

INTENDED USE

The ZEUS IFA *Borrelia burgdorferi* IgG/IgM Test System is designed for the qualitative and semi-quantitative presumptive detection of total (IgG and IgM) antibodies to *Borrelia burgdorferi* in human serum. This Test System should only be used for patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented by testing with a standardized Western blot procedure. Positive supplemental results are supportive evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease.

SIGNIFICANCE AND BACKGROUND

B. burgdorferi is a spirochete that causes Lyme disease. The organism is transmitted by ticks of the genus *Ixodes*. In endemic areas, these ticks are commonly found on vegetation and animals such as deer, mice, dogs, horses, and birds. *B. burgdorferi* infection shares features with other spirochetal infections (diseases caused by three genera in humans: *Treponema*, *Borrelia*, and *Leptospira*). Skin is the portal of entry for *B. burgdorferi* and the tick bite often causes a characteristic rash called *erythema migrans* (EM). EM develops around the tick bite in 60% to 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids. Lyme disease occurs in stages, often with intervening latent periods and with different clinical manifestations.

In Lyme disease, there are generally three stages of disease, often with overlapping symptoms. Symptoms vary according to the sites affected by the infection such as joints, skin, central nervous system, heart, eye, bone, spleen, and kidney. Late disease is most often associated with arthritis or CNS syndromes. Asymptomatic subclinical infection is possible and infection may not become clinically evident until the later stages.

Patients with early infection produce IgM antibodies during the first few weeks after onset of EM and produce IgG antibodies more slowly (1). Although IgM only may be detected during the first month after onset of illness, the majority of patients develop IgG antibodies within one month. Both IgG and IgM antibodies can remain detectable for years. Isolation of *B. burgdorferi* from skin biopsy, blood, and spinal fluid has been reported (2). However, these direct culture detection methods may not be practical in the large-scale diagnosis of Lyme borreliosis. Serological testing methods for antibodies to *B. burgdorferi* include indirect fluorescent antibody (IFA) staining, immunoblotting, and enzyme immunoassay (EIA).

B. burgdorferi is antigenically complex with strains that vary considerably. Early antibody responses often are to flagellin which has cross reactive components. Patients in early stages of infection may not produce detectable levels of antibody. Also, early antibiotic therapy after EM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus, serological tests for antibodies to *B. burgdorferi* are known to have low sensitivity and specificity and because of such inaccuracy, these tests cannot be relied upon for establishing a diagnosis of Lyme disease (3, 4).

In 1994, the Second National Conference on Serological diagnosis of Lyme disease recommended a two-step testing system toward standardizing laboratory serologic testing for *B. burgdorferi*. Because EIA and IFA methods were not sufficiently specific to support clinical diagnosis, it was recommended that positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested, or supplemented, by using a standardized Western Blot method (second step) for detecting antibodies to *B. burgdorferi* (Western Blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM). Two-step positive results provide supportive evidence of exposure to *B. burgdorferi*, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis.

PRINCIPLE OF THE ASSAY

The ZEUS IFA *B. burgdorferi* IgG/IgM Test System is designed to detect circulating antibodies to the *B. burgdorferi* spirochete in human sera. The assay is particularly useful for, but not limited to, the diagnosis of Lyme disease in its later stages. The assay employs *B. burgdorferi* immobilized on a glass slide and fluorescein-labeled anti-human immunoglobulin and involves two steps:

1. Human sera are reacted with the spirochetes immobilized on the slides. Antibodies in the sera will bind to the spirochetes and remain attached after rinsing.
2. Fluorescein-labeled anti-human immunoglobulin is added in the second step and will bind to the antibodies causing the spirochetes to fluoresce. The intensity of staining is graded on a scale of 1+ to 4+ or as negative. Negative sera lacking antibodies will not show fluorescence.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAve Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •	1. <i>B. burgdorferi</i> Antigen Substrate Slides: Ten, 10-well Slides containing fixed <i>Borrelia burgdorferi</i> (strain B31) organisms, with desiccant pouch.
CONJ	2. Conjugate: Goat anti-human IgG/IgM labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL +	3. Positive Control (Human Serum): Will produce staining of the <i>B. burgdorferi</i> organisms. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL -	4. Negative Control (Human Serum): Will produce no staining of the <i>B. burgdorferi</i> organisms. One, 0.5mL, green-capped, vial. Ready to use.
DIL SPE	5. SAve Diluent®: Three, 30mL, green-capped, bottles containing phosphate-buffered-saline. Ready to use. NOTE: The SAve Diluent® will change color when combined with serum.
BUF PBS	6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED	7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAve Diluent® (Product #: FA005CC), Mounting Media (Product #: FA9309S), and PBS (Product #: 0008LTS).
2. Test System also contains a Component Label containing lot specific information inside the Test System box.

