QUIDEL | Sofia 2 Lyme FIA

FOR USE WITH SOFIA 2
CLIA Complexity: Waived for Finger-stick Whole Blood

For *in vitro* diagnostic use.

℞ ONLY

A Certificate of Waiver is required to perform the test in a CLIA waived setting. To obtain a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA or from your state health department.

Laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1). Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

INTENDED USE

The Sofia 2 Lyme FIA employs immunofluorescence for the rapid differential detection of human IgM and IgG antibodies to *Borrelia burgdorferi* from finger-stick whole blood specimens from patients suspected of *B. burgdorferi* infection of at least 2 weeks' duration. The Sofia 2 Lyme FIA is intended for use as an aid in diagnosis of Lyme disease. A negative result does not preclude infection with *B. burgdorferi*. 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. The assay is to be performed on the Sofia 2 instrument. Professional guidelines should be consulted regarding testing and treatment for Lyme disease when acute *B. burgdorferi* infection is suspected.

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 migrans (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 2 Lyme FIA is an immunofluorescence-based, lateral flow assay for detection of IgM and/or IgG antibodies to *Borrelia burgdorferi* in patient finger-stick whole blood 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 (25 µL finger-stick whole blood) is collected using the Capillary Tube. After draining below the fill line, the Capillary Tube is inserted into the filled Reagent Tube. The Reagent Tube is then inverted and shaken to mix the sample. Two drops of the 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 for an automatically defined development time (WALK AWAY Mode) or pre-incubated on the bench top prior to loading into Sofia 2 (READ NOW Mode). Sofia 2 scans the test strip, analyzes the fluorescent signal, and then displays two (2) test results: IgM (⊕, ⊖, or ⊗) and IgG (⊕, ⊖, or ⊗).

REAGENTS AND MATERIALS SUPPLIED

15-Test Kit:

- Individually Packaged Test Cassettes (15): *Borrelia burgdorferi* antigens and anti-human IgM/IgG
- Blood Collection, Preparation Kits (15)
  - 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)
- QC Card (located on kit box)

MATERIALS REQUIRED BUT NOT SUPPLIED IN KIT

- Sofia 2
- Calibration Cassette (supplied with Sofia 2)
- Timer or watch for use in READ NOW Mode
- Lancets

WARNINGS AND PRECAUTIONS

- 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 Cassettes, Capillary Tubes, Reagent Tubes, solutions, or Controls.

- The user should never open the foil pouch of the Test Cassette or Capillary Tube exposing it to the ambient environment until 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 2 to identify the type of test being run.
- Do not attempt to scan a Test Cassette more than one time. The barcode on the Test Cassette contains a unique identifier that will prevent Sofia 2 from performing a second read on a previously scanned cassette. An error message will be displayed if a Test Cassette is scanned more than once on the same Sofia 2.
- As the detection reagent is a fluorescent compound, no visible results will form. Sofia 2 must be used for result interpretation.
- 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 2 and the Test Cassette: Sofia 2 Calibration Check Procedure, Built-in Procedural Control features, and External Controls.

Sofia 2 Calibration Check Procedure

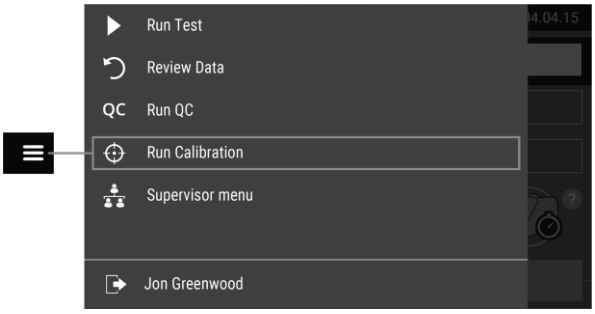
Note: This is a "Calibration Check" procedure.

The Calibration Check Procedure should be performed every 30 days. Sofia 2 can be set to remind the user to complete the Calibration Check Procedure.

