

BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDE CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following *Borrelia burgdorferi* Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slides	Code No.	REF
<i>Borrelia burgdorferi</i> (Lyme Disease)	BB-6112	
Σ Number of Tests	12-Well	

INTENDED USE

The Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative presumptive determination of *Borrelia burgdorferi* IgG or IgM antibodies in human serum.

Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of active infection, reinfection, or reactivation of the latent microbe and as a determination of immunological experience with *Borrelia burgdorferi*. Results should be supplemented by a second assay method, such as Western Blot, to provide supplementary serological evidence of the presence or absence of a *Borrelia burgdorferi* infection.

SUMMARY AND EXPLANATION

Lyme borreliosis (Lyme disease) is caused by the spirochete *Borrelia burgdorferi* sensu lato and transmitted primarily through the bite of the deer tick, *Ixodes scapularis* in the Midwest and Northeast, or *Ixodes pacificus* in the West.⁶ Lyme borreliosis has become the most commonly diagnosed tick-borne illness in the United States.¹

Symptoms of Lyme borreliosis demonstrate wide variations, but progress in three stages consisting of cutaneous involvement, cardiac and neurologic abnormalities, and arthritic involvement.^{2,3} Primary and secondary erythema migrans is characterized by a most distinctive cutaneous involvement, an expanding red rash with a pale center, known as erythema migrans (EM). This rash will develop at or near the site of the tick bite in approximately 60% of Lyme patients⁴ usually 3-14 days after the patient has been exposed to the spirochete.⁵ The primary skin lesion may not always take the classical pattern, but instead may appear as an erythematous plaque which extends its margins.⁵ During early infection patients may complain of low or moderate fever, headache, fatigue, arthralgias, stiff neck and myalgias. However, primary disease may also be asymptomatic and the EM may either not be developed or missed entirely by the patient.

Early disseminated disease complications may evolve a few weeks to months following the initial EM, and late disseminated disease manifestations occur months or years after the onset of infection if the primary disease is untreated with appropriate antimicrobial therapy.^{3,5,7} Clinical manifestations and complications of these later stages include skin disorders, arthritis, neurologic disorders, and cardiac involvement.^{3,5,8}

The manifestations of Lyme borreliosis may be numerous and varied, and may mimic other infectious and noninfectious diseases including influenza, meningitis, rheumatoid arthritis, psychosis, and multiple sclerosis.^{5,7,9} Consequently, laboratory diagnosis has assumed an increasingly important role in the diagnosis of the disease. Since traditional cultural methodologies have not been very useful for the identification of *B. burgdorferi*, the detection of a specific antibody response to antigens of *B. burgdorferi* is often required for diagnosis. However, just as clinical and physical findings may be problematic, immunoserologic test results may also provide inconclusive information due to complex and unpredictable immune responses during the various stages of Lyme borreliosis, common antigens shared by *B. burgdorferi* and other microorganisms, and the immunologic reactions of various autoantibodies with *B. burgdorferi* antigens.⁷

Immunologic response in individuals with early, primary disease tend to be directed to 23, 39 and 41-kDa flagellin antigen and are of the IgM class.^{5,7} These antibodies may be detectable within one to two weeks following the tick bite, but are generally delayed for four to six weeks. IgM antibodies may persist for months even if appropriate antimicrobial agents are administered. IgG immunologic responses appear to be directed to other immunogens of *B. burgdorferi* during later stages of Lyme borreliosis. These immunogens include 18, 23, 28, 30, 39, 41, 45, 58, 66, and 93-kDa proteins; IgM antibodies may also be produced to these components.^{5,7} With an increased complexity of antigenic determinants, a decreased specificity of immunoserologic assays may be expected.

However, antibody concentrations are usually quite elevated during the later stages of the disease, and the results are less likely to be interpreted as cross-reactions.⁷

The usual method of establishing *B. burgdorferi* infection is by serologic testing. The most commonly used assays are indirect immunofluorescence assays (IFA) and enzyme linked immunosorbent assays (ELISA).⁵ Infected humans produce IgG and IgM antibodies that recognize *B. burgdorferi* antigens.⁵ The bulk of the IgG-reactive antibodies are of the IgG1 and IgG3 subclasses.⁵ The IFA procedure uses whole borrelial organisms.

