Immunetics® C6 Lyme ELISA™ kit
Cat. No. – DK-E352-096-A
96 Tests
For In Vitro Diagnostic Use

Intended Use:

The Immunetics® C6 Lyme ELISA™ kit is intended for use in the presumptive detection of IgG and IgM antibodies to *Borrelia burgdorferi* (*B. burgdorferi)* in human serum. The assay should only be used on samples from patients with clinical history, signs or symptoms consistent with *B. burgdorferi* infection, including individuals who have received the licensed recombinant OspA Lyme disease vaccine (Lymerix). Positive or equivocal results should be supplemented by testing with a standardized Western Blot (second step) method. Positive Western Blot results provide evidence for exposure to or infection with *B. burgdorferi*. The diagnosis of Lyme disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi*. Negative results (either first or second step) should not be used to exclude Lyme disease.

Summary:

Lyme disease is a multi-system illness caused by infection with the spirochete *B. burgdorferi*[1,2]. Transmission of the disease occurs through the bite of any of several species of *Ixodes* ticks, which are found in the United States[3-5], Europe[6], Russia, and other countries in Asia.

As it progresses through various stages, Lyme disease produces an array of clinical symptoms[2,7]. The first sign of infection (primary stage) is the development of a circular skin rash, termed 'erythema migrans' (EM) at the bite site. This occurs in 60-80 % of patients within a few days or weeks of the initial infection; some patients do not develop or may overlook it. General flu-like symptoms (headache, abdominal pain, and fatigue) frequently accompany this rash. Weeks to months later, the disease evolves into its secondary stage. This stage is usually characterized by musculoskeletal pain or arthritis, neurological abnormalities and/or cardiac complications. Brief arthritic attacks affecting large joints become more persistent with time and may resolve into a chronic condition in late infection (tertiary stage).

Serological testing has been demonstrated to be useful in detecting the antibody response to *B. burgdorferi*[8,9]. In most Lyme disease patients, a specific IgM antibody response peaks between three to six weeks after infection, but may persist throughout the course of the disease[10,11]. Subsequently, specific IgG antibodies are produced which rise in titer more slowly than the IgM antibodies. Although the IgG response may not be detectable for several weeks, it generally increases during the manifestation of arthritic symptoms (tertiary stage) and may remain elevated for years after clinical remission[10,11]. IgG and IgM antibodies to *B. burgdorferi* can be detected by first step assays such as ELISA. According to CDC/ASTPHLD recommendations[12], samples yielding equivocal or positive results by ELISA or other first step assays should be re-tested using a standardized Western Blot assay.

The antigen used in the Immunetics® C6 Lyme ELISA™ kit is a synthetic peptide (C6 peptide*) derived from the VlsE protein, which has been shown to be both specific and highly immunogenic[13-16]. The peptide sequence is conserved and equally antigenic in humans infected with *Borrelia burgdorferi* or with European genospecies including *Borrelia afzelii* and *Borrelia garinii*[16]. As the antigen represents a defined sequence within the protein, potential cross-reactivity with unrelated and partially related antigens found in other organisms is greatly reduced. Likewise, cross-reactivity in individuals vaccinated with the licensed recombinant OspA Lyme disease vaccine (Lymerix) is not observed.

Protected by patents US 6,475,492, EP1171605, AU767955, CA2370493

Principle:

The Immunetics® C6 Lyme ELISA™ kit is based on a synthetic peptide antigen (C6 peptide) in microwell ELISA format. The antigen is derived from the VlsE protein of *B. burgdorferi*. In the assay procedure, diluted serum samples are added to and incubated in wells of an antigen-coated microwell plate. Antibodies specific to the C6 peptide in the serum sample are bound by the immobilized antigen and unbound antibodies are removed by wash steps. The bound antibodies are detected by addition of a horseradish peroxidase-conjugated (HRP) goat anti-human IgG/IgM conjugate. After removal of excess conjugate by further wash steps, a chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) is added. A blue-green product is produced in wells where antibodies have been bound to

the antigen. The color development reaction is quenched by addition of dilute sulfuric acid, after which optical absorbance at 450 nm is measured in each well using an ELISA microplate reader.

