CLIA Waiver Determination Decision Summary

A. Document Number:

CW170015

B. Parent Document Number:

K173496

C. Purpose of the Submission:

To obtain CLIA waiver for the Sofia 2 Lyme FIA for use with the Sofia 2 Analyzer. This was a Dual Submission, tracked as K173496 and CW170015.

D. Measurand (analyte):

IgM and/or IgG antibodies to *Borrelia burgdorferi*

E. Sample Type:

Finger-stick whole blood

F. Type of Test:

Fluorescent immunoassay (FIA), bi-directional lateral flow format

G. Applicant:

Quidel Corporation

H. Proprietary and Established Names

Sofia 2 Lyme FIA
Sofia Lyme Control Set

I. Test System Description:

1. Overview

The Sofia 2 Lyme FIA is an immunofluorescence-based, lateral flow assay for detection of IgM and/or IgG antibodies to *Borrelia burgdorferi* in patient specimens. Reagents for the assay are ready-to-use and provided in the kit.

The assay uses a bidirectional test strip format to detect both IgM and IgG antibodies to *B. burgdorferi*. One side of the test strip detects IgM antibodies to *B. burgdorferi* and the other side of the test strip detects IgG antibodies to *B. burgdorferi*. The test strip is contained in a plastic Test Cassette.

To perform the test, the patient finger-stick whole blood specimen is obtained with the provided Capillary Tube which also serves to separate the cells from the plasma. The user inserts the Capillary Tube into the Reagent Tube filled with the Reagent Solution and shakes the tube vigorously. Two drops of diluted sample are dispensed into the round sample well located near the center of the Test Cassette.

The Test Cassette is loaded into Sofia 2 in either the READ NOW Mode or WALK AWAY Mode. In READ NOW Mode, the user allows the cassette to develop on the countertop for 15 minutes. In WALK AWAY Mode, the user adds the specimen to the cassette, and immediately inserts the cassette into Sofia 2. Sofia 2 scans the test strip at 3, 5, 8, 10, and 15 minutes and reports the results either at 15 minutes or earlier if both IgM and IgG positive results are received. This feature allows for earlier read times.

The kit contains the following test components:

- Individually Packaged Test Cassettes (25): *Borrelia burgdorferi* antigens and anti-human IgM/IgG
- Blood Collection, Preparation Kits (25)
    - Capillary Tube
    - Reagent Tube
    - Reagent Solution: Ampoules with salt solution
- One (1) Bottle Positive Control: *B. burgdorferi* IgM and IgG positive plasma diluted 1:10 in 1xPBS with microcide
- One (1) Bottle Negative Control: *B. burgdorferi* negative serum diluted 1:10 in 1xPBS with microcide
- Package Insert (1)
- Quick Reference Instructions (1)

Calibration cassette for the Sofia 2 analyzer is provided separately.

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. When the test is complete, the results will be displayed on the Sofia 2 screen.

The Sofia 2 screen will display results for the procedural controls as being ✓ or ✗ and will provide a ⊕ or ⊖ result for the detection of IgM and/or IgG antibodies to *B. burgdorferi*. If the procedural controls are ✗, retest the patient's sample with a new Test Cassette.

J. Demonstrating "Simple":

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

- The test only requires a small volume of capillary whole blood specimen.
- The whole blood separation device and reagent are provided for sample collection and addition.
- The test requires only basic specimen and reagent handling to obtain accurate test results.
- The provided reagent is premeasured and provided in single-use vials.
- The test cartridges are unitized and contain all the reagents required for analysis.
- The test 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 displayed 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 trouble-shooting 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 2 Lyme 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 (K173496).

2. The Sofia 2 Lyme FIA with 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 prevent Sofia 2 from performing a test if the internal temperature of the device falls above or below the operating limits (15°C - 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. If the calibration fails the system goes into an error mode, and a message is displayed to contact Quidel Technical Support.
   - 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 2 Lyme 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 Error* 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 Control

- 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.

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 solution (prepared with diluted human serum or plasma that contains Lyme IgM and Lyme IgG antibodies) and one negative control solution (prepared with diluted human serum or plasma that does not contain Lyme IgM and IgG antibodies) 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 the external controls do not perform within specification, the instrument will not proceed.

