



Sofia[®]
Legionella FIA

FOR USE WITH SOFIA AND SOFIA 2

For *in vitro* diagnostic use.

Rx ONLY



INTENDED USE

The Sofia Legionella FIA employs immunofluorescence for qualitative detection of *Legionella pneumophila* serogroup 1 antigen in human urine specimens. It is designed to test specimens from patients with symptoms of pneumonia. Test results are to be used as an aid in diagnosis of *Legionella pneumophila* serogroup 1 infection. A negative result does not preclude infection with *Legionella pneumophila* serogroup 1. Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures.

The Sofia Legionella FIA may be used with Sofia or Sofia 2.

SUMMARY AND EXPLANATION

Legionella pneumophila (*L. pneumophila*) are gram-negative, obligate aerobic bacteria that are the causative agents of Legionnaires' disease in humans.^{1,2} Legionnaires' disease is a form of severe pneumonia that was named after the outbreak, which occurred in Philadelphia in the summer of 1976, resulting in 221 cases of which 34 were fatal.³ *L. pneumophila* can also bring about an "influenza-like" condition referred to as Pontiac fever.^{1,2} Patients suffering Legionnaires' disease may experience an assortment of symptoms including fever, non-productive cough, headache, diarrhea and delirium.¹


L. pneumophila is one of approximately 50 species of bacteria within the genus *Legionella* and family *Legionellaceae*; furthermore, there are at least 15 distinctive serogroups within the species of *L. pneumophila*.^{1,3,4} Structurally, *Legionella* bacteria are coccobacillary with measurements of 2-20 µm in length and 0.3-0.9 µm in width; the infectious form of the bacteria is motile due to a single flagellum.^{3,5} Water is the natural environment of all *Legionella* bacteria with the exception of *L. longbeachae*, which is frequently isolated from potting soil. Around 20 species of *Legionella* have been reported to be pathogenic in humans, with *L. pneumophila* comprising the largest percentage of human infections at 80%; minor contributors include: *L. longbeachae*, 3.2%; *L. bozeman*, 2.4%; and *L. dumoffii* and *L. feeleii* combined at 2.2%.⁶ There is some variation in the contribution of the different species to global infection; for example, in the United States, 90% of *Legionella* infections arise from *L. pneumophila* serogroup 1 and in Australia, approximately 30% are due to infection with *L. longbeachae*.^{3,7}

PRINCIPLE OF THE TEST

The Sofia Legionella FIA test employs immunofluorescence technology that is used with Sofia or Sofia 2 for the rapid detection of *Legionella pneumophila* serogroup 1 antigen.

The patient's urine sample is added to the Test Cassette. *Legionella pneumophila* serogroup 1 antigens, if present, bind to the detection particles. When the sample migrates up the test strip to the test line, the antigen-conjugate complex is bound to the capture antibody, forming a fluorescent line. If antigens are not

present, the fluorescent microparticles will not be trapped by the capture antibodies nor detected by Sofia or Sofia 2.

An internal control line helps ensure that the test has been executed properly, that the kit reagents are performing appropriately, and that adequate flow has occurred through the test strip during a test run. Another fluorescent line should form at the control position on the test strip each time a specimen or control is tested. If no control line is detected, the test will be reported as invalid by Sofia or  by Sofia 2.

Note: Depending upon the user's choice, the Test Cassette is either inserted into Sofia or Sofia 2 for automatically timed development (WALK AWAY Mode) or placed on the counter or bench top for a manually timed development and then placed into Sofia or Sofia 2 to be scanned (READ NOW Mode). READ NOW Mode allows for batch testing.

Sofia or Sofia 2 will scan the test strip and measure the fluorescent signal by processing the results using method-specific algorithms. Sofia or Sofia 2 will display the test results (Positive, Negative, or Invalid) on the screen.

REAGENTS AND MATERIALS SUPPLIED

25-Test Kit:

- Individually Packaged Test Cassettes (25): Rabbit polyclonal anti-*Legionella*
- Small, Clear 120 µL Fixed Volume Pipettes (25)
- *Legionella* Positive Control (1): Solution contains buffer with non-infectious *Legionella* antigen
- Negative Control (1): Solution contains buffer with non-infectious *Streptococcus C* antigen
- Package Insert (1)
- Quick Reference Instructions (1)
- QC Card (located on kit box)
- Printer Paper (1)

MATERIALS NOT SUPPLIED IN KIT

- Timer or watch for use in Read Now Mode
- Sofia or Sofia 2
- Specimen container
- Calibration Cassette (supplied with the Sofia Installation Pack or Sofia 2)

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.⁸
- Use of Nitrile or Latex (or equivalent) gloves is recommended when handling patient samples.⁸
- Do not reuse the used Test Cassette or Fixed Volume Pipettes.
- 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.
- Rheumatoid-like factors have been associated with the occurrence of false positives with immunoassays for the detection of *Legionella* antigen in urine. Where a false positive is suspected, it is recommended to heat the urine specimen at 95°C to 100°C for 5 minutes followed by a 15-minute centrifugation step (1000 X g), and repeat testing with the processed specimen in the Sofia assay.^{9,10}

