INSTRUCTIONS FOR USE

EN

Anti-Borrelia VlsE1/pepC10 IgG/IgM

REF 3Z9661
SM3Z9661
3Z9661B

IVD

CE

Rx Only

Σ 96

Σ 480

INTENDED USE

The Anti-Borrelia VlsE1/pepC10 IgG/IgM is intended for the qualitative detection of IgG and IgM class antibodies to VlsE1 and pepC10 antigens from *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of having Lyme disease.

Positive and equivocal test results with the Anti-Borrelia VlsE1/pepC10 IgG/IgM 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 or IgM Western blot testing;

or

(2) Modified two-tier test methodology (MTTT) using one or more of the following three ELISA-based assays:

- Anti-B. burgdorferi IgG/IgM Test System
- Anti-B. burgdorferi IgM Test System
- Anti-B. burgdorferi IgG 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 BACKGROUND

Borrelia burgdorferi is a spirochete that causes Lyme disease. The organism is transmitted by ticks of the genus *Ixodes*. In endemic areas, these ticks are commonly found 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 widespread 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). Although IgM only may be detected 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 which has cross reactive components. Patients in early stages of infection may not produce detectable levels of antibody. Also, 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 exclusively 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 (9,10,11) 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.

Various antigens have been tested in recent years to improve the serological diagnosis of Lyme disease. One such attempt has been to test for antibodies towards VlsE1 and pepC10 antigens. This assay detects IgG and IgM antibodies towards both VlsE1 and pepC10 antigens.
The Anti-Borrelia VlsE1/pepC10 IgG/IgM is designed to detect IgG and IgM class antibodies in human sera to VlsE1 and pepC10 antigens. 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 and IgM is added to the wells and the plate is incubated. The Conjugate will react with IgG and/or IgM antibodies 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.

PRINCIPLE OF THE ASSAY

The Anti-Borrelia VlsE1/pepC10 IgG/IgM is designed to detect IgG and IgM class antibodies in human sera to VlsE1 and pepC10 antigens. 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 and IgM is added to the wells and the plate is incubated. The Conjugate will react with IgG and/or IgM antibodies 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.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator and SAVe Diluent®.

Kit Component	Quantity Σ 96	Quantity Σ 480	Description
PLATE	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated VlsE1 and pepC10 antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG and IgM in 15mL, white-capped bottle(s). Ready to use.
CTRL +	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s). 21X concentrate.
CAL	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s). 21X concentrate.
CTRL -	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s). 21X concentrate.

DIL | SPE	1	4	SAVe Diluent®: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN | TMB	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN | STOP	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH | 10X	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) 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.**

NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

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).

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 remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, 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 SAVe Diluent®, Controls, and Calibrator 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. Allow 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 Test System.
27. 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.
28. 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 Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI 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, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (6, 7). Do not use if there are any added anticoagulants or preservatives. 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 10 days. If a delay in testing is anticipated, store test sera 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 (8).

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.

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

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. Ensure that the samples are properly mixed. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Use a different pipette tip for each sample.
5. Add 100µL of SAVe 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.
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 1. and 2. 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 the 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 the 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 the 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 of 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 minutes.

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.
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 CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor*: The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System 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/OD Ratios*: Calculate the Index Value/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
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/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 VlsE1 and pepC10 detected. If exposure to *B. burgdorferi* is suspected, a second sample should be collected and tested two to four weeks later.

b. An OD ratio ≥1.10 indicates that antibodies specific to *B. burgdorferi* were detected. This indicates presumptive evidence of probable exposure. The specimen should be tested by the second step IgG and/or IgM Western blot.
c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) indicate that antibodies specific to *B. burgdorferi* were detected. This indicates presumptive evidence of probable exposure. The specimen should be tested by the second step IgG and/or IgM Western blot.