3. Flex Studies

The operational limits of the Sofia 2 Lyme 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 venous whole blood specimens collected from individuals confirmed to be Lyme IgM and IgG negative with the Sofia 2 Lyme FIA on the Sofia 2 analyzer. Negative whole blood specimens were not pooled. The negative samples were then spiked with commercially available Lyme positive samples at the following IgM and IgG concentration: C_{95} and 2-3 X LoD, unless stated otherwise. Negative samples consisted of individual Lyme IgM and IgG negative whole blood.

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 negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples were tested in Walk Away mode on ten 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 2 Lyme 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, 2, or 3 minutes and were then placed horizontally until the 15-minute incubation time was complete. Five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples 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 2 Lyme 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 negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples 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 dropped, and all samples tested with the dropped cartridges generated the expected results.

d. Dispense volume - varying the sample volume applied to the test cassette

A dispense volume study was conducted using five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples with the Sofia 2 Lyme FIA on the Sofia 2 analyzer to evaluate the effect of dispensing too few drops (1 drop) or too many drops (3 drops); two drops is specified in the test procedure.

The negative sample produced 100% negative results when either 1, 2 or 3 drops were added to the assay. When 1 drop of the C_{95} and 2-3X LoD IgM were added, false negative results were obtained. The C_{95} and 2-3X LoD IgG samples were not affected when either 1, 2 or 3 drops were added to the assay and expected results were obtained. To mitigate the risk of obtaining inaccurate results, the assay procedure emphasizes that two drops must be dispensed.

e. Development/read time

The effect of varied incubation time (2 to 30 minutes) on the test results was evaluated prior to inserting the cassette into Sofia 2 in the Read Now mode. Five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples were tested on five different Sofia 2 instruments with results read after: 2, 5, 10, 15, 20, and 30 minutes incubation.

After 5 minutes and up to 30 minutes of incubation all samples generated expected results. After 2 minutes of incubation False negative results were obtained. To mitigate the risk of obtaining inaccurate results, the assay procedure emphasizes that the user must allow the test to develop for the full 15 minutes before inserting into the analyzer in the Read Now mode.

f. Early read study

This study was conducted with negative samples and 10 replicates of 2-3X LoD IgM and IgG positive samples. The test devices were read in (a) Read Now mode, then (b) inserted into the Sofia 2 and read after 2 minutes and subsequently at additional time intervals. The following read times were compared between the two modes: 3, 5, 8, 10 and 15 minutes. The results showed that expected results were obtained for all negative samples at all time points, while the positive samples generated positive results only after 8 minutes of incubation when using either operational mode. No difference in result interpretation was found between the Read Now and the Walk Away modes.

g. Varying whole blood sample volumes

A whole blood sample volume study was conducted for the Sofia 2 Lyme FIA on the Sofia 2 Analyzer to evaluate the effect of different whole blood sample volumes (10, 20, 25 (control), 30, 40, 50 µL) using five negative samples and five

replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples.

All testing gave the expected results with the exception of the 10 µL volume with the C_{95} IgM sample that generated 3 false negative results. To mitigate the risk of obtaining inaccurate results, the provided capillary has a black line indicating the level that the FS blood must reach and the assay procedure emphasizes that the blood sample must be filled to the black line.

h. Varying the reagent volume

A reagent volume study was conducted using five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples with the Sofia 2 Lyme FIA on the Sofia 2 analyzer to evaluate the effect of dispensing varying volumes of reagent solution; the following volumes were evaluated: (100, 200, 225, 250 (control), 275, 300 and 400 µL. With the exception of 100 µL which generated invalid results for all samples tested, expected results were obtained for all samples with all other tested reagent volumes.

i. Effect of substituting water or saline for the Running Buffer

A flex study was conducted to determine the effect of using water or saline instead of the provided running buffer on the performance of Sofia 2 Lyme FIA. Five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples were tested with water, saline, and the running buffer.