- Do not write on the barcode of the Test Cassette. This is used by Sofia or 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 or 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.
- As the detection reagent is a fluorescent compound, no visible results will form on the test strip. Sofia or Sofia 2 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

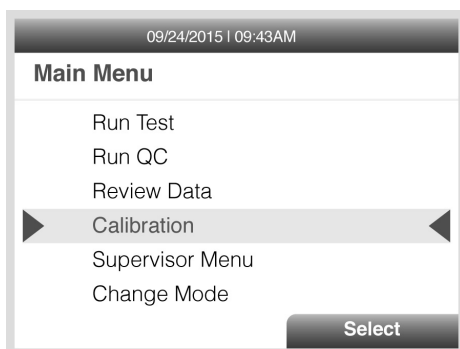
There are three types of Quality Control for Sofia or Sofia 2 and the Test Cassette: Calibration Check Procedure, Built-in Procedural Control features, and External Controls.

Sofia Calibration Check Procedure

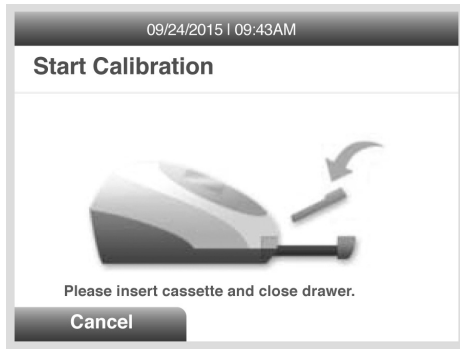
The Calibration Check Procedure is a required function that checks the Sofia optics and calculation systems using a specific Calibration Cassette. A Calibration Cassette is shipped with the Sofia Installation Pack.

Important: Ensure that the Calibration Cassette is stored in the provided storage pouch between uses in order 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 and 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.

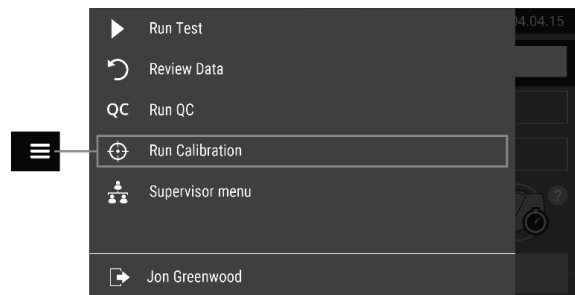
Sofia 2 Calibration Check Procedure

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.

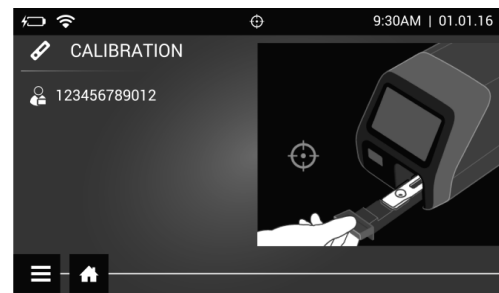
The Calibration Check is a required function that checks Sofia 2 optics and calculation systems using a specific Calibration Cassette. This Calibration Cassette is supplied with Sofia 2. Refer to the Sofia 2 User Manual for details regarding the Calibration Check Procedure.

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. 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

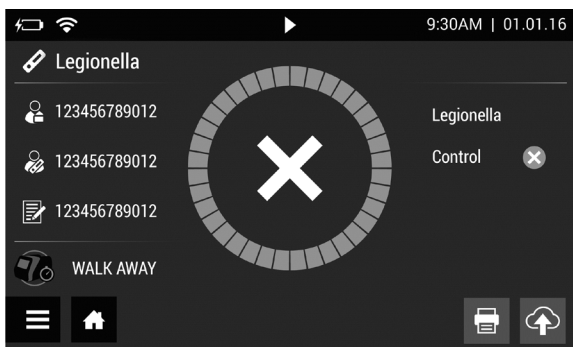
The Sofia Legionella FIA contains a built-in procedural control feature. Each time a test is run, the procedural control area is scanned by Sofia or Sofia 2 and the result is displayed on the Sofia or Sofia 2 screen.

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 or Sofia 2 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 or Sofia 2 after the Test Cassette has developed for 10 minutes. If the test does not flow correctly, Sofia or Sofia 2 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 on Sofia.



For example: This display shows an invalid result on Sofia 2.

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 per this Package Insert (as follows), or the Sofia or Sofia 2 User Manual.

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 kit box).
3. Sofia or Sofia 2 will prompt the user to select the desired mode (WALK AWAY or READ NOW) and then to run the External Controls.
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 **3 drops** of the Positive Control solution to a Test Cassette sample well. Then follow the Sofia or Sofia 2 screen instructions for developing and analyzing the Positive Control Cassette.
 - b. Prepare a **Negative Control Cassette** by adding **3 drops** of the Negative Control solution to a Test Cassette sample well. Then follow the Sofia or 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 “Passed” or “Failed” on Sofia or  or  on Sofia 2.

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 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 “Skip” on the Sofia display or  on the Sofia 2 display to skip the Control test that previously passed. The QC Results will show a skipped Control test as “unknown” on Sofia or  on Sofia 2.