3. **MTTT (2-EIA) Interpretation for IgG/IgM:**
In addition to being used in the standard two-tier testing (STTT) method, this device may be used in the 2-EIA or modified two-tier testing (MTTT) protocol for the detection of IgG/IgM antibodies to *B. burgdorferi* in the following way.
   a. The samples must be tested with the Anti-Borrelia VlsE1/pepC10 IgG/IgM.
   b. All the positive and equivocal samples must then be tested with the Anti-B. burgdorferi IgG/IgM 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/IgM antibodies and exposure to *B. burgdorferi.*
4. **MTTT (2-EIA) Use and Interpretation for IgM Antibody Detection:**
In addition to being used in the standard two-tier testing (STTT) method, this device may be used in the 2-EIA or modified two-tier testing (MTTT) protocol for the detection of IgM antibodies to *B. burgdorferi* in the following way.
   a. The samples must be tested with the Anti-Borrelia VlsE1/pepC10 IgG/IgM.
   b. All the positive and equivocal samples must then be tested with the Anti-B. burgdorferi IgM 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 IgM antibodies and exposure to *B. burgdorferi.*
5. **MTTT (2-EIA) Use and Interpretation for IgG Antibody Detection:**
In addition to being used in the standard two-tier testing (STTT) method, this device may be used in the 2-EIA or modified two-tier testing (MTTT) protocol for the detection of IgG antibodies to *B. burgdorferi* in the following way.
   a. The samples must be tested with the Anti-Borrelia VlsE1/pepC10 IgG/IgM.
   b. All the positive and equivocal samples must then be tested with the Anti-B. 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.*

LIMITATIONS OF THE ASSAY

1. The MTTT study was conducted using the Anti-Borrelia VlsE1/pepC10 IgG/IgM as the first-tier assay and the Anti-B. burgdorferi IgG/IgM Test System or the Anti-B. burgdorferi IgM Test System or the Anti-B. burgdorferi IgG Test System as the second-tier assay with testing performed in that order. The performance characteristics of the device have not been established for the alternate order of testing or for the use of other EIA assays in the MTTT (2-EIA) procedure.
2. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.
3. Do not perform as a screening procedure for the general population. The predictive value of a positive or negative result depends on the prevalence of analyte (antibodies present to VlsE1 and pepC10 antigens) in a given patient population. Test only when clinical evidence suggests the diagnosis of *Borrelia* infection or related etiological conditions observed by the physicians.
4. Hemolytic, icteric, or lipemic samples and specimens with abnormal IgG and RF antibody concentrations may interfere with the outcome of this assay. Avoid the use of these types of specimens.
5. Interpret test results of specimens from immunosuppressed patients with caution.
6. Performance characteristics of this device have not been established for matrices other than serum.
7. Performance characteristics of this device have not been established with specimens containing heterophile antibodies, which are known to cause false positive results in various immunoassays.
8. Specimens known to contain potentially cross reactive antibodies to *B. burgdorferi* with infections to tick-borne relapsing fever, rickettsial diseases, ehrlichiosis, babesiosis, and leptospirosis have not been tested, therefore the performance of this device is unknown if there is any cross-reactivity with these antibodies.
9. No fresh samples were tested in the prospective studies.
10. This test will not distinguish results that are both IgG and IgM positive from results that are either IgG or IgM positive.

EXPECTED RESULTS

Demographics and Age Distribution:

Internal and external investigators assessed the device's performance with 775 masked samples prospectively collected from patients between the ages of 3 and 105 that were submitted for *Borrelia* antibody testing. Site One, the manufacturer's research facility, tested 575 samples acquired from the New York region. Site Two, a hospital laboratory in the northeast, tested 100 samples acquired in California. Site Three, a reference laboratory located in the northeast, tested 100 samples acquired in Pennsylvania. Internal and external

investigators also assessed the device's performance with varying populations. Available patient demographics, quantity of samples tested and the number of samples which tested positive for each population are summarized in Table 1.

Table1: Anti-Borrelia VlsE1/pepC10 IgG/IgM Demographics

Populations	Number Tested	Gender		Age Range	Positive/Tested
		Male	Female		
Prospective	775	297	474	3 - 105	63/775
Characterized	100	58	42	3 - 91	92/100
Endemic Controls	200	78	122	5 - 99	10/198
Non-Endemic Controls	200	100	100	18 - 88	3/200

PERFORMANCE CHARACTERISTICS

1. **Clinical Studies and Method Comparison With A Commercially Marketed ELISA Predicate Device:**

The clinical studies consisted of 1314 serum samples evaluated at three sites located in the United States. All serum samples evaluated for concordance were tested with the Multi-Lyte VlsE1/pepC10 IgG/IgM reference assay. The following populations were tested at a total of three clinical sites: Site One was the manufacturer's research facility. Site Two was a hospital laboratory located in the northeast and Site Three was a reference laboratory also located in the northeast.

a. **Characterized Samples (100 Samples):**

One hundred characterized samples were acquired and tested. All samples were from patients with a history of Borreliosis. Symptoms included tick exposure, EM rash, myalgias, arthralgias, fever, headache or stiff neck.

