B antigens)
- 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 the Sofia 2 analyzer is the design of the optical detection system. While the original Sofia uses a motorized optics unit to collect fluorescent signal data as it performs a series of scans across the longitudinal axis of the test strip, the Sofia 2 captures a still image of the entire test strip window using a CMOS camera. To emulate the Sofia, the Sofia 2 analyzer converts pixels captured by the CMOS camera to digital data, which is then analyzed in an equivalent manner to that used by the 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. Both the original Sofia and the Sofia 2 analyzer instruments utilize the same fail-safe and failure alert mechanisms, the same calibration and assay-specific cartridges, and the same ultraviolet light-emitting diodes (UV LEDs) to excite the fluorophore. The test procedure for performing the assay remains unchanged. The test cassettes used with both instruments are identical.

The Sofia 2 has a USB port for connection of an external printer provided by Quidel which allows the user to print patient test results and quality control testing results, if desired.

The built in internal barcode scanner in the Sofia 2 enables the user to enter data such as user ID numbers, patient ID numbers, and order numbers without having to manually enter the information on the Sofia 2 touch screen.

## J. Demonstrating "Simple"

The Sofia Influenza A+B FIA with Sofia 2 was designed to be simple and easy to use by incorporating the following features:

- The test uses direct unprocessed nasal and 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 7$^{th}$ 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 Influenza A+B 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).

The sponsor provided detailed software validation and verification documentation, including requirements related to assay performance when using Sofia 2. The instrument software was reviewed under the parent 510(k) submission (K162438).

2. The Sofia Influenza A+B 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 are designed to 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.
   - Temperature sensor is designed to prevent the test from proceeding when the internal temperature of the analyzer is less than 15°C or greater than 35°C.
   - Calibration is required every 30 days to prevent signal drift and the instrument will not proceed until the calibration status is updated. 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. If the calibration fails, the system goes into an error mode and a message is displayed to contact Quidel Technical Support.
   - Internal barcode reader is designed to read the assay cassette barcode:
     - The instrument will not proceed if the cassette is not in the correct orientation (or is absent) and the barcode cannot be read;
     - The calibration will not proceed if a patient test cassette is present instead of the calibration cassette.
     - The instrument will not proceed if the assay cartridge has previously been used;
     - The instrument will not proceed if the assay cartridge is expired.
   - Power-on Self-Test (POST) is initiated each time the instrument is started and it checks for the integrity of the optics, the ambient temperature, the clock functionality, the integrity of the memory and the functionality of the electronic sensors. All measurements must be within predetermined specifications; otherwise the instrument will not proceed.

   External Controls

   One Positive Control Swab (coated with non-infectious recombinant influenza A and influenza B antigens) and one Negative Control Swab (coated with heat-inactivated, non-infectious Streptococcus C antigen) are included in each reagent kit. Each control is processed using a separate test cassette. If one or both of the External Controls do not perform within specifications, the instrument will not proceed.

In-test Strip Controls

- The Negative Control Line (NC) 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 (PCZ) 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 is used by the Sofia 2 to accurately orient the position of the image with respect to the PCZ and NC.

Dark Image Check

This function is used during each measurement cycle and is designed to detect leakage of ambient light. If any of these are detected the Sofia 2 will generate an internal error and will not allow the test to proceed.

Self-checks

The analyzer has an internal function of on-going internal performance monitoring and if the data indicate that performance is degrading, the user will be instructed to contact Quidel's Technical Support, in which case the company will either repair the instrument electronics or replace the entire unit.

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

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

| | User Action | Expected Results |
|---|---|---|
| 1 | Expired calibration | Error message:<br>*Calibration due 0 days*<br>Testing does not proceed |
| 2 | Pressing the power switch briefly (1 second) while the test is running in Walk Away mode (to simulate an inadvertent action) | The test continues |
| 3 | 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 |
| 4 | Ambient temperature outside of the instrument specifications (below and above the range limits) | Error message:<br>*Temperature Out of Range*.<br>Testing does not proceed |
| 5 | 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 |
| 6 | Open the drawer while the test cassette | Error message: |

