**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**

**A. 510(k) Number:** K163713

**B. Purpose for Submission:**

To obtain a substantial equivalence determination for the Sofia Lyme FIA for use with Sofia

**C. Measurand:**

IgM antibodies to *Borrelia burgdorferi* proteins

IgG antibodies to *Borrelia burgdorferi* proteins

**D. Type of Test:**

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

**E. Applicant:**

Quidel Corporation

**F. Proprietary and Established Names:**

Sofia Lyme FIA

Sofia Lyme Control Set

**G. Regulatory Information:**

1. Regulation section:

   21 CFR 866.3830

2. Classification:

   Class II

3. Product code:

   LSR; Reagent, Borrelia Serological Reagent

   JTT; Quality Control Material (assayed and unassayed)

4. Panel:

   Immunology and Microbiology Devices

## H. Intended Use:

1. Intended use(s):

   **Sofia Lyme FIA:**
   The Sofia Lyme FIA employs immunofluorescence for the rapid differential detection of human IgM and IgG antibodies to *Borrelia burgdorferi* from serum and plasma specimens from patients suspected of *B. burgdorferi* infection. This qualitative test is intended for use as an aid in the diagnosis of Lyme disease. A negative result does not preclude infection with *B. burgdorferi*. All positive results for IgM and/or IgG should be further tested by a corresponding second-tier western blot assay. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures.

   **Sofia Lyme Control Set:**
   The Sofia Lyme Control Set is intended for use as assayed quality control materials to verify the performance of the Sofia Lyme FIA test system.

2. Indication(s) for use:

   Same as Intended Use(s)

## I. Device Description:

The Sofia 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*.

To perform the test, the patient specimen (30 µL serum/plasma) is added to a pre-filled Reagent Vial. The diluted sample (100 µL) is then dispensed into the round sample well located near the center of the Test Cassette. The Test Cassette is loaded into the Sofia instrument for an automatically defined development time (WALK AWAY Mode) or pre-incubated on the bench top prior to loading into Sofia (READ NOW Mode). Sofia scans the test strip, analyzes the fluorescent signal, and then displays two (2) test results: IgM (positive, negative or invalid) and IgG (positive, negative or invalid). As an option, the results are automatically printed on the integrated printer.

Each Sofia Lyme FIA kit will contain one Positive and one Negative Control—each provided in separate dropper bottles. The external controls will be available separately as well in a Sofia Lyme Control Set. The Positive and Negative QC controls are formulated with patient Lyme IgM and IgG positive serum that are diluted into 1X PBS, and 0.3% Microcide is added to the solution as an antimicrobial. To use External Controls, two drops from the Negative or Positive Control are added to the round sample well located near the center of the cassette. The cassette is then treated in the same manner as a cassette containing a patient sample. The Positive and Negative Control each require their own cassette.

**J. Substantial Equivalence Information:**

1. Predicate device name(s): Vidas Lyme IgM
   Vidas Lyme IgG

2. Predicate 510(k) number(s): K122979
   K122986

3. Comparison with predicate:

| **Item**<br>**Features** | **Proposed Device**<br>**Sofia Lyme FIA with Sofia** | **Predicate IgM Device**<br>**BioMérieux Vidas Lyme IgM (**K122979) | **Predicate IgG Device**<br>**BioMérieux Vidas Lyme IgG** (K122986) |
|---|---|---|---|
| Intended Use: Sofia Lyme FIA | Sofia Lyme FIA:<br>The Sofia Lyme FIA employs immunofluorescence for the rapid differential detection of human IgM and IgG antibodies to *Borrelia burgdorferi* from serum and plasma specimens from patients suspected of *B. burgdorferi* infection. This qualitative test is intended for use as an aid in the diagnosis of Lyme disease. A negative result does not preclude infection with *B. burgdorferi*. All positive results for IgM and/or IgG should be further tested by a corresponding second- tier western blot assay.<br>Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures. | The VIDAS Lyme IgM (LYM) assay is an automated qualitative enzyme immunoassay intended for use on the instruments of the VIDAS family in the presumptive detection of human IgM antibodies to *Borrelia burgdorferi* in human serum (plain or separation gel) or plasma (sodium heparin or lithium heparin). It should be used to test patients with a history and/or symptoms of infection with *B. burgdorferi*. All VIDAS Lyme IgM positive and equivocal specimens should be further tested with a Western Blot IgG assay to obtain supportive evidence of infection with *B. burgdorferi*. | The VIDAS Lyme IgG (LYG) assay is an automated qualitative enzyme immunoassay intended for use on the instruments of the VIDAS family in the presumptive detection of human IgG antibodies to *Borrelia burgdorferi* in human serum (plain or separation gel) or plasma (sodium heparin or lithium heparin). It should be used to test patients with a history and/or symptoms of infection with *B. burgdorferi*. All VIDAS Lyme IgG positive specimens should be further tested with a western blot IgG assay to obtain supportive evidence of infection with *B. burgdorferi*. |

