FIRST TO Know®

Syphilis Test Package Insert

For In Vitro Diagnostic Use | For Over the Counter (OTC) Use

INTENDED USE

The First To Know® Syphilis Test is a qualitative rapid membrane immunochromatographic assay for the detection of *Treponema pallidum* (syphilis) antibodies in human whole blood (capillary). This test is intended for over the counter (OTC) consumer use in individuals suspected of syphilis. Positive test results with the First To Know® Syphilis Test alone are not sufficient to diagnose syphilis infection and must be followed by additional laboratory testing through a health care provider to confirm a diagnosis of syphilis.

This test is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop or change any treatments without a healthcare provider.

Results of testing with the First To Know® Syphilis Test will likely be positive for individuals previously diagnosed with syphilis, even if they were successfully treated. The First To Know® Syphilis Test cannot determine whether there has been re-infection with syphilis.

SUMMARY AND EXPLANATION

The First To Know® Syphilis Test is a single-use, lateral-flow, visually-read test intended for over-the-counter (OTC) use. All components of the test are contained in the cassette; no additional reagents are required to run the test. Procedure controls are intrinsic to the cassette. A single [~40 µL] drop of human capillary whole blood is added to the cassette. No software or instrumentation is required for use of the test. There are no accessories required to use the test. The test is a rapid chromatographic immunoassay that delivers results in fifteen minutes.

'Test' and 'Control' Lines on each cassette are visually read for this qualitative test. The control band and the test band may differ in color intensity. The intensity of the test band will vary depending on the concentration of syphilis-specific antibody present in the specimen. However, a quantitative value for syphilis cannot be determined by this qualitative test. The color intensity of the bands will increase slowly with time due to sample evaporation; the test result can be read as early as 15 minutes and must be read within 30 minutes to be valid.

PRINCIPLE OF THE PROCEDURE

The First To Know® Syphilis Test is a qualitative rapid membrane immunochromatographic assay for the detection of *Treponema pallidum* (syphilis) antibodies in human whole blood (capillary). To run the test, a fingerstick (capillary) blood sample is collected into the fill zone of the test cassette. The sample flows into a dry porous test strip composed of a plasma-separating membrane and a series of analytical membranes. The sample first passes through the plasma-separating membrane, which binds the erythrocytes in whole blood sample to prevent them from interfering with the test. The membrane also contains two separate colloidal gold conjugate materials: syphilis recombinant protein conjugated with colloidal gold and Rabbit IgG conjugated with colloidal gold which are solubilized in the plasma as it continues to move into the device. If positive, the syphilis specific antibody in the test sample binds to the recombinant syphilis antigen absorbed to gold in the upstream region of the test strip and the complex is captured by immobilized syphilis antigen striped at the test band location, as it flows downstream. The appearance of a visible test band indicates the sample contains a detectable level of syphilis antibody. Rabbit IgG conjugated with colloidal gold will flow past the test band region and bind to the polyclonal anti-rabbit antibody in the control band location of the analytical membranes, resulting in the appearance of a procedural control line.

WARNINGS & PRECAUTIONS

- Syphilis must be treated by a doctor to be cured. Treatment for syphilis infection requires a prescription for antibiotics.
- Please check the expiration date before you use the kit. Do not use this kit after its expiration date to avoid the risk of incorrect result.
- To prevent contamination, leave the test device in pouch until you are ready to use it.
- If you have a condition that makes it difficult to use the test (e.g., problems with vision,handling the test components, or understanding test instructions or results), please contact a healthcare provider for testing.
- Do not use this test if it has been stored outside the acceptable temperature of 59°F-86°F (15°C-30°C). Do not freeze.
- Use only the components included in this single use kit for performing the test. Do not use the kit if any of the components are missing or damaged.
- Dispose of the used and leftover kit contents in household trash.
- This kit contains small parts as well as single-use lancets used to pierce the skin. Keep kit components out of reach of children.
- Please discuss any symptoms that you are experiencing with a healthcare provider.
- Do not read the result after 30 minutes. An incorrect result may be obtained from reading a result before 15 minutes or after 30 minutes.