| | | |
|---|---|---|
| | is inserted in the Walk Away mode (during incubation) | *Test cancelled*<br>Testing/Results analysis does not proceed |
| 7 | Defective cassette is used (bent, scratched, etc.) that may result in false peaks | Invalid result<br>(The algorithm looks for lines in specific locations and uses a series of conditional checks to verify that the peak parameters are valid for size, shape and location) |
| 8 | Inserting a previously used cassette (barcode reader/barcode functionality) | Error message:<br>*Cassette cannot be reused*<br>Testing does not proceed |
| 9 | Inserting an expired cassette (barcode reader/barcode functionality) | Error message:<br>*The Cassette is past the expiration date. Please retest with a non-expired cassette.*<br>Testing does not proceed |
| 10 | Inserting a cassette for an incorrect assay (barcode reader/barcode functionality) | Error message:<br>*Cassette not Valid for Current Test*<br>Testing does not proceed |
| 11 | Inserting a cassette with an unreadable barcode (barcode reader/barcode functionality) | Error message:<br>*Unreadable Cassette Barcode*<br>Testing does not proceed |
| 12 | Attempt to run test with no cassette present | Error message:<br>*Unreadable Cassette Barcode*<br>Testing does not proceed |
| 13 | Attempt to start the test with the drawer open. | Error message: *Drawer Open*<br>Testing does not proceed. User informed with animation to insert cassette and close drawer. |
| 14 | Light leak in the instrument and power on the instrument (functionality of the Power On Self-Test (POST)) | Error message:<br>*Power On Self Test Error*<br>Testing does not proceed |
| 15 | Condensation inside the instrument and power on the instrument (functionality of the POST) | Error message:<br>*Power On Self Test Error*<br>Testing does not proceed or Invalid result |
| 16 | Using a damaged Calibration cassette | Calibration fails, testing does not proceed or error message:<br>*Unreadable Cassette barcode*<br>Testing does not proceed |

All studies generated the expected error messages confirming the effectiveness of the fail-safe mechanisms built into the analyzer's software.

3. Flex Studies

The operational limits of the device were evaluated in a series of experiments under "stress" conditions of use.

The test samples were prepared in a contrived negative matrix of 0.5% mucin in M5 VTM. Positive samples were prepared by spiking with commercially sourced influenza A (A/California/07/2009) or influenza B (B/Malaysia/2506/04) virus to prepare test samples at low positive concentrations (approximately 2-3x the assay LoD); negative samples consisted of the contrived negative matrix.

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 (fore, aft, left, and right) were evaluated by placing the Sofia 2 analyzers on a benchtop tilted at 15° and testing each sample in five individual replicates at each position. No failures were observed and all samples generated expected results.

b. Movement of the cassette during analysis

   This study evaluated the effect of moving the test cassette into vertical position during the incubation time. Samples were first added to the Sofia influenza A+B 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. No failures were observed and all samples generated expected results.

c. Inadvertent dropping of the test cassette

   This study evaluated the potential of invisible damage to the test cassette when inadvertently dropped from a bench top height or from a high storage shelf height.

   i. Un-pouched cassettes were dropped from 3 feet (workbench height) onto a hard surface (tiled floor) prior to use in the assay. After dropping, each cassette was briefly examined for damage. Only visually undamaged cassettes were to be tested.

   ii. Pouched cassettes were dropped from a height of 8 feet (high storage shelf height) onto a hard surface (tiled floor) prior to use in the assay. After dropping, each cassette was briefly examined for damage. Only visually undamaged cassettes were to be tested.

   One cassette dropped from the height of 3 feet was visibly damaged and was not used

for testing. One invalid result was observed with a device dropped from the height of 3 feet; all other tests generated expected results. There were no false positive or false negative results observed during the study.

d. Varying the sample volume applied to the test strip

This study evaluated the effect of varied sample volume (outside of the 120 µL delivered with the fixed-volume transfer pipette) on the performance of the assay. Ten different volumes were evaluated, ranging from 40 µL to 360 µL. The data showed that 80 µL to 160 µL sample volumes generate expected results. 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 clear caution directing the operator to use the provided transfer pipette and not to pour the sample onto the sample pad.