| **Item**<br>**Features** | **Proposed Device**<br>**Sofia Lyme FIA with Sofia** | **Predicate IgM Device**<br>**BioMérieux Vidas Lyme IgM (**K122979) | **Predicate IgG Device**<br>**BioMérieux Vidas Lyme IgG** (K122986) |
|---|---|---|---|
| Intended Use: Sofia Lyme Control Set | Sofia Lyme Control Set: The Sofia Lyme Control Set is intended for use as assayed quality control materials to verify the performance of the Sofia Lyme FIA test system. | | |
| Instrument | Sofia | VIDAS and miniVIDAS | VIDAS and miniVIDAS |
| Analyte | Human IgM and IgG antibodies against *B. burgdorferi* proteins | Human IgM antibodies against *B. burgdorferi* proteins | Human IgG antibodies against *B. burgdorferi* proteins |
| Automated Analysis | Yes | Yes | Yes |
| Read Results | Read results on instrument screen or print with optional printer | Result report is printed | Result report is printed |
| Read Result Time | 10 Minutes | 27 minutes | 27 minutes |
| Specimen Types | Human serum and plasma | Human serum and plasma | Human serum and plasma |
| Qualitative | Yes | Yes | Yes |
| Test Principle | Immunofluorescence Device | Immunofluorescence Device | Immunofluorescence Device |
| Format | Lateral-flow Bi-directional Test Cassette | Enzyme-linked fluorescent assay (ELFA) | Enzyme-linked fluorescent assay (ELFA) |
| Antibodies Used | Monoclonal anti-human IgG and polyclonal anti- human IgM | Anti-human IgM antibodies | Anti-human IgG antibodies |
| Antigens Used | Recombinant Proteins and synthetic peptides of B. burgdorferi | Recombinant proteins of B. burgdorferi | Recombinant proteins of B. burgdorferi |
| Detection Method | Polystyrene microparticles dyed with Europium chelate | Alkaline phosphatase/4-MUP | Alkaline phosphatase/4- MUP |
| Storage | Room Temperature (15-30°C) | 2-8°C | 2-8°C |
| Running Buffer Solution | One pre-filled vial containing PBS | Sample diluent and wash buffer | Sample diluent and wash buffer |

| Item<br>Features | Proposed Device<br>Sofia Lyme FIA with Sofia | Predicate IgM Device<br>BioMérieux Vidas Lyme IgM (K122979) | Predicate IgG Device<br>BioMérieux Vidas Lyme IgG (K122986) |
|---|---|---|---|
| Quality Control Features | Built-in features include:<br>• Built-in procedural control zone scanned by the analyzer to determine whether adequate flow occurred on the IgG side of the assay.<br>• Built-in reference control line scanned by the analyzer to determine whether adequate flow occurred on the IgM side of the assay.<br>• Analyzer prevents use of expired cartridge from being read by the reader<br>• Cassette must be properly inserted | • One positive and one negative control are included and must be tested after opening a new kit to monitor reagent failure. | • One positive and one negative control are included and must be tested after opening a new kit to monitor reagent failure. |

**K. Standard/Guidance Document Referenced (if applicable):**

Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of Antibodies to *Borrelia burgdorferi*

**L. Test Principle:**

Immunofluorescence

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

*a. Precision/Reproducibility:*
The precision study evaluated the within laboratory precision / repeatability of the Sofia Lyme FIA using serum samples.