LIMITATIONS

- Results of testing with the First To Know® Syphilis Test will likely be positive for individuals previously diagnosed with syphilis, even if they were successfully treated. The First To Know® Syphilis Test cannot determine whether there has been re-infection with syphilis, and testing must be performed by a healthcare provider to detect syphilis re-infection.
- A positive test result does not preclude the possibility of co-infection with additional pathogens.
- A negative test result does not preclude the possibility of infection with syphilis or other bacteria or viruses.
- The First To Know® Syphilis Test is not intended to replace routine healthcare during pregnancy regardless of test results.Testing for syphilis should be performed by a healthcare provider for women who are known to be pregnant or if pregnancy is suspected.
- The test is not a substitute for visits to a healthcare provider. The result obtained with this test should not be used to start, stop, or change any course of treatment unless advised by a healthcare provider.
- The First To Know® Syphilis Test is for fingerstick capillary blood only.
- Anti-Epstein Barr Virus (EBV) and Rheumatoid Factor positive (RF+) showed cross reactivity with 1 out of 10 samples.
- Gamma-globulin may cause invalid results at concentrations at 60 mg/mL or greater.
- Accurate results are dependent on adequate product storage and adherence to the specimen collection and testing procedures. Failure to follow test procedures can lead to incorrect results.
- Anyone with recent sexual contact with a person known to have a sexually transmitted infection, should visit a healthcare provider for treatment and evaluation as soon as possible (https://www.cdc.gov/std/treatment-guidelines/syphilis.htm).

COMMON QUESTIONS & ANSWERS

General Syphilis Questions & Answers

1. **What's syphilis?** Syphilis is a type of infection that spreads through sex. It starts with a small sore around private parts or in the mouth. Even if you don't see any sores, you can still spread it to others. There are other infections like chlamydia and gonorrhea that can look like syphilis, but they are caused by different bacteria and may require other testing and/or treatment.
2. **What are the symptoms of syphilis?** Infected people may experience painless ulcers, sores, vaginal discharge or wart-like growths on genitals, rectum or mouth. People may also experience rashes, small bumps or ulcers on their skin, palms or soles. Additional common symptoms include fatigue, itching, sore throat, swollen lymph nodes, weight loss, or rectal lining inflammation.
3. **What puts me at risk for syphilis?** Risk of syphilis results from unprotected sexual contact with one or multiple partners who is/are currently infected with syphilis. It is possible for a person to be infected with syphilis without having symptoms (i.e.; "asymptomatic") and still transmit the infection to another.
4. **How can I protect myself from being infected with syphilis?** If you are sexually active, doing the following can lower your chances of getting syphilis: (1) being in a long-term, mutually monogamous relationship with a partner who has been tested and has negative STD test results, or (2) using latex condoms the right way every time you have sex.
5. **When should I test for syphilis?** If you're sexually active and have symptoms or have had unprotected sex, you should get tested.

First To Know® Test Questions & Answers

1. **How soon after a risk event can I test myself?** Most people develop antibodies between 10 days and 3 weeks after symptoms appear.
2. **What does the test do?** The test looks for specific antibodies in your blood. Those antibodies are created by your immune system in response to the bacteria that cause syphilis. People infected with syphilis and people who have recovered from past syphilis infections have those antibodies.
3. **Who should use this test?** Anyone who thinks they might have syphilis due to symptoms or having unprotected sex with someone who has syphilis.
4. **How many drops of blood will it take to fill the test?** Most tests fill with 1-3 drops of blood.
5. **The drop of blood is still not enough to refill the test. What should I do?** If you still do not get enough blood to fill the Fill Zone, you can use the extra lancet that is provided to prick a second finger. A different finger is recommended.
6. **Why do I need to tap the test twice?** Tapping the test twice ensures the blood flows into the test and removes any air bubbles.