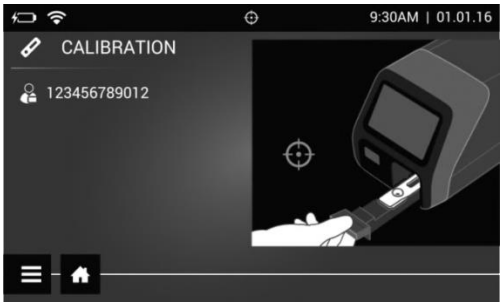
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

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

1. To check the calibration of Sofia 2, select "Run Calibration" from the Main Menu.



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



Sofia 2 indicates when the Calibration Check is completed, ✓ or ✗. Select ⌂ to return to the Run Test screen.

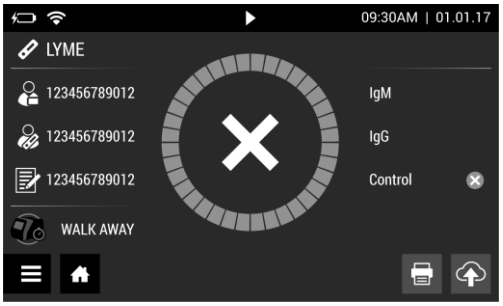
NOTE: If the Calibration Check does not pass, notify the on-site Supervisor or contact Quidel Technical Support for assistance Monday through Friday from 7:00 a.m. to 5:00 p.m. Pacific Time at 800.874.1517 (in the U.S.); 858.552.1100 (outside the U.S.); Fax: 858.455.4960; customerservice@quidel.com (Customer Service); technicalsupport@quidel.com (Technical Support); or contact your local distributor.

Built-in Procedural Controls

The Sofia 2 Lyme FIA contains a built-in procedural control feature. Each time a test is run, the procedural control area is scanned by Sofia 2 and the result is displayed on the Sofia 2 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 2 with each test result.

A ✓ 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 2 after the Test Cassette has developed for 15 minutes. If the test does not flow correctly, Sofia 2 will indicate that the result is ✗.** 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.

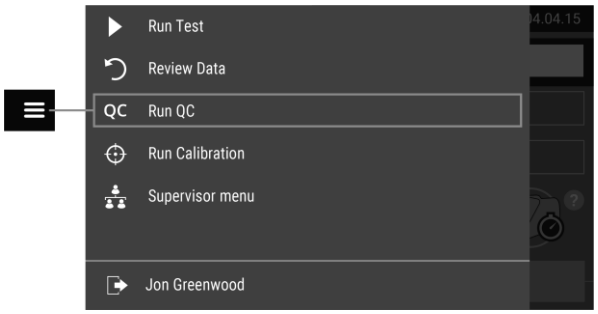
Quidel recommends that Positive and Negative External controls be run:

- 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.

To test External Controls, follow the instructions below.

External Quality Control Test Procedure

1. From the main menu, select Run QC.

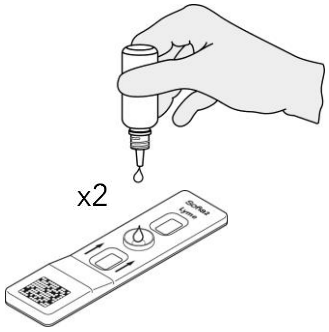


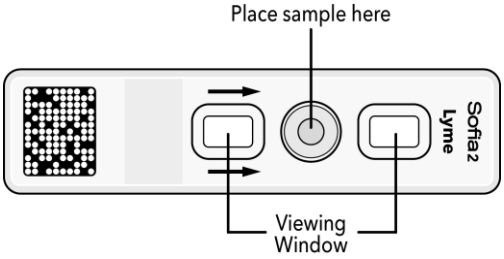
2. Following the prompt on the screen, scan the QC Card (located on the assay kit box).

3. Sofia 2 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 on page 8 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 the round Test Cassette sample well. Then follow the Sofia 2 screen instructions for developing and analyzing the Positive Control Cassette.





b. Prepare a ***Negative Control Cassette*** by adding **2 drops** of the Negative Control solution (blue cap) to the round Test Cassette sample well. Then follow the Sofia 2 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 ✔ or ✖.

Do not perform patient tests or report patient test results if either of the QC test results are ✖. If the control is ✖, retest starting with Step 1 and a new Test Cassette or contact Quidel Technical Support before testing patient specimens.

If both the Positive and Negative Controls fail, repeat testing with new 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 ⏭ on the Sofia 2 display in order to skip the Control test that previously passed. The QC Results will be blank for a skipped control test.

Additional External Controls may be obtained separately by contacting Quidel Customer Support Services at 800.874.1517 (in the U.S.) or 858.552.100 (outside the U.S.).