Testing procedures should be consistent with a recommended two-step algorithm. A positive first-step serologic test should be considered presumptive for the presence of antibodies and may indicate infection with *B. burgdorferi*. A positive supplemental second-step test (Western Blot) result provides additional serological evidence of infection with *B. burgdorferi*. Negative results from either a first-step or second-step assay should not be used to exclude Lyme borreliosis.²⁹

PRINCIPLE OF THE IFA PROCEDURE

The Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons¹⁶ and further developed by Riggs, et al.¹⁷ The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman IgG (or IgM) antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with a suspension of *Borrelia burgdorferi* microorganisms (Strain B31) fixed onto each well.

STORAGE AND STABILITY

The Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower  until labeled expiration date. 

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test. **IVD**
- Substrate slides are for single use only and must not be used more than once. **ⓧ**
- Use with non-Bion reagents could result in erroneous results.
- Care should be taken when handling substrate slides due to sharpe edges.
- The antigenic substrates have been fixed and contain no detectable *Borrelia burgdorferi* agents. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
- Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
- Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
- Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
- Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
- Antigen substrate slides should not be used beyond stated expiration date.
- Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
- Incubation times or temperatures other than those specified may give erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of detergents.
- Care should be taken to avoid splashing or generation of aerosols.
- Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
- Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membrane.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a "film" over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 14-28 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.^{18,19,20}

MATERIALS PROVIDED

The Bion BORRELIA BURGDORFERI ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. **LOT**

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. Borrelia burgdorferi Positive Human Control Serum
3. Borrelia burgdorferi Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium
6. IgG Binding Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable test tubes (12 x 75 mm or comparable) and rack
2. Disposable serological pipettes
3. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl with disposable pipette tips
4. Pasteur pipettes and bulbs
5. Moist chambers
6. Plastic squeeze wash bottle
7. Coplin jars or staining dishes with slide racks
8. 24 x 60 mm #1 coverslips
9. Felt tip marking pen
10. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490 nm excitation filter and 515-520 nm barrier filter, and optics to give a total magnification of 400X. The excitation wavelength of FITC is 490 nm and the emission wavelength is 520 nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

SCREENING:

Each laboratory should establish its own protocol for the preparation of serum screening dilutions.

IgG Screening:

For IgG testing it is suggested that a 1:80 dilution of each patient's serum be made as follows:

- a. Prepare a 1:20 dilution by adding 0.05 ml (50 µl) of patient's serum to 0.95 ml of PBS in tube #1. Mix.
- b. Prepare a 1:80 dilution by adding 0.1 ml (100 µl) of tube #1 to 0.3 ml of PBS in tube #2. Mix. Use tube #2 for the screening dilution.

IgM Screening:

When testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating IgM from IgG. Suggested methodologies are ion exchange chromatography²¹ or IgG immunoprecipitation.^{22,23} The screening test is then run using the IgM eluate.

IgG Binding Reagent Code No. GBR-9982 available from Bion.

SEMI-QUANTITATION:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titrating protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

IgG Testing:

The following fourfold serial titration is suggested:

- a. Prepare a 1:20 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.95 ml of PBS in tube #1.
- b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.
- c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.
- d. Do not use tube #1. Starting with tube #2, apply to substrate slide.

IgM Testing:

The following twofold titration is suggested:

- a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned above. This will be designated as tube #1.
- b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
- c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

These titrations will have the following dilutions:

Fourfold (IgG)	Twofold (IgM)
(Tube #1 = 1:20, not used)	Tube #1 = 1:10
Tube #2 = 1:80	Tube #2 = 1:20
Tube #3 = 1:320	Tube #3 = 1:40
Tube #4 = 1:1280	Tube #4 = 1:80
Tube #5 = 1:5120	Tube #5 = 1:160

2. SLIDE PREPARATION

Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. SPECIMEN APPLICATION

Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. INCUBATION 1

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

NOTE: For IgM testing, incubate the substrate slides in a moist chamber at 35-37°C for 90 minutes.

5. RINSE 1

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide.

