QUIDEL | Sofia® Lyme FIA

FOR USE WITH SOFIA ONLY

For *in vitro* use only, Rx only.

INTENDED USE

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.

SUMMARY AND EXPLANATION

Lyme disease (LD) is the most common tickborne disease in North America and Europe.[1] In the United States, LD is caused by the bacterium, *Borrelia burgdorferi*, transmitted through the bite of an infected blacklegged tick.[1,2]

Patients infected with *B. burgdorferi* may experience symptoms associated with three stages: early localized disease, early disseminated disease, and late persistent disease.[1] The most characteristic symptom of early localized disease is the appearance of erythema migrants (EM) on the skin, which appears in up to 80% of cases.[1,3] EM may also be accompanied by flu-like symptoms (headache, abdominal pain, and fatigue) days or weeks after infection.[3] In the second stage, early disseminated disease, untreated patients may begin to see neurological and rheumatological manifestations, and less commonly, dermatological, cardiac, or ophthalmological manifestations. These symptoms generally appear weeks to months after infection.[1] If the disease continues to be left untreated, late persistent disease may also follow months or years later.[3] In late stage disease, patients may see continued progression of manifestations in the joints, heart, skin, and nervous system.[2]

Early detection and treatment of LD can help resolve symptoms and prevent progression of the disease.[1] The primary means of identifying *B. burgdorferi* infection is detection of the body's IgM and IgG antibody response using immunoassay.[3] Detection of IgM antibodies to *B. burgdorferi* is generally most significant in the earlier stages of the disease. Conversely, detection of IgG antibodies has proven to be significant for longer periods, as the antibodies may remain detectable years after infection.

PRINCIPLE OF THE TEST

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. 100 µL of the diluted sample 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.

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Test Cassettes (25): *Borrelia burgdorferi* antigens and anti-human IgM/IgG
- Reagent Vials, filled with 0.3 mL reagent solution (25)
- 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)
- QC Card (located on kit box)
- Printer Paper (1)

MATERIALS REQUIRED BUT NOT SUPPLIED IN KIT

- Sofia
- Sofia Installation Pack
- Calibration Cassette (supplied with the Sofia Installation Pack)
- Timer or watch for use in READ NOW Mode
- Calibrated micropipette – for measuring 30 µL and 100 µL volumes
- Blood collection tubes

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.[4]
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.[4]
- Do not reuse the used Test Cassette or Reagent Vial.
- The user should never open the foil pouch of the Test Cassette exposing it to the ambient environment until the Test Cassette is ready for immediate use.
- Discard and do not use any damaged Test Cassette or material.
- To obtain accurate results, the Package Insert instructions must be followed.
- The Calibration Cassette must be kept sealed in the provided foil storage pouch between uses.
- Inadequate or inappropriate specimen collection, storage, and transport may yield false test results.
- Specimen collection and handling procedures require specific training and guidance.
- Do not write on the barcode of the Test Cassette. This is used by Sofia to identify the type of test being run and to identify the individual Test Cassette to prevent a second read of the Test Cassette by the same Sofia.

- As the detection reagent is a fluorescent compound, no visible results will form. Sofia must be used for result interpretation.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

KIT STORAGE AND STABILITY

Store the kit at room temperature, 59°F to 86°F (15°C to 30°C), out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

QUALITY CONTROL

There are three types of Quality Control for Sofia and the Test Cassette: Sofia Calibration Check Procedure, Built-in Procedural Control features, and External Controls.

Sofia Calibration Check Procedure

The Calibration Check Procedure is a required function that checks the Sofia optics and calculation systems using a specific Calibration Cassette. A Calibration Cassette is shipped with the Sofia Installation Pack.

Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses to protect it from exposure to light.

1. To check the calibration of Sofia, select “Calibration” from the Main Menu.

2. Following the prompts, insert the Calibration Cassette into Sofia and close the drawer. Sofia performs the Calibration Check automatically with no user input required.

Sofia indicates when the Calibration Check is completed. Select OK to return to the Main Menu.

NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.552.7905; technicalsupport@quidel.com (Technical Support); or contact your local distributor.

Built-in Procedural Controls

The Sofia Lyme FIA contains a built-in procedural control feature. Each time a test is run, the procedural control area is scanned by Sofia and the result is displayed on the Sofia screen.

The manufacturer's recommendation for daily control is to document the results of these built-in procedural controls for the first sample tested each day. This documentation is automatically logged in Sofia with each test result.