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

SPECIMEN COLLECTION AND STORAGE

Urine specimens should be collected in standard specimen containers. Boric acid may be used as a preservative. If specimens cannot be tested soon after collection, they may be stored at room temperature (15°C to 30°C) and tested within 24 hours of collection. Alternatively, specimens may be refrigerated at 2°C to 8°C and tested anytime up to 14 days. Longer storage at –20°C for up to 20 days is acceptable. Make sure to fully thaw frozen samples before testing.

TEST PROCEDURE

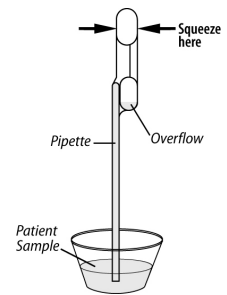
The user should never open the foil pouch of the Test Cassette exposing it to the ambient environment until it is ready for immediate use.

Expiration date: Check expiration 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 or Sofia 2 is set to the desired Mode: **WALK AWAY** or **READ NOW**. See the “Using Sofia and Sofia 2” section for more information.
2. Fill the provided Small, Clear 120 µL Fixed Volume Pipette with the patient urine sample.

To fill the Fixed Volume Pipette with the patient sample:

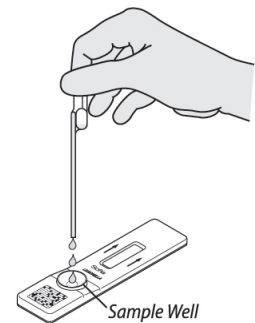
- a. **FIRMLY** squeeze the top bulb.
- b. Still squeezing, place the Pipette tip into the sample.
- c. With the Pipette tip still in the liquid sample, release pressure on bulb to fill the Pipette.



3. Firmly squeeze the top bulb to empty the contents of the Fixed Volume Pipette into the Test Cassette sample well. Extra liquid in the overflow bulb is OK.

NOTE: The Fixed Volume Pipette is designed to collect and dispense the correct amount of liquid sample. Discard the Pipette in your biohazard waste.

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



USING SOFIA AND SOFIA 2

WALK AWAY/READ NOW Modes

Refer to the Sofia or Sofia 2 User Manual for operating instructions.

Sofia and 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 or Sofia 2. The user then returns after 10 minutes to get the test result. In this mode, Sofia or Sofia 2 will automatically time the test development before scanning and displaying the test result.

READ NOW Mode

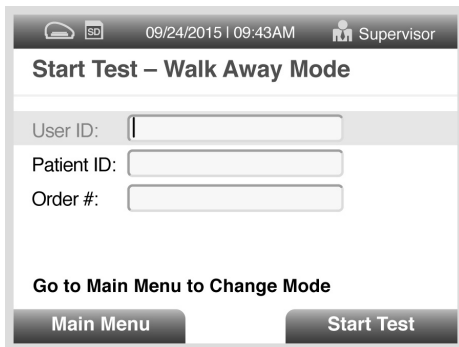
Allow the test to develop for the full 10 minutes BEFORE placing it into Sofia or Sofia 2.

The user must first place the Test Cassette onto the counter or bench top for 10 minutes (outside of Sofia or Sofia 2) and manually time this development step. Then, the user inserts the Test Cassette into Sofia or Sofia 2. In READ NOW Mode, Sofia or Sofia 2 will scan and display the test result within 1 minute. **Note:** Results will remain stable for an additional 10 minutes after the recommended development time of 10 minutes.

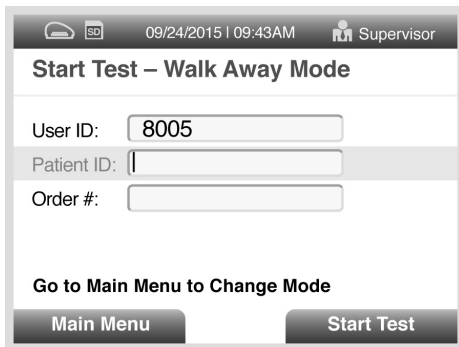
RUN TEST WITH SOFIA

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

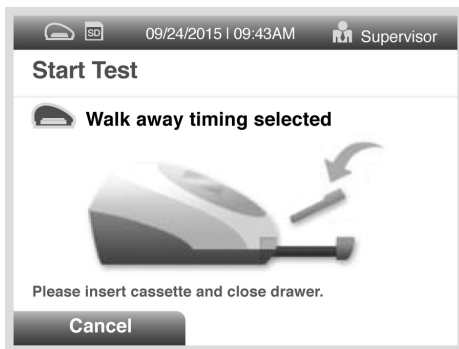
NOTE: If you mistakenly scan the incorrect barcode, use the Arrow Buttons on the Sofia key pad to re-highlight the field. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.



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



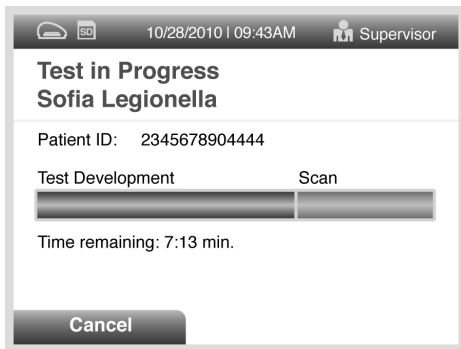
3. Press Start Test and the Sofia drawer will automatically open.