6. WASH 1

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. CONJUGATE APPLICATION

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. INCUBATION 2

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

9. RINSE 2

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. WASH 2

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. COVERSIP

Remove slides one at a time from the last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. READ

Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:²⁴

- 4+ = Maximal fluorescence; brilliant yellow-green.
- 3+ = Less brilliant yellow-green fluorescence.
- 2+ = Definite but dull yellow-green fluorescence.
- 1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence Microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control

Using a negative control serum on Bion BORRELIA BURGDORFERI SUBSTRATE SLIDES, the Borrelia burgdorferi microorganisms should exhibit less than 2+ fluorescence.

Positive Control

Using a positive control serum on Bion BORRELIA BURGDORFERI SUBSTRATE SLIDES, the Borrelia burgdorferi microorganisms should exhibit apple-green fluorescence at an intensity of 2+ or greater. Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

SENSITIVITY CONTROL

A titrated control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above, and one twofold or fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered to be negative for Borrelia burgdorferi antibodies if the organisms exhibit less than 2+ fluorescence.

A sample is considered negative for significant IgG Borrelia burgdorferi antibodies if the organisms exhibit less than 2+ fluorescence at a serum dilution of 1:80 and all greater dilutions.

A sample is considered negative for IgM Borrelia burgdorferi antibodies if the organisms exhibit less than 2+ fluorescence at a serum dilution of 1:10 and all greater dilutions.

Negative samples may exhibit fluorescent staining of the Borrelia burgdorferi organisms slightly greater than the negative control, but less than 2+ fluorescence.

POSITIVE

A serum dilution is considered positive for Borrelia burgdorferi antibodies if well defined specific fluorescent staining of the complete spirochetal microorganism is observed at an intensity of 2+ or greater.

A sample is considered positive for Borrelia burgdorferi IgG antibodies if well defined smooth, homogeneous fluorescent staining of the complete spirochetal microorganism is observed at an intensity of 2+ or greater at a serum dilution of 1:80 or greater.

A sample is considered positive for Borrelia burgdorferi IgM antibodies if well defined specific heterogeneous (rough) fluorescent staining of the spirochetal microorganism is observed at an intensity of 2+ or greater at a serum dilution of 1:10 or greater.

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 2+ apple-green fluorescent intensity is detected in the stained *B. burgdorferi* organisms. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:

1:80 = 4+
1:320 = 3+
1:1280 = 1+
1:5120 = +/-

The extrapolated endpoint is reported as 640.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

Most clinical investigators agree that a serum specimen should be reported as positive serological evidence of *B. burgdorferi* antibodies if it demonstrates an IgG titer of 1:320 or greater in a single serum sample, or a fourfold change in titer between two samples.^{12,15} Titers of 1:40 to 1:160 have been seen in patients without detectable disease. IgG titers less than 1:80 are considered negative for *B. burgdorferi* antibodies.

Positive results from a single assay should only be interpreted as a presumptive determination of antibodies to *B. burgdorferi*. A second assay method (Western Blot) should be performed and results from both assays reported to the clinician. When reporting positive results from only one assay before or in the absence of confirming results by a second assay, the likelihood of misdiagnosis of Lyme disease may increase. This may mislead the clinician from the true cause of illness and potentially expose the patient to inappropriate and/or toxic therapy.

Negative results should be reported as "No detectable antibodies to *B. burgdorferi*." However, this may represent primary infection with the humoral response not yet developed to detectable levels. If infection with *B. burgdorferi* is still suspected, a second specimen should be obtained 14-28 days later and the paired specimens tested simultaneously, looking for a seroconversion.

LIMITATIONS OF THE PROCEDURE

1. *Borrelia burgdorferi* antibody test results do not provide definitive results for establishing or ruling out a diagnosis of Lyme disease and should always be used in conjunction with information available from clinical evaluation and other diagnostic information (history of exposure, symptoms, signs, etc.).
2. A single serological IgG antibody titer to *B. burgdorferi* should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific *B. burgdorferi* antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.
5. In some instances, high IgG or IgM antibody levels in the first of paired specimens may prevent the detection of increases in total antibody, resulting in apparently stationary total antibody titer.
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Positive test results from cord blood or neonates should be interpreted with caution. The presence of *B. burgdorferi* IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection.²⁶
9. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
10. If both the positive and negative control substrate cells are not visible

when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.