Each negative or spiked positive serum sample was tested in duplicate with a total of 24 different runs (two runs per day over a total of 12 days) at one site (Quidel), utilizing two operators and two Sofia Analyzers during the study. Each sample was tested a total of 48 times over the course of the study. The testing days spanned across two calibration events. See summary tables below

**Sofia Lyme FIA Precision – Within Run**

| Sample | IgM Positive | | IgM % Positivity | IgG Positive | | IgG % Positivity |
|---|---|---|---|---|---|---|
| | Run 1 | Run 2 | Total (n=96) | Run 1 | Run 2 | Total (n=96) |
| Negative ($C_0$) | 0/48 | 0/48 | 0% | 0/48 | 0/48 | 0% |
| High Negative ($C_5$) | 3/48 | 5/48 | 8.33% | 2/48 | 5/48 | 7.29% |
| Low Positive ($C_{95}$) | 48/48 | 48/48 | 100% | 48/48 | 46/48 | 97.92% |
| Moderate Positive (2-3X) | 48/48 | 48/48 | 100% | 48/48 | 48/48 | 100% |

**Sofia Lyme FIA Precision – Between Operators**

| Sample | % IgM Positivity | | IgM % Positivity | % IgG Positivity | | IgG % Positivity |
|---|---|---|---|---|---|---|
| | Op. 1 | Op. 2 | Total (n=96) | Op. 1 | Op. 2 | Total (n=96) |
| Negative ($C_0$) | 0/48 | 0/48 | 0% | 0/48 | 0/48 | 0% |
| High Negative ($C_5$) | 3/48 | 5/48 | 8.33% | 3/48 | 4/48 | 7.29% |
| Low Positive ($C_{95}$) | 48/48 | 48/48 | 100% | 47/48 | 47/48 | 97.92% |
| Moderate Positive (2-3X) | 48/48 | 48/48 | 100% | 48/48 | 48/48 | 100% |

The reproducibility study demonstrated inter-laboratory reproducibility with a panel of test samples at various concentrations of IgM and IgG antibodies to *B. burgdorferi*. See summary of results below.

**Sofia Lyme FIA IgM and IgG Reproducibility Study Inter-laboratory Agreement**

| Site | IgM | | | | IgG | | | |
|---|---|---|---|---|---|---|---|---|
| | Negative | High Negative | Low Positive | Moderate Positive | Negative | High Negative | Low Positive | Moderate Positive |
| 1 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 27/30 | 30/30 |
| 2 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 26/30 | 30/30 |
| 3 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 30/30 | 25/30 | 30/30 |
| Total | 90/90 | 90/90 | 90/90 | 90/90 | 90/90 | 90/90 | 78/90 | 90/90 |
| % Overall Agreement (95% CI) | 100% (95.1-100%) | 100% (95.1-100%) | 100% (95.1-100%) | 100% (95.1-100%) | 100% (95.1-100%) | 100% (95.1-100%) | 86.7% (78.0-92.4%) | 100% (95.1-100%) |

b. *Linearity/assay reportable range: Not applicable*

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Not applicable (N/A)

d. *Detection limit:* Not applicable

e. *Analytical specificity:*

This study evaluated the analytical specificity of the Sofia Lyme FIA using serum obtained from asymptomatic (healthy, normal) populations in both endemic and non-endemic regions. Individuals were split evenly between endemic and non-endemic populations. The specificity of the Sofia Lyme FIA and the predicate device is summarized below.

**Summary of Analytical Specificity - IgM**

| Assay | N | Negative | Equivocal | Positive | % Specificity |
|---|---|---|---|---|---|
| **Sofia Lyme FIA IgM** | 200 | 180 | N/A | 20 | 90.0% |
| **Predicate IgM** | 200 | 181 | 16 | 3 | 90.5% |

**Summary of Analytical Specificity - IgG**

| Assay | N | Negative | Positive | % Specificity |
|---|---|---|---|---|
| **Sofia Lyme FIA IgG** | 200 | 180 | 20 | 90.0% |
| **Predicate Lyme IgG** | 200 | 197 | 3 | 98.5% |

f. *Assay cut-off:* Not applicable

g. *Interfering Substances:*

A study was performed to assess whether common endogenous and exogenous substances would interfere with the Sofia Lyme FIA. The substances and concentrations tested are in the table below. There was no evidence of interference for the listed substances at the tested levels.