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.



- Sofia will start automatically and display the progress. In WALK AWAY Mode, the test results will be displayed on the screen within 10 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, 13 seconds remaining. Sofia will read and display the results after 10 minutes.

INTERPRETATION OF RESULTS USING SOFIA

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 *Legionella pneumophila* serogroup 1. If the procedural controls are “invalid,” retest the patient’s sample with a new Test Cassette.

Positive Results:



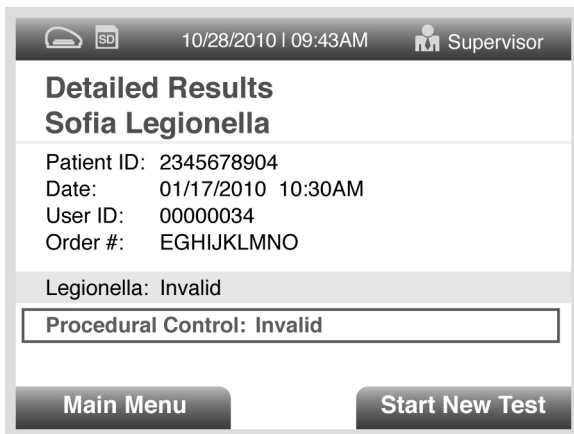
For example: This display shows a valid positive result for *Legionella pneumophila* serogroup 1.

Negative Results:



For example: This display shows a valid **negative result for *Legionella pneumophila* serogroup 1.**

Invalid Results:



For example: This display shows an invalid result.

Invalid Results: If the test is invalid, a new test should be performed with a new patient sample and a new Test Cassette.

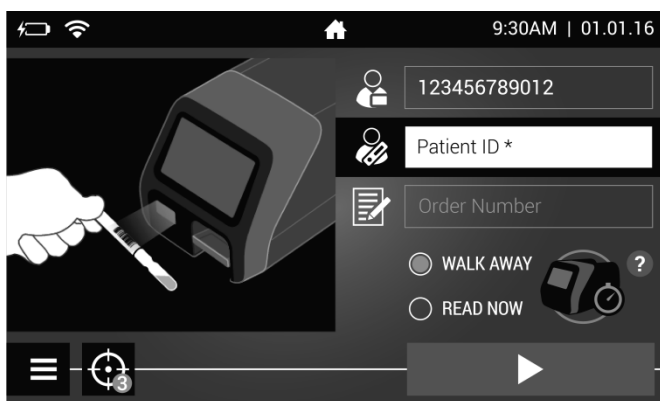
RUN TEST WITH SOFIA 2

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

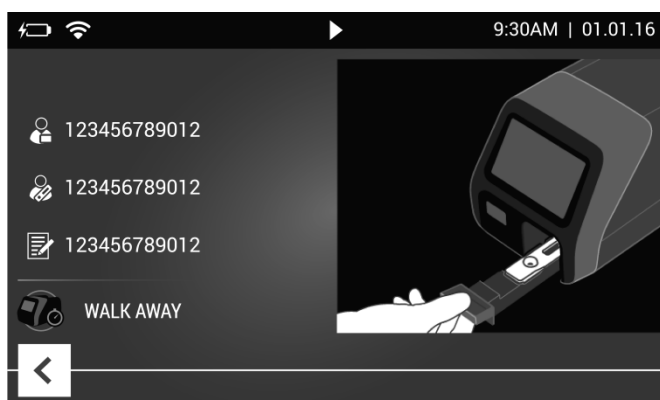
NOTE: If you mistakenly scan the incorrect barcode, select the field again to re-highlight it. Then simply rescan using the correct barcode, and the previous one will be overwritten with the correct barcode.



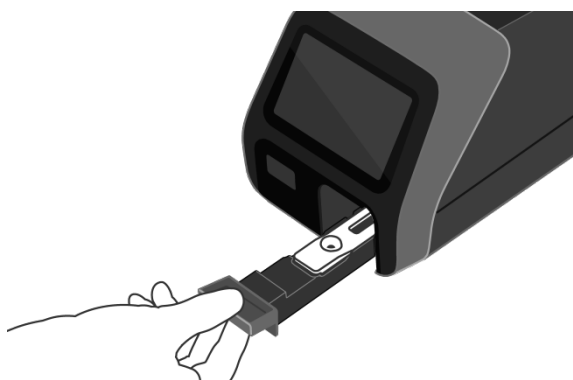
- Input the Patient ID and Order #, if applicable, using the barcode scanner or manually enter the data using the on-screen key pad.



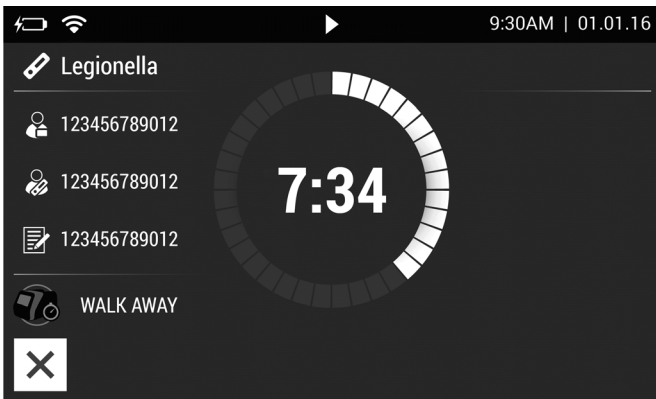
- Verify that the correct development mode, WALK AWAY or READ NOW, has been selected. Press ▶ and open the Sofia 2 drawer.