First To Know® Results Questions & Answers

1. **How quickly will I get my result?** Test results are available in 15 minutes and up to 30 minutes after taking the test.
2. **What does a positive result mean?** It means the test detected antibodies to the syphilis bacteria in the blood sample. You might have syphilis now or had it before.
3. **What should I do if I get a positive result?** See your doctor to confirm the result. If you can't see a doctor, contact your local health department (https://www.naccho.org/membership/lhd-directory). Treatment for syphilis infection is available but requires a consultation with a healthcare provider. Avoid sexual contact with others until after you have consulted with a doctor. You should notify anyone you have had sex with in the last 60 days (2 months) before receiving a positive test result or before your symptoms started. If you haven't had sex in the last 60 days, you should notify your most recent partner.
4. **What does a negative result mean?** It means the test did not detect antibodies to syphilis bacteria in the blood sample. There are a number of reasons your test may be negative:
    1. You are not infected with syphilis;
    2. You have been recently exposed/infected and tested too early to detect the antibodies in your blood;
    3. You have a different sexually transmitted infection that is not syphilis.
5. **What should I do if I get a negative result?** See your healthcare provider if you still have symptoms. It is possible you tested too early or have a different sexually transmitted infection.
6. **My test result is negative but I think I have been exposed. What should I do?** See your healthcare provider about your concern. Only a doctor can diagnose and treat syphilis or a sexually transmitted infection.
7. **When should I retest?** If you still think you have syphilis after a negative result, you can test again 10 days after symptoms show or 90 days after you were sexually active with someone that you believe had syphilis.
8. **What should I do if the result is invalid?** If you get an invalid result, please call our toll-free number at 1-844-207-3370.
9. **How accurate is this test?** A multi-site clinical study was conducted from September 1, 2021, to October 17, 2023, at six geographically diverse sites across the US. In this study, the First To Know® Syphilis Test was used by 1270 people, with results compared to a combination of three FDA-cleared tests. The First To Know® Syphilis Test correctly identified 93.4% of positive specimens (99 out of 106) and 99.5% of negative specimens (1158 out of 1164). Visit www.firsttoknow.com for more information.
10. **Other questions?** Contact us toll-free at 1-844-207-3370 or go to www.firsttoknow.com for more information.

FURTHER GUIDANCE

There is a possibility that you might have other infections such as chlamydia, gonorrhea, or HIV. These can be serious and need treatment, even if you feel okay. See your healthcare provider for further testing.

STORAGE AND STABILITY

- The kit can be stored at temperatures between 59-86°F (15-30°C).
- The test cassette must remain in the sealed pouch until use.
- DO NOT FREEZE.
- Do not use after the expiration date.

MATERIALS SUPPLIED

Each box contains the following materials needed to conduct the test:

- One (1) sealed aluminum pouch containing one (1) First To Know® Syphilis Test
- One (1) Instructions For Use
- One (1) Educational Guide
- Two (2) disposable, single-use sterile lancets
- One (1) alcohol swab
- One (1) bandage

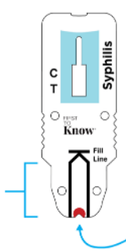
MATERIALS NOT SUPPLIED BUT NEEDED

- Watch or timer

DIRECTIONS FOR USE

PREPARATION

1. Before you start, lay out everything you need for the test.
2. Read the enclosed **Syphilis – Be The First To Know** Educational Guide before conducting the test.
3. Go to **firsttoknow.com** to watch a step-by-step video.
4. Make sure you have a watch or timer ready.
5. If you wear contacts or glasses, make sure that you are wearing them when you read the result.
6. Ensure you are hydrated by drinking a full glass of water prior to taking the test.
7. It is essential to get your blood flowing before you collect. Massage your hands under warm water.
8. Get your heart rate up. Run in place, do some jumping jacks, or shake your hands.
9. Remove test from pouch. Do not open test pouch until you are ready to begin your test.
10. Get familiar with the test. Locate the Sample Application Zone.



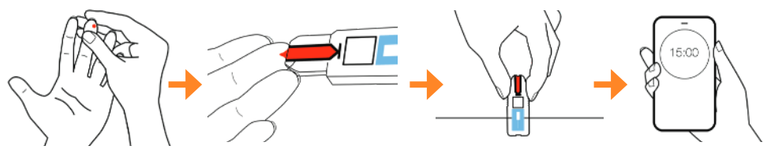
This is the Fill Zone. It is important to fill this part of the test completely. It may take several drops of blood.

This is the Sample Application Zone. This is where you will load the blood sample into the test.