A valid result obtained from the procedural control demonstrates that the test flowed correctly and the functional integrity of the Test Cassette was maintained. **The procedural control is interpreted by Sofia after the Test Cassette has developed for 10 minutes. If the test does not flow correctly, Sofia will indicate that the result is invalid.** Should this occur, review the procedure and repeat the test with a new patient sample and a new Test Cassette.

For example: This display shows an invalid result.

External Quality Control

External Controls are used to demonstrate that the reagents and assay procedure perform properly. Quidel recommends that Positive and Negative External controls be run:

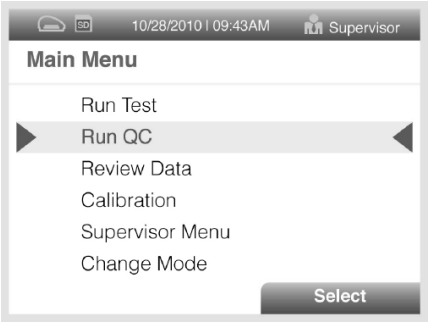
- Once for each new untrained operator
- Once for each new shipment of kits – provided that each different lot received in the shipment is tested
- As deemed additionally necessary by your internal quality control procedures, and in accordance with Local, State and Federal regulations or accreditation requirements

For information on how to obtain additional External Controls, contact Quidel Customer Support Services at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), or contact your local distributor.

To test External Controls, follow the instructions below.

EXTERNAL QUALITY CONTROL TEST PROCEDURE

1. From the main menu, select Run QC.



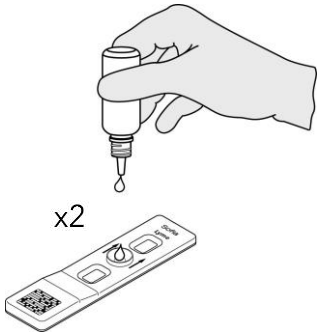
2. Following the prompt on the screen, scan the QC Card (located on the assay kit box).

3. Sofia will prompt the user to select the desired mode (WALK AWAY or READ NOW) and then to run the External Controls. A full description of each mode can be found in the "Using Sofia" section of this Package Insert.

4. Use the following procedure to test each of the Control solutions. **The Positive Control must be run first, followed by the Negative Control.**

a. Prepare a ***Positive Control Cassette*** by adding **2 drops** of the Positive Control solution (red cap) to a Test Cassette sample well. Then follow the Sofia screen instructions for developing and analyzing the Positive Control Cassette.

NOTE: The round Test Cassette sample well is located between the rectangular windows and has a green-tinted sample pad. DO NOT place sample into the left or right rectangular windows. When adding drops, hold the bottle vertically so that a complete drop forms.



b. Prepare a ***Negative Control Cassette*** by adding **2 drops** of the Negative Control solution (white cap) to a Test Cassette sample well. Then follow the Sofia screen instructions for developing and analyzing the Negative Control Cassette.

5. After both the Positive and Negative Controls have been run, the results will be displayed as "Passed" or "Failed."

Do not perform patient tests or report patient test results if either of the QC test results fail. If both the Positive and Negative Controls fail, repeat testing with both the Positive and Negative Controls a second time. If only a single Control fails, the user has the option of repeating both the Positive and Negative Controls or to repeat only the Control that failed. The user may select "Skip" on the Sofia display to skip the Control test that previously passed. The QC Results will show a skipped Control test as "unknown."

Repeat the test or contact Quidel Technical Support at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.).

SAMPLE COLLECTION AND STORAGE

Serum or Plasma

Collect and process serum or plasma per standard procedures. Samples may be stored refrigerated (2°C to 8°C) up to 48 hours, at room temperature (15°C to 30°C) up to 8 hours, and frozen (–20°C) up to 2 freeze/thaw

cycles. **Make sure sample is at room temperature before using in the test, and for samples that have been previously refrigerated or frozen, mix well by fully thawing and inverting the tube 10 times**.

Performance of the Sofia Lyme FIA has been established with the following tubes:

- Serum Separator Tube (RED)
- Serum Separator Tube (TIGER)
- Lithium Heparin
- Sodium Heparin

Note: EDTA is NOT compatible with Sofia Lyme FIA.

TEST PROCEDURE (SERUM/PLASMA)

Precautions

DO NOT open the foil pouch containing the Test Cassette until ready to test the sample. Place the Test Cassette on a clean and level surface.