The negative samples produced 100% negative results for all running buffer conditions. Using DI water will result in false negative results. To mitigate the risk of obtaining inaccurate results, the assay procedure clearly emphasizes that only the reagent solution provided in the kit must be used.

j. Sample and reagent order

A flex study to determine the effect of sample preparation and reagent order on the performance of the Sofia 2 Lyme FIA was conducted with five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples. Each whole blood sample was drawn into the capillary tube and then placed into an empty Reagent Tube, inverted and vigorously shaken up-and-down 5-times. The capillary tube was removed. A total of 250 µL of the Reagent Solution was then dispensed into the reagent tube and the capillary tube was re-inserted into the Reagent Tube, now with the Reagent Solution, then inverted and vigorously shaken up-and-down 5-times. Two drops of mixed sample were dispensed into the round sample port of the test device and incubated for 15 minutes on the benchtop before being inserted into the Sofia 2 instrument (Read Now Mode). All samples generated expected results. Changing the sample and reagent order had no effect on the performance of the whole blood separation device.

k. Mix and match lot study

Two lots each of the Running Buffer, the Separation Device and the cassettes were used in this study to test five negative samples and five replicates each of C95 and 2-3X LoD IgM and IgG positive samples with each condition. Expected results were obtained for all situations tested and demonstrated that mixing lots of the kit components did not affect the performance of the Sofia 2 Lyme FIA assay.

Specimen Integrity/Handling and Assay Stability

a. Whole blood collection time

A whole blood collection time study was conducted using five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples with the Whole Blood Separation Device (also called the Capillary Tube) prior to shaking the sample in the Whole Blood Separation Device (pre-processing). The study also evaluated the effect of the amount of time whole blood is in solution in the Whole Blood Separation Device after shaking in the device and before adding the sample to the Sofia 2 Lyme FIA (post-processing). The following time periods were evaluated per processing condition: 0 (immediately), 5 min, 10 min, 20 min, 1 hour, 4 hours, and 24 hours.

The negative samples remained 100% negative up to 24 hours for both the pre-processing and post-processing stages. For the pre-processing stage (time that the whole blood can be stored in the Whole Blood Separation Device prior to shaking), the whole blood sample is stable for up to 20 minutes.

For the post-processing stage (time that the whole blood can be stored in the Whole Blood Separation Device after shaking), the Whole blood sample is stable up to 1 hour. To mitigate the risk of obtaining inaccurate results, the assay procedure clearly states to immediately process the whole blood samples.

b. Hematocrit study

The effect of different hematocrit levels on Sofia 2 Lyme was evaluated with five negative samples and five replicates each of C95 and 2-3X LoD IgM and IgG positive samples. The following hematocrit levels were tested: 30%, 40%, 50%, and 60%. All samples generated expected results at the tested hematocrit levels.

c. Effect of hemolysis study

The effect of hemolysis on the assay performance was evaluated with five negative samples and five replicates each of C95 and 2-3X LoD IgM and IgG positive samples tested with each condition.

The test samples were either vortexed, freeze-thawed or rapidly shaken to produce

hemolysis, before dispensing onto Sofia 2 Lyme FIA device. The IgM containing test samples generated false negative results after freeze-thaw and one false negative after shaking. All other samples tested under the described conditions generated expected results.

When testing IgM and IgG whole blood samples at different hemolysis conditions, 100% of negative samples were negative, 100% of the positive IgM C95 and 2-3x LoD samples were positive, and 100% of the positive IgG 2-3x LoD samples were positive. The freeze-thaw condition, which resulted in gross hemolysis generated false negative results for the low positive IgM samples (IgM C95) and the shaking resulted in one false negative result with IgM C95 sample. Gentle inversion and vortexing of the whole blood samples had no effect on the Sofia IgM and IgG results.

The results showed that gross hemolysis may affect the Sofia 2 IgM results. This error is mitigated through a caution in the test procedure that if either Viewing Window on the cassette is light-to-dark red due to excess hemolysis, the test should be repeated with a new patient sample. In addition, occurrence of hemolysis of the collected FS specimens is unlikely due to the emphasis for immediate processing of the specimen collected with the capillary.