DIRECTIONS

Please refer to illustrations below.

1. **Remove cap from lancet.** Twist the gray cap of lancet 5-6 times, then pull it outward to remove. DO NOT PUSH THE RELEASE TAB YET.
2. **Sanitize finger.** Wipe the end of the selected finger with the alcohol swab provided.
3. **Prick fingertip.** Firmly press the lancet to the finger in an off-center position, then press the release tab. Try not to use the center or top of the finger, since these are the most sensitive areas.
4. **Fill test.** Massage the finger beginning at the base of the palm until a large, full drop of blood appears. Touch one or more drops of blood to the Sample Application Zone until the Fill Zone is full. If you can, stand up and hang your hand below your arm for better blood flow.
5. **Apply bandage.** Quickly put on the adhesive bandage.
6. **Tap test twice.** Hold the Sample Application Zone facing up and tap the opposite end of the test twice on a hard surface. Then, lay the test on the flat surface.
7. **Set a timer.** Set a timer for 15 minutes.
8. **Read test result.** Read the result after 15 minutes and before 30 minutes.
9. **Throw away test.** All used test components should be disposed of in your household waste.

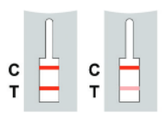


INTERPRETATION OF RESULTS

In order to visually read the result, the user views the test strip and distinguishes between the Test Line (labeled with a "T") and the Control Line (labeled with a "C") in order to interpret a positive, negative or invalid result. The user is guided on how to read the result with a detailed graphic on how to read and interpret the result.

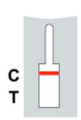
Read test result. Read the result after 15 minutes and before 30 minutes. If you see two lines, the test result is positive. One line may be lighter; they do not have to match.

Understanding a Positive Result



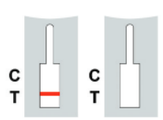
It means the test detected antibodies to the syphilis bacteria in the blood sample. You might have syphilis now or had it before. See your healthcare provider. For additional information, see Common Questions & Answers.

Understanding a Negative Result



It means the test did not detect antibodies to the syphilis bacteria in the blood sample. See your healthcare provider if you still have symptoms. It is possible you tested too early. For additional information, see Common Questions & Answers.

Understanding an Invalid Result



If no control "C" line appears, the test is invalid. You must retest using a new test. If you get an invalid result, please call our toll-free number at 1-844-207-3370.

EXPECTED VALUES

The observed positivity rate of the First To Know® Syphilis Test during the clinical study was 8.3%.

PERFORMANCE CHARACTERISTICS

CLINICAL TESTING

A multi-site study, conducted from September 1, 2021 to October 17, 2023 at six (6) geographically diverse clinical sites, enrolled 1,424 subjects from low prevalence clinics. Lay users performed the First To Know® Syphilis Test using self-drawn fingerstick blood and were assessed on their ability to execute the test and interpret results in a simulated home environment. A usability questionnaire was given to all participants to evaluate the ease of use.

Fingerstick blood was collected per the First To Know® Syphilis Test instructions. Venous whole blood samples were also collected and tested by a reference lab using three FDA-cleared comparator tests (two treponemal, one non-treponemal) where the "comparator positive" was established following the 2 out of 3 rule (i.e., if 2/3 are positive, then the comparator diagnosis is positive). Results of First To Know® Syphilis Test were compared to these reference tests to establish Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA).

Of the 1,424 prospectively enrolled subjects, 1,270 were included in the final PPA/NPA calculations. In the final set, 1,158 and 99 were negative or positive respectively by both the First To Know® Syphilis Test and comparator method; 6 were positive by the First To Know® Syphilis Test and negative by the comparator method, and 7 were negative by First To Know® Syphilis Test and positive by the comparator method.

The First To Know® Syphilis Test demonstrated an overall PPA of 93.4% (lower bound of the 95% confidence interval 87.0%) and a NPA of 99.5%.