e. Varied development/read time in Read Now mode

This study evaluated the effect of incorrect timing of the incubation interval (outside of the specified 15 minutes) on the performance of the assay. The study was conducted at ambient temperature utilizing the Read Now mode of the Sofia 2 analyzer. Each sample was tested in five replicates at 0, 2, 5, 8, 10, 15, 20, and 30 minutes after the sample was added to the cassette. All samples generated expected results when the Read Time was as early as 5 minutes and as late as 30 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. Mixing lots of reagents and external controls

Three lots of negative and positive external controls were run with two different lots of Sofia Influenza A+B FIA kits on the Sofia 2 analyzer. For each lot of controls, 10 individual positive and negative control swabs were run (total of 60 samples), using five Sofia 2 analyzers. All tests generated expected results.

g. Previously conducted flex studies

Flex studies that examined additional sources of errors related to the steps of the test procedure were previously conducted in support of the CLIA waiver applications for the Sofia Influenza A+B FIA for use with Sofia analyzer (K112177/A003 and K112177/A004) and were not repeated for this application. Those studies evaluated such factors as the effect of incorrect volume of the Reagent Solution used for rehydration of the lyophilized extractant, the stability interval of the rehydrated extraction reagent prior to sample addition, and the effect of improper storage conditions for the cassettes (-20°C and 55°C). Those studies demonstrated that the test is robust and not vulnerable to the evaluated errors.

h. Previously conducted field study

A separate field study was previously conducted to evaluate the ability of untrained operators to use the correct test procedure corresponding to either the swab specimens or to the liquid specimens. In that study, the user must use the provided pink fixed-volume pipette to add the liquid sample (a nasal wash or aspirate) into the Reagent Tube and mix the contents. In the next step the user is required to use the clear fixed-volume pipette to transfer the sample from the Reagent Tube into the sample well of the assay cartridge. In that study nine CLIA waived operators (intended users) at 3 CLIA waived sites tested a total of 300 samples provided as randomized panels of swab samples intermixed with liquid samples. The data from the study was reviewed under K112177/A004 and demonstrated that the users were able to select the appropriate test procedure, depending on the sample type and to obtain expected test results.

Specimen Integrity and Handling

a. Effect of mucin on the Early Read feature

The effect of mucin that may be present in the sample matrix on the assay performance was evaluated by testing negative samples consisting of two types of viscous viral transport media (M4-RT and M6) supplemented with porcine mucin at several dilutions, ranging from 0.20% to 0.80% (w/v). Generally, the increased mucin concentration made the sample more viscous, resulting in slower flow, such that at the earliest 3 minute read time the signal in the device window did not have the necessary features for the search algorithm to find a valid image, resulting in several invalid results at the 3-minute (187 sec) read at the highest mucin concentration (0.8%) in 50% M4-RT. All valid results obtained with the negative samples were 100% negative.

b. Previously conducted studies

Flex studies that examined factors related to the specimen integrity and handling were previously conducted in support of the CLIA waiver applications for the Sofia Influenza A+B FIA for use with Sofia analyzer (K112177/A003 and K112177/A004) 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), stability period of the extracted specimens prior to adding to the test cassette, and varying the volume of the liquid sample (wash or aspirate) added to the Extraction Solution. All studies showed that the system is robust and not sensitive to errors related to deviations from the specimen handling protocol.

Environmental Factors

a. Operational temperature and humidity

The study was designed to evaluate the effect of temperature and humidity outside of the expected normal conditions of use. Temperatures of 4°C and 40°C with humidity

up to 90% RH (relative humidity) were examined. All testing materials, including cassettes, extraction reagent, samples and Sofia 2 analyzers were equilibrated to the specified temperatures in an incubation chamber for 30 minutes prior to testing. The effect of the temperature and humidity was evaluated in different combinations:

i. Sofia 2 at ambient temperature (22.5° C) and assay reagents at 40°C/90% humidity (RH),
ii. Sofia 2 at 40°C/90% humidity (RH) and assay reagents at 40°C/90% humidity (RH)
iii. Sofia 2 at ambient temperature (22.5° C), and assay reagents at 4°C/ambient humidity,
iv. Sofia 2 at 4°C/ambient humidity, and assay reagents at 4°C/ambient humidity,

When Sofia 2 analyzers are left at temperatures significantly outside of the normal operating range (4°C with ambient humidity or 40°C with 90% humidity), Sofia 2 will not run; no test results were obtained at those conditions. In this situation, an error occurs and a message is displayed on the screen that the temperature is out of range.