Table 2: Characterized Samples - Summary of Comparative Testing Results

	Anti-Borrelia VlsE1/pepC10 IgG/IgM				Predicate AtheNA Multi-Lyte® *Borrelia* VlsE1/pepC10				Western Blot (IgG and/or IgM)			
Clinical Diagnosis	+	-	Total	Agreement with Clinical Diagnosis	+	-	Total	Agreement with Clinical Diagnosis	+	-	Total	Agreement with Clinical Diagnosis
Acute Localized	68	8	76	89.5% (68/76)	67	9	76	88.2% (67/76)	54	22	76	71.1% (54/76)
95% CI				80.5 - 95.3%				78.7 - 94.4%				59.5 - 80.9
Early Disseminated	3	0	3	100% (3/3)	3	0	3	100% (3/3)	3	0	3	100% (3/3)
95% CI				36.8 - 100%				36.8 - 100%				36.8 - 100%
Late Disseminated	21	0	21	100% (21/21)	21	0	21	100% (21/21)	20	1	21	95.2% (20/21)
95% CI				86.7 - 100%				86.7 - 100%				76.2 - 99.9%
Total	92	8	100	92.0% (92/100)	91	9	100	91.0% (91/100)	77	23	100	77.0% (77/100)
				84.8 - 96.5%				83.6 - 95.8%				67.5 - 84.8%

NOTE 1: 7/8 samples which tested negative by ZEUS ELISA were negative by western blot.

NOTE 2: 21/23 samples which were negative by blot testing had bands present but did not meet the criteria for a positive result.

b. **Prospectively Collected Population (775 Samples):**

Three hundred prospectively collected masked samples from patients with a Lyme antibody test ordered were tested. The samples were submitted for Lyme antibody testing, sequentially numbered, de-identified and archived. After procurement, Site One, the manufacturer's research facility, tested 100 samples acquired from the New York region. Site Two, a hospital laboratory in the northeast, tested 100 samples acquired in California. Site Three, a reference laboratory located in the northeast, tested 100 samples acquired in Pennsylvania. Sites One, Two and Three comprise Prospective Study I. Site One tested an additional 475 prospective samples and compared the results to the predicate device. These comprise Prospective Study II.

Table 3: Prospective Study I Samples

		Predicate AtheNA Multi-Lyte® *Borrelia* VlsE1/pepC10				
		Positive	Negative	Total	PPA or NPA	95% CI
Anti-Borrelia VlsE1/pepC10 IgG/IgM	Positive	2	1	3	22.2% (2/9)	2.8% - 60.0%
	Equivocal	0	1	1		
	Negative	7	289	296	99.3% (289/291)	97.5% - 99.9%
	Total	9	291	300		

Table 4: Prospective Study I Second-Tier Testing: Western blot testing was performed on the samples positive or equivocal by the test device and the predicate. The following results were obtained:

	Results	Sample Size (n)	Western Blot IgG/IgM	
			Positive	Negative
Anti-Borrelia VlsE1/pepC10 IgG/IgM	**Positive**	3	2	1
	Equivocal	1	0	1
Predicate	**Positive**	9	1	8

Table 5: Prospective Study II Samples

		Predicate AtheNA Multi-Lyte® *Borrelia* VlsE1/pepC10				
		Positive	**Negative**	**Total**	**PPA or NPA**	**95% CI**
Anti-Borrelia VlsE1/pepC10 IgG/IgM	**Positive**	40	20	60	93.0% (40/43)	80.9 -98.6%
	Equivocal	2	0	2		
	Negative	1	412	413	95.3% (412/432)	92.4 - 96.8%
	Site Total	43	432	475		

Table 6: Prospective Study II Second-Tier Testing: Western blot testing was performed on the samples positive or equivocal by the test device and the predicate. The following results were obtained:

