

B. burgdorferi EIA (IgG, IgM) TEST SYSTEM

EIA for the detection of IgG and IgM antibody
to *Borrelia burgdorferi*

REF 40-8696P

96 Tests

Store Kit at +2 to +8°C

Pour d'autres langues
Für andere Sprachen
Para otras lenguas
Per le altre lingue
Dla innych języków

Paraout linguas
Για τις άλλεςλώσσες
För andra språk
For andre språk



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INTENDED USE

The MarDx *B. burgdorferi* Enzyme Immunoassay (EIA) IgG & IgM Test System is a qualitative test intended for use in the presumptive detection of human IgG & IgM antibodies to *Borrelia burgdorferi* in human serum. This EIA system should be used to test serum from patients with a history and symptoms of infection with *B. burgdorferi*. All positive and equivocal specimens should be re-tested with a highly specific, second-tier test such as Western blot. Positive second-tier results are supportive evidence of infection with *B. burgdorferi*. The diagnosis of Lyme disease should be made based on history and symptoms (such as erythema migrans), and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi*. Negative results (either first or second-tier) should not be used to exclude Lyme disease. High Complexity Test. **For In Vitro Diagnostic Use**

SUMMARY AND PRINCIPLE

Lyme disease is a multisystem disease caused by the spirochete *Borrelia burgdorferi* (1). The disease has been documented in Europe since early this century. It was documented in the United States during an epidemic in 1975 among children in Old Lyme Connecticut who demonstrated arthritic symptoms. Steere, et al. recognized the disease as a unique clinical entity (2,3). The symptoms of Lyme disease have been mistaken with many diseases including: juvenile rheumatoid arthritis, lupus erythematosus, multiple sclerosis, Bell's palsy, rheumatic fever, Reiter's Syndrome, myocarditis and viral meningitis (4).

The spirochete is transmitted by ticks of the genus *Ixodes* from animal reservoirs such as deer, mice, dogs, horses and birds. The ticks are commonly found on vegetation in endemic regions especially in wooded areas common to the animal reservoir. The incidence of human infection coincides with the tick season from May through September (3,5).

Although the symptoms of Lyme Disease are varied and sometimes unclear, three distinct phases of the disease are recognized. Early manifestations include a single or multiple rash called erythema migrans (EM), a meningitis stage during the next weeks to months is often seen. Late manifestations are recognized to include arthritis or neurologic signs and symptoms. In asymptomatic or subclinical cases, symptoms of infection may not be evident until the later stages of disease (5).

Isolation of *B. burgdorferi* in culture is definitive evidence of active infection, but is not practical. Detection of specific antibodies is practical but an indirect marker of exposure. Patients produce IgM antibodies within a few weeks of the appearance of EM. Although only IgM antibodies may be detectable during the first month, IgG antibodies increase in most patients after approximately one month. Detectable levels of both IgG and IgM may persist for years (5,6).

B. burgdorferi strains exhibit considerable antigenic variation. Patients often develop early antibodies to the flagellar antigen which can be cross reactive. Patients in the early stage of disease and a portion of patients with late manifestations may not have detectable antibodies. Early antimicrobial treatment, after appearance of EM may lead to diminished antibody concentrations. Serologic tests have been shown to have low sensitivity and specificity and, therefore, cannot be relied upon for establishing a diagnosis of Lyme disease (6,7,8).

The Second National Conference on Serological Diagnosis of Lyme disease (1994) recommended the use of a two-tier test system for Lyme serology in which positive and equivocal samples from a sensitive first-tier test must be further tested by a more specific method such as Western blot (second tier). Positive results in the second tier test provide supportive evidence of exposure to *B. burgdorferi* which could support a clinical diagnosis of Lyme disease but should not be used as a criterion for diagnosis (9).

The MarDx EIA (IgG & IgM) test is an indirect enzyme immunoassay (EIA) technique utilizing antigens of *B. burgdorferi* (Strain B 31) bound to polystyrene microwells. The antibody from the patient sample, which is added to the microwells in the first step of this procedure, binds with the antigen. Following a rinsing period which removes unbound antibody, a peroxidase labeled antihuman IgG & IgM is added to each microwell. The conjugate will attach during the second incubation step only if human IgG & IgM antibody is present from the first step reaction. Following a second rinsing period which removes unbound peroxidase conjugate, a color indicator solution is added to the microwells which will react only in the presence of bound peroxidase. An acid solution is added after a specified period of time in order to stop the enzymatic conversion of the indicator solution for spectrophotometric analysis.