All samples **must be at room temperature** before beginning the assay.

Check expiration date on each individual test package or outer box before using. *Do not use any test past the expiration date on the label.*

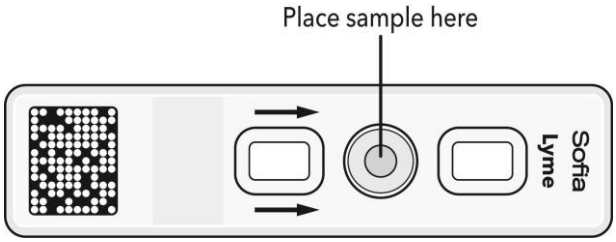
1. Verify that Sofia is set to the desired mode: **WALK AWAY** or **READ NOW**.
   See the "Using Sofia" section for more information.

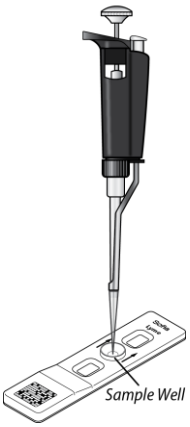
2. Fill a calibrated micropipette with **30 µL** of the patient serum/plasma sample.

3. Add patient sample to Reagent Vial and **gently mix**.

4. Fill a calibrated micropipette with **100 µL** of diluted sample from the Reagent Vial.

5. Dispense the contents of the micropipette into the round Test Cassette sample well.





6. Proceed to the next section, "Using Sofia," to complete the test.

USING SOFIA

WALK AWAY/READ NOW Modes

Refer to the Sofia User Manual for operating instructions.

Sofia may be set to two different modes (WALK AWAY and READ NOW). The procedures for each mode are described below.

WALK AWAY Mode

In WALK AWAY Mode, the user **immediately** inserts the Test Cassette into Sofia. The user then returns after 10 minutes to get the test result. In this mode, Sofia will automatically time the test development before scanning and displaying the test result.

READ NOW Mode

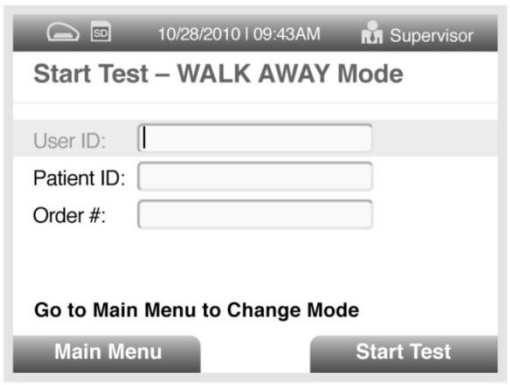
Allow the test to develop for the full 10 minutes BEFORE placing it into Sofia.

The user must first place the Test Cassette onto the counter or bench top for 10 minutes (outside of Sofia) and manually time this development step. Then, the user inserts the Test Cassette into Sofia. In READ NOW Mode, Sofia will scan and display the test result in approximately 1 minute. **Note:** Results will remain stable for an additional 10 minutes after the recommended development time of 10 minutes.

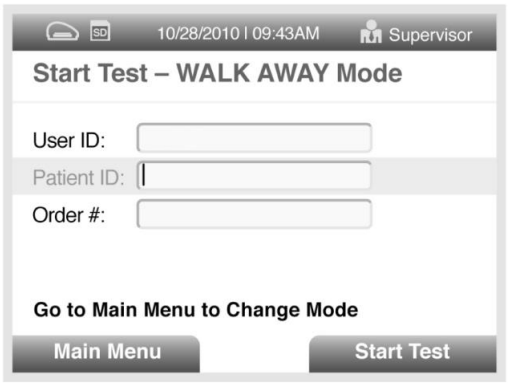
Run Test

1. Input the User ID using the barcode scanner or manually enter the data using the key pad.

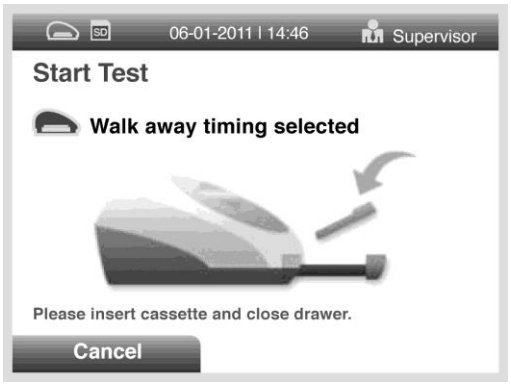
   NOTE: *If you mistakenly scan the incorrect barcode, use the Arrow Buttons on the Sofia key pad to re-highlight the field. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*