	Results	Sample Size (n)	Western Blot IgG/IgM	
			Positive	Negative
Anti-Borrelia VlsE1/pepC10 IgG/IgM	**Positive**	60	36	24
	Equivocal	2	2	0
Predicate	**Positive**	43	30	13

c. **CDC Characterized Lyme Panel (39 Samples):**

Forty-two samples of various reactivity were acquired from the CDC and evaluated internally at the manufacturer's site. Three samples were QNS or deteriorated and omitted from the calculations. From the remaining 39 samples, four samples were from normal blood donors and 35 samples were from patients diagnosed with Borreliosis. The results of the testing are presented here as a means of conveying further information on the performance of this assay with a characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Table 7: CDC Characterized Lyme Panel - Summary of Comparative Testing Results

	Anti-Borrelia VlsE1/pepC10 IgG/IgM				AtheNA Multi-Lyte® *Borrelia* VlsE1/pepC10				Western Blot (IgG and/or IgM)			
Time From Onset	+	-	Total	Agreement With Clinical Diagnosis	+	-	Total	Agreement With Clinical Diagnosis	+	-	Total	Agreement With Clinical Diagnosis
Normals	0	4	4	100% (4/4)	0	4	4	100% (4/4)	0	4	4	100% (4/4)
<1 Month	5	1	6	83.3% (5/6)	6	0	6	100% (6/6)	4	2	6	66.7% (4/6)
1 - 2 Months	5	1	6	83.3% (5/6)	6	0	6	100% (6/6)	5	1	6	83.3% (5/6)
3 - 12 Months	9	7	16	56.3% (9/16)	13	3	16	81.3% (13/16)	11	5	16	68.8% (11/16)
1 - 5 Years	4	0	4	100% (4/4)	1	3	4	25.0% (1/4)	4	0	4	100% (4/4)
>10 Years	3	0	3	100% (3/3)	1	2	3	33.3% (1/3)	3	0	3	100% (3/3)
Total	26	13	39	74.3% (26/35)	27	12	39	77.1% (27/35)	27	12	39	77.1% (27/35)

d. **Analytical Specificity:**

Testing of normal population was conducted on 200 samples acquired from individuals undergoing routine testing not infectious in nature in the New Jersey endemic area and 200 samples acquired from individuals undergoing routine testing not infectious in nature in the New Mexico non-endemic area.

Table 8: Analytical Specificity

Sample Type	Sample Size (n)	Negative	Equivocal	Positive	Positivity*
Endemic	200	189	1	10	5.0%
Non-endemic	200	197	0	3	1.5%

***Positivity with the predicate was found to be: endemic = 4.2%; non-endemic = 1.5%.**

e. **Precision and Reproducibility:**

Two separate studies were done to assess reproducibility; one was a five day, three site reproducibility study and the second was a 20 day single site repeatability study. The studies were conducted as follows: twelve samples were identified and/or

prepared (by ZEUS Scientific) for use in the two studies based upon their activity on the Anti-Borrelia VlsE1/pepC10 IgG/IgM. Selected samples were:

i. **Negative**: a sample with no analyte such that results of repeated testing of this sample are negative 100% of the time.
ii. **High Negative** (C5 concentration): a sample with an analyte concentration below the clinical cut-off such that results of repeated testing of this sample are negative approximately 95% of the time.
iii. **Low Positive** (C95 concentration): a sample with an analyte concentration above the clinical cut-off such that results of repeated testing of this sample are positive approximately 95% of the time.
iv. **Moderate Positive**: a sample with an analyte concentration such that results of repeated testing of this sample are positive 100% of the time.

Assay reproducibility was evaluated at three external clinical sites. To assess reproducibility, on each day of testing, each sample was diluted twice and then each dilution was run in triplicate. This was done twice per day by two different technicians and was repeated for five days. The results of the reproducibility study appear in Table 9. Assay repeatability was evaluated at the manufacturer site. On each day of testing, the samples were diluted twice and tested. This was repeated in a second run on the same day by a different technologist for a total of twenty days. The results of the repeatability study appear in Table 10.