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

The recommended operational parameters for the Sofia 2 analyzer and Sofia kits are between 15°C and 30°C. In this study, the performance of the Sofia 2 Lyme 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 (ambient temperature/ambient humidity) and at two temperatures outside of the instruments specifications (4°C/ambient humidity and 40°C/90% humidity) for 30 minutes prior to testing. Five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG

positive samples were tested in Read Now mode on five separate Sofia 2 analyzers kept at ambient temperature and humidity.

When the Sofia 2 internal temperature falls below 15°C or is higher than 31°C, the internal sensors prevent the instrument from operating.

When the cassettes were processed at 4°C/ambient humidity, the results for the C_{95} IgM and IgG samples generated false results. All other samples, including the negatives, processed under these conditions generated expected results. When the cassettes were processed at 40°C/90% humidity, all samples, including the negatives, generated expected results

b. System and device temperature study

This study evaluated the effects of the analyzer and assay when performed at different temperatures within the labeled range of 15°C – 31°C.

Operating and testing the Sofia 2 Lyme FIA at 15°C and 31°C did not have an impact on the percent positivity. All whole blood sample levels gave expected results at 15°C and 31°C when compared to the room temperature condition. The Sofia 2 Lyme FIA operates properly from 15-31°C.

c. System temperature and Read Now vs. Walk Away modes

All test materials including the Sofia Lyme FIA test cassette and the Sofia 2 analyzer were equilibrated to ambient, 15°C, or 30°C for at least 30 minutes prior to testing. Testing was performed in Read Now mode and Walk Away mode. Ten negative and ten IgM and IgG positive sample 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.

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

Sample-loaded cassettes with five negative samples and five replicates each of C_{95} and 2-3X LoD IgM and IgG positive samples 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. No failures were observed as a result of the incubation of the Sofia 2 Lyme FIA test cassette in direct environmental sunlight, and all samples generated the expected results.

The flex studies demonstrated that through the combination of built in fail-safe mechanisms and explicit cautions in the labeling a risk of false results due to environmental stresses or user errors is effectively minimized.

L. Demonstrating Accuracy

Studies were conducted at CLIA Waived sites with untrained operators to demonstrate the accuracy of the Sofia 2 Lyme FIA.

1. Clinical Performance

A prospective study with Sofia 2 Lyme FIA was performed using matched blood specimens (finger-stick and serum/plasma) samples collected from 525 subjects across 11 sites located in Lyme endemic regions throughout the United States. The subject population included 324 prospective subjects suspected of and exhibiting symptoms of Lyme disease (Arm 1) and 201 subjects who were previously diagnosed as having Lyme disease (Arm 2). Sofia 2 Lyme FIA finger-stick whole blood testing was performed immediately after collection at each of the 11 clinical sites by 24 untrained operators. The results obtained with the Sofia 2 Lyme FIA IgM and IgG tests were compared to a composite reference test result obtained by testing matched serum or plasma specimens for Lyme IgM and IgG (Tier 1 testing), followed by supplementary testing with Western blot (Tier 2 testing) of all Tier 1 positive and equivocal samples. The composite ELISA result was based on test results from three different FDA cleared IgM and IgG ELISA assays, where the final ELISA result was determined by "≥2 out of 3" rule. Percent agreements were calculated using the following formulas.

1st Tier Positive % Agreement vs. Composite ELISA Result	$\frac{\text{Sofia 2 Lyme positive samples}}{\text{Composite ELISA positive samples}}$
2nd Tier Positive % Agreement vs. Western Blot	$\frac{\text{Sofia 2 Lyme positive and Tier 2 Western blot positive samples}}{\text{Composite ELISA positive and Tier 2 Western blot positive samples}}$
Negative % Agreement vs. Composite ELISA Result	$\frac{\text{Sofia 2 Lyme negative samples}}{\text{Composite ELISA negative samples}}$

The first tier and second tier IgM summary table (Arm 1, Arm 2, and combined) with the positive and negative percent agreement of Sofia 2 Lyme FIA compared to the composite ELISA result and the Tier 2 positive percent agreement with the supplementary Western blot are presented in the Table below.

