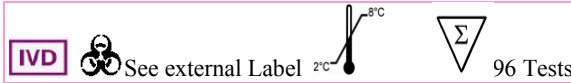


AccuDiag™
Lyme Disease IgG / IgM
ELISA

REF 1423-2



Test	Lyme Disease IgG / IgM
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative: Positive, Negative Control & Cut Off
Sample	10µL serum
Total Time	~ 95 min
Shelf Life	22 Months from the manufacturing date
Specificity	95%
Sensitivity	92%

INTENDED USE

The Diagnostic Automation, Inc. Borrelia burgdorferi IgG/IgM Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative presumptive detection of total (IgG and IgM) antibodies to Borrelia burgdorferi in human serum. This ELISA 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.

SUMMARY AND EXPLANATION

Borrelia burgdorferi is a spirochete that causes Lyme disease. Ticks of the genus Ixodes transmit the organism. In endemic areas, these ticks reside 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 chronicum migrans (ECM). ECM 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 three 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 ECM and produce IgG antibodies more slowly (1). Tests only detect IgM

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 (ELISA).

B. burgdorferi is antigenically complex with strains that vary considerably. Early antibody responses often are to flagellin that has cross-reactive components. Patients in early stages of infection may not produce detectable levels of antibody. In addition, early antibiotic therapy after ECM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus, serological tests for antibodies to B. burgdorferi have low sensitivity and specificity and because of such inaccuracies, health care professionals do not rely on these tests to establish 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 ELISA and IFA methods were not sufficiently specific to support clinical diagnosis, it was recommended that positive or equivocal results from a sensitive ELISA 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. These results could support a clinical diagnosis of Lyme disease, but scientists suggest avoiding the use as a sole criterion for diagnosis.

TEST PRINCIPLE

The Diagnostic Automation Inc.. Borrelia burgdorferi ELISA test system is designed to detect IgM and IgG class antibodies to *Borrelia burgdorferi* in human sera. Wells of plastic microwell strips are sensitized by passive absorption with *Borrelia burgdorferi* antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM/IgG is added to the wells and the plate is incubated. The Conjugate will react with IgM and/or IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

SPECIMEN COLLECTION AND PREPARATION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease (current edition).
2. 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.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.



4. 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 delay in testing is anticipated, store test sera at 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 (17).

MATERIALS AND COMPONENTS

Materials provided with the test kits

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v): Controls, Calibrators and Sample Diluent.

1. **Plate.** 96 wells configured in twelve 1x8-well strips coated with inactivated B. burgdorferi B31 strain) antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate.** Conjugated (horseradish peroxidase) goat anti-human IgM / IgG. Ready to use. One, 15 mL vial with a white cap.
3. **Positive Control** (Human Serum). One, 0.35 mL vial with a red cap.
4. **Calibrator** (Human Serum). One, 0.5 mL vial with a blue cap.
5. **Negative Control** (Human Serum). One, 0.35 mL vial with a green cap.
6. **Sample Diluent.** One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate- buffered-saline, (pH 7.2 ± 0.2). Green solution, ready to use. Note: Shake Well Before Use. (Product #: 1423-2Z). Preservative added.
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
8. **Stop solution:** One 5, mL (red cap) containing 1M H₂SO₄, 0.7M HCl. Ready to use.
9. **Wash buffer concentrate (10X):** dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

PRECAUTIONS

1. For In Vitro Diagnostic Use.

2. Normal precautions exercised in handling laboratory reagents should be followed. 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.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS 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 Biosafety 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.
5. The sample diluent, controls, wash buffer, and conjugate 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.
6. 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 refrigerated temperature immediately after use.
7. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.



- 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 kit.

- Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
- Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
- Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

ASSAY PROCEDURE

- Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
- Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/ Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2°and 8°C.

ABBREVIATED TEST PROCEDURE

- Dilute Serum 1:21.
- Add diluted sample to microwell - 100µL/well.
- *Incubate 25 ± 5 minutes.*
- Wash.
- Add Conjugate - 100µL/well.
- *Incubate 25 ± 5 minutes.*
- Wash.
- Add TMB - 100µL/well.
- *Incubate 10 - 15 minutes.*
- Add Stop Solution - 50µL/well - Mix.
- READ within 30 minutes.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

- Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent. Of the Negative Control, Calibrator, Positive Control, and each patient serum.
- To individual wells, add 100µL of each diluted control, calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
- Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
- Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
- Wash the microwell strips 5 times.

A. Manual Wash Procedure:

- Vigorously shake out the liquid from the wells.
- Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
- Repeat steps a. and b. for a total of 5 washes.
- Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

- Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
- Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes.
- Wash the microwells by following the procedure as described in step 7.
- Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.