SAMPLE COLLECTION AND STORAGE

Finger-stick Whole Blood

The pad (or the palmar surface of the distal segment) of the middle finger or ring finger of the non-dominant hand are the preferred sites for collecting a finger-stick whole blood sample. Avoid the side or tip of the finger.[5]

Instruct the patient to rest the arm in a downward position for at least 30 seconds to allow blood to flow to fingertips. A warming pad, or holding the patients hand under warm water, may be needed if patient has cold hands. Cleanse the puncture site with alcohol and let the skin air-dry before proceeding. Puncture the skin with the lancet. Discard the lancet in a sharps disposal container. Wipe away the first drop of blood with dry gauze.

NOTE: DO NOT massage or apply strong repetitive pressure ("milking") to the punctured finger. This may contaminate the sample with tissue-fluid and affect the test result.

Collect the finger-stick whole blood using the Capillary Tube and **immediately** test the sample as described in the Test Procedure.

TEST PROCEDURE

Instructional video available online at **quidel.com/sofia2lyme.**

Precautions

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

All materials and samples should be brought to room temperature if they were stored refrigerated. Perform all testing in the recommended temperature range of 59°F to 86°F (15°C to 30°C) and relative humidity range of 20% to 85% non-condensing.

Expiration Date: 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.*

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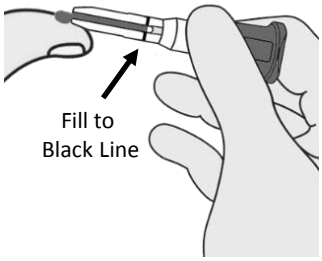
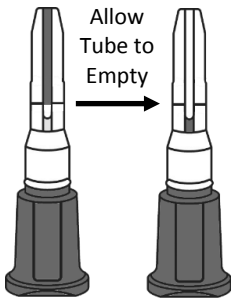
1. Verify that Sofia 2 is set to the desired mode: **WALK AWAY** or **READ NOW**. See the "Using Sofia 2" section for more information.

2. Hold the provided Capillary Tube **horizontally** and touch the tip to the patient blood sample. Blood will automatically draw into the Capillary Tube. **Fill to the black line.**

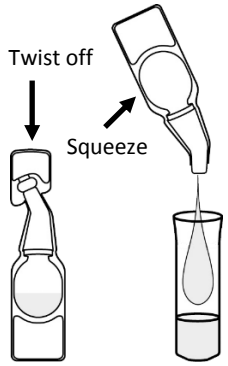


3. Stand Capillary Tube on a flat surface or hold vertically to allow the blood to drain below the fill line.

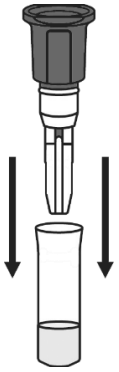


4. Dispense ALL of the Reagent Solution into the Reagent Tube.

   NOTE: Only use the Reagent Solution provided in the kit.

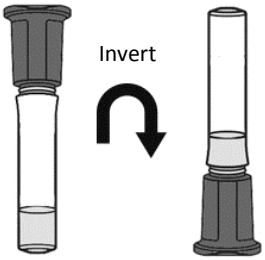


5. Insert Capillary Tube into Reagent Tube. Ensure that it is sealed tightly.



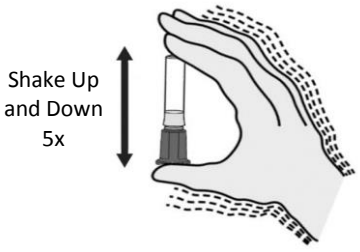
6.

   a) **Immediately invert** the tube.



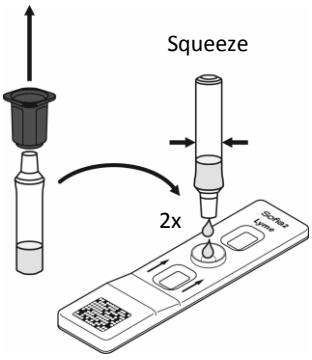
   b) Vigorously shake **up and down** 5 times.