Sofia 2 Lyme FIA IgM Results

Study Population	Number of Subjects	1st Tier Positive % Agreement vs. Composite ELISA Result	2nd Tier Positive % Agreement vs. Western Blot	Negative % Agreement vs. Composite ELISA Result
Arm 1	324	83.1% (64/77) (73.1-90.0%)	90.6% (48/53) (79.3-96.3%)	76.5% (189/247) (70.8-81.4%)
Arm 2	201	76.7% (79/103) (67.6-83.9%)	91.3% (42/46) (79.1-97.1%)	63.3% (62/98) (53.4-72.2%)
Total	**525**	**79.4% (143/180) (72.9-84.7%)**	**90.9% (90/99) (83.4-95.3%)**	**72.8% (251/345) (67.8-77.2%)**

The first tier and second tier IgG summary table (Arm 1, Arm 2, and combined) with the positive and negative percent agreement of Sofia 2 Lyme FIA compared to the composite ELISA result and Tier 2 positive percent agreement with the supplementary Western blot is presented below.

Sofia 2 Lyme FIA IgG Results

Study Population	Number of Subjects	1st Tier Positive % Agreement vs. Composite ELISA Result	2nd Tier Positive % Agreement vs. Western Blot	Negative % Agreement vs. Composite ELISA Result
Arm 1	324	84.3% (43/51) (71.7-92.1%)	95.8% (23/24) (78.1->99%)	84.2% (230/273) (79.4-88.1%)
Arm 2	201	93.4% (85/91) (86.1-97.2%)	95.7% (45/47) (85.0-99.6%)	72.7% (80/110) (63.7-80.2%)
Total	**525**	**90.1% (128/142) (84.0-94.1%)**	**95.8% (68/71) (87.8-99.0%)**	**80.9% (310/383) (72.9-84.7%)**

2. Performance with Analyte Concentrations Near the Assay Cutoff

Studies were conducted to demonstrate that untrained intended users could perform the test consistently and accurately using weakly reactive samples. The study consisted of three (3) distinct CLIA-waived sites, with three (3) operators at each site, where the Sofia 2 Lyme FIA with Sofia 2 was evaluated using coded randomized panels of simulated whole blood samples, including one (1) weak positive (C_{95} - a concentration at the assay cutoff) and one (1) high negative (C_5 - a concentration just below the assay cutoff) each for IgM and IgG. There were a total of 81 aliquots of each of the IgM samples and 72 aliquots of each of the IgG samples distributed to the sites. The results from the study are shown below.

Sofia 2 Lyme FIA with Sofia 2 Performance Near the Cutoff

Percent Agreement with Expected Results*					
Sample Level	Site 1	Site 2	Site 3	Overall	**Overall 95% CI**
IgM High Negative (C_5)	100% (27/27)	100% (27/27)	100% (27/27)	100% (81/81)	94.6-100%
IgM Weak Positive (C_{95})	88.9% (24/27)	96.3% (26/27)	92.6% (25/27)	92.6 (75/81)	84.5-96.9%
IgG High Negative (C_5)	100% (24/24)	95.8% (23/24)	100% (24/24)	98.6% (71/72)	91.8->99.9%
IgG Weak Positive (C_{95})	100% (24/24)	87.5% (21/24)	91.7% (22/24)	93.1% (67/72)	84.4-97.4%

*The expected results for "Weak Positive" samples are "Positive," while the expected results for "High Negative" samples are "Negative."

There were no significant differences observed in the results between sites or between operators when testing weakly reactive samples for IgM or for IgG. The study results demonstrated that untrained users were able to perform the test correctly and the test provided the expected results for samples with antibody concentrations near the assay cutoff.

M. Proposed Labeling

The labeling includes Package Insert, Operator Manual for the Sofia 2, Quick Reference Instructions (QRI) and a QuickStart Guide for the Sofia 2 analyzer. The QRI and the QuickStart Guide are written in simple language (at 7th grade reading level) and contain pictorial descriptions of the individual steps. 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.

