Borrelia VlsE1/pepC10 IgG/IgM Test System

IVD

REF 3Z9661

INTENDED USE

The ZEUS ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System 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 with a history of Lyme Borreliosis. All positive and equivocal specimens should be tested with a second-tier test such as Western Blot, which if positive, is supportive evidence of infection with *Borrelia burgdorferi*. Diagnosis of Lyme Borreliosis should be made based on the presence of *B. burgdorferi* antibodies, history, symptoms, and other laboratory data. Negative first or second tier results should not be used to exclude *Borreliosis*. This kit is for *in vitro* diagnostic use.

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 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). 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 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.

Various antigens have been tested in recent years to improve in assisting in the 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.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA *Borreliai* VlsE1/pepC10 IgG/IgM Test System 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, Calibrators and SAVe Diluent®.**

Symbol		No.	Component
PLATE		1.	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		2.	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG and IgM. One, 15mL, white-capped bottle. Ready to use.
CONTROL	+	3.	Positive Control (Human Serum): One, 0.35mL, red-capped vial.
CAL		4.	Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
CONTROL	-	5.	Negative Control (Human Serum): One, 0.35mL, green-capped vial.
DIL	SPE	6.	SAVe Diluent®: One, 30mL, green-capped, bottle 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	7.	TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	8.	Stop Solution: One, 15mL, red-capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	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 Test System Lot Number dependent and may be used interchangeably with the ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ELISA Test System utilizing Product No. 005CC.**
2. **Test System also contains:**
   a. **Component Label containing lot specific information inside the Test System box.**
   b. **Package Insert providing instructions for use.**

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, 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 on 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 Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
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

Temperature	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.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe 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

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 48 hours. If delay in testing is anticipated, store test sera at at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

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. **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. Ensure that the samples are properly mixed. 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 5X.
   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 Reagent Blank well, at the same rate and in 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 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 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 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 2.

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) 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.

LIMITATIONS OF THE ASSAY

1. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. 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.
3. 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.
4. Interpret test results of specimens from immunosuppressed patients with caution.
5. Performance characteristics of this device have not been established for matrices other than serum.
6. 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.
7. 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.
8. No fresh samples samples were tested in the prospective studies.
9. 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 1, the manufacturer's research facility, tested 575 samples acquired from the New York region. Site 2, a hospital laboratory in the northeast, tested 100 samples acquired in California. Site 3, 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: ZEUS ELISA *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

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 1 was the manufacturer's research facility. Site 2 was a hospital laboratory located in the northeast and site 3 was reference laboratory also located in the northeast.

1. Characterized Samples (100)
2. Prospective Population (775)
3. CDC Lyme Panel (39)
4. Analyitical Specificity: Endemic and Non-Endemic Control Samples (200 each group)
5. Precision and Reproducibility
6. Cross Reactivity (Site 1 only)
7. Interferring Substance (Site 1 only)

1. **Characterized Samples:**
   100 characterized samples were acquired and tested at the manufacturer's facility. 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

	ZEUS ELISA *Borrelia* VlsE1/pepC10 IgG/IgM				Predicate VlsE1/pepC10 IgG/IgM				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 ere 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.

2. **Prospectively Collected Population:**
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 1, the manufacturer's research facility, tested 100 samples acquired from the New York region. Site 2, a hospital laboratory in the northeast, tested 100 samples acquired in California. The third site, a reference laboratory located in the northeast, tested 100 samples acquired in Pennsylvania. Sites 1, 2 and 3 comprise Prospective Study I. Site 1 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 Multi-lyte VlsE1/pepC10 IgG/IgM				
		Positive	Negative	Total	PPA or NPA	95% CI
ZEUS ELISA 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%
	Site 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
ZEUS ELISA 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 Multi-lyte VlsE1/pepC10 IgG/IgM				
		Positive	Negative	Total	PPA or NPA	95% CI
ZEUS ELISA 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
ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM	Positive	60	36	24
	Equivocal	2	2	0
Predicate	Positive	43	30	13

3. **CDC Characterized Lyme Panel:**
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, 4 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

	ZEUS ELISA *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)

4. **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%.**

5. **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, Inc.) for use in the two studies based upon their activity on the **ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System**. Selected samples were:
a. **Negative**: a sample with no analyte such that results of repeated testing of this sample are negative 100% of the time.
b. **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.
c. **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.
d. **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: Reproducibilty 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%

8. **Cross Reactivity:**
A study was conducted to assess cross reactivity with the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System 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

ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Cross Reactivity Study	
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

9. **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), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 10 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples were chosen based on their performance on the ZEUS ELISA Borrelia VlsE1/pepC10 IgG/IgM Test System: 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% with the exception of 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% with the exception of the sample with the low spike of hemoglobin which exhibited a reduction of signal of 22.2%.

REFERENCES

1. Steere AC, *et al*: J. Infect. Dis. 154:295-300, 1986.
2. Rosenfeld MEA:Serodiagnosis of Lyme disease. J. Clin. Microbiol. 31:3090-3095, 1993.
3. Steere AC, *et al*:The Spirochetal Etiology of Lyme Disease. N. Engl. J. Med. 308:733-740, 1983.
4. Bakken LL, Callister SM, Wand PJ, and Schell RF:Interlaboratory Comparison of Test Results for Detection of Lyme Disease by 516 Patients in the Wisconsin State Laboratory of Hygiene/College of American Pathologists Proficiency Testing Program. J. Clin. Microbiol. 35:537-543, 1997.
5. 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.

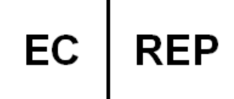
CE

ZEUS Scientific, Inc.
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111
International: +1 908-526-3744
Fax: +1 908-526-2058

ZEUS ELISA and SAVe Diluent® are trademarks of ZEUS Scientific, Inc.

Customer Service E-mail: orders@zeusscientific.com
Technical Service E-mail: support@zeusscientific.com
Website: www.zeusscientific.com

©2012 ZEUS Scientific, Inc. All Rights Reserved.



EMERGO EUROPE
Molenstratt 15
2513 BH, The Hague
The Netherlands
Tel: +31 (0)70 345 8570
Fax: +31 (0)70 346 7299