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








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



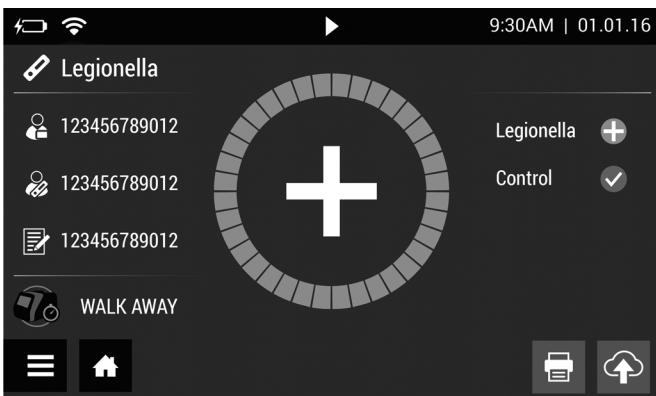
For example: This display shows that the test in WALK AWAY Mode has 7 minutes, 34 seconds remaining. Sofia 2 will read and display the results after 10 minutes.

INTERPRETATION OF RESULTS ON SOFIA 2

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.

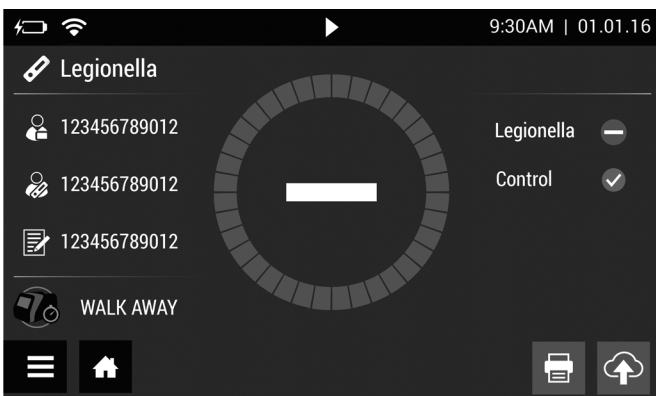
The Sofia 2 screen will display results for the procedural controls as being  or  and will provide a  or  result for the detection of *Legionella pneumophila*. If the procedural controls are , retest the patient's sample with a new Test Cassette.

Positive Results:



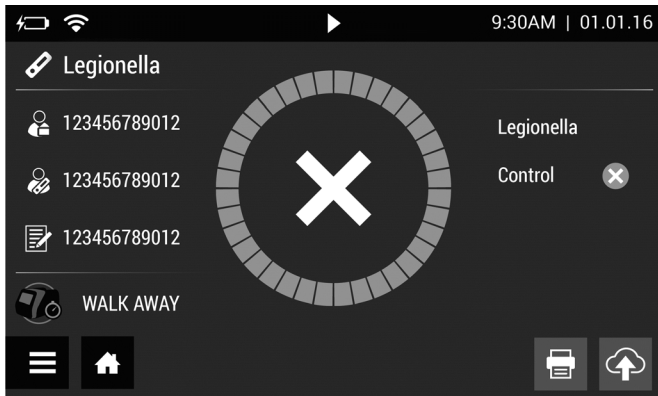
For example: This display shows a valid positive result for *Legionella pneumophila* serogroup 1.

Negative Results:



For example: This display shows a valid negative result for *Legionella pneumophila* serogroup 1.

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

- The contents of this kit are to be used for the qualitative detection of *Legionella pneumophila* serogroup 1 antigen from urine specimens.
- This test detects both viable (live) and non-viable *Legionella pneumophila* serogroup 1. Test performance depends on the amount of antigen in the specimen.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the Test Procedure may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- Positive test results do not identify specific *Legionella pneumophila* serogroups.
- Negative test results are not intended to rule in other non-*Legionella pneumophila* bacterial or viral infections.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low *L. pneumophila* activity when prevalence is moderate to low.

EXPECTED VALUES

The rate of positivity observed in *Legionella* testing will vary depending on the handling of specimens, detection method utilized, time of year, and disease prevalence.

SOFIA LEGIONELLA FIA PERFORMANCE CHARACTERISTICS ON SOFIA

Sofia Legionella FIA Performance vs. Comparator

The performance of the Sofia Legionella FIA was compared to a commercially available rapid *Legionella pneumophila* serogroup 1 antigen test using a blinded panel of 149 clinically acquired positive and negative urine specimens. Each specimen was evaluated using (1) the Sofia Legionella FIA per the Package Insert instructions, (2) the comparator test per the Package Insert instructions and interpreted at the indicated read time of 15 minutes, and (3) the comparator test at a second read time of 60 minutes. The results are presented in Table 1.

