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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
Borrelia burgdorferi (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 patients[4] 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 Coons[16] 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

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed and contain no detectable Borrelia burgdorferil agents. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing or generation of aerosols.
15. 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.
16. 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 Miicrobiological 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 burgdoferi 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 chromatography[21] 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 titering 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.

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	100%	71%
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 BURDORFERI-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 Ig M results of these 46 specimens comparing the Bion BORRELIA BURGDORFERI-G and -M ANTIBODY TEST SYSYTEMS 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

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