AccuDiag™ Lyme Disease IgG ELISA Kit

REF 1428-P1

IVD See External Label 2-8°C 96 Tests

Lyme Disease IgG ELISA	
Principle	**Indirect ELISA**
Detection	**Qualitative**
Sample	**10 µL serum/plasma**
Incubation Time	**70 minutes**
Sensitivity	**96.4%**
Specificity	**91.7%**
Shelf Life	**12 Months from the manufacturing date**

PRODUCT FEATURES

- **Very easy to use with little training**
- **Highly specific and consistent Assay**
- **Provides accurate results quickly**
- **Reading of results both visually and as absorbance data**

INTENDED USE

The The Diagnostic Automation *Borrelia burgdorferi* IgG ELISA Test System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative presumptive detection of IgG antibodies to Borrelia burgdorferi in human serum. This test should only be used for patients with signs and symptoms that are consistent with Lyme disease. Positive and equivocal test results with the ELISA *Borrelia burgdorferi* IgG Test System for the presence of *Borrelia burgdorferi* antibodies must be confirmed through additional testing by one of the following approaches:

(1)Standard two-tier test methodology (STTT) using IgG Western blot testing following current guidelines;

or

(2)Modified two-tier test methodology (MTTT) using the DAI ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System.

Positive test results by either the STTT or MTTT methodology are supportive evidence for the presence of antibodies and exposure to *Borrelia burgdorferi*, the cause of Lyme disease. A diagnosis of Lyme disease should be made based on the presence of *Borrelia burgdorferi* antibodies, history, symptoms, and other laboratory data.

SIGNIFICANCE AND SUMMARY

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 migrans* (EM). EM develops around the tick bite in 60 - 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids.

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

Patients with early infection produce IgM antibodies during the first few weeks after onset of EM and produce IgG antibodies more slowly (1). 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 (EIA).

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 EM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus, serological tests for antibodies to *B. burgdorferi* are known to have low sensitivity and specificity and because of such inaccuracy, these tests cannot be relied upon for establishing a diagnosis of Lyme disease (3, 4).

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

detecting IgM). Two-step positive results provide supportive evidence of exposure to *B. burgdorferi*, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis. This scenario is commonly referred-to as the Standard two-tier testing (STTT) protocol. Recent studies (17, 18, 19) have demonstrated that using a second ELISA test in place of the Borrelia immunoblot can result in a modified two-tier testing (MTTT) protocol with performance that is comparable to the STTT protocol.

ASSAY PRINCIPLE

The Diagnostic Automation B. *burgdorferi* ELISA test system is designed to detect IgG class antibodies to *B. burgdorferi* in human sera. The sensitized wells of plastic microwell strips are prepared by passive absorption with *Borrelia burgdorferi* whole cell 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 IgG (Fc chain specific) is added to the wells and the plate is incubated. The Conjugate will react with 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 & PREPARATION

1. DAI recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease.
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 (6, 7). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera. 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 (16).
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° and 8°C, for no longer than 10 days. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

REAGENTS

Materials provided with the kit

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

1. **Plate:** 96 wells configured in twelve 1x8-well strips coated with inactivated *B. burgdorteri* 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 IgG. Ready to use. One, 15mL, white-capped vial.
3. **Positive Control** (Human Serum): One, 0.35mL, red-capped vial.
4. **Calibrator** (Human Serum): One, 0.5mL, blue-capped vial.
5. **Negative Control** (Human Serum): One, 0.35mL, green-capped vial.
6. **Sample Diluent**: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Green solution. Ready to use.
7. **TMB:** One, 15mL amber-capped, amber bottle containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. **Stop solution:** One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
9. **Wash buffer concentrate (10X):** Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle 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.**

Notes:

1. ***The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.***
2. ***Package insert providing instructions for use.***

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm. ***NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.***
2. Pipettes capable of accurately delivering 10 - 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. (i.e.: 10% household bleach, 0.5% sodium hypochlorite.)