**Substances Evaluated for Interference with Sofia Lyme FIA**

| Interfering Substance | Concentrations Tested |
|---|---|
| Bilirubin | 15 mg/dL |
| Hemoglobin | 20 g/dL |
| Lipids | 750 mg/dL |
| Albumin | 5.0 g/dL |
| Acetylsalicylic Acid | 3.62 mmol/L |
| Amoxicillin | 206 µmol/L |
| Azithromycin | 15.3 µmol/L |
| Ceftriaxone | 1460 µmol/L |
| Cefuroxime Axetil | 1416 µmol/L |
| Doxycycline Hyclate | 67.5 µmol/L |
| Erythromycin | 81.6 µmol/L |
| Ibuprofen | 2425 µmol/L |
| Minocycline | 10.33 µmol/L |
| Penicillin G | 33.67 µmol/L |
| Phenoxymethylpenicillin | 14.27 µmol/L |
| Prednisolone | 8.31 µmol/L |
| Tetracyclines | 34 µmol/L |

*h. Cross-Reactivity:*

The cross-reactivity of the Sofia Lyme FIA was evaluated with 17 disease state sample types for possible interference with the Sofia Lyme FIA assay. Samples were characterized and obtained from commercial vendors and did not include any first or second tier Lyme testing results. The total number of each disease state sample type, as well as the number of positives observed with the Sofia Lyme FIA is summarized in the table below.

**Evaluation of Conditions that May Cross-React with Sofia Lyme FIA**

| Disease State Diagnosis | # of Samples | IgM Positive Results | IgG Positive Results |
|---|---|---|---|
| **Anti-Nuclear Antibodies** | 10 | 4/10 | 1/10 |
| **Babesiosis** | 12 | 3/12 | 4/12 |
| **Chronic Fatigue Syndrome** | 12 | 2/12 | 2/12 |
| **Cytomegalovirus** | 11 | 2/11 | 1/11 |
| **Epstein Barr Virus** | 10 | 4/10 | 0/10 |
| **Fibromyalgia** | 10 | 1/10 | 0/10 |
| ***H. pylori*** | 10 | 4/10 | 0/10 |
| **HIV** | 11 | 2/11 | 0/11 |
| **Influenza** | 11 | 0/11 | 0/11 |
| **Leptospirosis** | 6 | 1/6 | 2/6 |
| **Lupus** | 20 | 8/20 | 4/20 |
| **Multiple Sclerosis** | 10 | 1/10 | 1/10 |
| **Parvovirus B19** | 15 | 4/15 | 1/15 |
| **Rheumatoid Factor** | 10 | 0/10 | 0/10 |
| **Rickettsia** | 3 | 0/3 | 0/3 |
| **Rocky Mountain Spotted Fever** | 10 | 0/10 | 0/10 |
| **Syphilis** | 28 | 8/28 | 4/28 |

*i. Quality Controls/ Matrix Effects*

The Sofia Lyme External positive and negative quality controls are individual bottles manufactured to generate positive IgG and IgM results, and negative IgG and IgM results. The Sofia Lyme QC materials simulate the composition of patient samples as closely as possible in order to minimize matrix effects and correctly reflect the expected performance with patient samples. The positive and negative QC controls are formulated with patient serum samples that are diluted into 1X PBS, filled into dropper bottles and added dropwise to the Sofia Lyme FIA test device. Unlike a patient sample, 0.3% Microcide is added to the external control solutions to act as an antimicrobial.

The purpose of this study was to evaluate matrix effects other than the analyte that may influence the measured value of the analyte in the positive and negative external quality controls. This protocol is based on the FDA Guidance for Industry and FDA Staff Assayed and Unassayed Quality Control Material: June 7, 2007.

This study addressed the following potential effects:

- Determine if there was any bias of the external control matrix relative to the natural sample. Demonstrate that the manufacturing formulation that includes 0.3% Microcide did not alter the properties of the sample and still reflects similar performance compared to natural human specimens.

- Demonstrate that the external quality control solution result output was similar to the sample result output regardless of the preparatory and operational steps that are required.

- Demonstrate the precision of the manufactured positive and negative controls over a span of 5 days.

Study results are summarized in the tables below.