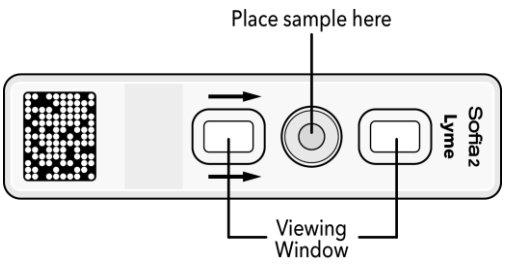
   NOTE: After shaking, immediately proceed to Step 7 and transfer the sample to the Test Cassette. Sample preparation should not be interrupted.



7. Remove the purple cap from the Reagent Tube and carefully dispense **2 drops** into the round Test Cassette sample well.

   NOTE: Solution may remain clear or change color. If either Viewing Window is light-to-dark red due to excess hemolysis, repeat test with a new patient sample.





8. Proceed to the next section, “Using Sofia 2,” to complete the test.

USING SOFIA 2

WALK AWAY/READ NOW Modes

Refer to the Sofia 2 User Manual for operating instructions.

Sofia 2 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 2. Sofia 2 scans the Test Cassette periodically during the test development time. Positive test results will be displayed between 3 and 15 minutes. Negative test results will be displayed at 15 minutes.

READ NOW Mode

Allow the test to develop for the full 15 minutes BEFORE placing it into Sofia 2.

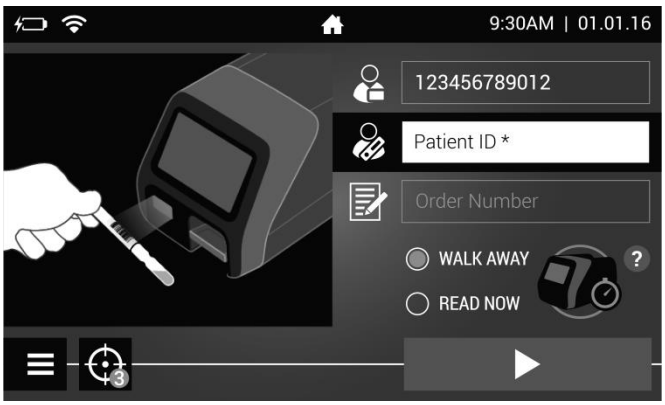
The user **MUST** first place the Test Cassette onto the counter or bench top for **15 minutes** (outside of Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia 2. In READ NOW Mode, Sofia 2 will scan and display the test result within 1 minute.

Run Test

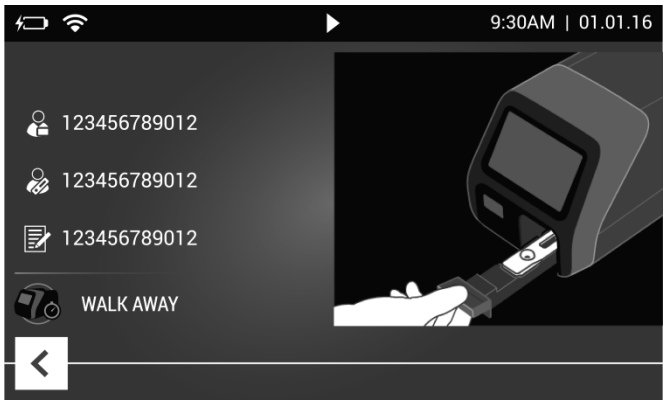
1. If necessary, input the User ID using the barcode scanner or manually enter the data using the touchscreen.

   NOTE: *If you mistakenly scan the incorrect barcode, select the field again to re-highlight it. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.*

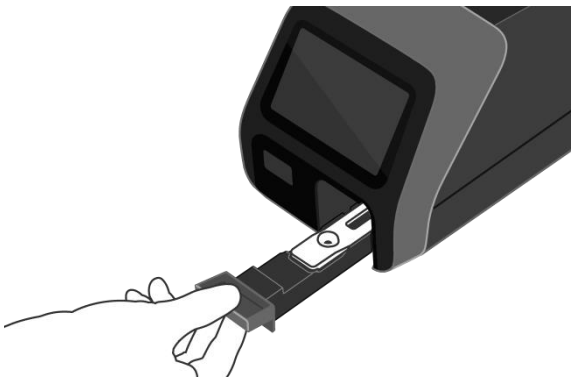
2. If necessary, input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the touchscreen.



