ID-FISH TECHNOLOGY INC.
Detecting Pathogen-Specific Antibodies

iDart™ Lyme IgG ImmunoBlot Kit

(Part # LGIBK04)

INTENDED USE

The iDart™ Lyme IgG ImmunoBlot Kit is an immunoblot assay intended for the in vitro qualitative detection of IgG antibodies to *Borrelia burgdorferi* in human serum. The iDart Lyme IgG ImmunoBlot Kit is intended to detect antibodies to LSA and multiple other *B. burgdorferi* antigens following a modified two-tier test methodology. Positive results from the iDart Lyme IgG ImmunoBlot Kit are supportive evidence for the presence of antibodies and exposure to *B. burgdorferi*. Negative results do not preclude infection with *B. burgdorferi*. iDart™ Lyme IgG ImmunoBlot Kit is intended to aid in the diagnosis of Lyme disease and the test kit should only be used on samples from patients with clinical history, signs and symptoms consistent with Lyme disease. The iDart Lyme IgG Immunoblot Kit is not intended as a screen for asymptomatic patients.

Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures.

For in vitro diagnostic use only
For professional use only
For prescription use only

SUMMARY AND EXPLANATION

Borrelia burgdorferi sensu lato (BB) are the causative agents of Lyme disease – the most common tick-borne disease in North America and Europe. In US, BB species, B. burgdorferi (B31 strain and 297 strain), B. spielmanii, B. californiensis, B. bissettii, B. mayonii and B. carolinensis are known to cause human infections [1], whereas, in Europe, at least six species of BB (B. burgdorferi sensu stricto, B. garinii, B. afzelii, B. valaisiana, B. lusitaniae and B. spielmanii) are known to be pathogenic to humans [2, 3]. The presence of a characteristic 'bulls-eye' Erythema Migrans (EM) rash is generally considered the earliest and best indicator of acute infection. However, the rash may be absent or may go unrecognized in 20 – 40% of the patients [4]. If the initial infection goes untreated, patients can develop disseminated Lyme disease characterized by cardiac, musculoskeletal, and neurological manifestations months to years after the initial tick bite [5,6,7,8,9,10,11]. Diagnosis at this stage can be even more difficult, since the history of the rash and tick bite may be lacking and the symptoms are shared with a number of other diseases [6, 8, 10, and 12]. Direct detection of the agent of Lyme disease using microscopy, culture, nucleic acid amplification and antigen detection have limited sensitivity and/or specificity, except early in the disease when an EM rash is present [4,13]. Therefore, the clinical diagnosis of Lyme disease in the US [13] and Europe [14] is usually supported by antibody detection using a two-tiered testing system. In this system, an Enzyme-linked Immunosorbent Assay (EIA) or Immunofluorescent Antibody (IFA) test is performed as a screen, followed by Western Blot (WB) confirmatory testing if the result obtained by EIA or IFA is indeterminate or positive [13]. The Centers for Disease Control and Prevention (CDC) guidelines for interpretation of the Western blot are based on the publication of Engstrom et al. [15] and Dressler et al. [16], and have been the standard for WB interpretation since the Dearborn conference in1995 [13]. Two-tiered serologic testing has a reported sensitivity of 30 to 40% during the first week after presentation of the EM rash and 29 to 78% in convalescent stages after treatment [4, 17]. Antibody response increases over time and the reported sensitivity in patients with neurological involvement or Lyme disease arthritis is 87% and 97% respectively [4, 15, and 16]. Pathogens that cause diseases such as anaplasmosis, babesiosis and ehrlichiosis are transmitted by the same tick that transmits BB. Thus, Lyme disease patients may harbor these other tick-borne diseases. Therefore, it is important to determine which antibodies are specific for Lyme disease [6, 12, 18, 19, 20, 21, 22, 23]. False positive IgM and IgG results have been reported in patients with illnesses such as rheumatoid arthritis, infectious mononucleosis, autoimmune diseases, bacterial endocarditis, syphilis, other spirochetal infections and Helicobacter pylori infections [16, 24].

The **iDart™ Lyme IgG ImmunoBlot** Kit is intended for the qualitative detection of IgG antibodies to *Borrelia burgdorferi* in human serum.

