ID-FISH TECHNOLOGY INC.
Detecting Pathogen-Specific Antibodies

iDart™ Lyme IgM ImmunoBlot Kit

(Part # LMIBK04)

INTENDED USE

The iDart™ Lyme IgM ImmunoBlot Kit is an immunoblot assay intended for the *in vitro* qualitative detection of IgM antibodies to *Borrelia burgdorferi* in human serum. The iDart™ Lyme IgM ImmunoBlot Kit is intended to detect antibodies to Lyme Screen Antigen (LSA) and multiple other *B. burgdorferi* antigens following a modified two-tier test methodology. Positive results from the iDart™ Lyme IgM 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 IgM 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 IgM 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 followed by Western Blot (WB) 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 in 1995 [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 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].

PRINCIPLES OF THE PROCEDURES

The iDart™ Lyme IgM 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* infection 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 anti-human IgM 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 IgM antibodies to *B. burgdorferi* antigens.

REAGENTS AND MATERIALS

Table 1: iDart™ Lyme IgM ImmunoBlot Kit (50 assays per kit)		Part No. LMIBK04
	Volume/Quantity	
Lyme IgM ImmunoBlot strips	50 strips	LMIBS03
IB Sample diluent	60 ml	IBSD03
IB Wash Buffer	60 ml	IBWB03
Milk powder	0.75 g package	Milk03
Lyme IgM IB Conjugate	60 ml	LMIBC03
IB Phosphatase Substrate	60ml	IBPS03
Lyme IgM IB Positive Control	60 µl	LMIBP03
Lyme IgM IB Negative Control	60 µl	LMIBN03
Lyme IgM IB Package Insert	1 each	LMIBPI
Lyme IgM IB Reading Guide	1 each	LMIBRG

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 IgM 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 kits are 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:

IgM Positive	All bands are present
IgM Negative	No Test Band is present. Primary control band (C1) and secondary control band (C2) 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 IgM ImmunoBlot strip is valid ONLY if both the primary control and the appropriate secondary control (IgM) 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 1 ml of sample diluent into each channel with strip while rocking on rocking platform at 20 rpm.
   v. Add 20 µl of patient serum to each respective patient strip channel
   vi. Add 20 µl of positive serum control to the positive control strip channel
   vii. Add 20 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 1 ml Lyme IgM 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 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™ IgM ImmunoBlot Kit Interpretation Criteria

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

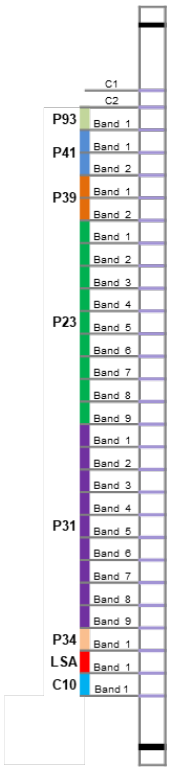

Figure 1. Schematic drawing of iDart Lyme IgM ImmunoStrip (not in scale)

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. False positive results for iDart™ Lyme IgM ImmunoBlot Kit may occur due to cross-reactivity to other conditions such as mononucleosis, *H. pylori* infection, and parvovirus-19 infection.
10. Treat all samples as potentially infectious.

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PERFORMANCE CHARACTERISTICS

1. **Precision/Reproducibility**
The iDart™ Lyme IgM 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 coded samples containing different levels of anti-B. burgdorferi IgM: negative, high negative, low positive, moderate positives, and high positive samples . This study generated 90 replicates per sample. There was 100% agreement on all test results across runs, days and sites (See Table 4).

Table 4. Reproducibility Study Summary - Overall – All Sites – 6 operators/5 days/ 3 replicates

Sample #	Sample Type	IgM	# of Samples (+)	Expected Result	% samples matched to expected result
MA	High Positive	P	90/90	P	100%
MB	Moderate Positive	P	90/90	P	100%
MC	Moderate Positive	P	90/90	P	100%
MD	Low Positive	P	90/90	P	100%
ME	High Negative	N	0/90	N	100%
MF	Negative	N	0/90	N	100%

2. **Cross Reactivity**
A cross-reactivity study was performed on specimens known to contain potentially cross-reactive antibodies to Lyme infection. A total of 243 serum samples from patients with bacterial or viral infections, as well as sera from patients with diagnoses that could be confused with Lyme disease, were tested. Based on the data presented in Table 5, the iDart™ Lyme IgM ImmunoBlot Kit demonstrated 97.94% specificity to samples containing antibodies to non-*Borrelia* pathogens or autoimmune diseases.(See Table 5).

Table 5. iDart™ Lyme Borrelia IgM ImmunoBlot Kit - Analytical Specificity

Source	Disease State	N (243)	IgM Positive	% Cross- reactivity
CDC	Fibromyalgia	15	0	0%
	Mononucleosis	15	1	6.67%
	Multiple sclerosis	15	0	0%
	Rheumatoid arthritis	11	0	0%
	Severe periodontitis	14	0	0%
	Syphilis	14	0	0%
	Leptospira	10	2*	20.00%*
IGeneX, Inc.	Rheumatoid Factor	5	0	0%
	ANA	5	0	0%
	Bartonella	11	0	0%
	Bartonella & Anaplasma	1	0	0%
	Bartonella &TBRF Borrelia	1	0	0%
	Babesiosis	8	0	0%
	Babesiosis & Tick-Borne Relapsing Fever	2	0	0%
	Babesiosis & Rickettsiosis	1	0	0%
	Tick Borne Relapsing Fever	10	0	0%
	Tick-Borne Relapsing Fever & Anaplasma	1	0	0%
	Tick-Borne Relapsing Fever & Ehrlichiosis	1	0	0%
	Tick-Borne Relapsing Fever & Rickettsia	1	0	0%
	Anaplasmosis	8	0	0%
	Anaplasmosis & Ehrlichiosis	1	0	0%
	Ehrlichiosis	4	0	0%
	Rickettsiosis	11	0	0%
New York Biologics (NY)	HIV	6	0	0%
	HCV	5	0	0%
	HSV1	7	0	0%
	CMV	11	0	0%
	EBV	9	0	0%
Kamineni Life Sciences Pvt. Ltd, Hydrabad (India)	Pregnant women	11	0	0%
	H. pylori	9	1	11.11%
Warde Medical Laboratory (MI)	Parvovirus-19	10	1	10.00%
	Varicella-zoster virus	10	0	0%