REAGENTS SUPPLIED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Purified *B. burgdorferi* antigen (strain B31, washed, concentrated and sonicated in PBS buffer) coated microassay plate: 96 wells, configured in a full plate, stored in a foil pouch with a desiccant. Product number 40-8208/FP.
2. *B. burgdorferi* EIA IgG+IgM Positive Control: human serum. Proclin (0.1%) and/or Sodium Azide (< 0.1%) added as a preservative. Product number 40-8006L, one vial, 250 µL.
3. *B. burgdorferi* EIA IgG+IgM Calibrator: human serum. Proclin (0.1%) and/or Sodium Azide (< 0.1%) added as a preservative. Product number 40-8007L, one vial, 250 µL.
4. *B. burgdorferi* EIA Negative Control: human serum. Proclin (0.1%) and/or Sodium Azide (< 0.1%) added as a preservative. Product number 40-8001L, one vial, 250 µL.
5. Peroxidase Conjugate, Anti-Human IgG & IgM. Goat anti-human IgG & IgM, containing Proclin (0.03%) as a preservative. Product number 40-8025P, one bottle, 13.5 ml.
6. EIA Color Developer: Tetramethylbenzidine (TMB). Product number 40-1006, one bottle, 13.0 ml.
7. Stop Solution: Contains a H₃PO₄ solution. Product number 40-1004, one bottle, 15 ml.
8. Serum Diluent. Contains Proclin (0.1%) as a preservative. Product Number 40-8012, one bottle, 100 ml.
9. 10x Wash Solution. Contains PBS, Tween-20. Product number 40-1013, one bottle, 100 ml.

PRECAUTIONS

1. For In Vitro Diagnostic use.
2. The preservatives used in the reagents may be toxic if ingested. Do not mouth pipette.
3. This kit contains 1 molar phosphoric acid as a stop solution. Good laboratory procedure should be exercised when using acid solutions. Avoid contact with skin, eyes, or clothing. Use copious amount of water to wash exposed areas.
4. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
5. The human serum components used in the preparation of the Controls in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and were found to be negative. Because no test methods can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
6. The Centers for Disease Control and Prevention, and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2 (10).
7. Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations may significantly alter the results of this test.
8. All reagents must be brought to +20 to +25°C before performing this test procedure. Temperatures above or below the recommended range may result in substantial variation of the test results.
9. If less than a full plate is to be used, center the strips within the plate frame to secure the strips during the wash steps.
10. Do not interchange kit components from one kit lot with another kit lot.
11. All unused microwells must be stored in desiccation at +2 to +8°C between use.
12. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results.
13. Wells are to be washed five times.
14. If a sodium hypochlorite (bleach) solution is used as a disinfectant, do not expose the work area during the test procedure to avoid interference with the enzyme activity.
15. Assays designed for antibody detection of *B. burgdorferi*:
 - a. Do not indicate when exposure occurred.
 - b. Do not indicate that active replication is occurring or not occurring.
 - c. Frequently yield false-negative and false-positive results.
 - d. Do not have an established role in detecting such antibodies in serum collected during late manifestations of infection.
 - e. Do not often detect antibodies at clinical presentation during early Lyme disease, even when erythema migrans and isolation of *B. burgdorferi* in culture have been determined.
16. The concentrations of anti-*B. burgdorferi* antibody in a given specimen determined by assays from different manufacturer's may vary due to differences in assay methods and reagent specificity.



Xi – Irritant

R36/38: Irritating to eyes and skin.

S26, 36, 45: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. In case of accident or if you feel unwell seek medical advice immediately.

STORAGE AND STABILITY

1. Store kit at +2 to +8°C.
2. Bring all components to room temperature prior to use.
3. Refer to the expiration date on all reagents.
4. Refer to the expiration date on the microwell strips.
5. Immediately return kit to refrigeration after use.
6. All unused opened microwells must be stored in desiccation at +2 to +8°C between use.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtubes.
2. Microtube rack.
3. Storage container, 1.0 L, plastic or glass.
4. Pipetors, 10 µL, 100 µL, and 1.0 ml capacity.
5. Pipetor, 100 µL capacity, 8 channel (optional).
6. Waste container.
7. Timer, 30 minute.
8. Plastic squeeze bottle, 500 ml.
9. Absorbent paper (towels).
10. Distilled water.
11. EIA plate reader with 450 nm filter.
12. Desiccant storage for open microwell strips.