Reagents Supplied:

1. Microwell Plate (Part # CB-P005-096). 96 wells, provided in twelve strips each containing 8 wells, coated with *Borrelia burgdorferi* synthetic peptide antigen. Stored in a re-sealable foil pouch with desiccant.
2. Positive Control (0.300 mL) (Part # CB-L018-300). Human source material containing a high titer of antibodies to *B. burgdorferi*, with Gentamicin and ProClin added as preservatives.
3. Cutoff Calibrator (0.500 mL) (Part # CB-L019-500). Human source material containing antibodies to *B. burgdorferi*, with Gentamicin and ProClin added as preservatives. Used to calibrate cutoff value of the assay.
4. Negative Control (0.300 mL) (Part # CB-N023-300). Human source material containing no detectable antibodies to *B. burgdorferi*, with Gentamicin and ProClin added as preservatives.
5. Conjugate (25 mL) (Part # CB-A028-025). Ready to use goat anti-human IgG/IgM-horseradish peroxidase conjugate.
6. Sample Diluent (60 mL) (Part # CC-S004-060). Ready to use dilution buffer containing Gentamicin and ProClin as preservatives.
7. TMB ELISA Substrate (25 mL) (Part # CC-S003-025). Ready to use solution containing tetramethylbenzidine (TMB).
8. 10X Wash Buffer Concentrate (60 mL × 3) (Part # CC-B001-060). Contains phosphate-buffered saline and Tween-20 detergent, with ProClin added as a preservative.
9. Stop Solution (30 mL) (Part # CC-S005-030). Ready to use solution of 1N sulfuric acid.

Precautions:

1. The human source materials used in this kit have tested negative by FDA approved methods for antibodies to HIV-1 and HIV-2, Hepatitis C and Hepatitis B surface antigen. However, because no test methods can offer complete assurance that those infectious agents are absent, all controls and test specimens should be handled as if capable of transmitting infectious agents. They should be considered as potentially infectious materials and handled at the Biosafety Level 2 as recommended in the CDC/National Institutes of Health manual "*Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition", 2009.
2. Wear proper personal protection while running the assay. Do not allow reagents to come into contact with skin, eyes or mouth, as irritation may result. Wash thoroughly with water in the event of any contact. Do not mouth pipette reagents.
3. Cap controls tightly after use to minimize evaporation.
4. Stop solution contains sulfuric acid. Waste containing stop solution should be brought to neutral pH before disposal. Caution: The addition of sodium hypochlorite (bleach) to solutions containing sulfuric acid will produce toxic chlorine gas.
5. Use fresh pipet tips for pipetting each sample and reagent. Re-use of pipet tips may introduce contamination.
6. Dispose of used assay reagents and samples by proper biohazard procedures.
7. Components of kit lots should be used only in the combination supplied, with the exception of 10X Wash Buffer which may be used interchangeably between lots. Do not use kit components beyond the expiration date on the label.
8. All kit components should be brought to room temperature (19 – 26 °C) prior to starting the assay.
9. Use only distilled or deionized water for preparation of buffers in the assay.
10. Wear gloves when handling microwell plate strips.
11. For optimal, reproducible and accurate performance of the test, follow instructions in package insert. Deviations from instructions in this insert may lead to false results.
12. Do not pre-wash wells with wash buffer or sample diluent.
13. Do not mix bottle caps of TMB ELISA Substrate or conjugate.

Material Required but not Supplied:

1. Graduated cylinders to measure liquid
2. 1 liter flask for Wash Buffer
3. Pipettors to dispense 10 µL to 200 µL (multichannel pipettor recommended or 10 µL, 100 µL and 200 µL pipettes)
4. Uncoated microwell plate or test tubes for sample dilution
5. Distilled or deionized water
6. Timer (0 – 60 minutes)
7. ELISA reader with 450 nm filter and a 590 – 650 nm reference filter
8. Vacuum aspirator or disposal means for assay solutions (multichannel aspirator recommended)
9. Absorbent towels

Optional Equipment:

1. Automated plate washer

Storage and Shelf Life of Reagents:

1. Store unopened kit between 2 – 8 °C. Expiration date is shown on the kit label.
2. Store opened kit components between 2 – 8 °C. Components must be used within 60 days of opening.
3. Unused microplate strips should be stored between 2 – 8 °C sealed within the original foil pouch with desiccant.
4. Kit components including Controls, HRP Conjugate, TMB ELISA Substrate, Stop Solution, Sample Diluent and 10X Wash Buffer Concentrate should be stored between 2 – 8 °C.
5. 1X (working) Wash Buffer solution can be stored at room temperature (19 – 26 °C) for up to 60 days.
6. TMB ELISA Substrate should be stored in light protective bottles that do not contain metallic ions. The shelf life of the substrate is based on storage in the plastic amber bottle that is provided with the kit.
7. Do not use kit components beyond the expiration date.

Specimen Collection:

1. Fresh human serum specimens should be collected and either stored at 2 – 8 °C if testing will take place within 10 days, or frozen.
2. Hemolyzed or lipemic sera, and sera subject to multiple freeze-thaw cycles may yield anomalous results.
3. Centrifuge sera for 1 minute at 10,000 rpm in a microcentrifuge to remove any visible precipitate prior to testing.
4. Do not test samples showing evidence of microbial contamination.
5. The assay is intended for use with human serum. The use of plasma with this assay has not been established.

Reagent, Control, and Sample Preparation:

1. 1X Wash Buffer. To prepare once, add 60 mL of 10X Wash Buffer Concentrate to 540 mL deionized/distilled water in a 1-liter flask and stir thoroughly. **Note:** On removal of the 10X Wash Buffer Concentrate from refrigeration, undissolved salts may be present. Allow the reagent to reach room temperature and shake the bottle to dissolve the salts.
2. Controls. Centrifuge Controls at 10,000 × g for 10 seconds before using to deposit all liquid at the bottom of the tube. (Prior to dispensing, if Controls are noticed to be excessively cloudy, have evidence of clotting or are difficult to pipette, centrifuge a second time for 1 minute at 10,000 rpm.) Dispense 200 µL of Sample Diluent into 5 clean test tubes or uncoated microplate wells. Add 10 µL of Negative Control to one tube or well; add 10 µL of Positive Control to another tube or well; add 10 µL of Calibrator to each of three tubes or wells and mix thoroughly.
3. Patient samples. Dispense 200 µL of Sample Diluent into each of a series of tubes or uncoated microplate wells, sufficient for the number of samples to be tested. Add 10 µL of each sample to the corresponding tube or well and mix thoroughly.

Assay Procedure:

Bring all assay reagents to room temperature (19 – 26 °C) before beginning the assay. All steps are performed at room temperature (19 – 26 °C).

1. Record the sample identity for each well on the provided record sheet to determine the number of strips necessary to perform the assay. Five wells will be needed for controls and calibrators. One well will be needed for each sample.
2. Remove the microplate frame containing the microplate strips from the foil pouch. Remove unneeded microplate strips from the frame and reseal unused strips in the foil pouch with desiccant. Microplate frame should be retained at the end of the assay to be used with the remaining microplate strips.
3. Add 100 µL of diluted Positive Control to one microwell and 100 µL of diluted Negative Control to another microwell.
4. Add 100 µL of diluted Calibrator to each of three microwells.
5. Add 100 µL of each diluted patient sample to microwells.
6. Incubate for 30 minutes.
7. Aspirate wells. If using manual or semi-automated washing manifold, wash three times as follows. Dispense approximately 150 µL (half of well volume) of 1X Wash Buffer into each well, then aspirate. Refill wells with approximately 300 µL of 1X Wash Buffer (full volume of well) and aspirate a second time. Refill wells with 300 µL of 1X Wash Buffer and aspirate a third time. Make sure that all wells have been aspirated after the third (final) wash step. (If an automated plate washer is used, wash four times with each wash consisting of 300-350µL of 1X Wash Buffer. After the final wash for both manual and automated washing, tap the plate on absorbent towels to remove all residual liquid.)
8. Dispense 100 µL of Conjugate into each well.