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank 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° - 8°C.
3. 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.

4. 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.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 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	

7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure:**
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps a. and b. for a total of 5 washes.
      4. 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 disinfectant at the end of the day's 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.
8. Add 100µL of the Conjugate to each well, including Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20-25°C) for 25 ±5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20-25°C) for 10 - 15 minutes.
13. 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. 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.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes after the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell – 100µl/well.
3. → *Incubate 25 ±5 minutes.*
4. Wash.
5. Add Conjugate – 100µl/well.
6. → *Incubate 25 ±5 minutes.*
7. Wash.
8. Add TMB – 100µl/well.
9. → *Incubate 10 – 15 minutes.*
10. Add Stop Solution – 50µl/well – Mix.
11. READ within 30 minuites.

RESULTS

Calculations:

a. ***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.

b. ***Cutoff OD Value***: To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
(CF x mean OD of Calibrator = cutoff OD value)

c. ***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 b.

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

INTERPRETATION

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

a. An OD ratio <0.90 indicates no significant amount of antibodies to *B. burgdorferi* IgG detected. If exposure to *B. burgdorferi* is suspected, a second sample should be collected and tested two to four weeks later (15).

b. An OD ratio >1.10 indicates that antibodies specific to B.burgdorferi were detected. This indicates presumptive evidence of probable exposure. 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. Do not report results until the supplemental testing is completed.

c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen by the second step IgG and/or IgM Western blot.

- **MTTT (2-EIA) Use and Interpretation for IgG Antibody Detection:**

In addition to being used as the first-tier immunoassay in the standard two-tier testing (STTT) method, this device may be used as a second-tier assay in the 2- EIA or modified two-tier testing (MTTT) protocol in the following way.

a. The samples must be tested first with the DAI ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System.
b. All the positive and equivocal samples must then be tested with this ELISA *Borrelia burgdorferi* IgG Test System.
c. Positive and equivocal results from the second-EIA testing should be reported as positive and interpreted as supportive evidence for the presence of IgG antibodies and exposure to *B. burgdorferi.*

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included in each assay.
2. 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.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

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

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
   c. 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 but will not ensure precision at the assay Cutoff.
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.

EXPECTED RANGES OF VALUES

Only 10 - 40% of patients with EM alone have detectable levels of antibody to *B. burgdorferi* (1, 12, and 13). The IgM response usually peaks from three to six weeks following infection and is often not detectable during the first two weeks of infection. The IgG antibody response is frequently not detectable for four to six weeks after infection. A more complete serological picture may be obtained by testing acute and convalescent sera. Most patients (94-97%) with neurological complications and essentially all patients with arthritis have elevated IgG titers to the spirochete (12, 14). In later stages, a positive antibody test may help distinguish Lyme disease from viral meningitis or unexplained nerve palsies. A positive antibody test may be particularly useful in differentiating Lyme arthritis from rheumatoid arthritis, juvenile arthritis and Reiter's Syndrome (11). Patients without signs or clinical features of Lyme disease should test negative with the DAI ELISA *B. Burgdorferi* IgG kit.

PERFORMANCE CHARACTERISTICS

1. **Comparative Studies:**
The Diagnostic Automation, Inc. *B. burgdorferi* antibody IgG ELISA test system was compared to a commercially available indirect fluorescent antibody (IFA) test procedure for detection of IgG antibodies to *B. burgdorferi*. Two hundred seven (207) serum samples were tested by the two procedures. The results of this study are summarized in Table 1 below:

Table 1

		B. Burgdorferi IFA Procedure	
		Positive	**Negative**
DAI ELISA B. Burgdorferi IgG Test System	**Positive**	54	12
	Negative	2	133
	Equivocal*	1	5

Relative Sensitivity = 96.4 % (54/56)
Relative Specificity = 91.7 % (133/145)
Concordance = 93.0 % (187/201)

*Equivocal samples were excluded from the calculations for sensitivity, specificity, and concordance.