REAGENT PREPARATION

1. Prepare 1X wash solution to be used in the assay from the 10X Wash Solution provided. On removal of the 10X wash concentrate from refrigeration, undissolved salts may be present. Allow the reagent to reach room temperature and shake the bottle to dissolve the salts. Do not dispense from the reagent until all salts are dissolved.
2. In order to prepare the 1X wash solution, dilute 1 part of concentrate with 9 parts of distilled water in a clean plastic squeeze bottle. For each 8 well strip to be used, prepare a minimum of 50 ml.
3. The Color Developer is ready to use
4. The Serum Diluent is ready to use.
5. The Conjugate is ready to use.

SPECIMEN COLLECTION AND STORAGE

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.¹¹ Early separation from the clot prevents hemolysis of serum.
3. The CLSI (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A4. 2010)¹¹ provides the following general recommendations for storing blood specimens, recognizing that there can be several exceptions:
 - Separated serum should remain at room temperature for no longer than eight hours. If assays will not be completed within eight hours, serum should be refrigerated (2 to 8 °C).
 - If assays are not completed within 48 hours, or the separated serum will be stored beyond 48 hours, serum should be frozen at or below -20 °C.
4. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.¹¹
5. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.¹¹

PREPARATION OF SPECIMENS AND CONTROLS

A Positive Control, three Calibrators, a Negative Control, and a reagent blank must be run in each microplate tested. Dilute kit controls at the same time as patient specimens for each run. The reagent blank is prepared from Serum Diluent without the addition of human serum. Prepare the controls and patient specimens in appropriately marked pre-dilution tubes as follows:

1. Add 1.0 ml of Serum Diluent to each tube.
2. Add 10 µl of each serum to the appropriate tube and mix thoroughly.
3. Place each tube in an appropriate holding rack.
4. Specimens, controls and calibrators are now diluted 1:100 and are ready for application to the Borrelia sensitized microwell plates.

TEST PROCEDURE

1. Remove the full plate from its foil pouch. If less than a full plate is used, strips must be centered within the plate frame to secure the strips during the wash steps. Place unused strips back into the foil pouch and seal tightly. Unused strips must be placed in desiccation at +2 to +8°C.
2. Add 100 µL of each control, calibrator, sample and reagent blank dilution into wells and record on EIA worksheet.
3. Incubate at (20-25°C) for 30 minutes.
4. Shake out contents of the wells. Refill each well with wash solution
5. Repeat Step 4 four times. Blot strips dry on absorbent paper.
6. Add 100 µL of conjugate into each well.
7. Incubate at (20-25°C) for 30 minutes.
8. Repeat Steps 4 and 5.
9. Add 100 µL of Color Developer into each well.
10. Incubate at (20-25°C) for 10 minutes. Positive wells will develop a blue color.
11. Add 100 µL of Stop Solution to each well. Mix by gently tapping side of plate to evenly disperse Stop Solution. Do not create bubbles. Positive wells will develop a yellow color.
12. Incubate for 2 minutes.

13. Read the test strips by an EIA plate reader at 450nm within 30 minutes after addition of stop solution.

QUALITY CONTROL

A spectrophotometer or EIA plate reader with a 450 nm filter is needed for the analysis of the color intensity.

1. Read the plate at 450 nm. Print out the optical density values (OD₄₅₀).
2. Round the blank well OD to 2 digits to the right of the decimal and subtract from all test ODs. (0.795 = 0.80, 2.333 = 2.33).
3. Round all test ODs as above.
4. Calculate the mean OD of the three Calibrator wells. If any of the three wells has an OD more than 10% from the mean, disregard that value and recalculate the mean from the remaining two wells. If the ODs from the remaining 2 wells are not within 10% of the mean, assay results are invalid and must be repeated.
5. The Cut-Off is determined by multiplying the rounded mean of the Calibrator ODs (from step 4 above) by the Correction Factor value printed on the Calibrator vial label. Calibrator Mean (Step 4) X Correction Factor = Cut-Off Value. Round the Cut-Off Value.
6. To calculate a Lyme Index Value (LIV), divide the rounded OD of the sample by the rounded Cut-Off value.
7. The assay is considered valid and reportable when:
 - a. The High Positive Control has a Lyme Index Value greater than 1.50.
 - b. The Negative Control has a Lyme Index Value less than 0.60.