PRINCIPLES OF THE PROCEDURES

The **iDart™ Lyme IgG ImmunoBlot** test is a line blot assay [25]. The recombinant Borrelial proteins, along with 2 controls proteins are dispensed onto a nitrocellulose membrane by spraying.
During the test procedure, if antibodies to *Borrelia burgdorferi* are present in the human serum sample, they will bind to the antigens sprayed onto the nitrocellulose strips. After removing serum and unbound antibodies by washing, the nitrocellulose strip is incubated with an antihuman IgG antibody conjugated with Alkaline Phosphatase.
After removing the unbound conjugated antibody by a washing step, visualization of the antigen-antibody complex is accomplished by the addition of a substrate 5-bromo, 4-chloro, 3-indolylphosphate (BCIP) and nitroblue tetrazolium (NBT) which forms a strong bluish-purple reaction product by the action of alkaline phosphatase. The reaction is stopped by washing the nitrocellulose strip with distilled or deionized water. Depending on the observed band pattern one can interpret the presence or absence of specific IgG antibodies to Lyme infection.

REAGENTS AND MATERIALS

Table.1 iDart™ Lyme IgG ImmunoBlot Kit (50 assays per kit)		Part No. LGIBK04
	Volume/Quantity	
Lyme IgG ImmunoBlot strips	50 strips	LGIBS03
IB Sample diluent	55ml	IBSD03
IB Wash Buffer	60ml	IBWB03
Milk powder	0.75g package	Milk03
Lyme IgG IB Conjugate	60ml	LGIBC03
IB Phosphatase Substrate	60ml	IBPS03
LYME IgG IB Kit Positive Control	60µl	LGIBP03
LYME IgG IB Kit Negative Control	60µl	LGIBN03
LYME IgG IB Package Insert	1 each	LGIBGPI
LYME IgG IB Reading Guide	1 each	LGIBGRG

Equipment required and may be purchased from ID-FISH:

- ImmunoBlot Incubation Tray

Materials required, but not provided

- Pipettor 10 µl, 200 µl and 1000 µl
- Platform Rocker

PRECAUTIONS

For *in vitro* diagnostic use only.

Safety precautions

1. Do not handle nitrocellulose strips with bare hands. Wear clean gloves and use forceps when handing strips.
2. Establish biosafety precautions in handling human blood specimens and microbiological hazards. All reagents should be handled as potentially infectious material.
3. Material Safety Data Sheets are available upon request.
4. Follow standard biological safety precautions. Do not eat, drink, smoke, apply cosmetics, insert contact lenses, store or prepare food within the designated work area.
5. Dispose of reagents in accordance with federal, state and local regulations.

Technical Precautions

1. Do not use product after the expiration dates printed on labels.
2. Do not use product for any use other than intended use stated on package insert.
3. Avoid microbial and chemical contamination of product.
4. Product is only valid when stored properly under conditions stated on the package insert.

STORAGE AND STABILITY

iDart™ Lyme IgG ImmunoBlot Kit and reagents are stable until the expiration dates marked on the packaging and container(s) when stored as specified.
Store kit components at 2-8°C.
Allow test components to equilibrate to room temperature (15–30°C or 59-86°F) prior to use. Opened kit is stable with In-Use life of 6 months.

QUALITY CONTROL

Control material should be tested in accordance with the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
To monitor the assay, reagent performance and day-to-day variation, a positive control serum for LYME along with a negative control serum should be included with each run (provided in kit).

	Test run is valid only when:
IgG Kit Positive	All bands are present
IgG Kit Negative	No Test Band is present. 2° control band and 1° control band are present.

Record control reactions and document control failures. Do not use kit if controls do not perform accurately and the assay is invalid.

- The result of an iDart Lyme IgG ImmunoBlot strip is valid ONLY if both the primary control and the appropriate secondary control (IgG) are clearly visible.
- A test procedure is valid ONLY if the negative serum control is negative and positive serum control is positive.