2. Input Patient ID and/or Order # using the barcode scanner or manually enter the data using the key pad.

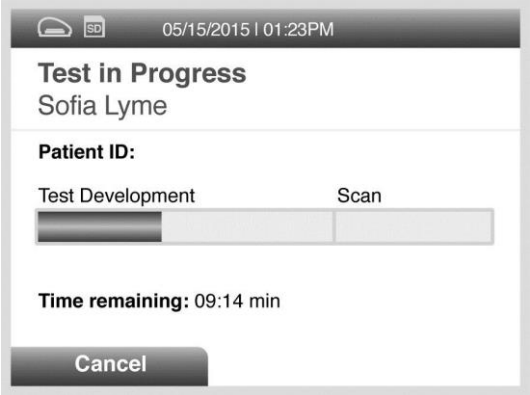


3. Press Start Test and the Sofia drawer will automatically open.



4. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Insert the prepared patient Test Cassette into the drawer of Sofia and gently close the drawer.

5. Sofia will start automatically and display the progress as shown in the example below. In WALK AWAY Mode, the test results will be displayed on the screen in approximately 10 minutes. In READ NOW Mode, the test results will be displayed on the screen in approximately 1 minute. See Interpretation of Results section.



For example: This display shows that the test in WALK AWAY Mode has 9 minutes, 14 seconds remaining. Sofia will read and display the results after 10 minutes.

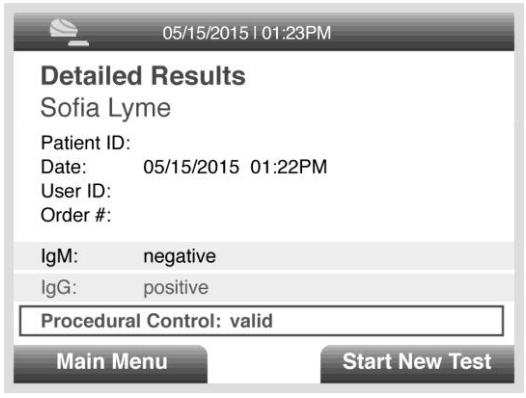
INTERPRETATION OF RESULTS

When the test is complete, the results will be displayed on the Sofia screen. The results will be automatically printed on the integrated printer if this option is selected. Test Lines, which are fluorescent, cannot be seen with the naked eye.

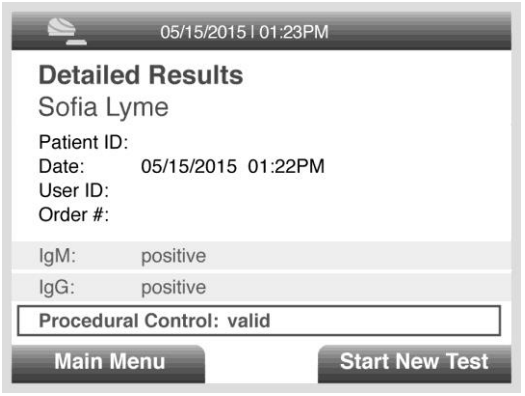
The Sofia screen will display results for the procedural controls as being “valid” or “invalid” and will provide a positive or negative result for the detection of IgM and/or IgG antibodies to *B. burgdorferi*. If the procedural controls are “invalid,” retest the patient’s sample with a new Test Cassette.

Positive Results:

This display shows a valid positive result for IgM antibodies to B. burgdorferi. Interpret to be presumptive of an acute infection to B. b. Test the specimen with IgM Western blot.



This display shows a valid positive result for IgG antibodies to B. burgdorferi. Interpret to be presumptive of a past exposure to B. b. Test the specimen with IgG Western blot.



This display shows a valid positive result for IgM and IgG antibodies to B. burgdorferi. Interpret to be presumptive of a recent exposure to B. b. Test the specimen with IgM and IgG Western blot.

Negative Results:

This display shows a valid negative result for antibodies to B. burgdorferi. Interpret to be presumptive of no evidence of B. b. infection.

Invalid Results:

This display shows an invalid result.

If the test is invalid, a new test should be performed starting with Step 1 and a new Test Cassette.