Note: The controls in this kit are intended to monitor for substantial assay failure and will not control precision at the Cut-Off.

INTERPRETATION OF RESULTS

A Lyme Index Value of 1.2 or greater should be considered presumptive evidence of exposure to *B. burgdorferi*.

REPORTING

Positive - Lyme Index of greater than or equal to 1.2.

A positive result is presumptive evidence of exposure to *B. burgdorferi*. The result should be supplemented by a second tier test that is more specific for antibodies to *B. burgdorferi* and should not be reported until second-step testing is complete. A positive result is not an indication of when exposure occurred.

Negative - Lyme Index of less than 1.0.

No detectable antibodies to *B. burgdorferi*. A negative result indicates that there was not serologic evidence of exposure to *B. burgdorferi* at the time the specimen was collected. A negative result should not be the basis for excluding *B. burgdorferi* as the cause of illness, especially if blood was collected within 2 weeks of when symptoms began.

If Lyme disease is strongly suspected, a second specimen should be collected 2 to 4 weeks after the first specimen and tested then.

Equivocal - Lyme Index of greater than or equal to 1.0 and less than 1.2.

An equivocal result is presumptive evidence of possible exposure to *B. burgdorferi*. The result should be supplemented by a second tier test that is more specific for antibodies to *B. burgdorferi* and should not be reported until second-step testing is complete.

LIMITATIONS OF PROCEDURE

1. Sera from patients with other pathogenic spirochetal diseases such as syphilis, yaws, pinta, leptospirosis, and relapsing fever may give false positive results. Sera from patients with mononucleosis or lupus erythematosus may also give false positive results. In cases where false positive results occur, clinical, epidemiologic and laboratory workups should be carried out. Although the clinical picture is quite different between active syphilis and Lyme disease, an easy means of differentiating these two diseases is by the use of the VDRL or RPR tests. In active syphilis, the VDRL or RPR are positive. In Lyme disease, the VDRL and RPR are negative.
2. Antibiotic therapy given early in the disease may prevent the development of an antibody response.
3. The evaluation of all test results must include the clinical history presented by the patient, the patients exposure in endemic regions for Lyme disease, epidemiological data, other test results, and any other potential spirochetal diseases. Positive test results in patients for which a history of exposure to Lyme disease, or symptoms or clinical findings consistent with Lyme disease do not exist may have a low predictive value.
4. Negative results early in disease have a low predictive value. Retesting may be warranted if symptoms consistent with Lyme disease persist.
5. Positive or equivocal first tier test results should not be reported until second-tier testing of the specimen is performed using a method that is more specific such as Western blot.
6. The use of this assay has not been evaluated for individuals who have received Lyme disease vaccine.

EXPECTED VALUES

In patients without present or past infection with *B. burgdorferi*, test results should be negative except for patients with cross-reacting antibodies (See Limitations).

In patients with Lyme disease, the test results are dependent on the stage of the disease. IgG antibody titers can be detected within two or three weeks following onset of disease. As the disease progresses, the chances of a positive test increases. Persons presenting within 3 weeks of onset of EM are positive for IgG 25% of the time. Persons with second stage symptoms are almost always positive as are persons with third stage symptoms.

IgG testing is most useful to detect ongoing or past cases of Lyme disease. For early detection, a test which detects IgM class antibodies is recommended. IgM antibodies rise quickly after infection, reaching their peak within 3 weeks and declining rapidly. IgG antibodies start rising 2 to 3 weeks after infection and persist while symptoms are present and drop slowly during recovery (5,6).

Positive results with Lyme Index Values between 1.0 and 1.4 are in the lowest third of all positives, results with indexes between 1.4 and 2.4 are in the middle third of all positives. Results with indexes greater than 2.4 are in the highest third.