Table 1
Sofia Legionella FIA Compared to a Commercially Available Rapid *Legionella pneumophila* Serogroup 1 Antigen Test

		Comparator Test (15-minute read time)	Comparator Test (60-minute read time)
Sofia Legionella FIA (10-minute read time)	Sensitivity	38/38 = 100% (95% CI = 91-100%)	47/47 = 100% (95% CI = 92-100%)
	Specificity	93/112 = 83% (95% CI = 75-89%)	93/102 = 91%* (95% CI = 84-96%)

*Of the 9 results that were positive by Sofia Legionella FIA and negative by the Comparator Test, 2 specimens tested positive by a second commercially available rapid *Legionella pneumophila* serogroup 1 antigen test. With discrepant result resolution, sensitivity = **100% (49/49)** and specificity = **93% (93/100)**.

Reproducibility Studies

The reproducibility of the Sofia Legionella FIA was evaluated at two different sites. Two different operators at each site tested a series of coded, contrived samples, prepared in negative clinical matrix, ranging from negative to moderate positive for *L. pneumophila*. Testing occurred on 5 different days spanning over approximately a 1 week period. The inter-laboratory agreement (Table 2) for negative samples was 100%, and 100% for positive samples. The intra-laboratory agreement (Table 3) for all samples was 100%.

Table 2
Sofia Legionella FIA Reproducibility Study Inter-Laboratory Agreement

Laboratory Site	No Bacteria Negative*	<i>Legionella</i> High Neg* (0.3x LOD)	<i>Legionella</i> Low Pos** (1-3x LOD)	<i>Legionella</i> Mod Pos** (5-10x LOD)
1	30/30	30/30	30/30	30/30
2	30/30	30/30	30/30	30/30
Total	60/60	60/60	60/60	60/60
% Overall Agreement with Expected Result (95% CI)	100% (94-100%)	100% (94-100%)	100% (94-100%)	100% (94-100%)

*Percent agreement for this sample was the number that tested negative divided by the number of known negatives.

**Percent agreement for this sample was the number that tested positive divided by the number of known positives.

Table 3
Sofia Legionella FIA Reproducibility Study Intra-Laboratory Agreement

Lab. Site	No Bacteria Negative*	<i>Legionella</i> High Neg* (0.3x LOD)	<i>Legionella</i> Low Pos** (1-3x LOD)	<i>Legionella</i> Mod Pos** (5-10x LOD)	% Overall Agreement with Expected Result (95% CI)
1	30/30	30/30	30/30	30/30	100% (120/120) (96-100%)
2	30/30	30/30	30/30	30/30	100% (120/120) (96-100%)

*Percent agreement for this sample was the number that tested negative divided by the number of known negatives.

**Percent agreement for this sample was the number that tested positive divided by the number of known positives.

Limit of Detection

The limit of detection (LOD) for the Sofia Legionella FIA was determined using two strains of *Legionella pneumophila* serogroup 1 (Table 4).

Table 4
Limit of Detection with Human Isolates of *Legionella pneumophila*

Strain	Pontiac/Non-Pontiac	Minimum Detectable Level (cfu/mL)*
<i>L. pneumophila</i> Serogroup 1 Knoxville Strain	Pontiac	2.31x10 ³
<i>L. pneumophila</i> Serogroup 1 Camperdown Strain	Non-Pontiac	8.43x10 ⁴

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL (cfu=colony forming unit).

Analytical Reactivity

Analytical reactivity was demonstrated using a total of 5 additional strains of *Legionella pneumophila* Serogroup 1 and also *Legionella pneumophila* serogroups 3, 4, and 6. (Table 5). The Sofia Legionella FIA detected all of the strains examined.

Table 5
Limit of Detection with Human Isolates of *Legionella pneumophila* Serogroups 1, 3, 4, and 6

Strain	Pontiac/Non-Pontiac	Minimum Detectable Level (cfu/mL)*
<i>L. pneumophila</i> Serogroup 1 Allentown Strain (ATCC 43106)	Pontiac	7.43x10 ⁴
<i>L. pneumophila</i> Serogroup 1 France 5811 Strain (ATCC 43112)	Pontiac	1.95x10 ⁴
<i>L. pneumophila</i> Serogroup 1 Heysham Strain (ATCC 43107)	Non-Pontiac	1.68x10 ⁴
<i>L. pneumophila</i> Serogroup 1 Philadelphia Strain (ATCC 33152)	Pontiac	6.93x10 ³
<i>L. pneumophila</i> Serogroup 1 Pontiac Strain (ATCC 13395)	Pontiac	6.94x10 ⁴
<i>L. pneumophila</i> Serogroup 3, (CCUG 30657)	--	9.00 x 10 ⁴
<i>L. pneumophila</i> Serogroup 4, (CCUG 13398T)	--	7.60 x 10 ⁷
<i>L. pneumophila</i> Serogroup 6 (CCUG 13440)	--	7.40 x 10 ⁵

*The levels of bacteria were determined by limiting dilution, bacterial culture, and colony counting to give cfu/mL (cfu=colony forming unit).