LIMITATIONS

- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- False positive results may occur if samples are read before the minimum 10 minute development time.
- Antibody detection methods do not provide definitive results for establishing or ruling out a diagnosis of Lyme disease.
- A negative result in the Sofia Lyme FIA does not rule out the possibility of *B. burgdorferi* infection in a patient.
- Positive results in the Sofia Lyme FIA must be interpreted with caution. Cross-reactivity may be observed with certain diseases. Positive results for IgM and/or IgG should be further tested by a corresponding second-tier western blot assay. Refer to the Cross-reactivity section of the package insert.

EXPECTED VALUES

The rate of positivity observed in Lyme testing may vary depending on the time of year, the geographical location and disease prevalence for the season. Available patient demographics (age and gender), the number of samples tested and the number of samples that were positive for Sofia Lyme FIA are summarized in Table 1.

Table 1
Sofia Lyme FIA IgM/IgG Study Results

Study	Samples Tested			Age Range	Positive IgM/ Total Tested	Positive IgG/ Total Tested
	Total	Male	Female			
Prospective	678	297	381	4-89	124/678	101/678
Retrospective	52	26	26	6-90	32/52	32/52
Sensitivity	95*	58	35	16-74	61/95	76/95
Endemic Negative	100	59	41	20-64	8/100	11/100
Non-Endemic Negative	100	50	50	19-60	12/100	9/100

*Two (2) samples do not have age information.

PERFORMANCE CHARACTERISTICS

CDC Lyme Panel

A blinded serum panel consisting of 280 samples was obtained from the CDC and was evaluated internally using the Sofia Lyme FIA. The Lyme disease samples are from physician-diagnosed patients with various stages of the disease. Early Lyme EM positive samples were collected days to weeks after disease onset. Late Lyme samples were collected months to years post disease onset. Samples from control individuals include sera from patients with lookalike or cross-reactive diseases/syndromes (syphilis, rheumatoid arthritis, fibromyalgia, mononucleosis, multiple sclerosis and severe periodontitis) and from healthy controls that are from individuals residing in Lyme disease endemic and non-endemic regions who have no prior history of physician-diagnosed

Lyme disease. The summarized results in Tables 2 and 3 are presented to provide additional information about the performance of the Sofia Lyme FIA compared to Western Blot with a blinded, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Table 2
Sofia Lyme FIA tested with CDC Panel – IgM Results

Clinical Status		Sofia Lyme IgM			Western Blot Lyme IgM		
	n	Pos	Neg	% Agreement with clinical Status	Pos	Neg	% Agreement with clinical Status
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%

Table 3
Sofia Lyme FIA tested with CDC Panel – IgG Results

Clinical Status		Sofia Lyme IgG			Western Blot Lyme IgG		
	n	Pos	Neg	% Agreement with clinical Status	Pos	Neg	% Agreement with clinical Status
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%

Analytical Specificity

To determine the analytical specificity of the Sofia Lyme FIA, 200 seemingly healthy individuals with no known history of physician-diagnosed Lyme disease were evaluated. Half of the samples (100) were collected from a non-endemic Lyme region while the other half of the samples (100) were collected from an endemic Lyme region of the United States. Samples were tested on Sofia Lyme FIA and the predicate Lyme IgM and IgG assays. The Analytical Specificity results for Sofia Lyme FIA and the predicate devices are summarized in Table 4.

Table 4
Sofia Lyme FIA and Predicate Analytical Specificity

	n	Sofia IgM	Predicate IgM*		Sofia IgG	Predicate IgG
Endemic	100	92.0%	95.0%		89.0%	97.0%
Non-Endemic	100	88.0%	86.0%		91.0%	100.0%
Total	**200**	**90.0%**	**90.5%**		**90.0%**	**98.5%**

*Includes positive and equivocal results.

Sensitivity

To assess the sensitivity of the Sofia Lyme FIA, 95 well-characterized clinically or culture confirmed Lyme disease serum samples were tested on Sofia Lyme and compared to the predicate IgM and IgG assays. The samples were blinded and tested on both Sofia Lyme FIA and the predicate Lyme IgM and Lyme IgG tests. The Sofia results are compared to those obtained with predicate Lyme IgM and IgG assays. Results for IgM and IgG are shown in Tables 5 and 6.

Table 5
Lyme IgM Results for Sofia Compared to Predicate Assay

		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.