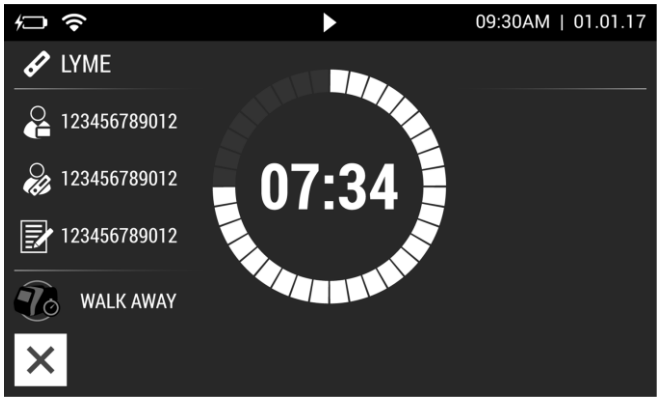
3. Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



4. Insert the prepared patient Test Cassette into the drawer of Sofia 2 and gently close the drawer.



5. Sofia 2 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 within 3-15 minutes. In READ NOW Mode, the test results will be displayed on the screen within 1 minute. See Interpretation of Results section.



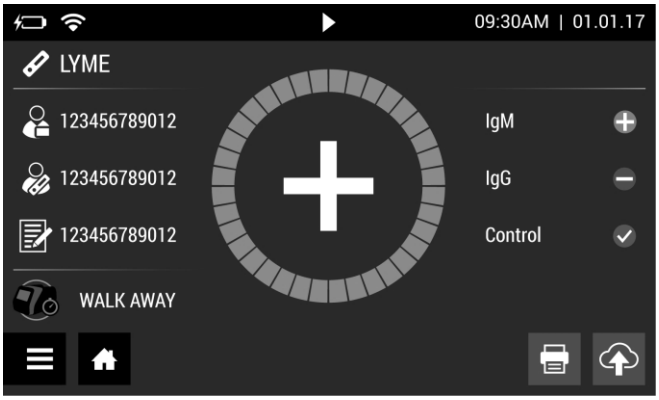
For example: This display shows that the test in WALK AWAY Mode has 7 minutes, 34 seconds remaining.

INTERPRETATION OF RESULTS

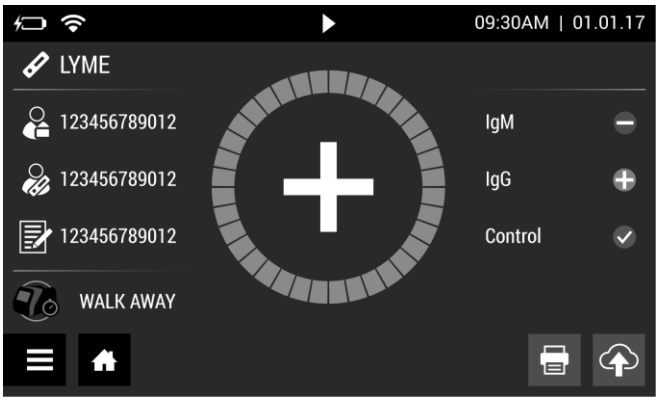
When the test is complete, the results will be displayed on the Sofia 2 screen. Test Lines, which are fluorescent, cannot be seen with the naked eye.

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.

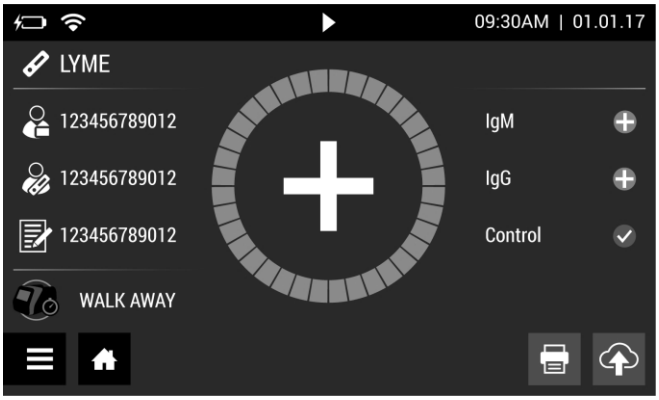
Positive Results:



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.