SPECIMEN COLLECTION AND HANDLING

Specimen Type
Serum. Minimal volume – 0.5 ml

Specimen collection

i. Special Requirement and Handling
   a. Handle all reagents and samples as if they are potentially infectious.
   b. Collect blood samples aseptically in a non-additive (red top) or Serum Separator (SST) vacutainer tube, using approved venipuncture techniques. Only qualified personnel may perform venipunctures.
   c. Allow the blood sample to clot at room temperature prior to centrifugation. Centrifuge the clotted blood sample at a speed and time that will separate the serum from the clot without lysing the erythrocytes.
   d. If using the non-additive (red top) tube, aseptically transfer the serum to a tightly closing sterile, screw top tube or equivalent shipping container.

ii. Mailing Containers and Shipping
   a. Sterile, screw top tube, Serum Separator Tube (SST) or equivalent, according to Federal transportation regulations, shipped second day air transportation.

iii. Criteria for Unacceptable Specimens
   a. Samples that are improperly labeled.
   b. Samples that is not sufficient in quantity for testing (less than 0.5 ml).
   c. Samples that are grossly hemolyzed or lipemic should not be used. Plasma samples are also acceptable for testing.

iv. Stability Time (Storage, Preservation. Etc.)
   a. Serum samples should be stored in the refrigerator at 2-8°C for no long than 5 days. Store samples frozen at -20°C or below with a single freeze-thaw cycle only if testing is delayed. (Avoid repeated freezing and thawing of specimen).

TEST PROCEDURE

Working stock reagent preparation

1x Sample Diluent working stock (1.5% milk) as needed:
Prepare as needed. Same day use only

	1x Sample Diluent	Milk powder
10 strips	10 ml	0.15 g
25 Strips	25 ml	0.375 g

1x IB Wash Buffer as needed:
Prepare as needed. Same day use only

	IB Wash Buffer	dH_2O
10 strips (150 ml)	9 ml	141 ml
25 Strips (375 ml)	22.5 ml	352.5 ml

1. Primary sample incubation
   i. Remove and label one strip for each patient serum.
   ii. Remove and label one strip for positive control serum and one strip for negative control serum
   iii. Place labeled strips into clean channels of the incubation tray.
   iv. Pipette 1ml of sample diluent into each channel with strip while rocking on rocking platform at 20rpm.
   v. Add 10 µl of patient serum to each respective patient strip channel
   vi. Add 10 µl of positive serum control to the positive control strip channel
   vii. Add 10 µl of negative serum control to the negative control strip channel.
   viii. Incubate on the rocking platform for 60 minutes.

2. Primary Wash
   i. Aspirate sample serum and controls from each channel completely.
   ii. Pipette 1.5 ml of wash butter into each channel with strip.
   iii. Incubate for 5 minutes while rocking at room temperature.
   iv. Aspirate wash buffer completely from each channel.
   v. Repeat wash 3 more time.

3. Secondary antibody incubation
   i. Pipette 1ml Lyme IgG IB Conjugate diluent into each channel.
   ii. Incubate on rocking platform for 60 minutes at room temperature.
   iii. Aspirate conjugate solution from each channel completely.

4. Secondary antibody Wash
   i. Pipette 1.5 ml of wash buffer into each channel with strip.
   ii. Incubate for 5 minutes while rocking at room temperature.
   iii. Aspirate wash buffer completely from each channel.
   iv. Repeat wash 3 more time.

5. Substrate Incubation
   i. Pipette 1 ml of substrate solution into each channel
   ii. Incubate on rocking platform, until the band on the C2 shows up, between 15 – 30 minutes.
   iii. Stop reaction by aspirating the substrate solution and adding 1.5 ml of dIH_2O into each channel.
   iv. Decant dIH_2O after 1 minute. Remove strips from channel and lay flat on paper towel to dry.

INTERPRETATION AND REPORTING OF RESULTS

1. Use the Reading Guide from each kit to locate and identify bands present on the strip.
2. Place positive and negative control strips beside the Reading Guide.
3. Read positive and negative control strips.
4. The positive and negative control strips of the run must be comparable to their previously established profiles with band intensity within +/-1 intensity due to subjectivity in reading.
5. All reportable bands should be present on positive control strip. If any of the reportable bands are absent on positive control strip, the test must be repeated.
6. If the negative control strip shows 2 or more reportable bands with intensity equal to or greater than 1+, the test must be repeated.
7. C1 and C2 bands must show on every sample test strip.
8. When analyzing sample test strips, it is helpful to place the sample strip against with the positive control strip and reading guide to facilitate the position assignment of each band.
9. Within each strip, C2 is the benchmark calibrator for test bands. It defines the 1+ colorimetric intensity for each strip.
10. The intensity of the bands on the sample test strip is then scored by comparing the intensity of the band to the intensity of C2 band within the same strip.