Leaving the assay reagents at temperatures and humidity levels outside of the expected normal conditions, as above, while the Sofia 2 analyzer is kept at ambient temperature and humidity had no effect on the assay performance; all samples tested generated expected results.

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

The performance of the assay using the two operational modes (the Read Now and the Walk Away modes) of the Sofia 2 analyzer was compared at three operating temperatures: 15°C, 22.5°C (ambient) and 30°C. All testing materials, including cassettes, extraction reagent, samples and Sofia 2 analyzers were equilibrated to the specified temperatures in an incubation chamber for 30 minutes. Ten samples were tested for each sample type (negative, low positive for influenza A and low positive for influenza B), at each temperature, and in each development mode. The test generated expected results for all samples at all testing temperatures in both modes.

c. Vibrations due to surrounding instrumentation

This study was designed to evaluate the effect of vibrations produced by laboratory equipment on the Sofia Influenza A+B FIA test cassettes run with the Sofia 2 analyzer. Individual Sofia 2 analyzers were placed on a benchtop at various distances relative to a lab centrifuge (21.5, 43, 65.5, 88 and 112 centimeters). Samples were loaded onto the cassettes and placed onto the Sofia 2 analyzers to run in the Walk Away mode while the centrifuge was running at 10,000 rpm. All tests gave expected results. One influenza B positive sample generated an invalid result and was repeated, giving the expected result. No failure points were recorded for all testing conditions of the vibration test.

d. Exposure to sunlight (environmental lighting)

The test samples were processed and the sample-loaded cassettes were allowed to incubate on the workbench, exposed to bright sunlight. The cassettes were incubated for 15 minutes in natural sunlight and the results were recorded using the Read Now mode. Two Sofia 2 analyzers produced "unreadable barcode detected" error message, and the cassette was tried again and passed after 4 tries. One replicate would not run after multiple tries with the "unreadable barcode detected" message so the replicate was re-run and passed. All tests generated expected results; no erroneous results were generated.

Hardware and Software

a. Early Read study with contrived positive samples

This study was designed to evaluate the viral concentrations of the influenza virus that can be detected when the test cassettes are imaged and interpreted by the Sofia 2 analyzer at 3, 5, 8, 10 and 15 minutes in Walk Away mode. The positive samples were prepared in VTM at concentrations ranging from 5 to 2000 $TCID_{50}$/mL for influenza A and from 3 to 2000 $TCID_{50}$/mL for influenza B. In this study 10 replicates of each positive sample and 20 replicates of each negative sample were tested using 20 Sofia 2 analyzers. The data showed that, depending on the viral load, samples with influenza A concentrations greater than 53 $TCID_{50}$/mL and with influenza B concentrations greater than 63 $TCID_{50}$/mL can be interpreted as positive as early as 3 minutes.

b. Early Read study with clinical samples

This study was designed to verify the performance of the Sofia 2 analyzer utilizing the multi-read function of the Sofia 2 analyzer. The study was conducted in the Walk Away mode using 20 Sofia 2 analyzers. A total of 30 clinical negative samples were tested with the Sofia Influenza A+B FIA assay on the Sofia 2 in Walk Away mode. In this study all samples generated the expected negative results at all read time-points, i.e., 3, 5, 8, 10, and 15 minutes.

c. 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. The variability in the signal was analyzed to determine the percentage change in signal over 3 reads per working day for 45 calendar days. The observed change in the signal was less than 5.5% across five Sofia 2 instruments.

The conducted flex studies demonstrated that the system is robust and is not sensitive to user errors or environmental stresses. The combination of built in fail-safe mechanisms

and explicit cautions in the labeling provide adequate controls to ensure that improper use of the device is not likely to yield erroneous results.

## L. Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

The accuracy of the Sofia Influenza A+B 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 K112277/A003 and K112277/A004) and on a method comparison study conducted with trained operators to demonstrate comparable performance between the Sofia 2 and the Sofia analyzers. An additional field study was conducted at CLIA waived sites to demonstrate that untrained operators can obtain accurate results when testing weakly reactive samples using the Sofia Influenza A+B FIA test with Sofia 2 analyzer.