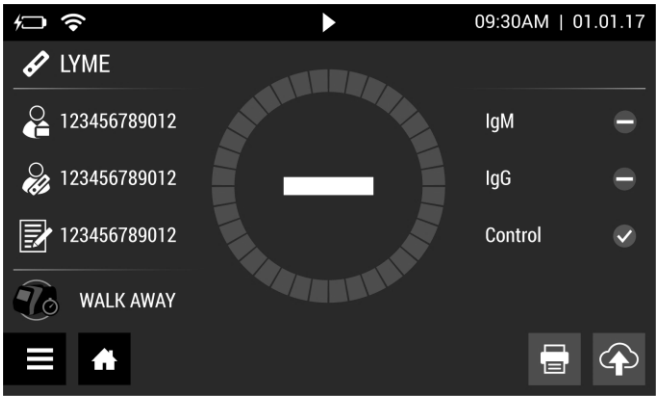


This display shows a valid positive result for IgG antibodies to B. burgdorferi. Interpret to be presumptive of a past exposure to B.b. Test the specimen with IgG Western blot.



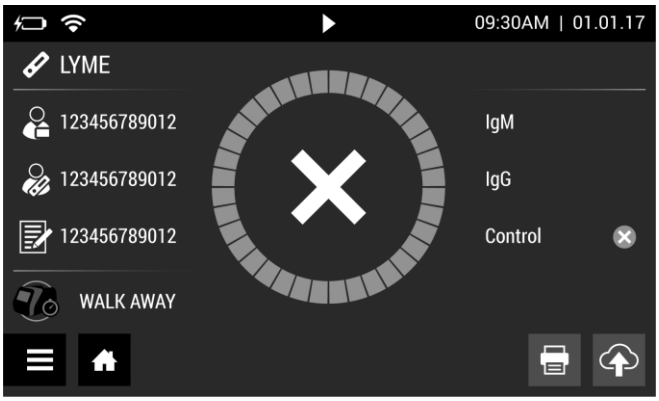
This display shows a valid positive result for IgM and IgG antibodies to B. burgdorferi. Interpret to be presumptive of a recent exposure to B.b. Test the specimen with IgM and IgG Western blot.

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.

Note: Like with all serological Lyme detection devices, false negative results may occur with Sofia 2 Lyme FIA when tick exposure is suspected to have occurred less than two weeks prior to presentation. Testing on a second draw of blood after one to two weeks is recommended.

LIMITATIONS

- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- False results may occur if samples are read before the minimum 15-minute development time in READ NOW Mode.
- Antibody detection methods do not provide definitive results for establishing or ruling out a diagnosis of Lyme disease.
- A negative result in the Sofia 2 Lyme FIA does not rule out the possibility of *B. burgdorferi* infection in a patient.
- Positive results in the Sofia 2 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 and Sofia 2 Lyme FIA are summarized in Table 1.

Table 1
Sofia 2 and Sofia Lyme FIA IgM/IgG Study Results

Study	Samples Tested			Age Range	Positive IgM/ Total Tested	Positive IgG/ Total Tested
	Total	Male	Female			
Prospective	324	165	159	4-84	122/324	86/324
Previously Diagnosed (Arm 2)	201	104	97	6-87	115/201	115/201
Sensitivity	95	58	35	16-74	61/95	76/95
Endemic Negative	100	72	28	18-69	14/100	5/100
Non-Endemic Negative	100	68	32	20-64	7/100	2/100

PERFORMANCE CHARACTERISTICS

Due to the inability to collect a finger-stick whole blood sample for certain studies, the performance characteristics are based on a combination of studies performed with either Sofia Lyme FIA or Sofia 2 Lyme FIA on either Sofia or Sofia 2 which are equivalent.

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 with Sofia. 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 with Sofia 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 with Sofia 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%	26	4	86.7%

Analytical Specificity

To determine the analytical specificity of the Sofia Lyme FIA with Sofia 2, 200 serum/plasma samples from 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 with Sofia 2 and Predicate Analytical Specificity

	n	Sofia IgM	Predicate IgM*		Sofia IgG	Predicate IgG
Endemic	100	86.0%	80.0%		95.0%	98.0%
Non-Endemic	100	93.0%	93.0%		98.0%	99.0%
Total	**200**	**89.5%**	**86.5%**		**96.5%**	**98.5%**

*Equivocal results were considered Positive.