9. Incubate for 20 minutes.
10. Aspirate wells. Perform four wash steps with 1X Wash Buffer as in step 7 above.
11. After the final aspiration, invert, shake out and blot the plate against absorbent towels to remove all residual liquid.
12. Dispense 100 µL of TMB ELISA Substrate into each well.
13. Incubate for 4 minutes. Please note: Optimal assay performance requires precise timing of the TMB ELISA Substrate incubation step.
14. Dispense 100 µL Stop Solution into each well in the same order as the TMB ELISA Substrate was dispensed in the previous step. Tap the plate gently to mix contents of wells. Read absorbance values within 5 minutes.
15. Read Absorbance at 450 nm with a reference filter of 650 nm using an ELISA plate reader. If the reader is not equipped with a 650 nm filter, the use of an alternate filter between 590 – 650 nm will provide equivalent results.

Quality Control:

1. Control values must be within the following ranges in order for the assay to be considered valid:
2. Negative Control A_{450} must be <0.18
3. Each Calibrator A_{450} must be between 0.400 and 2.00
4. Positive Control A_{450} must be >1.2
5. If any control A_{450} value is not within the above ranges, the assay should be repeated.

Calculations:

1. Calculate the mean value for the three Calibrator Controls. If any Calibrator absorbance value differs by more than 0.1 absorbance units from the mean, discard the data point that is farthest from the mean. Recalculate the mean from the two remaining data points. If the Calibrator absorbance values still differ by more than 0.1 absorbance units from the mean, the assay is invalid and must be re-run. The mean Calibrator absorbance value must be between 0.4 and 2.0 absorbance units.
2. Calculate the assay cutoff value by dividing the mean Calibrator value by 2.150 (Correction Coefficient).
3. Calculate the Lyme Index value (LI) for each patient sample by dividing the A_{450} of the sample by the cutoff value.

Interpretation of Results:

Lyme Index	Interpretation
≤ 0.90	Negative result. No antibody to *B. burgdorferi* detected in the present assay. This result does not exclude the possibility of *B. burgdorferi* infection, and where early Lyme disease is suspected, a second sample should be drawn 2 – 4 weeks later and re-tested.
0.91 – 1.09	Equivocal result. The imprecision inherent in any method implies a lower degree of confidence in the interpretation of samples with A_{450} values very close to the calculated cutoff value. For this reason an equivocal category has been designated. Equivocal samples should be tested with a supplemental assay such as a standardized Western Blot test in accordance with CDC/ASTPHLD recommendations.
≥ 1.10	Positive result. Antibody to *B. burgdorferi* detected in the present assay. All positive results should be supplemented by re-testing the corresponding serum samples on a standardized Western Blot test in accordance with CDC/ASTPHLD recommendations[12].

The cutoff is determined for each assay run by dividing the mean calibrator value by the correction coefficient. In this way, the cutoff is intended to compensate for run-to-run assay variations, which might otherwise affect sensitivity and specificity. The calibrator has been designed to yield an absorbance value in the linear portion of the C6 ELISA dose-response curve. The correction coefficient was determined by analysis of C6 ELISA results for 131 normal donors and 108 well-characterized Lyme disease patients. In assay runs using two separate kit lots, the correction coefficient (2.15) was determined as the value which yielded a cutoff which minimized the number of false positive and of false negative results.

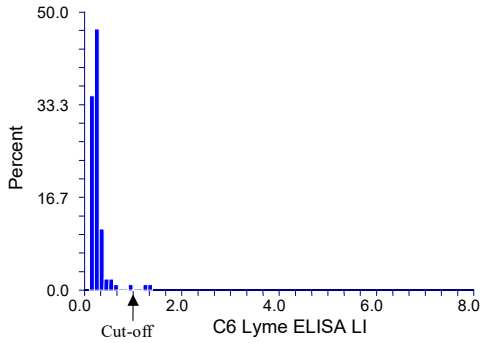
Limitations:

1. A negative result does not exclude the possibility of infection with *B. burgdorferi*. Patients in early stages of Lyme disease and those who have been treated with antibiotics may not exhibit detectable antibody titers. Patients with clinical history, signs or symptoms suggestive of Lyme disease should be re-tested in 2-4 weeks in the event that the initial test result is negative.
2. A positive result is not definitive evidence of infection with *B. burgdorferi*. It is possible that other disease conditions may produce artifactual reactivity in the assay. All equivocal or positive results should be supplemented by re-testing the corresponding serum samples on a standardized Western Blot test in accordance with CDC/ASTPHLD recommendations[12].
3. The C6 Lyme ELISA™ kit has been tested on serum samples from individuals vaccinated with a licensed OspA vaccine (Lymerix, , Lyme Disease Vaccine (Recombinant OspA) manufactured by GlaxoSmithKline Biologicals) ; see Expected Values for results. The performance of the test has not been determined on serum samples from recipients of other Lyme disease vaccines. 4. This assay should not be used to screen the general population. The predictive value of the assay is a function of the pre-test probability of Lyme disease in the population tested. Hence, only patients with clinical symptoms of Lyme disease or suspected exposure to *B. burgdorferi* should be tested.
5. Hemolyzed, lipemic, bilirubinemic or turbid samples may produce artifactual assay results. A fresh sample should be collected for re-testing.
6. Optimal performance requires strict adherence to the assay procedure described in the insert. Deviations from the procedure may lead to aberrant results.
7. False positive results may be obtained with sera from patients with diseases other than Lyme disease including syphilis, periodontal disease, rheumatoid arthritis, systemic lupus erythematosis, and other autoimmune diseases.

Expected Values:

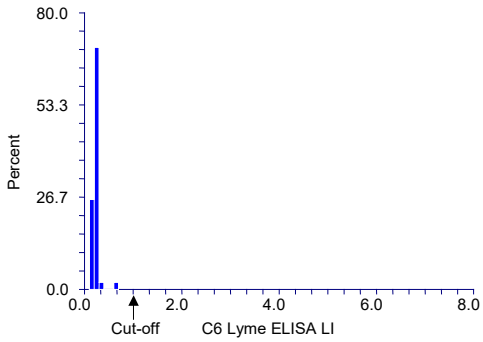
The distribution of LI values among normal, healthy blood donors is shown in the histogram in Figure 1. Of 98 serum samples from normal, healthy individuals residing in areas considered non-endemic for Lyme disease, 1 (1 %) was positive using the C6 Lyme ELISA™ kit. A population of 99 blood donor samples from an endemic area yielded 2 positive (2 %) and 1 equivocal (1 %) result. The overall frequency of positive or equivocal results in the combined population was 4 out of 198 (2 %).

Figure 1. Distribution of Lyme Index values in serum samples from normal individuals



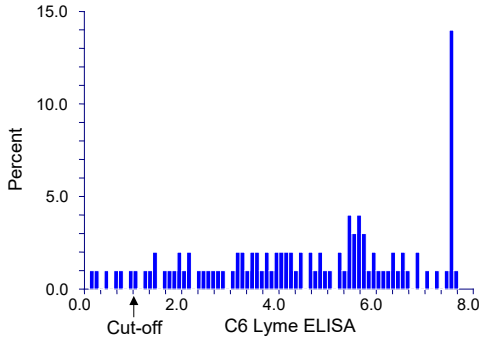
The response of the C6 Lyme ELISA™ kit in testing serum samples from individuals vaccinated with a licensed recombinant OspA vaccine against Lyme disease (Lymerix, manufactured by GlaxoSmithKline Biologicals) is shown in Fig. 2 below. Among 43 vaccinees, there were 0 positive or equivocal results.

Figure 2. Distribution of Lyme Index values in Lyme disease vaccine (Lymerix) recipients



In a group of 180 patients diagnosed with Lyme disease, including 141 sera which were positive by ELISA and Western Blot assays and 39 sera in a panel provided by the CDC, the C6 Lyme ELISA™ kit yielded 6 negative results (3 %); the distribution of LI values is shown in Fig. 3 below.

Figure 3. Distribution of Lyme Index values in Lyme disease patients



(Note: On the histogram, LI = 7.6 [equal to the maximum LI value observed for this panel] was assigned to all LI values derived from A-450 values which were off-scale, i.e. greater than the maximum readable value for the microplate readers used).

In a prospective population of 191 patient serum samples submitted for Lyme disease screening tests, 4 (2 %) were either positive or equivocal using the C6 Lyme ELISA™ (see Table 8 below).