Table 6
Lyme IgG Results for Sofia Compared to Predicate Assay

		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	100.0%	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	100.0%	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%

Prospective Clinical Study

A prospective study was performed using 678 serum samples collected from patients that were submitted for routine Lyme disease testing in the United States. These samples were prospectively collected from ten different clinical sites located in endemic areas of 4 states, as well as clinical laboratory remnant specimens that were collected from endemic areas of 6 states. Sofia Lyme FIA testing was performed at 3 laboratories, including 1 Quidel site. The predicate Lyme IgM and IgG assays and western blot Lyme IgM and IgG assays were performed at 2 distinct sites that were different from the 3 Sofia testing sites. First tier results for Sofia and the predicate assays are shown in Tables 7 and 8.

Retrospective Clinical Study

A retrospective study was performed using 52 serum samples obtained from a commercial vendor, which were previously characterized as positive for anti- *B. burgdorferi* antibodies. This serum sample set was not included in any other Sofia Lyme FIA testing. First tier results for Sofia and the predicate assays are shown in Tables 7 and 8.

Table 7

Lyme IgM Results for Sofia Compared to Predicate Assay

IgM Method Comparison (Prospective Study): 1st Tier PPA and NPA Analysis

		Predicate Lyme IgM				
		Positive	**Equivocal**	**Negative**	**% Agreement**	**95% CI**
Sofia IgM	**Positive**	37	20	67	**PPA**[2] = 56.4% (57 / 101)	46.7% - 65.7%
	Negative	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

IgM Method Comparison (Retrospective Study): 1st Tier PPA and NPA Analysis

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

[1]Of the 3 specimens that were Sofia Lyme FIA negative and predicate equivocal, 1 was negative by western blot

Table 8

Lyme IgG Results for Sofia Compared to Predicate Assay

IgG Method Comparison (Prospective Study): 1st Tier PPA and NPA Analysis

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

IgG Method Comparison (Retrospective Study): 1st Tier PPA and NPA Analysis

		Predicate Lyme IgG			
		Positive	**Negative**	**% Agreement**	**95% CI**
Sofia IgG	**Positive**	27	5	**PPA** = 100.0% (27 / 27)	89.2% - 100.0%
	Negative	0	20	**NPA** = 80.0% (20 / 25)	60.4% - 91.6%
Total		27	25		

Second-Tier Testing

As recommended by CDC guidelines, second tier Western blot testing was performed on all positive (and equivocal with the predicate assay) samples when tested by either Sofia or the predicate. The percent agreement between Sofia and the predicate Lyme test are shown in Tables 9 and 10.

Table 9

IgM Second Tier Testing

IgM Method Comparison (Prospective Study): 2st Tier PPA Analysis

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

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

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

[2]3 samples had insufficient volume to be tested on WB

IgM Method Comparison (Retrospective Study): 2st Tier PPA Analysis

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

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

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

Table 10

IgG Second Tier Testing

IgG Method Comparison (Prospective Study): 2st Tier PPA Analysis

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

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

[1]4 samples had insufficient volume to be tested on WB.

IgG Method Comparison (Retrospective Study): 2st Tier PPA Analysis

	Tier 1 +	IgG WB +	IgG WB -
Predicate IgG	27	8	19
Sofia IgG	32[1]	8	22
Predicate + Sofia IgG	27	8	19

1st Tier PPA (95% CI)	100.0% (85.2-100.0%)	27/27
2nd Tier PPA (95% CI)	100.0% (62.8-100.0%)	8/8

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

Precision

The precision of the Sofia Lyme FIA was evaluated at 1 Quidel site utilizing 2 operators and 2 Sofia instruments. Contrived samples were prepared at levels that ranged from negative to moderate positive for both IgM and IgG. Each sample was tested by 2 operators in duplicate with a total of 24 different runs (2 runs per day over a total of 12 days) for a total of 96 times over the course of the study. The within run and between operator results for negative samples were 0% positive, 7.3-8.3% for high negative, 97.9-100.0% for low positive, and 100.0% for moderate positive samples, see Tables 11 and 12 for results.