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. Benefit-Risk Considerations

Currently, there is no gold standard for diagnosing of Lyme disease (LD) and the treatment is largely based on patient symptoms and the clinical judgment of the clinician. The current CDC guidelines for diagnosing LD call for a 2-tier testing with (1) an ELISA test for antibodies to *B. burgdorferi* and (2) supplementary testing with Western blot of samples reactive by ELISA. A positive Lyme ELISA test alone is not confirmatory for *B. burgdorferi* infection. If a patient with suspected early LD has a negative serology, i.e., if the first-tier test (ELISA) yields negative results, the provider should either (a) consider an alternative diagnosis, or (b) in cases where the patient has had symptoms for less than or equal to 30 days (early LD), follow up with a convalescent serum. In the latter case, the

provider may treat the patient prophylactically with antibiotics.

The results of the testing with three different predicates demonstrated that Sofia 2 Lyme FIA performed as well or better when compared to each predicate separately for both IgM and IgG. Importantly, the Sofia 2 Lyme FIA detected more positives than the comparator method (composite ELISA) for both IgM (94 subjects) and IgG (73 subjects) and had an acceptable positive percent agreement with the 2nd tier test (90.9% for IgM and 95.8% for IgG), which is considered critical in evaluating the performance of Lyme tests. However, we must be reminded that the comparator method for the detection of Lyme is imperfect and, therefore, the calculated performance estimates for the Sofia 2 Lyme FIA must be evaluated with consideration of all criteria used for the determination of the CLIA waiver status of this device.

The sponsor (Quidel) has conducted clinical studies, including an evaluation of the device performance with weakly reactive samples, as well as analytical flex studies to demonstrate that the device is simple and has a low risk of erroneous results when used by untrained operators in CLIA waived settings. Review of the information provided supports the premise that the device meets the criteria for simplicity and it is designed to effectively mitigate errors that might result from mishandling of the device by operators not trained in laboratory procedures or from unintended environmental conditions that may be encountered in CLIA waived healthcare settings.

Benefits:

1. As a duplex device for the detection of IgM and IgG antibodies, the Sofia 2 Lyme FIA test can detect antibody over a broader range of disease states when compared with a single analyte test.

2. For patients suspected of Lyme disease, but without or after resolution of the erythema migrans (EM) rash, a positive IgG test provides valuable information to the physician allowing for differential diagnosis and informed treatment decisions.

3. The test is performed on finger-stick (FS) whole blood specimen eliminating the need for a phlebotomist to be present at the site of patient care, which would otherwise require that the patient go to another location to have the blood drawn. The use of the FS specimen also facilitates testing in young children.

4. The Sofia test system has been used successfully in CLIA waived settings since 2011, with tests for detection of influenza A and B, Strep A and RSV.

5. Allowing the use of the Sofia 2 Lyme FIA in CLIA waived settings may facilitate access to testing.

Risks:

1. A false negative result may occur if the test is performed within the first two weeks of infection when antibodies have not become detectable yet. Due to the biology of the Lyme disease, this risk is well understood by the clinicians testing for Lyme. A false negative result might preclude the physician from starting an antibiotic regimen. However, this is the same risk as currently exists with the laboratory-based Tier 1 Lyme tests; therefore this is not a new risk. Nevertheless, the advantage is that the doctor can counsel the patient at the time of the visit to come back for a second sample draw, to see if the antibodies appear after a certain time.

   This risk has been mitigated by stating in the intended use statement in the labeling that the test should be used on "patients suspected of acute *B. burgdorferi* infection of at least 2 weeks' duration." Moreover, the intended use statement clearly indicates that "Professional guidelines should be consulted regarding testing and treatment for Lyme disease when acute *B. burgdorferi* infection is suspected."

2. A false positive result will initiate antibiotic treatment. However, treating with antibiotics if infection with *B. burgdorferi* is suspected is the current practice when the serology test results are not available. This risk has been mitigated by expanding the intended use statement in the labeling to indicate that "Positive results must be confirmed by testing with a corresponding second-tier *B. burgdorferi* Western blot assay. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures."

P. Conclusion

In conclusion, the device is simple to use and is unlikely to be affected if the test is performed incorrectly by untrained users. The information submitted in this CLIA waiver application supports a CLIA waiver approval decision.