PRECAUTIONS

1. For *In Vitro* diagnostic use.

- Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
- The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
- The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
- Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
- Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate.
- The SAVE Diluent®, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may be toxic if ingested.
- Dilution or adulteration of these reagents may generate erroneous results.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross contamination of reagents and/or samples could cause erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Avoid splashing or generation of aerosols.
- Do not expose reagents to strong light during storage or incubation.
- Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
- Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
- Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
- Do not apply pressure to slide envelope. This may damage the substrate.
- The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
- Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
- Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
- Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

- Small serological, Pasteur, capillary, or automatic pipettes.
- Disposable pipette tips.
- Small test tubes, 13 x 100mm or comparable.
- Test tube racks.
- Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
- Cover slips, 24 x 60mm, thickness No. 1.
- Distilled or deionized water.
- Properly equipped fluorescence microscope.
- 1 Liter Graduated Cylinder.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

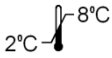
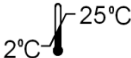
Transmitted Light			
Light Source: Mercury Vapor 200W or 50W			
Excitation Filter	Barrier Filter	Red Suppression Filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten – Halogen 100W			
KP490	K510 or K530	BG38	
Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

- ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
- Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (6, 7). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
- Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and

give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (11).

STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAVE Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

- Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
- Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.** Prepare a 1:16 dilution (e.g.: 10µL of serum + 150µL of SAVE Diluent® or PBS) of each patient serum. **The SAVE Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.** Using two-fold dilutions, continue to dilute each patient serum to 1:256.
Dilution Options:
 - Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVE Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected endpoint titer for each lot of Positive Control.
 - When titrating patient specimens, initial and all subsequent dilutions should be prepared in SAVE Diluent® or PBS only.
- Each patient serum should be screened using the 1:128 and 1:256 dilutions. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient in the appropriate wells.
- Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
- Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
- Wash Slides for two, 5 minute intervals, changing PBS between washes.
- Rinse Slides for about 5 - 10 seconds in a gentle stream of distilled water as in step 5, and air dry. Slides must be completely dry before adding Conjugate.
- Place 20µL of Conjugate on each well.
- Repeat steps 4 - 7.
- Place a small amount (4 - 5 drops) of Mounting Media between the two rows of offset wells and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

QUALITY CONTROL

- Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
- It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
 - Negative Control - characterized by the absence of fluorescence.
 - Positive Control - characterized by a 4+ to 3+ apple-green fluorescence staining of the *B. burgdorferi* organisms.
- Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTES:

- The intensity of the observed fluorescence may vary with the microscope and filter system used.**
- Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.**

INTERPRETATION OF RESULTS

- Negative:** No detectable antibody; result does not exclude *B. burgdorferi* infection. An additional sample should be tested within 4 - 6 weeks if an early infection is suspected (8). For the IFA test system, a negative reaction is equal to or less than 1+ fluorescence of a 1:128 dilution.
- Positive:** Antibody to *B. burgdorferi* presumptively detected. Per current recommendations, the result cannot be further interpreted without supplemental Western-blot testing. (Western-blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM). Results should not be reported until the supplemental testing is complete. For the IFA test system, a positive reaction will demonstrate 1+ or greater fluorescence staining at a dilution of 1:256 or greater.
- Borderline:** Current recommendations state that borderline results should be followed by supplemental Western-blot testing. The borderline result should be reported with the results from Western-blot testing. Results should not be reported until the supplemental testing is complete. For the IFA test system, a borderline specimen will demonstrate a 1+ or greater fluorescence at 1:128 but less than 1+ fluorescence at 1:256.

LIMITATIONS OF THE ASSAY

- Sera from patients with other spirochetal diseases (syphilis, yaws, pinta, leptospirosis, and relapsing fever), mononucleosis, and occasionally systemic lupus erythematosus may give false positive results. In cases where false positive reactions are observed, extensive clinical, epidemiologic and laboratory workups should be carried out to determine the specific diagnosis. False positive sera from syphilis patients can be distinguished from true Lyme disease positive patients by running an RPR or MHAATP assay on such specimens (9). Lyme sera will be negative in these tests (9, 10). Also, when titered in the FTA-ABS test, syphilis sera will show higher titers against the *T. pallidum* antigen than against the *B. burgdorferi* antigen (9).
- Patients with early Lyme disease may have undetectable antibody titers with this assay. In early Lyme disease IgG/IgM antibodies may not reach a level of diagnostic significance and may never do so when ECM is the sole manifestation (11). If Lyme disease is suspected early on, additional serum samples at varying intervals should be taken to demonstrate a rise in IgG titer.
- An alternative method for determining IgM antibodies may be more useful for diagnosing early Lyme disease or reinfection.
- Early antibiotic therapy may abort an antibody response to the spirochete.
- All data should be interpreted in conjunction with clinical symptoms of disease, epidemiologic data, and exposure in endemic areas.
- Screening of the general population should not be performed. The positive predictive value depends on the pretest likelihood of infection. Testing should only be performed when clinical symptoms are present or exposure is suspected.