Table 2. SCORING OF PROTEIN BANDS INTENSITY

Band Intensity	Indicated by
-	No band detected
+/- = I (*)	A mark on the strip but not strong enough to be a definite band or band intensity < calibration standard
+	A definite line or band intensity > or = to calibration standard

11. Each patient sample strip will be evaluated according to ID-FISH interpretation criteria (see Table 3. for details):

Table 3. iDart IgG ImmunoBlot test Interpretation Criteria

Positive	if **LSA** AND **one** or more bands from at least two of the following groups are present – P93, P41, P39, P23, P31 and P34 are present.
Negative	if the band pattern does not meet the positive criteria.

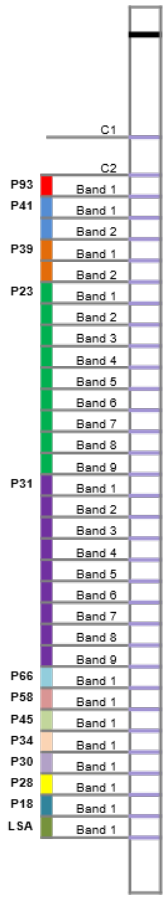

Figure 1. Schematic drawing of LYME IgG ImmunoStrip (not in scale)

LIMITATIONS AND INTERFERENCES

1. A negative result does not exclude the possibility of a LYME infection.
2. A positive result does not always indicate a current infection. It may reflect a past exposure to Borrelial antigens.
3. Serum from individuals with other spirochetal and tick-borne infections may have cross-reactive antibodies present to *B. burgdorferi* proteins.
4. Antibiotics therapy given to Lyme disease patients in early stages of the disease can suppress the development of specific Borrelia antibodies [26].
5. The performance of this assay, when testing sera from patients with any immune-deficient diseases such as HIV2, HTLV, etc. and sera from patients that have had immune-suppressive therapy with drugs or medications, is unknown.
6. The assay must be performed as outlined to obtain reproducible results. Test reagents must be stored as indicated.
7. Due to variations in test performance and the uncertainty associated with unreadable blots, it is recommended that all unreadable blots be repeated using original specimen.
8. The results of this test must be interpreted in relation to patient's clinical history, epidemiological data, stages of the disease or clinical symptoms and other laboratory results.
9. Treat all samples as potentially infectious.