Summary of First To Know® Syphilis Test Clinical Study Results

First To Know® Result		Comparator Result	
		Positive	Negative
	Positive	99	6
	Negative	7	1158
		106	1164

PPA = 99/106 = 93.4% (95% confidence: 87.0% - 96.8%)
NPA = 1158/1164 = 99.5% (95% confidence: 98.9% - 99.8%)

USABILITY STUDY

Test usability was assessed in two studies. For the first, all 1345 participants enrolled in clinical study were observed while performing testing and difficulties were noted. A second study enrolled 20 participants to assess lay users' execution of the First To Know® Syphilis Test workflow using the written instructions alone. Following both studies, a questionnaire was issued to participants to assess ease of use. The results of both studies demonstrated that the First To Know® Syphilis Test is easy to use by lay users.

User label comprehension was assessed using two sets of questionnaires. One questionnaire was issued to all 1345 participants following the clinical study. A second questionnaire was issued to all 20 participants following the second usability study plus an additional 20 participants (total of 40 participants). Both questionnaires were aimed at evaluating users' understanding of key communication messages (e.g., FAQ messages and warnings) found in the labeling. The results of user label comprehension demonstrated that the First To Know® Syphilis Test labeling is easy to understand by lay users.

ANALYTICAL TESTING

Hook Effect Study

The hook effect was determined by testing various concentrations of sample. For a hook effect to be present, signal intensity would have to increase with dilution, not decrease as expected from normal sample dilution. Results are summarized in the next column.

Results from Hook Effect Study

Dilution ratio	# Positive									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S1
Neat	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:4	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:8	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:16	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:32	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:64	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:128	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:256	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
1:512	2/2	2/2	0/2	0/2	0/2	0/2	2/2	0/2	2/2	2/2
1:1024	0/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2
1:2048	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

The First To Know® Syphilis Test does not exhibit a hook effect.

Cross Reactivity Study

The cross reactivity of the First To Know® Syphilis Test was tested with 20 different cross reactants in ten samples.

Of the 200 cross-reactant samples tested, two samples showed weak positive results, one for EBV IgM, and the other for Rh factors. Results are summarized below.

Result Summary from Cross Reactivity Study

Cross reactant Sample	Sample #s	Number of Positives	Percent Cross Reactive
Anti-Lyme Positive	1 - 10	0/10	0%
Anti-Gonorrhea Positive	11-20	0/10	0%
Anti-Chlamydia Trachomatis Positive	21-30	0/10	0%
Anti-Leptospirosis Positive	31-40	0/10	0%
Anti-Trichomonas Positive	41-50	0/10	0%
Anti-Toxoplasma gondii Positive	51-60	0/10	0%
Anti-CMV Positive	61-70	0/10	0%
Anti-EBV Positive	71-80	1/10	10%
Anti-HAV Positive	81 – 90	0/10	0%
Anti-HBV Positive	91 - 100	0/10	0%
Anti-HCV Positive	101 - 110	0/10	0%
ANA Positive	111 – 120	0/10	0%
Anti-HIV Positive	121 – 130	0/10	0%
Hemodialysis Patient	131 – 140	0/10	0%
Anti-HSV Positive	141 – 150	0/10	0%
Rh Factors Positive	151 – 160	1/10	10%
HAMA Positive	161 – 170	0/10	0%
Heterophile Antibodies Positive	171 – 180	0/10	0%
Anti-HPV Positive	181 – 190	0/10	0%
Anti-HTLV Positive	191 – 200	0/10	0%

The results of the study show 0% Reactivity for all cross reactants except for one each at 10% reactivity for Anti-Epstein Barr Virus (EBV) and Rheumatoid Factor positive (RF+).

Interference Study

An interference study was conducted to evaluate the effects of common interfering substances on the performance of the First To Know® Syphilis Test.

For each interference agent tested, interference was evaluated at the concentration listed in the table below. One interference agent (triglycerides) was not soluble at the recommended 20 times the intended testing concentration. As a result, the triglycerides were tested at 2.5 mg/mL rather than 5 mg/mL. Hemolysis was observed on 23 tests, and a yellow background was observed on two other tests. However, the hemolysis and yellow background did not adversely affect the results.

Gamma-globulin may cause invalid results at concentrations at 60 mg/mL or greater. All other potentially interfering substances tested did not show interference at the concentrations tested.