Sensitivity

To assess the sensitivity of the Sofia Lyme FIA with Sofia, 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 Lyme FIA with 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 Lyme FIA with 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%	45.4-100.0%	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%	71.8-100.0%	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 Method Comparison Study and Second Tier Testing

A prospective study with Sofia 2 Lyme FIA with Sofia 2 was performed using matched blood specimens (finger stick and serum/plasma) samples collected from 327 subjects suspected of and exhibiting symptoms of Lyme disease across 11 sites located in Lyme endemic regions throughout the United States. Sofia 2 Lyme FIA finger stick whole blood testing was performed immediately at each of the 11 clinical sites by CLIA-Waived operators. The predicate Lyme IgM and IgG assays and Western blot Lyme IgM and IgG assays were performed at central reference laboratories that were different from the Sofia 2 testing sites. Out of 327 samples tested, three (3) invalid Sofia 2 test results were obtained, therefore 324 evaluable subjects were included in the analysis.

First tier IgM results with positive and negative percent agreement for Sofia 2 Lyme FIA and the predicate assay are shown in Table 7.

Table 7
Lyme IgM Results for Sofia 2 Lyme FIA with Sofia 2 Compared to Predicate Assay
IgM Method Comparison: 1st Tier PPA and NPA Analysis

		Predicate Lyme IgM				
		Positive	**Equivocal**	**Negative**	**% Agreement**	**95% CI**
Sofia 2 IgM	**Positive**	57	18	47	**PPA** = 82.4% (75/91)	73.2%-89.0%
	Negative	5	11	186	**NPA** =79.8% (186/233)	74.2%-84.5%
Total		62	29	233		

Second tier IgM results - Western blot testing was performed on all positive (and equivocal with the predicate assay) samples when tested by either Sofia 2 or the predicate. The percent agreement between Sofia 2 and the predicate Lyme test for IgM results are shown in Table 8.

Table 8
IgM Second Tier Testing
IgM Method Comparison: 2st Tier PPA Analysis

	Tier 1 + or ±	IgM WB +	IgM WB -
Predicate IgM	91	51	40
Sofia 2 IgM	122	52	70
Predicate + Sofia IgM	75	48	27

1st Tier PPA (95% CI)	82.4% (73.2-89.0%)	75/91
2nd Tier PPA (95% CI)	94.1% (83.5-98.6%)	48/51

First tier IgG results with positive and negative percent agreement for Sofia 2 Lyme FIA and the predicate assay are shown in Table 9.

Table 9
Lyme IgG Results for Sofia 2 Lyme FIA with Sofia 2 Compared to Predicate Assay
IgG Method Comparison: 1st Tier PPA and NPA Analysis

		Predicate Lyme IgG			
		Positive	**Negative**	**% Agreement**	**95% CI**
Sofia 2 IgG	**Positive**	48	38	**PPA** = 88.9% (48/54)	77.5%-95.2%
	Negative	6	232	**NPA** = 85.9% (232/270)	81.2%-89.6%
Total		54	270		

Second tier IgG results - Western blot testing was performed on all positive samples when tested by either Sofia 2 or the predicate. The percent agreement between Sofia 2 and the predicate Lyme test for IgG results are shown in Table 10.

Table 10
IgG Second Tier Testing
IgG Method Comparison: 2st Tier PPA Analysis

	Tier 1 +	IgG WB +	IgG WB -
Predicate IgG	54	23	31
Sofia 2 IgG	86	23	63
Predicate + Sofia 2 IgG	48	22	26

1st Tier PPA (95% CI)	88.9% (77.5-95.2%)	48/54
2nd Tier PPA (95% CI)	95.7% (77.3->99.9)	22/23

Precision

The precision of the Sofia Lyme FIA with Sofia 2 was evaluated at one Quidel site utilizing 2 operators and 2 Sofia 2 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 are shown in Tables 11 and 12.

Table 11
Sofia Lyme FIA with Sofia 2 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%	0/48	0/48	0%
High Negative (C_5)	0/48	0/48	0%	0/48	0/48	0%
Low Positive (C_{95})	48/48	48/48	100%	48/48	46/48	100%
Moderate Positive (2-3X)	48/48	48/48	100%	48/48	48/48	100%

Table 12
Sofia Lyme FIA with Sofia 2 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%	0/48	0/48	0%
High Negative (C_5)	0/48	0/48	0%	0/48	0/48	0%
Low Positive (C_{95})	48/48	48/48	100%	48/48	48/48	100%
Moderate Positive (2-3X)	48/48	48/48	100%	48/48	48/48	100%

Reproducibility

The reproducibility of the Sofia Lyme FIA with Sofia 2 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 2 weeks. See Table 13 for results.