SPECIFIC PERFORMANCE CHARACTERISTICS COMPARISON TO ELISA

MarDx Lyme Disease EIA (IgG&IgM) Test System was compared to three different EIA test systems to determine the accuracy in detecting antibodies to *B. burgdorferi*. One study utilized a commercially available EIA, while 2 studies utilized in-house EIA tests of reference laboratories. Specimens from endemic and non-endemic Lyme disease areas were run. The combined results of all studies are presented in Table 1.

TABLE 1
REFERENCE EIA

	POSITIVE	NEGATIVE	
MARDX			
POSITIVE	39	10	
NEGATIVE	6	155	
			95% CI*
Relative Sensitivity	87%	(39/45)	73-95
Relative Specificity	94%	(155/165)	89-97
Agreement	93%	(194/210)	88-96

*Confidence intervals calculated by exact method of Gardner et. al.

SPECIFIC PERFORMANCE CHARACTERISTICS COMPARISON TO IFA

MarDx Lyme Disease EIA Test System was also compared to two different immunofluorescent assays. One study utilized MarDx Lyme Disease IFA (IgG) and MarDx Lyme Disease IFA (IgM) tests. The second study utilized an in-house test at a reference laboratory. The combined results are presented in Table 2.

TABLE 2
RESULTS, MARDX EIA VS REFERENCE IFA

	POSITIVE	NEGATIVE	
MARDX			
POSITIVE	34	0	
NEGATIVE	5	131	
			95% CI
Relative Sensitivity	87%	(34/39)	73-96
Relative Specificity	100%	(131/131)	97-100
Agreement	97%	(165/170)	93-99

CLASS SPECIFIC SENSITIVITY

Three of the studies above utilized class specific reference assays that measure the level of IgG and IgM antibodies separately. Using these results the sensitivity of the MarDx Lyme Disease EIA (IgG&IgM) Test can be calculated by the antibody class present (Table 3).

TABLE 3
SENSITIVITY BY ANTIBODY CLASS

Antibody Class	MarDx Positive	Total Sensitivity	95% CI
IgM	9/11	82%	48-98
IgG	25/32	78%	60-91
IgG+IgM	14/14	100%	77-100

REPRODUCIBILITY

Within run variation was determined by testing one sample 30 times on the same assay plate. The between run variation was determined by running a low sample and a high sample once each on four different assay runs on four different days (Table 4).

TABLE 4

	Within Run	Between Run	
	Low	High	Low
Mean Index	0.82	1.77	0.92
Coefficient of Variation (CV) 5.0%		1.7%	8.7%

THE CDC LYME DISEASE SERUM PANEL STRATIFIED BY TIME AFTER ONSET

The following information is from a serum panel obtained from the CDC and tested by MarDx. The panel consists of 42 case-defined sera and 5 normal sera from individuals without Lyme disease. 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 the CDC.

TABLE 5 MarDx *B. burgdorferi* IgG/IgM ELISA Results

Time From Onset (Months)	Positive	Equivocal	Negative	Total	% Agreement with Clinical Diagnosis	95% CI
Normals	0	0	5	5	N/A	N/A
<1	2	0	3	5	40	5-85
1-2	9	0	1	10	90	56-100
3-12	7	4	8	19	58	34-80
> 12	8	0	0	8	100	63-100
Total	26	4	17	47		

Positive and equivocal results were considered 1-step positive for calculation of percent (%) agreement. Thirty sera from this panel were positive or equivocal by MarDx IgG/IgM EIA. Eight case-defined sera, negative by MarDx IgG/IgM EIA, were negative by IgG and IgM Western blot (CDC results). Two sera negative by MarDx IgG/IgM EIA were positive by IgG Western blot and two sera were positive by IgM Western blot. Five normal sera were negative by MarDx IgG/IgM EIA.

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Consult Instructions for Use

REF

Product Number

LOT

Lot Number

IVD

In Vitro Diagnostic Medical Device

EC REP

Authorized Representative in the European Community



Use By



Caution, consult accompanying documents



Temperature limitation



Manufacturer



Irritant - Precaution

CONTROL -

Negative Control

CONTROL +

Positive Control

CONJ

Conjugate

PLT

EIA Microwell Full Plate

CAL

Calibrator

SUBS TMB

EIA Color Developer

STP SOLN

Stop Solution

DIL SP

EIA Serum Diluent

BUF 10X

EIA 10X Wash Solution

CF

Correction Factor



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