Performance Characteristics:

Reactivity in Normal Population

One hundred ninety seven serum specimens were obtained from normal blood donors, comprising 99 sera from individuals residing in regions endemic for Lyme disease (northeastern U.S.) and 98 sera from individuals residing in areas considered non-endemic for Lyme disease (southwestern U.S.). Sera were tested on the C6 Lyme ELISA™ kit and on a whole cell sonicate (WCS) *B. burgdorferi* ELISA cleared for *in vitro* diagnostic use. Table 1 below summarizes the percent positive/equivocal results in each category, with corresponding number of specimens shown in parentheses (same format for all tables) and 95 % confidence interval (CI).

Table 1. Reactivity of C6 ELISA in Normal Population

	Endemic	Non-	Total	95 % CI
C6 Lyme ELISA Kit	3 % (3)	1 % (1)	2 % (4)	1-5 %
WCS *B. burgdorferi* ELISA	3 % (3)	8 % (8)	6 % (11)	3-10 %
197 (Samples)	99	98	197	

A panel of 50 sera was tested comprising 43 sera from individuals who were vaccinated with a licensed recombinant OspA vaccine (Lymerix, manufactured by GlaxoSmithKline Biologicals). The 43 individuals each received the full complement of 3 vaccine doses according to the manufacturer's recommended schedule at months 0, 1 and 12. Serum samples were taken in month 13 or later. Three additional sera in the panel were taken from individuals prior to vaccination, 3 sera were from individuals who were injected with a placebo in place of the vaccine and who subsequently became infected with *B. burgdorferi*, and 1 serum was from an individual who received the vaccine and likewise became infected with *B. burgdorferi*. Individuals participating in the trial were negative on Lyme disease ELISA tests prior to the start of the trial and were in good health. Serum samples were tested on the C6 Lyme ELISA™ kit and on the WCS *B. burgdorferi* Lyme ELISA. Overall results are presented in Table 2 below. The distribution of Lyme Index values in the vaccine recipient group is shown in the histogram in Figure 2. Results demonstrate that the C6 Lyme ELISA™ is non-reactive with sera from individuals vaccinated with a licensed recombinant OspA vaccine, while the WCS *B. burgdorferi* ELISA exhibits 100 % reactivity with the same specimens. The three pre-vaccination specimens yielded negative results on the C6 Lyme ELISA™ kit, while the four specimens representing individuals infected with *B. burgdorferi*, whether placebo or vaccine recipients, were all detected as positive.

Table 2. Reactivity of C6 Lyme ELISA™ kit in Lyme disease vaccine (Lymerix) recipients.

	Negative	Pos/Equiv	95 % CI (Negative)
C6 Lyme ELISA™ Kit	100 % (43)	0 % (0)	93-100 %
WCS *B. burgdorferi* ELISA	0 % (0)	100 % (43)	0-7 %
n = 43			

Sensitivity

The following information is from a serum panel obtained from the CDC and tested by C6 Lyme ELISA™ kit. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Table 3. CDC Panel.

Time after Onset	Positive	Equivocal	Negative	Total	% Agreement with Clinical Diagnosis
Normals	0	0	5	5	100 %
0-1 Month	5	0	0	5	100 %
1-2 Months	5	0	1	6	83 %
3-12 Months	16	1	3	20	85 %
>1 Year	8	0	0	8	100 %
Total	34	1	9	44	

The C6 Lyme ELISA™ kit yielded 90 % agreement with clinical diagnosis of Lyme disease (35 out of 39 Lyme disease patient sera detected as positive/equivocal).

Serum samples from 180 patients clinically diagnosed with Lyme disease and with positive ELISA results were tested on the C6 Lyme ELISA™ kit and on the WCS *B. burgdorferi* ELISA; 173 of these patients were also positive by Lyme IgG or IgM Western Blot using CDC criteria[12]. The overall sensitivities of detection on the C6 Lyme ELISA™ kit and the WCS ELISA are presented in Table 4.

Table 4. Overall sensitivity of C6 Lyme ELISA™ kit vs. WCS *B. burgdorferi* ELISA

	Sensitivity	95 % CI
C6 Lyme ELISA™ Kit	97 % (174)	93-99 %
WCS *B. burgdorferi* ELISA	94 % (169)	89-97 %
n = 180		

The sensitivities of detection of the 180 Lyme disease patient sera on the C6 Lyme ELISA™ kit and the WCS *B. burgdorferi* ELISA were subcategorized according to time after onset of disease, as shown in Table 5.