Table 13
Sofia Lyme FIA with Sofia 2 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	30/30	30/30
2	30/30	30/30	30/30	30/30	30/30	30/30	30/30	30/30
3	30/30	30/30	27/30	28/30	30/30	30/30	25/30	30/30
Total	**90/90**	**90/90**	**87/90**	**88/90**	**90/90**	**90/90**	**85/90**	**90/90**
% Overall Agreement (95% CI)	**100% (95.1-100%)**	**100% (95.1-100%)**	**96.7% (90.3-99.3%)**	**97.8% (91.8-99.9%)**	**100% (95.1-100%)**	**100% (95.1-100%)**	**94.4% (87.3-97.9%)**	**100% (95.1-100%)**

Matrix Equivalency

The Sofia 2 Lyme FIA is to be used only with a finger-stick whole blood sample.

The finger stick whole blood matrix on the Sofia 2 Lyme FIA was demonstrated to be equivalent to the serum/plasma matrix on the Sofia Lyme FIA. A field study was conducted using matched finger-stick whole blood, serum and plasma. The results are summarized below in Table 14 and show performance is equivalent between the different sample types.

Table 14
Matrix Equivalency

n=321	IgM			IgG		
	Finger-stick Whole Blood	Serum	Plasma	Finger-stick Whole Blood	Serum	Plasma
Positive	136	135	133	105	96	98
Negative	185	186	188	216	225	223
% Positivity	**42.4%**	**42.1%**	**41.4%**	**32.7%**	**29.9%**	**30.5%**

Assay Cutoff

An Assay Cutoff Study was performed to determine and confirm the two separate assay cutoff limits for the Sofia 2 Lyme FIA. One cutoff limit was established for Lyme IgG and one cutoff limit was established for Lyme IgM. The general procedure was to test matched finger stick whole blood, serum and plasma samples with the aim of setting the whole blood assay cutoff so that the clinical performance, percent positive agreement (PPA) and the percent negative agreement (NPA), of the whole blood assay is statistically similar to the FDA cleared Sofia Lyme FIA serum and plasma assay. After the cutoffs were established, the values were validated as part of the clinical trial as well as other analytical studies.

Interfering Substances

A study was performed to assess potential interfering substances with the Sofia Lyme FIA with Sofia. The substances and concentrations tested are described in Table 15, there was no interference or cross-reactivity results when tested with the Sofia Lyme FIA.

Table 15
Sofia Lyme FIA with Sofia 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 with Sofia 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 16.

Table 16
Sofia Lyme FIA with Sofia 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

Note: The results obtained with Leptospirosis (6) and Rickettsia (3) samples may not be conclusive due to low number of samples tested. See also the Limitations section.

CLIA Waiver Studies

Prospective Study

A prospective study with Sofia 2 Lyme FIA was performed using matched blood specimens (finger-stick and serum/plasma) samples collected from 528 subjects across 11 sites located in Lyme endemic regions throughout the United States. The subject population included prospective 324 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 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 the 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.

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 positive percent agreement with the supplementary Western blot are presented below in Table 17.

Table 17
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 the positive percent agreement with the supplementary Western blot are presented below in Table 18.

Table 18
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->99.9%)	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) (76.7-84.6%)**

Near the Cutoff Testing

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 each, where the Sofia 2 Lyme FIA with Sofia 2 was evaluated using coded randomized panels of simulated 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 below in Table 19.

Table 19
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."

Flex Studies

Using risk analysis as a guide, analytical flex studies were conducted using Sofia 2 Lyme FIA with Sofia 2. The studies demonstrated that the test and Sofia 2 are insensitive to stresses of environmental conditions and potential user errors.

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 Time. If outside 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 20319 – Sofia 2 Lyme FIA – 15 Test

IVD

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REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult instructions for use
IVD For *In Vitro* diagnostic use	 Contains sufficient for 15 determinations
CONT Contents/Contains	CONTROL + Positive control
CONTROL − Negative control	