11. Antigen substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
12. If testing for IgM specific antibodies, the presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.²⁷ Therefore, all sera should be treated by ion exchange chromatography²¹ or IgG immunoprecipitation^{22,23} before testing to eliminate possible RF interference.
13. If testing for IgM specific antibodies, high titers of specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites resulting in a false negative IgM reaction.²⁴ Therefore, all sera should be treated by ion exchange chromatography²¹ or IgG immunoprecipitation^{22,23} before testing to avoid this possible problem.
14. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1, 2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM.
15. IgG removal reagents may lower the total amount of IgM present in serum after treatment. The amount removed can be as high as 25% of total IgM present³⁰. The effects of IgG removal on the sample may vary by assay type performed (IFA, ELISA, etc.). The specific IgM may vary by assay specificity of related organism as well. Inherent cross-reactivity between related viruses, previous infections with related viral species, and bacterial infections is also possible³¹. It is suggested that paired serums be treated and processed for antibody titers at the same time to minimize processing differences. Serums tested for acute infection should be checked for the patient's clinical history and repeated if indeterminate. Paired samples are recommended for analysis.
16. IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relation to patient's clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.²⁵

SPECIFIC LIMITATIONS OF THE B. BURGORFERI ASSAY

1. False-positive IFA reactions have been reported from patients with other spirochetal diseases (syphilis, yaws, pinta, relapsing fever and leptospirosis).^{5,6} Lyme borreliosis patients occasionally have reactive FTA-ABS and treponemal agglutination tests for syphilis.^{5,6} False positive sera from syphilis cases may be distinguished from true Lyme positive sera by performing reagin antibody assays like the RPR card and VDRL assay.⁶ Lyme disease sera will be negative.⁶
2. Some patients with infectious mononucleosis and rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosus have false-positive test results to *B. burgdorferi*.^{5,13}
3. In the later stages of Lyme Disease, IgM antibody determinations tend to be less specific than those for IgG antibodies, but may be useful in early disease or when reactivation or reinfection is suspected.^{5,10}
4. Patients with early disease often have serum antibody titers below the diagnostic threshold for six weeks or more after onset.^{5,10,11} Only about 50 to 60% of patients with early disease (appearance of EM) have diagnostic titers as measured by either IFA or ELISA.^{5,10,11}
5. Antibiotic therapy early in first-stage disease may blunt antibody response to the point that diagnostic threshold levels are never attained.⁴
6. In cases of reinfection, antibody titers to *Borrelia burgdorferi* may show a fourfold rise from previous convalescent values.¹⁴
7. A positive result from one (first-step) serological test is only presumptive evidence of the presence of *B. burgdorferi* antibodies. Another assay (second-step) should be performed to increase specificity.
8. Screening of the general population should not be performed. The positive predictive value depends on the pretest likelihood of infection.

EXPECTED VALUES

Levels of specific IgM antibody usually peak during the third to sixth week after disease onset and then demonstrate a gradual decline over a period of months.^{5,10} Titers of specific IgG antibody are generally low during the first several weeks of illness, but become maximal months later during the arthritic involvement. The IgG response often remains elevated at titers of 1:128 or more for years.¹⁰

In a study conducted by the Centers for Disease Control, 100 normal control sera were tested by IFA assay. Eight sera had titers of 1:128 and the remaining had titers of 1:64 or less. In this same study, using 1:256 and greater as positive for Lyme disease, and excluding treponemal sera, the IFA assay had a specificity of 97% and a sensitivity of 87%. The sensitivity increased to 93% when dilutions of 1:64 or greater were considered. The high degree of cross-reactivity with other spirochetal diseases has been discussed by Mertz, et al.¹⁵ Antibody detection by IFA alone does not provide definitive results for establishing or ruling out a diagnosis of Lyme disease. A second assay should be performed to increase specificity.

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the performance characteristics for both relative specificity and sensitivity of the Bion BORRELIA BURGDORFERI-G ANTIBODY TEST SYSTEM, qualitatively and semi-quantitatively with another commercially available test system for the presence of IgG antibody to Borrelia burgdorferi. The panel consisted of twelve negative and eight positive specimens for Lyme borreliosis with antibody titers ranging from 1:64 to 1:4096. Qualitatively there was agreement in 15 of the 20 specimens (75%) between the BION Test System and the commercial test system. In addition, these twenty specimens were also evaluated by an independent, certified Immunology Reference Laboratory, in which there was agreement in 19 of the 20 specimens (95%) with the BION test system.¹⁹ See TABLE 1 for data.