Table 5. Sensitivity by Time after Onset

	0-1 mo.	95 % CI	1-2 mo.	95 % CI	3-12 mo.	95 % CI	>1 Year	95 % CI
C6 Lyme ELISA™	100 % (98)	96-100 %	78 % (7)	44-96 %	89 % (24)	71-97 %	98 % (45)	89-100 %
WCS ELISA	98 % (96)	93-100 %	78 % (7)	44-96 %	81 % (22)	64-92 %	96 % (44)	86-99 %
n =	98		9		27		46	

The sensitivity of the C6 Lyme ELISA™ kit in detection of antibodies to *B. burgdorferi* in Lyme patient sera was also evaluated with respect to symptom category. A comparison of sensitivity in the C6 Lyme ELISA™ kit and the WCS *B. burgdorferi* ELISA is shown in Table 6. Note: Some of the 180 patients tested exhibited multiple symptoms and are thus counted under more than one symptom category.

Table 6. Sensitivity by Symptom Category

	Erythema Migrans	95 % CI	Arthritic	95 % CI	Neurological	95 % CI
C6 Lyme ELISA™	96 % (110)	92-99 %	99 % (68)	93-100 %	96 % (24)	81-100 %
WCS ELISA	95 % (108)	89-98 %	99 % (68)	93-100 %	84 % (21)	65-94 %
n =	114		69		25	

The C6 Lyme ELISA™ kit was evaluated in comparison with the two-tier protocol recommended by the CDC[12] (samples found positive or equivocal on ELISA are retested by Western Blot). The C6 Lyme ELISA™ kit exhibited 97 % concordance with the results of Western Blot testing on the 180 Lyme disease patient sera (Table 7).

Table 7. C6 Lyme ELISA™ kit vs. Western Blot Results

		Western Blot	
		Pos	Neg
C6 Lyme ELISA™	Pos/Equiv	95 % 171/180	1.7 % 3/180
	Neg	1.1 % 2/180	2.2 % 4/180

Prospective Study Results

A prospective study was performed on 191 serum samples serially received at a reference laboratory for Lyme disease screening tests. Sera were tested on the C6 Lyme ELISA™ kit and the WCS *B. burgdorferi* ELISA (Tables 8A,B). Concordant results were found in 77.5 % of the specimens tested, while 22 % were found negative on the C6 Lyme ELISA™ kit and positive or equivocal on the WCS ELISA. The 4 samples found positive/equivocal by the C6 Lyme ELISA™ kit were re-tested by Lyme disease Western Blot (Immunetics® QualiCode™ *B. burgdorferi* IgG/IgM kits); results are shown in Table 8C.

Table 8A,B. Prospective Samples: C6 Lyme ELISA™ kit and WCS ELISA Results

A.

	Pos/Equiv	Neg
C6 Lyme ELISA™	2 % 4/191	98 % 187/191
WCS ELISA	24% 45/191	76% 146/191

B.

		WCS ELISA	
		Pos	Neg
C6 Lyme ELISA™	Pos/Equiv	1.5 % 3/191	0.5 % 1/191
	Neg	22 % 42/191	76 % 145/191

C. Lyme disease Western Blot Data for Prospective Samples found Pos/Equiv by C6 Lyme ELISA™ kit

Sample	IgG Western Blot		IgM Western Blot	
	Bands detected	Interp.	Bands detected	Interp.
1	None	Neg	p41, p39	Pos
2	p41	Neg	None	Neg
3	41, 39, 30, 23, 18	Pos	41, 39, 23	Pos
4	93, 66, 60, 58, 45, 41, 39, 34, 31, 30, 28, 23, 18	Pos	39	Neg

Twenty-three of the 43 discrepant samples were tested on Immunetics® QualiCode™ *B. burgdorferi* IgG and IgM Western Blots. Results (Table 9) indicate that the majority (19, or 83%) of sera tested, which were negative by C6 Lyme ELISA™ kit but positive by WCS ELISA, were negative by Western Blot. The 2 sera which were negative by C6 Lyme ELISA™ kit and positive by Western Blot included 1 serum which was positive by IgM Western Blot alone, and 1 serum which was positive by IgG Western Blot alone.