7. The performance characteristics of the ZEUS IFA *B. burgdorferi* IgG/IgM Test System are not established with samples from individuals vaccinated with *B. burgdorferi* antigens.

EXPECTED RESULTS

Most investigators agree that a cut-off dilution of 1:256 differentiates positive and negative results with a high degree of specificity, particularly in the later stages of Lyme disease (12). The 1:256 cut-off dilution was established in a study of 267 serum specimens. Of the 267 specimens, 177 were obtained from blood bank donors and 90 from non-Lyme disease patients (12 dermatologic, 2 cardiac, 26 neurologic, and 50 patients with rheumatic diseases). One of the normal serum samples showed a titer >1:256, while 9 produced titers \geq 1:256 for a total of 10 out of 177 specimens. One out of 90 specimens obtained from subjects with non-Lyme disease disorders was \geq 1:256; therefore, of the 267 sera tested, <5% (13) produced values >1:256.

PERFORMANCE CHARACTERISTICS

1. The ZEUS IFA *B. burgdorferi* IgG/IgM Test System was compared to an ELISA assay in a double blind study at a large reference laboratory. The results of this study are summarized in Table 1. The relative sensitivity and specificity of the IFA and ELISA assays in this study are based on specific diagnosis determined by clinical features and extensive laboratory workups of patients in each disease category, *i.e.*, all suspected Lyme disease patients demonstrated specific clinical features that were confirmed by positive assays for Lyme disease antibodies.

Table 1: Comparison of the ZEUS IFA *B. burgdorferi* IgG/IgM Test System and a reference ELISA Assay

Disease Category	Number Tested	IFA Positive	ELISA Positive
<i>B. burgdorferi</i> ECM	10	1	6
Neurologic	9	8	9
Arthritic	10	10	10
Autoimmune	112	5	2
Other Infectious Disease	106	23	16
The overall sensitivity for both methods was:*		66%	79%
The overall specificity for each method was:*		87%	92%

*As compared to clinical diagnosis and other clinical laboratory data.

2. These results show that the ZEUS IFA *B. burgdorferi* IgG/IgM Test System procedure is less sensitive in the early stages of Lyme disease (*B. burgdorferi*), particularly in those cases associated with erythema chronicum migrans. However, in complicated cases of Lyme disease, the sensitivity was virtually 100% for both methods. These results are consistent with the lower amount of specific IgG antibodies present in the early stages of Lyme disease. These results are consistent with routine referral specimens submitted for analysis at the reference laboratory. The study was conducted in a double-blind manner. The results of this study are shown in Table 2.

Table 2: Comparison of the ZEUS IFA *B. burgdorferi* IgG/IgM Test System and a reference IFA Procedure

Number Tested	Number Negative		Number Positive	
	ZEUS IFA	Reference IFA	ZEUS IFA	Reference IFA
50	38	38	12	12

The results of this study show 100% agreement between the two procedures in detecting positive and negative sera using a 1:256 dilution as a cut-off point.

3. In a study conducted by the CDC (10), the IFA and ELISA methods for detecting antibodies in serum against *B. burgdorferi* were compared employing serum specimens obtained during different stages of Lyme disease. The results are shown in Table 3.

Table 3:

Disease Stage	IFA Positive	ELISA Positive
ECM	50	50
Carditis	100	100
Neuritis	92	100
Arthritis	100	97
More than one complication	71	80

These results are similar to those reported in Table 1 above and further substantiates that the IFA assay for detection of antibodies associated with Lyme disease is a reliable, sensitive method, particularly in cases of complicated late stage Lyme disease.

4. The following information is from a serum panel obtained from the CDC and tested in-house at ZEUS Scientific, on the ZEUS IFA *B. burgdorferi* IgG/IgM Test System. The results are presented as a means to convey further information on the performance of this assay with a masked characterized serum panel. This does not imply an endorsement of the assay by CDC. Table 4 shows the results of this study.

Table 4: The CDC *B. burgdorferi* Disease Serum Panel Stratified by Time After Onset

Time from Onset	Positive	+/-	Negative	Total	% Agreement with Clinical Diagnosis
Normals	2	1	1	4	33%
< 1 month	5	0	1	6	83%
1-2 months	7	0	2	9	78%
3-12 months	13	2	4	19	76%
> 1 year	1	4	3	8	25%
Total	28	7	11	46	69%

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ZEUS Scientific, Inc.
 200 Evans Way, Branchburg, New Jersey, 08876, USA
 Toll Free (U.S.): 1-800-286-2111, Option 2
 International: +1 908-526-3744
 Fax: +1 908-526-2058
 Website: www.zeusscientific.com
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EC	REP	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands
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