RESULTS

A. Calculations:

1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

$$(CF \times \text{mean OD of Calibrator} = \text{cutoff OD value})$$

3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:	
Mean OD of Calibrator	0.793
Correction Factor (CF)	0.25
Cut off OD	0.793 x 0.25 = 0.198
Unknown Specimen OD	0.432
Specimen Index Value or OD Ratio	0.432 / 0.198 = 2.18

B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

- An OD ratio <0.90 indicates no significant amount of IgM antibodies to B. burgdorferi detected. An additional sample should be tested within four to six weeks if early infection is suspected (5).
- An OD ratio >1.10 is presumptively positive for IgG/M antibody to B. burgdorferi. 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 completed.
- Current recommendations state that equivocal results should be followed by 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. This equivocal result should be reported with results from Western Blot testing. Results should not be reported until the supplemental testing is completed.

PERFORMANCE CHARACTERISTICS

A. Comparative Study

The Diagnostic Automation, Inc. B. burgdorferi antibody IgG/IgM ELISA test system was compared to a commercially available, and a reference IFA B. burgdorferi assay for the detection of antibodies in two, double blind clinical studies.

The first study compared the Diagnostic Automation Inc. B. burgdorferi ELISA test system to a commercially available IFA B. burgdorferi test system for the detection of antibodies in 199 serum samples randomly at a large medical center on the east coast. The results of this double blind study are shown in Table 1:

Table 1

		Diagnostic Automation, Inc. B. burgdorferi ELISA test system	
		Pos.	Neg.
B. burgdorferi IFA Procedure	Pos.	58	5
	Neg.	7	129

Analysis of the data in Table 1 reveals a sensitivity of 92 %, a specificity of 95 %, and an overall concordance of 94%.

The second study compared the Diagnostic Automation, Inc. B. burgdorferi ELISA test system to a reference IFA B. burgdorferi test procedure for the detection of IgG and IgM antibodies in 263 serum samples randomly processed at a larger reference laboratory. The results of the double blind study are shown in Table 2:

Table 2

		Diagnostic Automation, Inc. B. burgdorferi ELISA	
		Pos.	Neg.
Reference B. burgdorferi IFA for IgG/ IgM	Pos.	11	2
	Neg.	8	242

Statistical analysis of the data in Table 2 show a sensitivity of 85 %, and a specificity of 97%. The overall concordance was 96%.

In both clinical studies all discrepant results were repeated, and identical results were obtained. In addition, IgM positive/IgG negative serum samples (7) were identified as positive with the Diagnostic Automation, Inc. B. burgdorferi ELISA test system, and pooled IgM positive /IgG negative reference sera were identified as positive with the Diagnostic Automation, Inc. B. burgdorferi ELISA test system. These results indicate that the Diagnostic Automation, Inc. B. burgdorferi ELISA test system is capable of detecting both IgG and IgM class specific antibodies against B. burgdorferi in individual microtiter wells.

Table 3 shows test results obtained using a serum panel from the CDC. 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 3

The CDC B. burgdorferi Disease Serum Panel Stratified by Time After Onset					
Time from onset	Pos	+ / -	Neg.	Total	% agreement with clinical diagnosis
Normals	1	1	3	5	75%; 3/4
< 1 month	6	0	0	6	100%; 6/6
1-2 months	7	0	1	8	88%; 7/8
3-12- months	18	0	2	20	90%; 18/20
> 1 year	8	0	0	8	100%; 8/8
Total	40	1	6	47	93% (39/42 pos. and 3/4 Neg.)

B. Reproducibility

The intra- and inter-assay variation was determined by running 8 replicates of positive, borderline, and negative samples on three consecutive days. The results of these assay are as follows:

	Intra-assay						Inter-assay	
	Run # 1		Run # 2		Run # 3			
	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV
Negative	0.42	16.6%	0.49	5.7%	0.49	5.7%	0.47	7.0%
Positive	1.65	6.8%	1.63	3.1%	1.64	3.7%	1.64	0.01%
Positive	1.20	2.5%	1.02	7.8%	1.30	6.1%	1.20	2.20%
Borderline	0.76	15.4%	0.77	5.5%	0.93	2.9%	0.82	9.5%

C. Cross Reactivity

Sera from patients with North American borreliosis, yaws, pinta, leptospirosis, autoimmune diseases, and syphilis may cross react.

QUALITY CONTROL

- Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
- Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
- The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

OD Range	
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥ 0.500

- The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
- The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
- If the above conditions are not met the test should be considered invalid and should be repeated.