Table 9. Prospective Samples: ELISA vs. Western Blot Results

		WB-Pos	WB-Neg
C6 Lyme ELISA™	Pos/Equiv	1	1
	Neg	2	19
WCS ELISA	Pos/Equiv	3	19
	Neg	0	1

Reproducibility

Reproducibility was tested on a panel of 9 specimens, including the kit Positive, Negative and Calibrator controls and 6 specimens representing 2 negative, 2 weakly reactive and 2 positive sera. Reproducibility was assessed in four analyses, intra-assay, inter-assay, inter-lot and inter-site. Results from each of these analyses are summarized in the following tables, which list the average Lyme Index (LI) value, the standard deviation (SD), and coefficient of variation (CV).

Table 10. Intra-Assay Reproducibility

Specimen	Avg LI	SD	CV
Positive	4.880	0.481	9.85
Negative	0.266	0.042	15.71
Cutoff	2.150	0.316	14.69
1	4.518	0.212	4.69
2	2.690	0.236	8.77
3	2.374	0.278	11.72
4	1.164	0.057	4.87
5	0.723	0.035	4.82
6	0.219	0.023	10.30
n =	10	10	10

Table 11. Inter-Assay Reproducibility

Specimen	Kit 1 Avg LI	Kit 2 Avg LI	Kit 3 Avg LI	SD	CV
Positive	5.301	4.689	5.543	0.395	7.62
Negative	0.192	0.248	0.234	0.030	13.38
Cutoff	2.150	2.15	2.150	0.093	4.31
1	3.605	4.57	5.516	0.938	20.55
2	2.999	2.866	3.052	0.146	4.91
3	2.447	2.498	2.500	0.499	23.13
4	1.473	1.392	1.553	0.479	26.02
5	0.785	0.879	0.848	0.055	6.60
6	0.188	0.215	0.222	0.046	21.97
n =	10	10	10	30	30

Table 12. Inter-Lot Reproducibility

Specimen	Lot 1 Avg LI	Lot 2 Avg LI	Lot 3 Avg LI	SD	CV
Positive	5.244	5.244	4.961	0.375	7.47
Negative	0.279	0.279	0.305	0.032	11.25
Cutoff	2.150	2.161	2.15	0.215	9.97
1	4.868	4.868	5.111	0.302	6.25
2	2.690	2.865	3.021	0.228	7.97
3	2.374	2.405	2.576	0.209	8.24
4	1.164	1.457	1.569	0.183	13.07
5	0.723	0.827	0.930	0.099	11.95
6	0.219	0.259	0.268	0.029	11.55
n =	10	10	10	30	30

Table 13. Inter-Site Reproducibility

Specimen	Site 1 Avg LI	Site 2 Avg LI	Site 3 Avg LI	SD	CV
Positive Control	4.377	4.880	4.893	0.405	8.559
Negative Control	0.239	0.266	0.152	0.057	25.95
Cutoff Calibrator	2.149	2.150	2.149	0.245	11.30
1	4.410	4.518	5.008	0.338	7.26
2	2.864	2.690	3.409	0.378	12.70
3	2.485	2.374	2.886	0.316	12.31
4	1.471	1.164	1.758	0.267	18.37
5	0.920	0.723	0.987	0.128	14.61
6	0.206	0.219	0.132	0.045	24.24
N =	10	10	10	30	30

Cross-Reactive Conditions

Sera from 178 individuals with disease conditions other than Lyme disease were tested for cross-reactivity with the C6 Lyme ELISA™ kit. Results for fourteen conditions are provided in Table 14.

Table 14. Cross-Reactive Conditions: C6 Lyme ELISA™ kit Results

Disease Condition	N	Pos/Equi
ANA	18	1
Bilirubinemic	5	0
H. pylori	18	0
Hemolyzed	4	1
HIV	18	0
Lipemic	5	0
Mononucleosis	17	0
Multiple sclerosis	20	0
Periodontal	4	1
Relapsing Fever	18	0
Rheumatoid Arthritis	16	1
SLE	10	1
Syphilis	20	1
Tularemia	5	0

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