REFERENCES

1. Mathiesen DA, Oliver JH Jr, Kolbert CP, Tullson ED, Johnson BJ, Campbell GL, et al. Genetic heterogeneity of Borrelia burgdorferi in the United States. J Infect Dis. 1997; 175: 98-107.
2. Wilhelmsson P, Fryland L, Borjesson S, Nordgren J, Bergstrom S, Ernerudh J, et al. Prevalence and diversity of Borrelia species in ticks that have bitten humans in Sweden. J Clin Microbiol. 2010; 48: 4169-4176.
3. Fingerle V, Schulte-Spechtel UC, Ruzic-Sabljic E, Leonhard S, Hofmann H, Weber K, et al. Epidemiological aspects and molecular characterization of Borrelia burgdorferi s.l. from southern Germany with special respect to the new species Borrelia spielmanii sp Nov. Int J Med Microbiol. 2008; 298: 279-90.
4. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev. 2005; 18: 484-509.
5. Fallon BA, Keilp JG, Corbera KM, Petkova E, Britton CB, Dwyer E, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology. 2008; 70: 992-1003.
6. Hsieh YF, Liu HW, Hsu TC, Wei JC, Shih CM, Krause PJ, et al. Serum reactivity against Borrelia burgdorferi OspA in patients with rheumatoid arthritis. Clin Vaccine Immunoblot. 2007; 14: 1437-1441.
7. Logigian EL, Steere AC. Clinical and electrophysiologic findings in chronic neuropathy of Lyme disease. Neurology. 1992; 42: 303-311.
8. Marcus LC, Steere AC, Duray PH, Anderson AE, Mahoney EB. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis. Demonstration of spirochetes in the myocardium. Ann Intern Med. 1985; 103: 374-376.
9. Nadelman RB, Wormser GP. Lyme borreliosis. Lancet. 1998; 352: 557-565.
10. Stanek G, Strle F. Lyme borreliosis. Lancet. 2003; 362: 1639-1647.
11. Steere AC. Lyme disease. N Engl J Med. 2001; 345: 115-125.
12. Krause PJ, McKay K, Thompson CA, Sikand VK, Lentz R, Lepore T, et al. Disease- specific diagnosis of coinfecting tick-borne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease. Clin Infect Dis. 2002; 34: 1184–1191.
13. Centers for Disease Control and Prevention (CDC). Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep. 1995; 44: 590-591.
14. Wilske B, Fingerle V. [Diagnosis of Lyme borreliosis. How to corroborate suspected borreliosis]. MMW Fortschr Med. 2000; 142: 28-31.
15. Engstrom SM, Shoop E, Johnson RC. ImmunoBlot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol. 1995; 33: 419-427.
16. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis. 1993; 167: 392-400.
17. Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for Lyme disease. Clin Infect Dis. 2008; 47: 188-195.
18. Benach JL, Coleman JL, Habicht GS, MacDonald A, Grunwaldt E, Giron JA. Serological evidence for simultaneous occurrences of Lyme disease and babesiosis. J Infect Dis. 1985; 152: 473-477.
19. De Martino SJ, Carlyon JA, Fikrig E. Coinfection with Borrelia burgdorferi and the agent of human granulocytic ehrlichiosis. N Engl J Med. 2001; 345: 150-151.
20. Nadelman RB, Horowitz HW, Hsieh TC, Wu JM, Aguero-Rosenfeld ME, Schwartz I, et al. Simultaneous human granulocytic ehrlichiosis and Lyme borreliosis. N Engl J Med. 1997; 337: 27-30.
21. Shah JS, Liu S, Du Cruz I, Poruri A, Maynard, R, Shkilna M, et al. Line immunoblot assay for tick-borne relapsing fever and findings in patient sera from Australia, Ukraine and the USA. Healthcare 2019; 7(4): 121.
22. Shah JS, Burrascano JJ, Ramasamy R. Recombinant protein immunoblots for differential diagnosis of tick-borne relapsing fever and Lyme disease. *J Vector Borne Dis*. 2023;60(4):353-364. doi:10.4103/0972-9062.383641
23. Stricker RB, Harris NS, Yong DC, Winger EE. Clinical and seroepidemiologic characteristics of Babesia WA-1 coinfection in patients with Lyme disease in California. J Invest Med. 2003; 51: S145.
24. Reed KD. Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol. 2002; 40: 319-324.
25. Liu S, Cruz ID, Ramos CC, Taleon P, Ramasamy R, Shah J. Pilot Study of Immunoblots with Recombinant *Borrelia burgdorferi* Antigens for Laboratory Diagnosis of Lyme Disease. *Healthcare*. 2018; 6(3):99. https://doi.org/10.3390/healthcare603009
26. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. 1996. Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J Clin Microbiol 34: https://doi.org/10.1128/jcm.34.1.1-9.1996

PERFORMANCE CHARACTERISTICS

1. **Precision/Reproducibility**
The **iDart™ Lyme IgG ImmunoBlot Kit** was tested in a blind study to evaluate reproducibility across 3 separate sites each with 2 operators over 5 days, 2 runs a day, using a panel of blinded and coded samples of negative, moderate negative, high negative, low positive, moderate positive and high positive samples for IgG. It is concluded that there was 100% agreement of all bands among all runs, all days and across 3 sites for the **iDart™ Lyme IgG ImmunoBlot Kit** (See Table 3).

Sample #	**Sample Type**	**IgG**	**# of Samples (+)**	**Expected Result**	**% agreement with expected result**
GA	High Positive	P	90/90	P	100%
GB	Moderate Positive	P	90/90	P	100%
GC	Negative-1	N	0/90	N	100%
GD	Negatbie-2	N	0/90	N	100%
GE	Negative-3	N	0/90	N	100%
GF	Low Positive	P	90/90	p	100%

2. **Cross Reactivity**
A cross reactivity study was performed on specimens known to contain potentially cross-reactive antibodies to Lyme infection. Serum samples from patients with bacterial/viral infections and sera from patients with diagnoses that can be confused with the late manifestations of Lyme disease were tested. Based on the data presented in Table 4, there was no cross-reactivity with antibodies to all non- Borrelia pathogens or autoimmune diseases tested with the iDart™ Lyme IgG ImmunoBlot Kit.