1. Clinical Performance of the Sofia Influenza A+B FIA used with Sofia

   The sensitivity and specificity of the Sofia Influenza A+B FIA used with Sofia analyzer was demonstrated in the CLIA waiver clinical study conducted during the spring of 2011 with 1973 patients. The results of the Sofia Influenza A+B FIA with Sofia, when used by operators representative of those encountered at CLIA waived sites, were compared to results obtained by viral culture (Comparator Method). The sensitivity of the assay with nasal and nasopharyngeal swabs was demonstrated to be 93% [(219/235), 95% CI: (89% -96%)] for influenza A and 90% [(188/209), 95% CI: (85% - 93%)] for influenza B. The specificity of the assay (with nasal and nasopharyngeal swabs) was demonstrated to be 95% [(1014/1072), 95% CI: (93% - 96%)] for influenza A and 96.4% [(1058/1098), 95% CI: (95% - 97%)] for influenza B. The sensitivity of the assay with nasopharyngeal washes and aspirates was demonstrated to be 99% [(68/69), 95% CI: (92% - 100%)] for influenza A and 88% [(46/52), 95% CI: (77% - 95%)] for influenza B. The specificity of the assay (with nasopharyngeal washes and aspirates) was demonstrated to be 96% [(554/580), 95% CI: (93% - 97%)] for influenza A and [96% (575/597), 95% CI: (94% - 98%)] for influenza B.

   The review of the CLIA waiver application for the original Sofia Influenza A+B FIA with Sofia included additional analytical reactivity data generated in a third party study conducted in 2011, using a protocol developed by CDC and BARDA, comparing analytical reactivity among eleven legally marketed antigen based rapid influenza detection tests (RIDTs). The results of this study showed that for both influenza A and influenza B, the Sofia Influenza A+B FIA with the Sofia analyzer demonstrated increased reactivity when compared to the reactivity of some of the older tests evaluated in the study.

   The decision to grant CLIA waiver for the Sofia Influenza A+B FIA with Sofia in 2011 included consideration of all the information provided in the CLIA waiver application to FDA, as well as a risk/benefit assessment and the public health needs for improved RIDTs. FDA determined that the improved performance of the Sofia Influenza A+B FIA with Sofia over the earlier influenza rapid tests combined with an automated reader

feature represented an overall improvement over existing devices of this type and provided an important diagnostic tool to physicians in CLIA waived settings.

2. Comparison of Sofia 2 vs. Sofia analyzers

The equivalence of performance of the Sofia Influenza A+B FIA when used with the Sofia 2 or Sofia analyzers was demonstrated in a study conducted in support of the K162438. The study was conducted at three clinical testing sites with panels of known positive and negative clinical samples and contrived samples prepared in viral transport media. 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. Fifteen Sofia and fourteen Sofia 2 analyzers were used across the three testing sites. The testing of all samples was conducted in Walk Away Mode. An appropriate regression analysis of the numeric values obtained on Sofia 2 vs. Sofia for samples at viral concentrations near the assay cutoff was performed and showed a minimal bias between the results from the two instruments.

The qualitative performance of the Sofia Influenza A+B FIA assay using Sofia 2 was compared to the performance of the assay when using Sofia. Performances were compared for influenza A positive, influenza B positive, and influenza negative samples separately. The positive percent agreement (PPA) and the negative percent agreement (NPA) between results obtained with the Sofia Influenza A+B FIA on the Sofia 2 and those on the Sofia were calculated. The summary of the results is shown in the table below.

**Table 2. Performance of the Sofia Influenza A+B FIA using Sofia 2 vs. Sofia**

| | PPA | 95% CI | NPA | 95% CI |
|---|---|---|---|---|
| Influenza A | 97.7% (304/311) | 95.4% - 98.9%. | 96.6% (423/438) | 94.4% - 97.9% |
| Influenza B | 98.1% (154/157) | 94.5% - 99.4%. | 99.2% (587/592) | 98.0% - 99.6%. |

Additional details of the comparison study may be found in the Decision Summary for K162438 at https://www.accessdata.fda.gov/cdrh_docs/reviews/K162438.pdf.