Analytical Specificity

Cross Reactivity

The Sofia Legionella FIA was evaluated with a total of 29 bacterial and fungal microorganisms and 6 viral isolates. Bacterial and fungal isolates were evaluated at 1x10⁸ cfu/mL. Viral isolates were evaluated at concentrations ranging from 1.40x10⁶-1.40x10⁷ TCID₅₀/mL. None of the organisms or viruses examined showed any sign of cross reactivity in the assay (Table 6). Flow of the sample and detection of the Control Line by Sofia were also not affected.

Table 6
Analytical Specificity and Cross Reactivity

Organism/Virus	Concentration*	Result
<i>Acaligenes faecalis</i>	1x10 ⁸ cfu/mL	Negative
<i>Bacillus cereus</i>	1x10 ⁸ cfu/mL	Negative
<i>Bacillus subtilis</i>	1x10 ⁸ cfu/mL	Negative
<i>Candida albicans</i>	1x10 ⁸ cfu/mL	Negative
<i>Candida parapsilosis</i>	1x10 ⁸ cfu/mL	Negative
<i>Citrobacter freundii</i>	1x10 ⁸ cfu/mL	Negative
<i>Enterobacter aerogenes</i>	1x10 ⁸ cfu/mL	Negative
<i>Enterobacter cloacae</i>	1x10 ⁸ cfu/mL	Negative
<i>Enterococcus faecalis</i> (Group D <i>Streptococcus</i>)	1x10 ⁸ cfu/mL	Negative
<i>Enterococcus faecium</i>	1x10 ⁸ cfu/mL	Negative

Organism/Virus	Concentration*	Result
<i>Escherichia coli</i>	1x10 ⁸ cfu/mL	Negative
<i>Haemophilus influenza</i>	1x10 ⁸ cfu/mL	Negative
<i>Klebsiella pneumoniae</i>	1x10 ⁸ cfu/mL	Negative
<i>Moraxella osloensis</i>	1x10 ⁸ cfu/mL	Negative
<i>Morganella morganii</i>	1x10 ⁸ cfu/mL	Negative
<i>Nocardia asteroides</i>	1x10 ⁸ cfu/mL	Negative
<i>Proteus mirabilis</i>	1x10 ⁸ cfu/mL	Negative
<i>Proteus vulgaris</i>	1x10 ⁸ cfu/mL	Negative
<i>Pseudomonas aeruginosa</i>	1x10 ⁸ cfu/mL	Negative
<i>Serratia liquefaciens</i>	1x10 ⁸ cfu/mL	Negative
<i>Serratia marcescens</i>	1x10 ⁸ cfu/mL	Negative
<i>Staphylococcus aureus</i>	1x10 ⁸ cfu/mL	Negative
<i>Staphylococcus epidermidis</i>	1x10 ⁸ cfu/mL	Negative
<i>Staphylococcus saprophyticus</i>	1x10 ⁸ cfu/mL	Negative
<i>Streptococcus pyogenes</i> (Group A)	1x10 ⁸ cfu/mL	Negative
<i>Streptococcus agalactiae</i> (Group B)	1x10 ⁸ cfu/mL	Negative
<i>Streptococcus anginosus</i> (Group F)	1x10 ⁸ cfu/mL	Negative
<i>Streptococcus dysgalactiae</i> (Group G)	1x10 ⁸ cfu/mL	Negative
<i>Streptococcus pneumoniae</i>	1x10 ⁸ cfu/mL	Negative
Adenovirus	1.40x10 ⁷ TCID ₅₀ /mL	Negative
Coxsackievirus	1.40x10 ⁷ TCID ₅₀ /mL	Negative
Influenza A	1.40x10 ⁷ TCID ₅₀ /mL	Negative
Influenza B	1.40x10 ⁷ TCID ₅₀ /mL	Negative
Parainfluenza virus	1.40x10 ⁶ TCID ₅₀ /mL	Negative
Respiratory Syncytial Virus	1.40x10 ⁷ TCID ₅₀ /mL	Negative

*The levels of bacteria/fungi were determined by limiting dilution, culture, and colony counting to give cfu/mL (cfu=colony forming unit). Virus concentrations were determined by viral cell culture and DFA to give pfu/mL (pfu=plaque forming unit). Pfu/mL was translated into TCID₅₀/mL per ATCC conversions (TCID₅₀/mL=50% tissue culture infectious dose).

Interfering Substances

The following substances commonly found in urine were evaluated and did not interfere with the Sofia Legionella FIA at the levels tested (Table 7).

Table 7
Non-interfering Substances

Substance	Concentration
Amphotericin B	0.055 mg/mL
Ascorbic acid	1.0 mg/mL
Beet root	0.01% v/v
Bilirubin	0.2 mg/mL
Biotin	< 714 ng/mL

Substance	Concentration
Caffeine, purified	0.4% v/v
Chlorophyll	0.81 mg/mL
Ciprofloxacin	0.22 mg/mL
Erythromycin	0.067 mg/mL
Glucose	20 mg/mL
Itraconazole	0.22 mg/mL
Miconazole	5% v/v
Oxalic acid	0.01% v/v
Prednisone	0.22 mg/mL
Protein (BSA)	5 mg/mL
Rifampicin	0.09 mg/mL
Urea	20 mg/mL
Vaginal contraceptive gel with nonoxynol-9, 4%	0.05% v/v
Water-10 based personal lubricant (KY Jelly)	2.5% v/v
Erythrocytes	10 ⁶ /mL
Leukocytes	1.25x10 ⁵ /mL
Tobacco	0.40% v/v
Whole Blood	10% v/v
Rheumatoid Factor*	0.012 units/mL

*Rheumatoid Factor interfered at concentrations >0.025 units/mL. Refer to the Warnings and Precautions Section of this Package Insert.