Table 9: Reproducibility Results

Panel Member		Sample Size (n)	Mean IV	Within-Run		Within -Day		Between-Run		Between-Site		Total	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 1	Moderate +	180	2.33	0.12	5.0	0.14	6.0	0.09	4.0	0.15	6.3	0.15	6.5
Sample 2	Moderate +	180	2.23	0.16	6.9	0.18	8.2	0.10	4.6	0.19	8.6	0.19	8.6
Sample 3	Moderate +	180	2.56	0.14	5.3	0.17	6.4	0.11	4.3	0.19	7.3	0.19	7.4
Sample 4	Low +	180	1.29	0.09	6.7	0.11	8.4	0.07	5.7	0.11	8.6	0.11	8.7
Sample 5	Low +	180	1.35	0.09	6.5	0.11	8.2	0.08	6.2	0.11	8.4	0.12	8.7
Sample 6	Low +	180	1.36	0.09	6.6	0.11	8.2	0.08	6.1	0.12	8.7	0.12	8.9
Sample 7	High -	180	0.74	0.05	6.6	0.06	7.8	0.04	4.9	0.06	8.1	0.06	8.1
Sample 8	High	180	0.61	0.05	7.6	0.05	9.0	0.04	5.8	0.05	9.0	0.06	9.1
Sample 9	High	180	0.60	0.04	7.5	0.06	9.3	0.04	6.2	0.06	9.6	0.06	10.2
Sample 10	Negative	180	0.25	0.03	11.5	0.04	14.6	0.03	10.1	0.04	15.2	0.04	15.8
Sample 11	Negative	180	0.40	0.03	6.8	0.03	8.3	0.02	5.8	0.04	9.1	0.04	10.5
Sample 12	Negative	180	0.30	0.02	7.7	0.03	9.7	0.02	7.4	0.03	10.3	0.03	10.9
Control	Negative	180	0.16	0.01	7.9	0.02	10.7	0.01	8.7	0.02	11.5	0.02	11.7
Calibrator	Positive	180	2.34	0.12	5.3	0.13	5.5	0.03	1.4	0.13	5.6	0.13	5.6
Control	Positive	180	4.23	0.26	6.0	0.28	6.6	0.12	2.8	0.34	8.0	0.34	8.1

Table 10: Repeatability Results

Panel Member		Sample Size (n)	Mean IV	Within-Run		Within -Day		Between-Run		Total	
				SD	% CV	SD	% CV	SD	% CV	SD	% CV
Sample 1	Moderate +	80	2.34	0.09	4.1	0.18	7.5	0.17	7.3	0.22	9.3
Sample 2	Moderate +	80	2.38	0.10	4.2	0.17	7.0	0.16	6.8	0.22	9.3
Sample 3	Moderate +	80	2.28	0.09	4.1	0.17	7.4	0.16	7.0	0.22	9.5
Sample 4	Low +	80	1.32	0.07	5.2	0.12	8.9	0.11	8.3	0.13	9.5
Sample 5	Low +	80	1.38	0.07	5.4	0.10	7.1	0.07	5.3	0.13	9.1
Sample 6	Low +	80	1.32	0.08	5.8	0.11	8.1	0.08	6.1	0.12	9.3
Sample 7	High -	80	0.72	0.04	5.2	0.05	7.2	0.04	5.7	0.08	10.9
Sample 8	High	80	0.65	0.03	4.2	0.05	7.3	0.04	6.8	0.08	12.3
Sample 9	High	80	0.61	0.05	7.3	0.07	11.0	0.06	9.2	0.09	14.5
Sample 10	Negative	80	0.26	0.02	7.2	0.03	13.4	0.03	13.1	0.05	18.7
Sample 11	Negative	80	0.42	0.02	3.8	0.03	6.4	0.02	6.1	0.06	13.2
Sample 12	Negative	80	0.32	0.03	7.9	0.04	11.5	0.03	8.7	0.06	20.0
Control	Negative	80	0.18	0.02	11.6	0.03	14.9	0.02	10.0	0.03	18.0
Calibrator	Positive	80	2.34	0.19	7.9	0.20	8.5	0.09	4.0	0.23	9.8
Control	Positive	80	4.41	0.15	3.4	0.31	6.9	0.31	6.9	0.34	7.7