Table 2 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 2: CDC B. burgdorferi Disease Serum Panel Stratified by Time After Onset

The CDC B. burdorferi Disease Serum Panel Stratified by Time After Onset					
Time from onset	**Pos**	**+ / -**	**Neg.**	**Total**	**% agreement with clinical diagnosis**
Normals	0	0	5	5	100%
< 1 month	1	1	4	6	20%
1-2 months	2	1	5	8	28%
3-12- months	8	3	9	20	47%
> 1 year	8	0	0	8	100%
Total	19	5	23	47	57%

2. **Precision and Reproducibility:**
To assess intra- and inter-assay variation of the test procedure, seven serum samples ranging from negative to high positive were tested by the Diagnostic Automation, Inc. Lyme IgG ELISA. Eight replicates of each sample were tested on three consecutive days. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. These data are shown below:

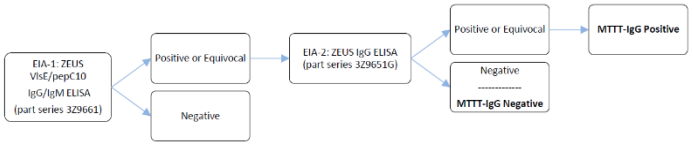
Table 3

	Intra-Assay (n= 8)						Inter-Assay (n= 3)	
	Day 1		Day 2		Day 3			
Sample #	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV
1	1.75	8.2%	1.76	6.0%	1.50	9.6%	1.67	7.2%
2	1.22	3.9%	1.21	6.5%	1.12	3.4%	1.18	3.8%
3	4.25	2.8%	4.30	6.7%	3.84	2.1%	4.13	5.0%
4	2.73	3.5%	3.14	2.1%	2.53	4.1%	2.80	9.1%
5	2.69	2.5%	2.97	7.1%	2.64	4.3%	2.77	15.2%
6	0.49	5.5%	0.60	5.1%	0.59	8.1%	0.56	8.9%
7	0.35	7.4%	0.43	2.1%	0.39	8.1%	0.39	8.4%

3. **MTTT (2-EIA) Performance Characteristics**
The following studies were conducted to determine the performance of the DAI ELISA Borrelia burgdorferi IgG Test System as a second-tier assay in the modified two-tier testing (MTTT) or the 2-EIA protocol.

a. **MTTT-IgG Method Comparison**: The DAI ELISA *B.burgdorferi* IgG Test System was utilized as the second-tier assay in a MTTT protocol as depicted in the flow chart below. The EIA used in the first-tier was DAI ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System. Performance of MTTT-IgG versus STTT was assessed using two separate cohorts; a retrospective cohort and a prospective cohort.

Flow Chart: MTTT-IgG Algorithm



b. **Retrospective Cohort:** The 356-sample retrospective cohort consisted of the 280 member CDC Premarketing Panel that was supplemented with an additional 46 Stage 2 Lyme Disease (LD) specimens and an additional 30 Stage 3 LD specimens. Therefore, the retrospective panel consisted of 166 cases of LD (60 Stage 1, 56 Stage 2 and 50 Stage 3), 90 specimens from diseases other than LD and 100 healthy controls (50 endemic and 50 non-endemic).

Initially, the 356 retrospective samples were tested with the first-tier assay, DAI ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System. There were 160 positive and 6 equivocal results. In the STTT-IgG protocol the samples that were positive or equivocal (n=166) were tested with *B. burgdorferi* IgG Western blot. In the MTTT-IgG protocol the samples (n=166) were tested on a second EIA, the DAI ELISA Borrelia burgdorferi IgG Test System. The second-tier EIA equivocal and positive results were considered positive. The equivocal and positive results were added together, and the results compared with the STTT-IgG positive results. Table 4 shows the outcome of MTTT-IgG as compared to the STTT-IgG protocol.