Table 4: iDart™ Lyme IgG ImmunoBlot Kit - Cross Reactivity

Source	**Disease State**	**N (376)**	**iDart Lyme IgG ImmunoBlot**			**% Cross- reactivity**
			LSA	**2+ Bands**	**IgG Positive**	
CDC	Fibromyalgia	15	0	0	0	0%
	Mononucleosis	15	0	1	0	0%
	Multiple sclerosis	15	0	0	0	0%
	Rheumatoid arthritis	15	0	0	0	0%
	Severe periodontitis	15	0	0	0	0%
	Syphilis	15	0	0	0	0%
IGeneX (CA)	Babesiosis	28	0	0	0	0%
	Bartonellosis	48	0	0	0	0%
	Ehrlichiosis	5	0	0	0	0%
	Anaplasmosis	7	0	0	0	0%
	Rickettsiosis	22	0	0	0	0%
	Tick Borne Relapsing Fever	14	0	0	0	0%
New York Biological (NY)	HIV*	12	0	0	0	0%
	RPR	23	0	2	0	0%
	HSV1	8	0	1	0	0%
	HSV2	2	0	0	0	0%
	CMV	13	0	0	0	0%
	EBV	27	0	0	0	0%
BEI	RSV	4	0	0	0	0%
	FLU	21	0	0	0	0%
Kamineni Life Sciences Pvt. Ltd, Hyderabad (India)	Pregnant women	12	0	0	0	0%
	H. pylori	10	0	0	0	0%
Warde Medical Laboratory (MI)	Parvovirus-19	10	0	0	0	0%
	Varicella-zoster virus	10	0	1	0	0%
CDC	Leptospira	10	0	0	0	0%
False Positive			0	5	0	0%
Agreement					**100%**	

3. **Interference from Endogenous Analytes**
The potential interfering effect of endogenous substances in patient samples when using the iDart Lyme IgG ImmunoBlot was evaluated using one positive, one low positive and one negative Borrelia IgG samples. Samples were spiked with the endogenous substances at the final concentrations listed in the table below. All samples were tested in singlicate. No interference was observed in he tested samples. .

Table 5. Effect of Interference Substances on iDart™ Lyme IgG ImmunoBlot Kit

Agent	**Concentration in serum**	**iDart™ Lyme IgG result**			**Effect on ImmunoBlot Kit**
		High Positive	Low Positive	Negative	
Bilirubin	1mg/dL (low)	Positive	Positive	Negative	No effect
Bilirubin	15mg/dL (high)	Positive	Positive	Negative	No effect
Albumin	3.5g/dL (low)	Positive	Positive	Negative	No effect
Albumin	5g/dL (high)	Positive	Positive	Negative	No effect
Cholesterol	150mg/dL (low)	Positive	Positive	Negative	No effect
Cholesterol	250mg/dL (high)	Positive	Positive	Negative	No effect
Triglycerides	150mg/dL (low)	Positive	Positive	Negative	No effect
Triglycerides	500mg/dL (high)	Positive	Positive	Negative	No effect
Hemoglobin	10g/dL (low)	Positive	Positive	Negative	No effect
Hemoglobin	20g/dL (low)	Positive	Positive	Negative	No effect

4. Method Comparison

The performance of the iDart™ Lyme IgG ImmunoBlot Kit for detection of Borrelial-specific antibodies was compared to an FDA-cleared EIA and immunoblot as part of the standard two-tier test methodology (STTT) .Results are summarized below. A total of 768 serum samples were procured from two vendors and tested at three clinical sites. Table 6 bellow summarizes the distribution of samples per testing site and cohort .

Table 6: Sample distribution by clinical site and cohort.

	Number of Samples	Sample Type	Vendors Providing Samples
Site 1	290	Prospectively banked – Cohort 1	Bay Area Lyme Foundation
Site 2	37	Prospective – Cohort 2	IGeneX Inc.
Site 2	230	Prospective – Cohort 3	IGeneX Inc.
Site 3	211	Prospective – Cohort 2	IGeneX Inc.

All samples were blinded, re-coded, and tested at the respective clinical sites as per the instructions for use for the iDart Lyme IgG ImmunoBlot Kit. Performance by cohort is summarized in tables 7 through 9.