f. **Cross Reactivity:**

A study was conducted to assess cross reactivity with the Anti-Borrelia VlsE1/pepC10 IgG/IgM using sera that were sero-positive to EBV VCA IgG, ANA, Syphilis, CMV IgG, CMV IgM, Rubella IgG, VZV IgM, Toxoplasma IgG, and RF. ELISA and micro-particle immunoassay test systems manufactured by various companies for commercial distribution were used to determine the sero-positivity of the samples. Additionally, samples requested to be obtained from patients diagnosed with RA, Parvovirus, fibromyalgia, multiple sclerosis and H. pylori were purchased commercially. Ten samples for each possible cross-reactant were tested. The cross-reactivity data has been summarized in the following Table 11. In total, 140 samples were tested for possible cross reactivity with 14 analytes. Three out of 140 samples tested positive. Further testing with Western Blot revealed that the one of the samples positive for H. pylori was blot positive (weak IgM) and the other sample was blot negative. The sample positive for Parvovirus was blot negative. Some anti-Borrelia activity is indicated but 2/3 samples do not meet the two-tier criteria for positivity.

Table 11: Cross Reactivity Results

Possible Cross-Reactants	Positive Results/Number Tested
EBV VCA IgG	0 / 10
ANA	0 / 10
Syphilis	0 / 10
CMV IgG	0 / 10
CMV IgM	0 / 10
Rubella IgG	0 / 10
Toxo IgG	0 / 10
VZV IgM	0 / 10
RF	0 / 10
RA	0 / 10
Parvovirus	1 / 10
Fibromyalgia	0 / 10
Multiple Sclerosis	0 / 10
H. pylori	2 / 10

g. **Interfering Substances:**

The effect of potential interfering substances was determined on samples using the investigational device with the following: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The quantity of analyte of each potential interfering substance is as follows:

Bilirubin: 1mg/dL (low), 15mg/dL (high)
Albumin: 3.5g/dL (low), 5g/dL (high)
Cholesterol: 150mg/dL (low), 250mg/dL (high)
Triglycerides: 150mg/dL (low), 500mg/dL (high)
Hemoglobin: 10g/dL (low), 20g/dL (high)
Intralipid: 300mg/dL (low), 750mg/dL (high)

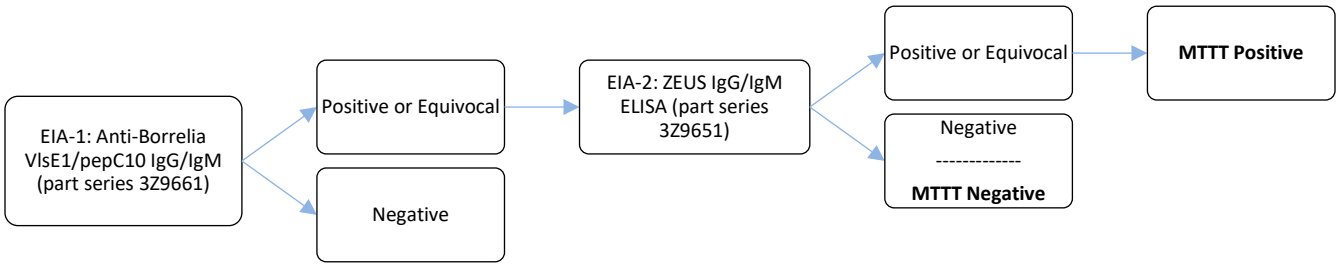
Three samples were chosen based on their performance on the Anti-Borrelia VlsE1/pepC10 IgG/IgM: positive, borderline and negative. The samples were exposed to the possible interfering substances and tested. All positive samples exhibited a change of signal <15%. All borderline samples showed a change of signal less than 15% except for the samples with the low and high spikes of hemoglobin which exhibited a reduction of signal greater than 15% (16.5% and 17.3% respectively). All negative samples showed a change of signal less than 15% except for the sample with the low spike of hemoglobin which exhibited a reduction of signal of 22.2%.

2. **MTTT (2-EIA) Performance Characteristics**

The following studies were conducted to determine the performance of the Anti-Borrelia VlsE1/pepC10 IgG/IgM as a first-tier assay in the modified two-tier testing (MTTT) or the 2-EIA protocol.