**External Controls – Evaluation of Matrix Effects**

| Matrix | Method | N | IgM % Positivity | IgG % Positivity |
|---|---|---|---|---|
| | | | Cutoff | Cutoff |
| **Negative QC Control** | **Dropper** | 100 | 0% | 0% |
| **Negative Patient Sample** | **Pipette** | 100 | 0% | 0% |
| **Positive QC Control** | **Dropper** | 100 | 100% | 100% |
| **Positive Patient Sample** | **Pipette** | 100 | 100% | 100% |

**External Controls – Precision**

| | | | # Positive Results | | % Positivity | |
|---|---|---|---|---|---|---|
| **QC Control** | **Date** | **n** | **IgM** | **IgG** | **IgM** | **IgG** |
| **Negative** | 8/22/2016 | 20 | 0/20 | 0/20 | 0% | 0% |
| | 8/23/2016 | 20 | 0/20 | 0/20 | 0% | 0% |
| | 8/24/2016 | 20 | 0/20 | 0/20 | 0% | 0% |
| | 8/25/2016 | 20 | 0/20 | 0/20 | 0% | 0% |
| | 8/26/2016 | 20 | 0/20 | 0/20 | 0% | 0% |
| | Total | 100 | 0/100 | 0/100 | 0% | 0% |
| **Positive** | 8/22/2016 | 20 | 20/20 | 20/20 | 100% | 100% |
| | 8/23/2016 | 20 | 20/20 | 20/20 | 100% | 100% |
| | 8/24/2016 | 20 | 20/20 | 20/20 | 100% | 100% |
| | 8/25/2016 | 20 | 20/20 | 20/20 | 100% | 100% |
| | 8/26/2016 | 20 | 20/20 | 20/20 | 100% | 100% |
| | Total | 100 | 100/100 | 100/100 | 100% | 100% |

In addition to the above studies, the external controls were used daily or as described in the package insert in the following studies, and performed as expected:

| | |
|---|---|
| Blood Tube Matrix Comparison | Precision |
| Reproducibility | Cross-Reactivity |
| Interfering Substances | Read Now vs Walk Away |
| Specimen Volume control | Development Time |
| Diluted Sample Volume | |

2. Comparison studies:

*a. Method comparison with predicate device: prospective*

A prospective study was performed on 678 samples to establish the clinical performance of the assay. Of these, 178 samples were prospectively-collected and 500 were frozen samples previously submitted for Lyme testing and sequentially retained without regard to results. At each of three laboratories, samples were tested in parallel using a commercially available Lyme IgM and IgG EIA method (predicate) and the Sofia Lyme FIA. Possible results for the predicate assay were negative, positive or equivocal. Possible results for the Sofia Lyme FIA were negative or positive. Positive Percent Agreement (PPA) was calculated between Sofia Lyme FIA positive results and predicate positive and equivocal results because the Centers for Disease Control (CDC) recommended 2-tier Lyme testing algorithm calls for first tier positive and equivocal results to be tested by a second tier western blot assay. Combined results from the three sites are shown below:

**Lyme IgM Results (Tier 1) for Sofia Compared to the Predicate Test -Prospective Specimens**

| | **Predicate Lyme IgM** | | | **% Agreement** | **95% CI** |
|---|---|---|---|---|---|
| | **Pos** | **Equivocal** | **Neg** | | |
| **Sofia IgM Pos** | 37 | 20 | 67 | PPA[2] = 56.4% (57 / 101) | 46.7% - 65.7% |
| **Sofia IgM Neg** | 7 | 37[1] | 510 | NPA = 88.4% (510 / 577) | 85.5% - 90.8% |
| **Total:** | 44 | 57 | 577 | | |

[1]Of the 37 specimens that were Sofia Lyme FIA negative and predicate equivocal, 34 were negative by western blot

[2] Additional Information: Four specimens that were negative by the predicate were positive by Sofia Lyme FIA and confirmed positive by western blot

**Lyme IgG Results (Tier 1) for Sofia Compared to Predicate Test -Prospective Specimens**

| | Predicate Lyme IgG | | % Agreement | 95% CI |
|---|---|---|---|---|
| | Pos | Neg | | |
| **Sofia IgG Pos** | 47 | 54 | PPA = 82.5% (47 / 57) | 70.4% - 90.4% |
| **Sofia IgG Neg** | 10 | 567 | NPA = 91.3% (567 / 621) | 88.8% - 93.3% |
| **Total:** | 57 | 621 | | |

**Second-Tier Testing**: In accordance with the CDC recommended 2-tier Lyme testing algorithm the Sofia Lyme FIA results and the predicate Lyme IgM and IgG positive and equivocal results were confirmed using a commercially available Lyme IgM or IgG western blot assay. A summary of second-tier testing results along with the PPA between Sofia Lyme FIA and the predicate for first and second tier testing are shown below.