Table 11
Sofia Lyme FIA Precision – Within Run

IgM or IgG Sample	IgM Positive		IgM % Positivity	IgG Positive		IgG % Positivity
	Run 1	Run 2	Total (n=96)	Run 1	Run 2	Total (n=96)
Negative	0/48	0/48	0.0%	0/48	0/48	0.0%
High Negative (C_5)	3/48	5/48	8.3%	2/48	5/48	7.3%
Low Positive (C_{95})	48/48	48/48	100.0%	48/48	46/48	97.9%
Moderate Positive (2-3X)	48/48	48/48	100.0%	48/48	48/48	100.0%

Table 12
Sofia Lyme FIA Precision – Between Operator

IgM or IgG Sample	% IgM Positivity		IgM % Positivity	% IgG Positivity		IgG % Positivity
	Operator1	Operator 2	Total (n=96)	Operator 1	Operator 2	Total (n=96)
Negative	0/48	0/48	0.0%	0/48	0/48	0.0%
High Negative (C_5)	3/48	5/48	8.3%	3/48	4/48	7.3%
Low Positive (C_{95})	48/48	48/48	100.0%	47/48	47/48	97.9%
Moderate Positive (2-3X)	48/48	48/48	100.0%	48/48	48/48	100.0%

Reproducibility

The reproducibility of the Sofia Lyme FIA was evaluated at 3 different laboratories, 1 of which was Quidel. Two operators at each site tested a series of coded, contrived samples ranging from negative to moderate positive IgM and IgG concentrations. Testing occurred for a minimum of 5 separate days, spanning a period of approximately 1 month. The inter-laboratory agreement for the IgM test on negative samples was 100.0%, 100.0% for high negative, 100.0% for low positive, and 100.0% for moderate positive samples. The inter-laboratory agreement for the IgG test on negative samples was 100.0%, 100.0% for high negative, 86.7% for low positive, and 100.0% for moderate positive samples, see Table 13 for results.

Table 13
Sofia Lyme FIA Reproducibility Study Inter-laboratory Agreement

Site	IgM Negative (C_0)	IgM High Negative (C_5)	IgM Low Positive (C_{95})	IgM Moderate Positive (2-3X LOD)	IgG Negative (C_0)	IgG High Negative (C_5)	IgG Low Positive (C_{95})	IgG Moderate Positive (2-3X LOD)
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)	**100.0% (95.1-100.0%)**	**100.0% (95.1-100.0%)**	**100.0% (95.1-100.0%)**	**100.0% (95.1-100.0%)**	**100.0% (95.1-100.0%)**	**100.0% (95.1-100.0%)**	**86.7% (78.0-92.4%)**	**100.0% (95.1-100.0%)**

Matrix Equivalency

Matched serum and plasma samples were collected from negative donors from a non-endemic region of the United States. Samples were contrived with IgM and IgG concentrations that covered the dynamic range of the assay. The comparative matrix for negative and positive IgM and IgG samples demonstrate that the performance with matched serum and plasma samples were acceptable when using four tube types: Serum

Separator Tube (RED), Serum Separator Tube (TIGER), Lithium Heparin, and Sodium Heparin. EDTA is not compatible with Sofia Lyme FIA.

Interfering Substances

A study was performed to assess potential interfering substances with the Sofia Lyme FIA. The substances and concentrations tested are described in Table 14, there was no interference or cross-reactivity results when tested with the Sofia Lyme FIA.

Table 14
Sofia Lyme FIA Interfering Substances

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
Penicillin Phenoxymethyl	14.27 µmol/L
Prednisolone	8.31 µmol/L
Tetracyclines	34 µmol/L

Cross-Reactivity

The cross-reactivity of the Sofia Lyme FIA was evaluated with 17 disease state sample types that have the potential to interfere 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 Table 15.

Table 15
Sofia Lyme FIA Cross-Reactivity

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

ASSISTANCE

If you have any questions regarding the use of this product or if you want to report a test system problem, please call Quidel Technical Support at 800.874.1517 (in the U.S.) or 858.552.1100, Monday through Friday, from 7:00 a.m. to 5:00 p.m., Pacific Standard Time. If outside of the U.S., contact your local distributor or technicalsupport@quidel.com. Test system problems may also be reported to the FDA through the MedWatch medical products reporting program (phone: 800.FDA.1088; fax: 800.FDA.0178; http://www.fda.gov/medwatch).

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REF 20298 – Sofia Lyme FIA – 25 Test

IVD

Quidel Corporation
10165 McKellar Court
San Diego, CA 92121
quidel.com

1313400EN00 (10/17)

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult instructions for use	IVD For *In Vitro* diagnostic use
 Contains sufficient for 25 determinations	CONT Contents/Contains
CONTROL + Positive control	CONTROL − Negative control