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

Flow Chart 1: MTTT IgG/IgM Algorithm



Retrospective Cohort Testing: 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, Anti-Borrelia VlsE1/pepC10 IgG/IgM. There were 160 positive and 6 equivocal results. In the STTT protocol the samples that were positive or equivocal (n=166) were tested with B. burgdorferi IgM and/or IgG Western blots. In the MTTT-IgG/IgM protocol the samples (n=166) were tested on a second EIA, the Anti-B. burgdorferi IgG/IgM 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. Table 12 shows the outcome of MTTT-IgG/IgM as compared to the STTT protocol.

Table 12: Comparison of MTTT-IgG/IgM and STTT (IgG and/or IgM) Results for Retrospective Cohort

	Stage I (n=60)		**Stage II (n=56)**		**Stage III (n=50)**		**Healthy Controls (n=100)**		**Disease Controls (n=90)**	
	STTT-IgG/IgM	MTTT-IgG/IgM	STTT-IgG/IgM	MTTT-IgG/IgM	STTT-IgG/IgM	MTTT-IgG/IgM	STTT-IgG/IgM	MTTT-IgG/IgM	STTT-IgG/IgM	MTTT-IgG/IgM
Positive	38	47	34	37	50	50	0	0	0	2
Negative	22	13	22	19	0	0	100	100	90	88
Sensitivity or PPA	**63.3%**	**78.3%**	**60.7%**	**66.1%**	**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%**	**97.8%**

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 13 below:

Table 13: 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, Anti-Borrelia VlsE1/pepC10 IgG/IgM. 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* IgM and/or IgG Western blots. In the MTTT-IgG/IgM protocol the samples (n=421) were tested on a second ELISA, the Anti-B. burgdorferi IgG/IgM 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 versus MTTT-IgG/IgM appears in Table 14 below:

Table 14: MTTT-IgG/IgM Method compared to STTT (IgG and/or IgM) Method in the Prospective Cohort

		STTT (IgG and/or IgM)		
		Positive	Negative	Total
MTTT-IgG/IgM	Positive	167	63**	230
	Negative	12*	2690	2702

	Total	179	2753	2932

Positive Agreement: 93.3% (167/179) 95% CI: 88.6 – 91.6.0%

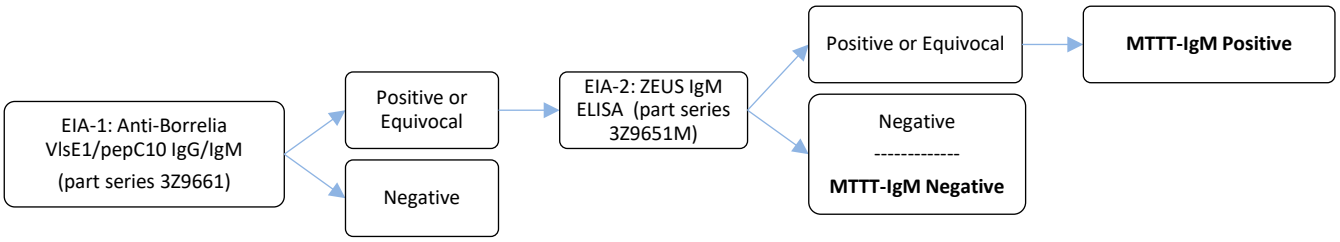
Negative Agreement: 97.7% (2690/2753) 95% CI: 97.1 – 98.2%

*Of the 12 samples that were STTT positive/MTTT negative, one of the 12 was confirmed to be a case of Stage 1 Lyme Disease. One sample had no clinical information available and the remaining ten did not have clinical information consistent with Lyme disease.

**Of the 63 samples that were MTTT positive/STTT negative, four samples were from confirmed cases of Lyme Disease (three Stage 1 and one late disease). Thirty two samples had no clinical information available and the remaining twenty seven specimens did not have clinical information consistent with Lyme disease.

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

Flow Chart 2: MTTT-IgM Algorithm



Retrospective Cohort Testing: 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, Anti-Borrelia VlsE1/pepC10 IgG/IgM. There were 160 positive and 6 equivocal results. In the STTT protocol the samples that were positive or equivocal (n=166) were tested with B. burgdorferi IgM Western blot. In the MTTT-IgM protocol the samples (n=166) were tested on a second EIA, the Anti-B. burgdorferi IgM 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. Table 15shows the outcome of MTTT-IgM as compared to the STTT protocol.