**Lyme IgM Results (Tier 2) for Sofia Compared to the Predicate Assay -Prospective Specimens**

| Assay | Tier 1 + or ± | IgM WB + | IgM WB - |
|---|---|---|---|
| **Predicate IgM** | 101[1] | 37 | 63 |
| **Sofia IgM** | 124[2] | 45 | 76 |
| **Predicate and Sofia IgM** | 57[1] | 33 | 23 |

[1]One sample had insufficient volume to be tested on WB insufficient
[2] Three samples had insufficient volume to be tested on WB insufficient

**Lyme IgM PPA for Sofia Compared to the Predicate Assay -Prospective Specimens**

| | PPA | N | 95% CI |
|---|---|---|---|
| **1st Tier PPA** | 56.4% | 57/101 | 46.7% - 65.7% |
| **2nd Tier PPA** | 89.2% | 33/37 | 74.7% - 96.3%% |

**Lyme IgG Results (Tier 2) for Sofia Compared to Predicate Test -Prospective Specimens**

| Assay | Tier 1 + | IgG WB + | IgG WB - |
|---|---|---|---|
| **Predicate IgG** | 57 | 28 | 29 |
| **Sofia IgG** | 101[1] | 25 | 72 |
| **Predicate and Sofia IgG** | 47 | 25 | 22 |

[1]Four samples had insufficient volume to be tested on WB insufficient

**Lyme IgG PPA for Sofia Compared to the Predicate Assay -Prospective Specimens**

| | PPA | N | 95% CI |
|---|---|---|---|
| **1st Tier PPA** | 82.5% | 47/57 | 70.4% - 90.4% |
| **2nd Tier PPA** | 89.3% | 25/28 | 72.0% - 97.1% |

*b. Method comparison with predicate device: retrospective*

Additionally, to supplement positive IgM and IgG results, 52 known positive samples were obtained from a commercial vendor, blinded and distributed to testing sites. Results of testing are summarized below:

**Lyme IgM Results for Sofia Compared to Predicate Test - Retrospective Specimens**

| | Predicate Lyme IgM | | | % Agreement | 95% CI |
|---|---|---|---|---|---|
| | Pos | Equivocal | Neg | | |
| **Sofia IgM Pos** | 17 | 11 | 4 | PPA* = 90.3% (28 / 31) | 74.3% - 97.4% |
| **Sofia IgM Neg** | 0 | 3[1] | 17 | NPA = 81.0% (17 / 21) | 59.4% - 92.9% |
| **Total:** | 17 | 14 | 21 | | |

[1]Of the three specimens that were Sofia Lyme FIA negative and predicate equivocal, 1 was negative by western blot

**Lyme IgG Results for Sofia Compared to Predicate Test - Retrospective Specimens**

| | Predicate Lyme IgG | | % Agreement | 95% CI |
|---|---|---|---|---|
| | Pos | Neg | | |
| **Sofia IgG Pos** | 27 | 5 | PPA =100% (27 / 27) | 89.2% - 100.0% |
| **Sofia IgG Neg** | 0 | 20 | NPA = 80.0% (20 / 25) | 60.4% - 91.6% |
| **Total:** | 27 | 25 | | |

**Second-Tier Testing**: In accordance with the CDC recommended 2-tier Lyme testing algorithm the Sofia Lyme FIA results and the predicate Lyme IgM and IgG positive and equivocal results were confirmed using a commercially available Lyme IgM or IgG western blot method. A summary of second-tier testing results along with the PPA between Sofia Lyme FIA and the predicate for first and second tier testing are shown below.

**IgM Second Tier Testing - Retrospective Specimens**

| Assay | Tier 1 + or ± | IgM WB + | IgM WB - |
|---|---|---|---|
| **Predicate IgM** | 31 | 22 | 9 |
| **Sofia IgM** | 32[1] | 20 | 11 |
| **Predicate and Sofia IgM** | 28 | 20 | 8 |

[1]One sample had insufficient volume to be tested on WB insufficient

**Lyme IgM PPA for Sofia Compared to the Predicate Assay -Retrospective Specimens**

| | PPA | N | 95% CI |
|---|---|---|---|
| **1st Tier PPA** | 90.3% | 28/31 | 74.3% - 97.4% |
| **2nd Tier PPA** | 90.9% | 20/22 | 71.0%-98.7% |

**IgG Second Tier Testing - Retrospective Specimens**

| Assay | Tier 1 + | IgG WB + | IgG WB - |
|---|---|---|---|
| **Predicate IgG** | 27 | 8 | 19 |
| **Sofia IgG** | 32[1] | 8 | 22 |
| **Predicate and Sofia IgG** | 27 | 8 | 19 |