Table 4: Comparison of MTTT-IgG and STTT (IgG) Results for Retrospective Cohort

	Stage I (n=60)		Stage II (n=56)		Stage III (n=50)		Healthy Controls (n=100)		Disease Controls (n=90)	
	STT T-IgG	MTT T-IgG	STT T-IgG	MTT T-IgG	STT T-IgG	MTT T-IgG	STTT-IgG	MTTT-IgG	STT T-IgG	MTT TT-IgG
Positive	19	36	24	35	50	50	0	0	0	0
Negative	41	24	32	21	0	0	100	100	90	90
Sensitivity or PPA	31.7%	60.0%	42.9%	62.5%	100%	100%	N/A	N/A	N/A	N/A
Specificity or NPA	N/A	N/A	N/A	N/A	N/A	N/A	100%	100%	100%	100%

c. **Prospective Cohort Testing:** A prospective cohort of serum samples sent to a laboratory for routine *Borrelia* serology was assembled. These specimens were collected from three different geographical locations in the US, all from areas endemic to LD. Two of the three sites (Massachusetts and Minnesota) collected the specimens and performed the respective ELISA testing. One site (Wisconsin) collected the specimens and sent them to the manufacturer for the respective ELISA testing. The three sites and their corresponding number of specimens have been summarized in Table 5 below.

Table 5: Summary of the Prospective Specimen Cohort

Geographic Location	Sample Size (n)
Massachusetts	900
Wisconsin	990
Minnesota	1042
Total	**2932**

Initially, the 2,932 prospective samples were tested with the first-tier assay, DAI ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System. There were 363 positive and 58 equivocal results. In the STTT protocol the samples that are positive or equivocal (n=421) are tested with B. burgdorferi IgG Western blot. In the MTTT-IgG protocol the samples (n=421) were tested on a second ELISA, the ELISA Borrelia burgdorferi IgG Test System. The second-tier EIA equivocal and positive results were considered positive. The equivocal and positive results were added together, and the results compared with the STTT positive results. A summary of the outcome of STTT (IgG) versus MTTT-IgG appears in Table 6 below.

Table 6: MTTT-IgG Method Compared to STTT (IgG) Method in the Prospective Cohort

		STTT (IgG)		
		Positive	Negative	Total
MTTT-IgG	Positive	115	77**	192
	Negative	10*	2730	2740
	Total	125	2807	2932

Positive Agreement: 92.0% (115/125) 95% CI: 85.9 – 95.6%

Negative Agreement: 97.3% (2730/2807) 95% CI: 96.6 – 97.8%
*Of the 10 samples that were STTT-IgG positive/MTTT-IgG negative, five did not have clinical information consistent with Lyme disease, one had clinical evidence of a past infection, three had clinical data consistent with Stage 1 LD and one had no clinical information available.
**Of the 77 samples that were MTTT-G positive/STTT-G negative, Twenty-four samples did not have clinical information consistent with Lyme disease, three had evidence of a past infection, two had clinical information consistent with Stage 1 Lyme disease and 48 had no clinical data available.

LIMITATIONS OF THE ASSAY

1. The MTTT study was conducted using the ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System as the first-tier assay and the ELISA *Borrelia burgdorferi* IgG Test System as the second-tier assay with testing performed in that order. The performance characteristics of the device are not established for changing the order of testing or for substituting other EIA assays in the MTTT (2-EIA) procedure.
2. Sera from patients with other spirochetal diseases (syphilis, yaws, pinta, leptospirosis, and relapsing fever), or infectious mononucleosis and systemic lupus erythematosus may give false positive results (8, 9). In cases where false positive reactions are observed, extensive clinical epidemiologic and laboratory workups should be carried out to determine the specific diagnosis. False positive sera from syphilis patients can be identified by running an RPR and a treponemal antibody assay on such specimens (10). True *B. burgdorferi* disease positive sera will be negative in these assays.
3. False negative results may be obtained if serum samples are drawn too early after onset of disease before antibody levels have reached significant levels (8). Also, early antibiotic therapy may abort an antibody response to the spirochete (11).
4. Interpret all data in conjunction with clinical symptoms of disease, epidemiologic data, exposure in endemic areas, and results of other laboratory tests.
5. 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 is suspected.
6. The performance characteristics of the DAI ELISA *B. burgdorferi* IgG Test System are not established with samples from individuals vaccinated with *B. Burgdorferi* antigens.