TABLE 1 - Summary of Relative IgG Comparison Testing

		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative		
OTHER KIT	Positive	3	0	3/3	
	Negative	5*	12		12/17

* 4 of the 5 were found positive and 1 was equivocal by an independent reference lab.

Semi-quantitatively there was no more than one twofold difference (+/-) in titer between fourteen of the specimens with the BION Test System, the commercially available test system and the Reference Laboratory. Six specimens showed results of fourfold or greater difference between the BION Test System and the commercial test system. The Reference Laboratory reported results that agreed within a twofold or less difference with the BION results in four of the six specimens. One strong positive specimen was reported fourfold higher on the BION Test System than in either the commercial test system or by the Reference Laboratory. However, all three assays reported the specimen as positive. One specimen was reported as equivocal by the Reference Laboratory due to an antibody titer that was considered close to the negative/positive cutoff value; whereas, this specimen was negative on the commercial test system and positive on the BION Test System.¹⁹

Interlot and intralot precision of the Bion BORRELIA BURGDORFERI-G ANTIBODY TEST SYSTEM was evaluated by testing 10 serum specimens for B. burgdorferi antibodies; 2 negative and 8 positive ranging in titer from 1:128 to 1:2040. They were tested on three different lots, and three times on the same lot. In each instance there was no more than a

twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology. None of the sera vacillated between a positive or negative result.¹⁹ TABLES 2 and 3 summarize these results.

TABLE 2
Summary of IgG Interlot Precision

Spec. #	Lot #1	Lot #2	Lot #3
1	<1:64	<1:64	<1:64
2	<1:64	<1:64	<1:64
3	1:128	1:128	1:128
4	1:512	1:512	1:512
5	1:256	1:256	1:512
6	1:1024	1:1024	1:1024
7	1:1024	1:2048	1:2048
8	1:512	1:1024	1:1024
9	1:1024	1:2048	1:2048
10	1:2048	1:2048	1:2048

TABLE 3
Summary of IgG Intralot Precision

Spec. #	1st Titer	2nd Titer	3rd Titer
1	<1:64	<1:64	<1:64
2	<1:64	<1:64	<1:64
3	1:128	1:128	1:128
4	1:512	1:256	1:256
5	1:256	1:256	1:256
6	1:1024	1:1024	1:1024
7	1:1024	1:1024	1:1024
8	1:512	1:1024	1:1024
9	1:1024	1:2048	1:2048
10	1:2048	1:2048	1:2048

Sera from 51 culture positive patients were tested to compare various test methodologies. The IFA assay using Bion BORRELIA BURGDORFERI-M ANTIBODY TEST SYSTEM appears to be the most sensitive overall with the P39 EIA the least sensitive. Fluorescence, EIA and Western Blot were comparable but had a number of equivocal reactions, particularly with sera from patients with secondary EM. TABLE 4 summarizes these results.^{7, 28}

TABLE 4 - Summary of Results of Sera from Culture Positive Patients Tested in Several B. burgdorferi Assays

Patient Group	# of Patients	Positive IgM IFA	Positive P39 EIA	Positive EIA	Positive Western Blot
Culture Positive					
Primary EM	19	8	1	5	2 (3)*
Secondary EM	32	32	0 (1)*	18 (9)*	20 (5)*

*Numbers in () are equivocal results

In addition, a control group of sera were tested by various test methodologies to determine cross-reactivity. The IFA assay using Bion BORRELIA BURGDORFERI-M ANTIBODY TEST SYSTEM and P39 EIA were highly specific in that none of the sera from other disease state patients or healthy persons were reactive. Fluorescence EIA gave positive reactions with a number of these same sera. None of the control group was tested by Western Blot. TABLE 5 summarizes these results.^{19, 20}

TABLE 5 - Summary of Results of Other Disease State and Normal Sera in Several B. burgdorferi Assays

Conditions	# of Patients	IgM IFA	P39 EIA	Fluorescence EIA
Infectious Mononucleosis	20	0	0	4
Rheumatoid Arthritis	19	0	0	3
Systemic Lupus	22	0	0	1
Syphilis	13	0	0	7
Streptococcal Sequelae	20	0	0	0
Healthy Subjects	16	0	0	0

A Lyme serum panel of 46 specimens was obtained from the Centers for Disease Control and Prevention (CDC). These specimens were tested in a blind study for IgG and IgM antibodies to B. burgdorferi using the Bion BORRELIA BURGDORFERI-G and -M ANTIBODY TEST SYSTEMS. TABLE 6 shows the number of specimens with detectable IgG and/or IgM antibodies to B. burgdorferi at various time intervals between onset and collection.^{19, 21}

SPECIFIC PERFORMANCE CHARACTERISTICS - (cont.)