Table 15: Comparison of MTTT-IgM and STTT (IgM) Results for Retrospective Cohort

	Stage I (n=60)		**Stage II (n=56)**		**Stage III (n=50)**		**Healthy Controls (n=100)**		**Disease Controls (n=90)**	
	STTT-IgM	MTTT-IgM	STTT-IgM	MTTT-IgM	STTT-IgM	MTTT-IgM	STTT-IgM	MTTT-IgM	STTT-IgM	MTTT-IgM
Positive	28	46	28	42	8	36	0	0	0	2
Negative	32	14	28	14	42	14	100	100	90	88
Sensitivity or PPA	**46.7%**	**76.7%**	**50.0%**	**75.0%**	**16.0%**	**72.0%**	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%**	**97.8%**

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. The three sites and their corresponding number of specimens have been summarized in Table 13.

Initially, the 2,932 prospective samples were tested with the first-tier assay, Anti-Borrelia VlsE1/pepC10 IgG/IgM. 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 IgM Western blot. In the MTTT-IgM protocol the samples (n=421) were tested on a second ELISA, the Anti-B. burgdorferi IgM 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 (IgM) positive results. A summary of the outcome of STTT (IgM) versus MTTT-IgM appears in Table 16 below:

Table 16: MTTT-IgM Method Compared to STTT (IgM) Method in the Prospective Cohort

		STTT (IgM)		
		Positive	Negative	Total
MTTT-IgM	Positive	101	126**	227
	Negative	4*	2701	2705
	Total	105	2872	2932

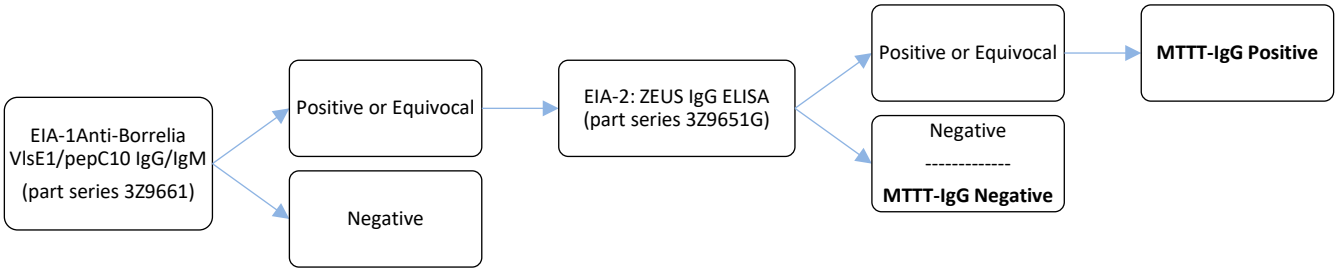
Positive Agreement: 96.2% (101/105) 95% CI: 90.6 – 98.5%
Negative Agreement: 95.5% (2701/2827) 95% CI: 94.7 – 96.2%

*Of the 4 samples that were STTT-IgM positive/MTTT-IgM negative, three did not have clinical information consistent with Lyme disease and one had no clinical information available.

**Of the 126 samples that were MTTT-M positive/STTT-M negative, twenty-eight samples did not have clinical information consistent with Lyme disease, two had evidence of a past infection, five had clinical information consistent with Stage 1 Lyme disease and 91 had no clinical data available.

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

Flow Chart 3: MTTT-IgG Algorithm



Retrospective Cohort Testing: 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, Anti-Borrelia VlsE1/pepC10 IgG/IgM. 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 Anti-B. 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. Table 17 shows the outcome of MTTT-IgG as compared to the STTT-IgG protocol.

Table 17: 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)**	
	STTT-IgG	MTTT-IgG	STTT-IgG	MTTT-IgG	STTT-IgG	MTTT-IgG	STTT-IgG	MTTT-IgG	STTT-IgG	MTTT-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%**

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. The three sites and their corresponding number of specimens have been summarized in Table 13.

Initially, the 2,932 prospective samples were tested with the first-tier assay, Anti-Borrelia VlsE1/pepC10 IgG/IgM. 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 Anti-B. 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. A summary of the outcome of STTT (IgG) versus MTTT-IgG appears in Table 18 below:

Table 18: 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.

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
CE	Conformity with Directive 98/79		

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