[1]Two samples had insufficient volume to be tested on WB insufficient

**Lyme IgG PPA for Sofia Compared to the Predicate Assay -Retrospective Specimens**

| | PPA | N | 95% CI |
|---|---|---|---|
| **1st Tier PPA** | 100.0% | 27/27 | 85.2% - 100.0% |
| **2nd Tier PPA** | 100.0% | 8/8 | 62.8% - 100.0% |

c. *Matrix comparison:*

A matrix comparison study was performed by testing replicates of donor blood. Ten donors were used for each concentration, with the exception of the negative IgG

sample, for which there were 21 donors. Five replicates from each donor were used.

The comparative matrix qualitative IgM and IgG results demonstrate that four (4) whole blood collection tube types, (serum (RED), serum (TIGER), sodium heparin, and lithium heparin tubes) are not significantly different when compared to the serum matrix IgM and IgG results.

The EDTA collection tube qualitative results were significantly different when compared to the serum matrix results. This difference was most pronounced in samples that were evaluated at the C5 level.

The IgM and IgG Matrix Equivalency summary tables for each collection tube including each level, the number and percentage (%) tested are shown below:

**Matrix Comparison – Blood Collection Tubes - IgM**

| Whole Blood Collection Tube | Number and Percentage (%) of Samples | | | |
|---|---|---|---|---|
| | Negative | C5 | C95 | 2-3X |
| **Serum (RED)** | 0 of 50 (0%) | 49 of 50 (98%) | 50 of 50 (100%) | 50 of 50 (100%) |
| **Serum (Tiger)** | 0 of 50 (0%) | 48 of 50 (96%) | 50 of 50 (100%) | 50 of 50 (100%) |
| **EDTA** | 0 of 50 (0%) | 40 of 50 (80%) | 50 of 50 (100%) | 50 of 50 (100%) |
| **Sodium Heparin** | 0 of 50 (0%) | 50 of 50 (100%) | 50 of 50 (100%) | 50 of 50 (100%) |
| **Lithium Heparin** | 0 of 50 (0%) | 46 of 50 (92%) | 50 of 50 (100%) | 50 of 50 (100%) |

**Matrix Comparison – Blood Collection Tubes - IgG**

| Whole Blood Collection Tube | Number and Percentage (%) of Samples | | | |
|---|---|---|---|---|
| | Negative | C5 | C95 | 2-3X |
| **Serum (RED)** | 0 of 105 (0%) | 36 of 50 (72%) | 44 of 50 (88%) | 50 of 50 (100%) |
| **Serum (Tiger)** | 0 of 105 (0%) | 44 of 50 (88%) | 45 of 50 (90%) | 50 of 50 (100%) |
| **EDTA** | 0 of 105 (0%) | 50 of 50 (100%) | 50 of 50 (100%) | 50 of 50 (100%) |
| **Sodium Heparin** | 0 of 105 (0%) | 34 of 50 (68%) | 46 of 50 (92%) | 50 of 50 (100%) |
| **Lithium Heparin** | 0 of 105 (0%) | 43 of 50 (86%) | 48 of 50 (96%) | 50 of 50 (100%) |

3. Clinical studies:

   *a. Clinical Sensitivity:*

Well-characterized clinically or culture confirmed Lyme disease serum samples were tested with the Sofia Lyme FIA. The results were compared to those obtained with the predicate Lyme IgG and IgM assays. The samples include the following patient populations:

- Category 1a: Initial (acute) samples from patients with documented erythema migrans (EM) or culture positive <1 month after symptom onset.
- Category 1b: Initial (acute) samples from patients with documented erythema migrans (EM) or culture positive 1-2 months after symptom onset.
- Category 1c: Initial (acute) samples from patients with documented erythema migrans (EM) or culture positive 2-3 months after symptom onset.
- Category 2: Convalescent samples from patients with documented erythema migrans (EM) or culture positive 3-12 months after appearance of symptoms.
- Category 3: Late Lyme (>1 year) neurological or Arthritic.

Results are reported as Lyme IgG and/or Lyme IgM positive or negative by Sofia for each sample tested. Results are summarized for each test method as "Sofia % Sensitivity" and "Predicate Device % Sensitivity" including 95% Confidence Interval for each method. Percent Sensitivity and 95% Confidence Interval are calculated for each sample sub-group as well as for all groups combined.