TABLE 6 – Summary of IFA Results at Time Intervals Between Onset and Collection

BION IgG / IgM Results	<1 Month	1-3 Months	3-6 Months	6-12 Months	>12 Months	TOTAL SPECIMENS
Negative / Negative	0	2	3	0	0	5
Negative / Positive	2	3	2	0	0	7
Equivocal / Negative	0	2	1	2	2	7
Equivocal / Positive	3	5	2	1	1	12
Positive / Negative	0	1	1	4	4	10
Positive / Positive	0	3	0	1	1	5
TOTAL SPECIMENS	5	16	9	8	8	46

The data presented in TABLES 7 and 8 shows the IgG and IgM results of these 46 specimens comparing the Bion BORRELIA BURGDORFERI-G and -M ANTIBODY TEST SYSTEMS with the Western Blot results obtained from CDC. The data in TABLE 9 shows these results comparing both of the Bion TEST SYSTEMS with the ELISA results obtained from CDC.^{19,21}

TABLE 7 – Summary of Comparison of IgG Results Between Western Blot and IFA

		BION IgG			Relative Sensitivity	Relative Specificity
		Positive	Negative	Equivocal	91.7%	100%
Western Blot	Positive	11	1	9*	11/12	
	Negative	0	16	9*		16/16

* Equivocal results were excluded from calculations

TABLE 8 – Summary of Comparison of IgM Results Between Western Blot and IFA

		BION IgM		Relative Sensitivity	Relative Specificity
		Positive	Negative	81%	80%
Western Blot	Positive	17	4	17/21	
	Negative	5*	20		20/25

* 3 of the 5 specimens were from patients who had positive culture for B. burgdorferi

TABLE 9 – Summary of Comparison of IgG and IgM Results Between ELISA and IFA

		BION IgG & IgM			Relative Sensitivity	Relative Specificity
		Positive	Negative	Equivocal	96.2%	81.8%
ELISA	Positive	25	1	6*	25/26	
	Negative	2	9	0		9/11
	Equivocal	2*	0	1*		