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening – strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
2°C – 8°C	Conjugate – DO NOT FREEZE.
2°C – 8°C	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent
2°C – 25°C	Stop Solution: 2-25°C Wash Buffer (1X): 20-25°C for up to 7 days, 2-8°C for 30 days. Wash Buffer (10X): 2-25°C

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The 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, handle these products 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).
5. 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.
6. 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.
7. The Sample Diluent, Controls, Conjugate and Wash Buffer 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the 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. Do not use reagents from other sources or manufacturers.
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. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a 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.
26. 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.

REFERENCES

1. Steere AC, Taylor E, Wilson ML, Levine JF, and Spielman A: Longitudinal assessment of the clinical and epidemiologic features of Lyme disease in a defined population. J. Infect. Dis. 154:295-300, 1986.
2. Rosenfeld MEA:Serodiagnosis of Lyme disease. J. Clin. Microbiol. 31:3090-3095, 1993.
3. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, and Marawista SE: The spirochetal etiology of Lyme disease. New Engl. J. Med. 308:733, 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 Testig Program. J. Clin. Microbiol. 35:537-543, 1997.
5. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens. Final Rule. Fed. Register 56:64175-64182, 1991.
6. Procedures for the collection of diagnostic blood specimens by venipuncture - Second edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
7. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
8. 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.
9. Magnarelli LA, Anderson JF, and Johnson RC: Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J. Infect. Dis. 156:183-188, 1987.
10. Hunter EF, Russell H, Farshy CE, *et al:* Evaluation of sera from patients with Lyme disease in the fluorescent treponeme antibody-absorption test for syphilis. Sex. Trans. Dis. 13:236, 1986.
11. Steere AC, Hutchinson GJ, Rahn DW, Sigal LH, Craft JE, DeSanna ET, and Malawist SE: Treatment of the early manifestations of Lyme disease. Ann. Intern. Med. 99:22, 1983.
12. Craft JE, Grodzicki RL, Shrestha M, Fischer DK, Garcia-Bianco M, and Steere AC: Antibody response in Lyme disease. Yale J. Biol. Med. 57:561, 1984.
13. Shrestha M, Grodzicki RL, and Steere AC: Diagnosing early Lyme disease. Am. J. Med. 78:235, 1985.
14. Reik L, Smith L, Khan A, and Nelson W: Demyelinating encephalopathy in Lyme disease. Neurology 35:267, 1985.
15. Barbour A:Laboratory Aspects of Lyme Borreliosis. Clin Micr. Rev. 1:399-414, 1988.
16. 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.
17. Branda JA, *et al.* Two-Tiered Antibody Testing for Lyme Disease With Use of 2 Enzyme Immunoassays, a Whole-Cell Sonicate Enzyme Immunoassay Followed by a VlsE C6 Peptide Enzyme Immunoassay. Clin Infect Dis **2011**; 53:541–547.
18. Mollins CR, *et al.* Lyme Boreliosis Serology: Performance of Several Commonly Used Laboratory Diagnostic Tests and a Large Resource Panel of Well Characterized Patient Specimens. J Clin Microbiol **2016**; 54:2726-2734.
19. Branda JA, *et al.* Advances in Serodiagnostic Testing for Lyme Disease Are at Hand. Clin Infect Dis 2018 Mar 19;66(7):1133-1139

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2023-09**
Brand Name	**AccuDiag™**
REF 1428-P1	**AccuDiag™ - Lyme Disease IgG ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands** www.cepartner4u.eu
Revision Date: 2020-01-27	