The data showed that the performance of the assay on both analyzers was comparable. Because the Sofia 2 and Sofia analyzers are similar in both design and function, with limited changes to the user interface and minimal changes to the test procedure in the QRI, an additional comparison of the Sofia Influenza A+B test with Sofia 2 vs Sofia in the hands of untrained operators was not needed.

3. Performance with Analyte Concentrations Near the Assay Cutoff:

A study was conducted at three geographically diverse CLIA waived healthcare provider sites. The study was designed to evaluate the ability of untrained operators in CLIA waived settings to obtain accurate results with weakly positive samples when testing with the Sofia Influenza A+B FIA using the Sofia 2 analyzer. A total of 10 operators participated in the study (3 operators each at two sites and 4 operators at one site) and a total of nine Sofia 2 analyzers were used across the three sites. The study was conducted on 10 testing days over a period of two weeks.

The operators were non-laboratorian personnel and included medical assistants, nurses and office staff. The work experience of the operators ranged from < 1 year to 13 years and their education level ranged from high school to college; none of the operators had experience with diagnostic testing other than simple CLIA waived tests. The operators performed the testing using the Quick Reference Instructions; no additional training was provided to the operators.

Each operator tested a coded panel of individual samples contrived at virus concentrations near the assay cutoff. The test samples were contrived by spiking inactivated strains of influenza A and influenza B into negative clinical matrix. The matrix was prepared by placing nasal swab specimens collected from human subjects into viral transport media. The selected virus strains were diluted with the clinical matrix to concentrations targeting the LoD of the assay. Three samples were prepared: a low positive sample for influenza A, a low positive sample for influenza B and a negative sample which consisted of the influenza-negative matrix.

All samples were coded and the operators at each site tested 24 replicates of each sample (a total of 72 samples per level across the 3 sites). The samples were blinded and randomized and the testing was incorporated into the daily workflow of each testing site.

Testing of two samples was repeated due to invalid results; both samples generated a valid result on the repeat testing and are included in the calculations of agreement with expected results.

**Table 3: Performance of the Sofia Influenza A+B FIA with Sofia 2 Testing Samples at Virus Concentrations Near the Assay Cutoff**

| Percent Agreement with Expected Results | | | | | |
|---|---|---|---|---|---|
| **Sample Type** | **Site 1** | **Site 2** | **Site 3** | **Overall** | **Overall 95% CI** |
| Influenza A Low Positive | 100% (24/24) | 95.8% (23/24) | 100% (24/24) | 98.6% (71/72) | 92.5% - 99.8% |
| Influenza B Low Positive | 100% (24/24) | 100% (24/24) | 95.8% (23/24) | 98.6% (71/72) | 92.5% - 99.8% |
| 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 obtained for each mode on the Sofia 2 analyzer are presented below.

**Table 4: Performance of the Sofia 2 Analyzer by Different Operational Modes**

| Percent Agreement with Expected Results | | | | | | |
|---|---|---|---|---|---|---|
| **Sample Type** | **Operational Mode** | **Site 1** | **Site 2** | **Site 3** | **% Overall Agreement** | **95% CI** |
| Influenza A Low Positive | Read Now | 12/12 | 11/12 | 12/12 | 97.2% (35/36) | 85.8% – 99.5% |
| | Walk Away | 12/12 | 12/12 | 12/12 | 100% (36/36) | 90.4% – 100% |
| Influenza B Low Positive | Read Now | 12/12 | 12/12 | 11/12 | 97.2% (35/36) | 85.8% – 99.5% |
| | Walk Away | 12/12 | 12/12 | 12/12 | 100% (36/36) | 90.4% – 100% |
| Negative | Read Now | 12/12 | 12/12 | 13/13 | 100% (37/37) | 90.6% - 100% |
| | Walk Away | 12/12 | 12/12 | 11/11 | 100% (35/35) | 90.4% – 100% |

There were no significant differences in the observed positivity of the device with weakly positive 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 operators were able to perform the test correctly and the test provided the expected results for samples with virus concentrations near the assay cutoff.

4. 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 7$^{th}$ 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 and the interpretation of results section for Sofia 2 shows the specific graphics that can be seen on the Sofia 2 screen designed to make the display more user-friendly.

5. Operator Questionnaire Results

The 10 operators who 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.

**M. Conclusion:**

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