* Equivocal results were excluded from calculations

- Barbour, A.G., Laboratory Aspects of Lyme Borreliosis, Clin. Micro. Rev. 1:399-414, 1988.
- Hunter, E.F., H. Russell, C.E. Farshy, et al., Evaluation of Sera from Patients with Lyme Disease in the Fluorescent Treponemal Antibody Absorption Test for Syphilis, Sex. Trans. Dis. 13:232-236, 1986.
- Mitchell, P.D., Marshfield Medical Center Laboratory, Marshfield, WI, Personal communication, 1988.
- Steere, A.C., W.P. Batsford, et al., Lyme Carditis: Cardiac Abnormalities of Lyme Disease, Ann. Internal. Med. 93:8-16, 1980.
- Johnson, R.C., Lyme Borreliosis: A Disease That Has Come Into Its Own, Lab. Management 26:34-40, 1988.
- Craft, J.E., R.L. Grodzicki and A.C. Steere, Antibody response in Lyme disease: Evolution of Diagnostic Tests, J. Infect. Dis. 149:789-795, 1984.
- Ackermann, R., J. Kabatzki, H.P. Boisten et al., Ixodes ricinus spirochete and European erythema chronicum migrans disease, Yale J. Biol. Med. 57:573-580, 1984.
- Magnarelli, L.A., J.A. Meegan, J.F. Anderson, and W.A. Chappell, Comparison of an indirect fluorescent antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease, J. Clin. Micro. 20:181-184, 1984.
- Russell, H., J.S. Sampson, G.P. Schmid et al., Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease, J. Infect. Dis. 149:465-470, 1984.
- Pfister, H.W., U. Neubert, B. Wilske, et al., Reinfection with Borrelia burgdorferi, Lancet ii:984-985, 1986.
- Mertz, L.E., G.H. Wobig, J. Duffy, and J.A. Katzman, Ticks, Spirochetes and new diagnostic tests for Lyme disease, Mayo Clin. Proc. 60: 402-406, 1985.
- Weller, T.H., A.H. Coons, Fluorescent Antibody Studies with Agents of Varicella and Herpes Zoster Propagated In Vitro, Proc. Soc. Exp. Biol. Med., 86:789-794, 1954.
- Riggs, J.L., R.J. Siewald, J.H. Burckhalter, C.M. Downs, T.G. Metcalf, Isothiocyanate Compounds as Fluorescent Labeling Agents for Immune Serum, Am. J. Pathol. 34:1081-1097, 1958.
- Goldman, M., Fluorescent Antibody Methods, New York, Academic Press, 1968.
- Kawamura, A. Jr., Fluorescent Antibody Techniques and Their Application, Baltimore, University Park Press, 1969.
- Nairn, R.C., Fluorescent Protein Tracing, Baltimore, Williams and Wilkins, 3rd Ed., 1969.
- Johnson, R.B., and R. Libby, Separation of Immunoglobulin M (IgM) Essentially Free of IgG From Serum in Systems Requiring Assay of IgM-Type Antibodies Without Interference From Rheumatoid Factor, J. Clin. Micro. 12:451-454, 1980.
- Gispen, R., J. Nagel, B. Brand-Saathof, S. DeGraff, Immunofluorescence Test for IgM Rubella Antibodies in Whole Serum After Absorption with Specific Anti-gamma Fc, Clin. Exp. Immunol., 22:431-437, 1975.
- Joassin, L., M. Reginster, Elimination of Nonspecific Cytomegalovirus Immunoglobulin M Activities in the Enzyme-Linked Immunosorbent Assay by Using Antihuman Immunoglobulin G, J. Clin. Micro. 23:576-581, 1986.
- Lyerla, H.C., F.T. Forrester, The Immunofluorescence (IF) Test, in: Immunofluorescence Methods in Virology, USDHHS, Georgia, 71-81, 1979.
- Data on file, Bion, Des Plaines, IL.
- Chernesky, M.A., C.G. Ray, T.F. Smith, Laboratory Diagnosis of Viral Infections, Cumitech 15, ASM, Washington, D.C., March 1982.
- Gardner, P.S., J. McQuillin, Rapid Virus Diagnosis: Application of Immunofluorescence, In: Detection of Virus specific IgM by Immunofluorescence, Butterworth, Boston, 259-287, 1980.
- Mitchell, P.D., K.D. Reed, T.L. Aspeslet, M.F. Vandermause, J.W. Melski, Comparison of Four Immunoserologic Assays for Detection of Antibodies to Borrelia burgdorferi in Patients with Culture-Positive Erythema Migrans, J. Clin. Micro., 32:1958-1962, 1994.
- "Recommendations for Test Performance and Interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease", Centers for Disease Control and Prevention, MMWR 1995; 44:590-591.
- Martens, T.B., T.D. Jaskowski, C.L. Mouritsen and H.R. Hill, An Evaluation of the Effectiveness of Three Immunoglobulin G (IgG) Removal Procedures for Routine IgM Serological Testing, Clin. & Diag. Lab. Immunol. Vol. 2, 98-100, Jan 1995.
- Gong, D.H., S.H. Kim, H.J. Kim, A. Lee and M.S. Han, Cross-Reactivity of Potentially Interfering Substances of other Infections, Laboratory Medicine and Quality Assurance (LMQA), Jan. 2022, 40-47.

BIBLIOGRAPHY

- CDC Update: Lyme disease and cases occurring during pregnancy-United States. Morbid. Mortal. Weekly Rep. 34:376-384, 1985.
- Habicht, G.S., G. Beck, and J.L. Benach, Lyme Disease, Scientific American 257:78-83, 1987.
- Steere, A.C., S.E. Malawista et al., The Clinical Spectrum and Treatment of Lyme Disease, Yale J. Biol. Med. 57:453-461, 1984.
- Steere, A.C., R.L. Grodzicki, et al., The spirochetal etiology of Lyme disease. N. Engl. J. Med. 308:733-740, 1983.

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