**IgM Results for each Clinical Characterization Category**

| | | Sofia IgM | | | | Predicate IgM | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Category** | **n** | **Pos** | **Neg** | **% Sens** | **95% CI** | **Pos** | **Equiv** | **Neg** | **%Sens[1]** | **95% CI** |
| **Acute, < 1 month, with EM** | 64 | 39 | 25 | 60.9% | 48.7-72.0% | 28 | 8 | 28 | 56.3% | 44.1-67.7% |
| **Acute, 1-2 months, with EM** | 4 | 3 | 1 | 75.0% | 28.9-96.6% | 2 | 0 | 2 | 50.0% | 15.0-85.0% |
| **Convalescent, 3-12 months, with EM** | 15 | 11 | 4 | 73.3% | 47.6-89.5% | 6 | 2 | 7 | 53.3% | 30.1-75.2% |
| **Late Lyme (>1 yr), Neuro or Arthritic** | 12 | 8 | 4 | 66.7% | 38.8-86.5% | 7 | 3 | 2 | 83.3% | 54.0-96.5% |
| **All Categories** | 95 | 61 | 34 | 64.2% | 54.2-73.1% | 43 | 13 | 39 | 58.9% | 48.9-68.3% |

[1]Of the 13 samples that were "equivocal" by the predicate, 12 of 13 were negative by FDA approved western blot.

**IgG Results for each Clinical Characterization Category**

| | | Sofia IgG | | | | Predicate IgG | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Category** | **n** | **Pos** | **Neg** | **% Sens** | **95%CI** | **Pos** | **Neg** | **% Sens** | **95%CI** |
| **Acute, < 1 month, with EM** | 64 | 50 | 14 | 78.1% | 66.5-86.6% | 28 | 36 | 43.8% | 32.3-55.9% |
| **Acute, 1-2 months, with EM** | 4 | 4 | 0 | 100% | 54.3-100.0% | 3 | 1 | 75.0% | 28.9-96.6% |
| **Convalescent, 3-12 months, with EM** | 15 | 10 | 5 | 66.7% | 41.5-85.0% | 6 | 9 | 40.0% | 19.8-64.3% |
| **Late Lyme (>1 yr), Neuro or Arthritic** | 12 | 12 | 0 | 100% | 78.4-100.0% | 10 | 2 | 83.3% | 54.0-96.5% |
| **All Categories** | 95 | 76 | 19 | 80.0% | 70.8-86.9% | 47 | 48 | 49.5% | 39.6-59.4% |

*b. Clinical specificity:* Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

CDC Lyme panel was provided and tested with the Sofia Lyme FIA. First tier testing on the panel provided by the CDC was completed with a total Lyme antibody assay. These assay results were reported as positive or negative only, the type of antibody (IgM or IgG) was not reported. As a result their ability to evaluate the performance of the Sofia Lyme FIA on the CDC provided panel is limited. Please note that provision of a panel for testing does not imply an endorsement of the assay by the CDC.

**Sofia IgM % Agreement with expected CDC result**

| | | Sofia IgM | | | Western Blot Lyme IgM | | |
|---|---|---|---|---|---|---|---|
| **Group ID** | **N** | **Pos** | **Neg** | **% Agreement** | **Pos** | **Neg** | **% Agreement** |
| **Negative Controls** | **190** | 33 | 157 | 82.6% | 0 | 190 | 100.0% |
| **Early Lyme EM Positive** | **60** | 49 | 11 | 81.7% | 31 | 29 | 51.7% |
| **Late Lyme** | **30** | 22 | 8 | 73.3% | 17 | 13 | 56.7% |

**Sofia IgG % Agreement with expected CDC result**

| | | Sofia IgG | | | Western Blot Lyme IgG | | |
|---|---|---|---|---|---|---|---|
| **Group ID** | **N** | **Pos** | **Neg** | **% Agreement** | **Pos** | **Neg** | **% Agreement** |
| **Negative Controls** | **190** | 25 | 165 | 86.8% | 0 | 190 | 100.0% |
| **Early Lyme EM Positive** | **60** | 49 | 11 | 81.7% | 19 | 41 | 31.7% |
| **Late Lyme** | **30** | 30 | 0 | 100.0% | 26 | 4 | 86.7% |

## N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

## O. Conclusion:

The submitted information in this premarket notification supports a substantial equivalence determination.