4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

LIMITATIONS OF PROCEDURE

1. Sera from patients with other spirochetal diseases (syphilis, yaws, pinta, leptospirosis, and relapsing fever), infectious mononucleosis, or systemic lupus erythematosus may give false positive results (6). Observations of false positive reactions require extensive clinical epidemiologic and additional laboratory workups to determine the specific diagnosis. Technicians can distinguish false positive sera from syphilis patients from true *B. burgdorferi* disease positive sera by running an RPR or MHATP assay on such specimens (7).
2. Drawing serum samples too early after onset of disease, before antibody levels have reached significant levels, results in false negative results (8). In addition, early antibiotic therapy may abort an antibody response to the spirochete (9).
3. Interpret all data in conjunction with clinical symptoms of disease, epidemiologic data, exposure in endemic areas, and results of other laboratory tests.
4. Do not perform screening of the general population. The positive predictive value depends on the pretest likelihood of infection. Only perform testing when clinical symptoms are present or exposure suspected.
5. Diagnostic Automation, Inc. did not establish performance characteristics of the Diagnostic Automation, Inc. ELISA *Borrelia burgdorferi* IgG/IgM Test System for samples from individuals vaccinated with *B. burgdorferi* antigens.

EXPECTED VALUES

Titers of IgM antibodies to *B. burgdorferi* peak three to six weeks after onset of ECM and gradually decline thereafter (10). Titers of IgG antibodies are low during ECM but increase in titer during the course of the disease, reaching peak titers when arthritis is present (10). IgG antibodies may remain elevated for years (11). Studies have shown that 90% or more of patients with ECM alone develop elevated titers of IgM antibodies (10, 12). In the absence of ECM, a positive ELISA test may distinguish early *B. burgdorferi* disease from other febrile illnesses (10). However, a much lower percentage of patients have elevated IgM antibodies when tested during the first three weeks after onset of ECM (6, 13). In these patients, obtaining a more complete serological picture by testing acute and convalescent sera is necessary. Most patients (94 - 97%) with neurological complications and essentially all patients with arthritis have elevated IgG titers to the spirochete (6, 12). In later stages, a positive antibody test may help distinguish *B. burgdorferi* disease from viral meningitis or unexplained nerve palsies. A positive antibody test may be particularly useful in differentiating *B. burgdorferi* arthritis from rheumatoid arthritis, juvenile arthritis, and Reiter's Syndrome (10). Patients without signs or clinical features of *B. burgdorferi* disease should test negative with the Diagnostic Automation, Inc. ELISA *Borrelia burgdorferi* IgG/IgM Test System.

STORAGE



1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.

7. Sample Diluent: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

REFERENCES

1. Steere AC, et al: *J. Infect. Dis.* 154:295-300, 1986.
2. Rosenfeld MEA: Serodiagnosis of Lyme disease. *J. Clin. Microbiol.* 31:3090-3095, 1993.
3. Steere AC, et al: The Spirochetal Etiology of Lyme Disease. *N. Engl. J. Med.* 308:733-740, 1983.
4. Bakken LL, Callister SM, Wand PJ, and Schell RF: Interlaboratory Comparison of Test Results for Detection of Lyme Disease by 516 Patients in the Wisconsin State Laboratory of Hygiene/College of American Pathologists Proficiency Testing Program. *J. Clin. Microbiol.* 35:537-543, 1997.
5. Barbour A: Laboratory Aspects of Lyme Borreliosis. *Clin. Micro. Rev.* 1:399-414, 1988.
6. Russel H, Sampson JS, Schmid GP, Wilkinson HW, and Plikaytis B: Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. *J. Infect. Dis.* 149:465, 1984.
7. Hunter EF, Russell H, Farshy CE, et al: Evaluation of sera from patients with Lyme disease in the fluorescent treponemal antibody-absorption test for syphilis. *Sex. Trans. Dis.* 13:236, 1986.
8. Shrestha M, Grodzick RL, and Steere AC: Diagnosing early Lyme disease. *Am. J. Med.* 78:235, 1985.
9. Steere AC, Hutchinson GJ, Rahn DW, Sigal LH, Craft JE, DeSanna ET, and Malawista SE: Treatment of the early manifestations of Lyme disease. *Ann. Intern. Med.* 99:22, 1983.
10. Craft JE, Grodzicki RL, Shrestha M, Fischer DK, Carcia-Bianco M, and Steere AC: Antibody response in Lyme disease. *Yale J. Biol. Med.* 57:561, 1984.
11. Dammin GJ: Lyme Disease: Its transmission and diagnostic features. *Lab Mgmt.* 24:33, 1986.
12. Steere AC, Malawista SE, Bartenhagen NH, Spieler PN, Newman JH, Rahn DW, Hutchinson GJ, Green J, Snyderman DR, and Taylor E: The Clinical Spectrum and Treatment of Lyme disease. *Yale J. Biol. Med.* 57:453, 1984.
13. Reik L, Smith L, Khan A, and Nelson W: Demyelinating encephalopathy in Lyme disease. *Neurology* 35:267, 1985.
14. Procedures for the collection of diagnostic blood specimens by venipuncture: NCCLS Procedure H3; Approved Standard.
15. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H1, Approved Guideline.
16. U.S. Department of Labor, Occupational Safety and Health Administration. Final Rule; 21CFR 1910.1030.
17. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4th Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.



ISO 13485 ISO 9001	
	
 Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA	
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EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
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