Potential Interference Agents

Interference Agent	Testing Concentration
Acetaminophen	0.2 mg/mL
Acetylcysteine	1.66 mg/mL
Acetylsalicylic acid (Aspirin)	0.65 mg/mL
Albumin	50 mg/mL
Ampicillin	0.2 mg/mL
Ascorbic acid	0.2 mg/mL
Bilirubin	2 mg/mL
Cefoxitin	0.66 mg/mL
Cholesterol	2.5 mg/mL
Cyclosporine	1.4 mg/mL
Doxycycline	0.03 mg/mL
Gamma-Globulin	60 mg/mL
Hemoglobin	20 mg/mL
Heparin	3000 U/L
Ibuprofen	0.5 mg/mL
K2EDTA	0.8 mg/mL
Levodopa	6.5 mg/mL
Methyldopa	0.015 mg/mL
Metronidazole	0.12 mg/mL
Phenylbutazone	5 mg/mL
Rifampin	0.064 mg/mL
Theophylline	0.04 mg/mL
Triglycerides	2.5 mg/mL

PRECISION/REPRODUCIBILITY STUDIES

The following precision/reproducibility studies were conducted with the First To Know® Syphilis Test: Lot-to-lot and site-to-site.

Lot-to-lot Precision

The study utilized K_2EDTA venous whole blood spiked with syphilis-positive pooled plasma or serum samples (≤ 10% of whole blood volume, not including natural serum in the blood) as the sample matrix. Test samples included a non-reactive sample, a high negative sample (C5), a low reactive sample (C95), and a moderately reactive sample. The study also included positive and negative control samples. At each of three (3) CLIA Waived sites, three (3) site operators performed tests with each of four (4) samples in duplicate with First To Know® Syphilis Tests from each of three (3) lots, each of three (3) days for a total of 162 observations per sample. In addition, each site operator performed tests of positive and negative controls for each of three (3) lots, each of three (3) days.

Results

For each lot, the percent positive at each sample antibody index (AI) was calculated and compared to the Acceptance Criteria above.

STUDY RESULTS – LOT-TO-LOT PRECISION

Sample Concentration	Lot 1135 Overall	Lot 1141 Overall	Lot 1150 Overall	All Lots Overall	Expected Percent Positive Results
Non-Reactive	0%	0%	0%	0%	0%
High Negative	0%	0%	0%	0%	0% to 5%
Low Reactive	100%	100%	98.1%	99.4%	>/=95%
Moderate Reactive	100%	100%	100%	100%	100%

The results of the lot-to-lot precision study met the acceptance criteria.

Site-to-site Reproducibility

The study used the same sample sets described above in the lot-to-lot study. At each of three (3) clinical sites, three (3) site operators performed tests with each of four (4) samples in triplicate with First To Know® Syphilis Tests from one (1) lot, each of five (5) days for a total of 135 observations per sample. In addition, each site operator performed tests of positive and negative controls from one (1) lot, each of five (5) days.

Results

For each lot, the percent positive at each sample antibody index (AI) was calculated and compared to the Acceptance Criteria above.

STUDY RESULTS – SITE-TO-SITE REPRODUCIBILITY

Sample Concentration	Site 1 Overall	Site 2 Overall	Site 3 Overall	All Sites Overall	Expected Percent Positive Results
Non-Reactive	0%	0%	0%	0%	0%
High Negative	0%	0%	0%	0%	0% to 5%
Low Reactive	100%	95.6%	100%	98.5%	>/=95%
Moderate Reactive	100%	100%	100%	100%	100%

The results of the site-to-site reproducibility study met the acceptance criteria.

FLEX STUDIES

The operational limits of the device were evaluated in a series of experiments simulating conditions of use outside of the intended use environment or in instances of user errors by testing. The results demonstrated that the test is robust to stresses of environmental conditions and potential user error.

ASSISTANCE

Contact us toll-free at 1-844-207-3370 or go to www.firsttoknow.com for more information.

Symbol Legend

REF	Catalog Number or Product Code		Do Not Use if Package is Damaged
	Consult Instructions for Use		Sufficient for <1> Test
	Do Not Reuse		Manufacturer
	Caution	IVD	In Vitro Diagnostics Medical Device

NOWDiagnostics®

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