SOFIA LEGIONELLA FIA PERFORMANCE CHARACTERISTICS ON SOFIA 2

Analytical Method Comparison of Sofia Legionella FIA with Sofia and Sofia 2 Comparative Performance

The performance of Sofia Legionella FIA when used with Sofia vs. Sofia 2 was compared using a panel of urine samples at one site. Negative urine samples were pooled and spiked with different concentrations of *Legionella* positive control stock solution. Panel members spanned a broad range of negative and positive samples distributed across the dynamic range of the assay.

The Sofia vs. Sofia 2 comparison results are shown below in Table 8. Positive agreement was 96.0% and negative agreement was 100%.

Table 8
Sofia Legionella FIA – Sofia vs. Sofia 2 Comparison

		Sofia		
		Pos	Neg	
Sofia 2	Pos	121	0	Positive Agreement = 96.0% (121/126) (95% CI = 91 - 98%)
	Neg	5	34	Negative Agreement = 100% (34/34) (95% CI = 90 - 100%)
	Total	126	34	Overall Agreement = 91.1% (155/170) (95% CI = 86% - 95%)

Sofia Legionella FIA performance vs. two Comparators

The performance of the Sofia Legionella FIA was compared to two commercially available rapid *Legionella* antigen tests using a panel of 102 frozen archival clinical specimens of 49 known positives and 53 known negatives. Each specimen was evaluated using the Sofia Legionella FIA per the Package Insert instructions and the Comparator Tests' Package Insert instructions. The results are presented in Tables 9 and 10.

Table 9
Sofia Legionella FIA Performance Compared to a Commercially Available Qualitative Test

		Comparator Test 1		
		Pos	Neg	
Sofia Legionella	Pos	49	2*	Positive Agreement = 100% (49/49) (95% CI = 93% - 100%)
	Neg	0	51	Negative Agreement = 96.2% (51/53) (95% CI = 87%-99%)
	Total	49	53	Overall Agreement = 98.0% (100/102) (95% CI = 93%-99%)

*There were 2 discordant Sofia 2 positive/Sofia negative results which were near the cutoff specimens.

Table 10
Sofia Legionella FIA Performance Compared to a Commercially Available Qualitative Test

		Comparator Test 2		
		Pos	Neg	
Sofia Legionella	Pos	50	1*	Positive Agreement = 100% (50/50) (95% CI = 92% - 100%)
	Neg	0	51	Negative Agreement = 98.1% (51/52) (95% CI = 90% - 100%)
	Total	50	52	Overall Agreement = 99.0% (101/102) (95% CI = 95%-100%)

*There was 1 discordant Sofia 2 positive/Sofia negative results which was a near the cutoff specimen.

Reproducibility Studies

The reproducibility of the Sofia Legionella FIA was evaluated at two different sites. Two operators at each site tested a series of coded, contrived samples, prepared in negative urine matrix, including negative, low positive, and moderate positive specimens. Testing occurred on 5 different days spanning over approximately 1 week. The inter-laboratory agreement (Table 11) for negative samples was 100%, and 100% for positive samples. The intra-laboratory agreement for all samples was 100%.

Table 11
Sofia Legionella FIA Reproducibility Study Inter-Laboratory Agreement

Laboratory Site	Negative*	1x LoD**	3x LoD**
1	15/15	15/15	15/15
2	30/30	30/30	30/30
Total	45/45	45/45	45/45
% Overall Agreement with Expected Result (95% CI)	100% (92 – 100)	100% (92 – 100)	100% (92 – 100)

*Percent agreement for this sample was the number that tested negative divided by the number of known negatives.

**Percent agreement for this sample was the number that tested positive divided by the number of known positives.

Table 12
Sofia Legionella FIA Reproducibility Study Intra-Laboratory Agreement

Laboratory Site	Negative*	1x LoD**	3x LoD**	% Overall Agreement with Expected Result (95% CI)
1	15/15	15/15	15/15	100% (45/45) (92 – 100%)
2	30/30	30/30	30/30	100% (90/90) (96 – 100%)

*Percent agreement for this sample was the number that tested negative divided by the number of known negatives.

**Percent agreement for this sample was the number that tested positive divided by the number of known positives.

ASSISTANCE

If you have any questions regarding the use of this product, please call Quidel’s Technical Support Number 800.874.1517 (n the U.S.) or 858.552.1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time, U.S.A. If outside the United States contact your local distributor or technicalsupport@quidel.com.

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1226110EN00 (05/21)

REF

Catalogue number



CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Intended use

R_x ONLY

Prescription use only



Consult instructions for use

IVD

For *In Vitro* diagnostic use



Contains sufficient for 25 determinations

CONT

Contents/Contains

CONTROL +

Positive control

CONTROL -

Negative control