False Positive			5	
Agreement			97.94%	

* Two Leptospira samples positive by iDart Lyme IgM testing were also positive for IgM with STTT

3. Interference from Endogenous Analytes

The potential interfering effect of endogenous substances in patient samples when using the iDart™ Lyme IgM ImmunoBlot was evaluated using one positive, one low positive and one negative *Borrelia* IgM 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 the tested samples. (See Table 6).

Table 6. Effect of Interference Substances on iDart™ Lyme IgM ImmunoBlot Kit

Agent	Concentration in serum	iDart™ Lyme IgM ImmunoBlot Kit result			Effect on iDart™ Lyme IgM ImmunoBlot Kit
		High Pos (mA)	Low Pos (mB)	Negative (mC)	
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 (high)	Positive	Positive	Negative	No effect

4. Fresh *versus* Frozen Sample Stability:

This study was conducted to support the use of frozen samples in the clinical and analytical validation studies. To evaluate the performance of the iDart™ IgM ImmunoBlot Kit when using fresh and frozen samples, a total of 63 decoded left-over patient serum samples were included in this study. Samples were tested fresh (stored at 2° – 8°C) and after freezing at -20°C for at least 2 days, and not more than 22 days. All IgM positive samples (N=21) remained positive, and all IgM negative samples (N=42) remained negative when tested with the iDart™ Lyme IgM ImmunoBlot Kit fresh and after storage at -20°C.

5. Method Comparison

The performance of the iDart™ Lyme IgM 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). A total of 997 serum samples were procured from a vendor and tested at three U.S. clinical sites. Samples were collected during year 2024 from different geographic regions in the US. All study samples were non-selected, left-over samples collected from patients with signs and symptoms consistent with Lyme disease and that were prescribed a Lyme test. Table 7 below summarizes the distribution of samples per testing site.

Table 7. Sample distribution by clinical site and cohort.

	Number of Samples	**Sample Type**	**Vendor Providing Samples**
Site 1	304	Prospectively collected serum samples	IGeneX Inc.
Site 2	357	Prospectively collected serum samples	IGeneX Inc.
Site 3	336	Prospectively collected serum samples	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 IgM ImmunoBlot Kit. Overall performance is summarized in table 8.

Table 8 - Performance Summary. iDart™ Lyme IgM ImmunoBlot Kit vs STTT

N=997		**STTT**	
		Positive (+)	Negative (-)
iDart™ Lyme IgM ImmunoBlot Kit	Positive (+)	60	18
	Negative (-)	6	913
	Total	66	931
	PPA (95% CI)	90.91% (81.55%– 95.77%)	
	NPA (95% CI)	98.07% (96.96%– 98.77%)	

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6. Analytical Specificity

Well-characterized serum samples collected from apparent healthy individuals from both endemic and non-endemic areas for Lyme Disease were tested with the iDart™ Lyme IgM ImmunoBlot Kit following the instructions for use. Table 9 and 10 below summarize the performance of iDart™ Lyme IgM ImmunoBlot Kit. when testing samples from endemic and non-endemic areas respectively.

Table 9. iDart™ IgM ImmunoBlot Kit - Analytical Specificity (Endemic) (N=177)

Sample Source	N	IgM	% Positive
CDC	50	1	2.00%
BAY AREA LYME FOUNDATION (NY, MA, WI)	127	0	0.00%
TOTAL	177	1	0.56%
Agreement		99.44%	

Table 10.iDart™ IgM ImmunoBlot Kit - Analytical Specificity (Non- Endemic) (N=127)

Sample Source	N	IgM	% Positive
CDC	45	1	2.22%
IGeneX, Inc.	82	0	0.00%
TOTAL	127	1	0.79%
Agreement		99.21%	

7. CDC panel

A reference Pre-Marketing Panel of 258 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 11).

Table 11. Performance on CDC Pre-Marketing Panel with respect to different Disease Stages. iDart™ Lyme IgM ImmunoBlot Kit vs STTT (N=258)

Disease Stage	Stage I		Stage II		Stage III		Overall		Healthy controls		Disease Controls	
N	50		9		20		79		95		84	
Test Kits	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT	iDart	STTT
Positive	33	28	8	7	11	9	52	44	2	1	1	3
Negative	17	22	1	2	9	11	27	35	93	94	83	81
Sensitivity	66.0%	56.0%	88. 9%	77. 8%	55.0%	45.0%	65.8%	55.7%				
Agreement									97.9%	98.9%	98.8%	96.4%

LABELS

Manufacturing part number

Lot number

In Vitro Diagnostics Use Only

Use By Date

Store Product Away from direct sunlight

Keep dry

Store product between 2°C to 8°C

For Professional Use Only

Instruction for product use

This product is sufficient for 50 assays

50

This product is non-sterile

Prescription Use Only

Authorized representative in the European Community

Biological material of human origin

Hazardous Substances – skin irritant