Table 7: Performance Summary on prospective banked samples from Bay Area Lyme Foundation (n=290). iDart Lyme IgG ImmunoBlot test versus STTT.

		STTT	
		Positive (+)	Negative (-)
iDart Lyme IgG ImmunoBlot	Positive (+)	19	36
	Negative (-)	1	234
	Total	20	270
	PPA (95% CI)	95.00% (76.39% – 99.11%)	
	NPA (95% CI)	86.67% (82.09% – 90.21%)	

Table 8: Performance Summary on samples from IGeneX Inc. Cohort 2 (n=248). iDart Lyme IgG ImmunoBlot test versus STTT.

		STTT	
		Positive (+)	Negative (-)
iDart Lyme IgG ImmunoBlot	Positive (+)	114	12
	Negative (-)	6	116
	Total	120	128
	PPA (95% CI)	95.00% (89.52% – 97.69%)	
	NPA (95% CI)	90.63% (84.33% – 94.56%)	

Table 9: Performance Summary on samples from IGeneX Inc. Cohort 3 (n=230). iDart Lyme IgG ImmunoBlot test versus STTT.

		STTT	
		Positive (+)	Negative (-)
iDart Lyme IgG ImmunoBlot	Positive (+)	10	7
	Negative (-)	1	212
	Total	11	219
	PPA (95% CI)	90.91% (62.27% – 98.38%)	
	NPA (95% CI)	96.80% (93.55% – 98.44%)	

5. Analytical Specificity

Table10 below shows the results of testing iDart™ Lyme IgG ImmunoBlot Kit on samples collected from a population of 313 apparently healthy individuals from endemic areas, and 112 samples collected from healthy individuals in non-endemic areas in the US.

Table 10: iDart Lyme IgG ImmunoBlot results for samples collected from healthy individuals in endemic areas.

Source	N (313)	IgG Positive
CDC	**62**	0
BAY AREA FOUNDATION (NY, MA, WI)	**251**	2
TOTAL	**313**	2
% Positive		**0.64%**

Table 11: iDart Lyme IgG ImmunoBlot results for samples collected from healthy individuals in non-endemic areas.

Source	N (112)	IgG Positive
CDC	**62**	0
CA	**50**	0
TOTAL	**112**	0
% Positive		**0.00%**

6. CDC panel

A reference Panel of 280 serum samples was received from CDC. These samples were from patients diagnosed with Lyme Disease at different stages (Stages 1, 2, and 3), Lyme disease look-like infections (infectious mononucleosis, multiple sclerosis, rheumatoid arthritis, fibromyalgia and severe periodontitis), and from healthy controls living in endemic and non-endemic regions of Lyme disease. Results are analyzed according to disease stages and compared to STTT (See Table 12).

Table 12. iDart Lyme IgG ImmunoBlot Kit performance using the CDC Reference Panel

Disease Stage	Stage I		Stage II		Stage III		Overall		Healthy controls		Disease Controls	
N	**60**		**10**		**20**		**90**		**100**		**90**	
Test Kit	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT
Positive	35	18	9	9	20	20	64	47	0	0	0	0
Negative	25	42	1	1	0	0	26	43	100	100	90	90
Sensitivity	58.33%	30.00%	90.00%	90.00%	100%	100%	71.11%	52.22%				
Agreement									100%	100%	100%	100%

LABELS

REF **Manufacturing part number**

LOT **Lot number**

IVD **In Vitro Diagnostics Use Only**

Use by Date

Store Product Away from direct sunlight

Keep dry

8°C
2°C **Store product between 2°C to 8°C**

For Professional Use Only

Instruction for product use

Σ
50 **This product is sufficient for 50 assays**

NON STERILE **This product is non-sterile**

Prescription Use Only

EC REP **Authorized representative in the European Community**

BIO **Biological material of human origin**

Hazardous Substances – skin irritant

ID-FISH TECHNOLOGY INC.
Detecting Pathogen Specific Antibodies

REF **LGIBK04**
LOT **24-IUO-001**
YYYY-MM-DD

iDart® Lyme IgG ImmunoBlot Kit

50 test strips Store at 2-8°C

ID-FISH Technologies, Inc.
556 Gibraltar Drive,
Milpitas, CA 95035, USA
www.idfishtechnology.com

Rx ONLY P IVD