510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K242872

B Applicant

ID-FISH Technology, Inc.

C Proprietary and Established Names

iDart Lyme IgM ImmunoBlot Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LSR	Class II	21 CFR 866.3830 - Treponema Pallidum Treponemal Test Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
To obtain a substantial equivalence determination for the iDart Lyme IgM ImmunoBlot Kit for qualitative detection of anti-*Borrelia burgdorferi* IgM antibodies in human serum.

B Measurand:
Anti-*Borrelia burgdorferi* IgM antibodies.

C Type of Test:
Immunoblot assay.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for 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.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

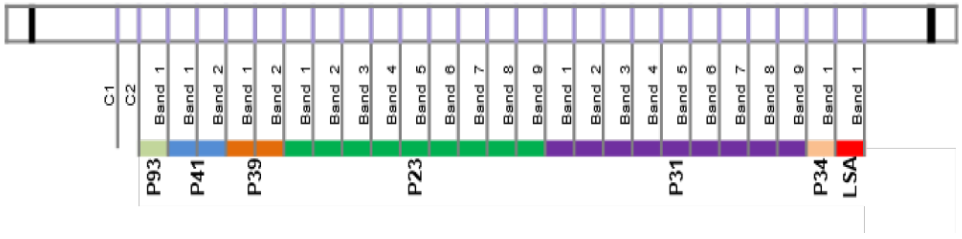
Not Applicable

IV Device/System Characteristics:

A Device Description:

The iDart Lyme IgM ImmunoBlot is a line immunoblot assay. Antigenic proteins specific for different *Borrelia* species that cause Lyme Disease are produced by recombinant DNA technology in *Escherichia coli*. The purified proteins are applied as discrete lines on a nitrocellulose membrane (test strip) along with two control proteins in the following order: Control 1 (C1: IgG/IgM), Control 2 (C2: Protein L), P93, P41, P39, P23, P31, P34, and LSA (a chimeric VlsE peptide termed the Lyme Screening Antigen).

Figure 1. Schematic of the iDart Lyme IgM ImmunoBlot strip.



B Principle of Operation:

During the test procedure, human serum is added to the test strip. Antibodies to *Borrelia burgdorferi*, if present, will bind to antigen lines on the test strip. After removing serum and unbound antibodies by washing, the test strip is incubated with alkaline phosphatase conjugated anti-human IgM antibody for detection of anti-*Borrelia burgdorferi* IgM antibodies in the patient sample.

After removing the alkaline phosphatase conjugated antibody by washing, the antigen-antibody complex is visualized as bands on the test strip by adding the alkaline phosphatase substrate 5-

Bromo, 4-chloro, 3-indolylphosphate (BCIP) and nitro blue tetrazolium (NBT) to form a blue-purple precipitate on the detected band(s). The reaction stops by washing the test strip with distilled or deionized water. A test strip reading guide included with the kit shows the location of specific antigens in the test strip. Every test strip has two functional control bands (C1 and C2). The test strip is only valid when both controls bands are visible after completion of the test. Any band found having a visual intensity equal to or greater than the C2 control band intensity is considered as a positive band.

Reagent and Materials

The following reagents are provided as part of the iDart Lyme IgM ImmunoBlot Kit.

Table 1. iDart Lyme IgM ImmunoBlot Kit, 50 assays per kit.

Component	*Packaging*	*Volume/ Quantity*	*Part No.*
Lyme IgM ImmunoBlot strips	50ml tube	50 strips	LMIBS03
IB Sample diluent	60ml bottle	55ml	IBSD03
IB Wash Buffer	60ml bottle	60ml	IBWB03
Milk powder	0.5ml microcentrifuge tube	0.75g	Milk03
Lyme IgM IB Conjugate	60ml bottle	60ml	LMIBC03
IB Phosphatase Substrate	60ml bottle	60ml	IBPS03
LYME IgM IB Positive Control	0.5ml microcentrifuge tube	120µl	LMIBP03
LYME IgM IB Negative Control	0.5ml microcentrifuge tube	120µl	LMIBN03
LYME IgM IB Package Insert	Paper	1 each	LMIBPI
LYME IgM IB Reading Guide	Laminated paper	1 each	LMIBRG

The following are materials and reagents required but not provided with the kit:

i. ImmunoBlot Incubation Tray (may be purchased from ID-FISH Technologies, Inc.)
ii. Pipettor 10, 200, and 1000µl
iii. Platform Rocker

Quality Controls

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, positive controls for anti-*Borrelia* antibodies along with a negative control must be tested with each run.

1. All reportable bands should be present on positive control strip. If any of the reportable bands are absent on positive control, the test must be repeated.
2. No test bands are present. If the negative control strip shows 2 or more reportable bands with intensity equal to or greater than C2 control band, the test must be repeated.
3. C1 and C2 control bands must show in every test strip.

Interpretation of Results

A test strip result is valid only if both internal controls bands (C1 and C2) are clearly visible, and the negative and positive serum controls results are comparable to the pre-established profiles. Within each strip, C2 is the benchmark calibrator for test bands. The intensity of the bands on the sample test strip is then scored by comparing the intensity of the reportable bands 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, band intensity < C2 control band
+	A definite line or band intensity ≥ to C2 control band

Table 3. Interpretation of results for iDart Lyme IgM ImmunoBlot test.

Test Result	Interpretation
Positive	**Positive or indetermined LSA** band AND one or more 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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Viramed Borrela All-In-One ViraChip Test Kit

B Predicate 510(k) Number(s):
K220016

C Comparison with Predicate(s):

Device & Predicate Device(s):	K242872	K220016
Device Trade Name	iDart Lyme IgM ImmunoBlot Kit	Viramed Borrelia All-In-One ViraChip Test Kit
General Device Characteristic Similarities		
Intended Use/Indications For 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	The Viramed Biotech AG Borrelia All-In-One ViraChip is an in vitro qualitative microarray assay for the detection of IgM and IgG antibodies to *Borrelia burgdorferi* in human serum. The assay is intended for testing serum samples from symptomatic patients or those suspected of Lyme Disease. It is intended to detect antibodies to VlsE and multiple other *B. burgdorferi* antigens following a modified two-tier test methodology. Positive results from the Viramed Biotech AG Borrelia All-In-One ViraChip are supportive evidence for the presence of antibodies and exposure to *B. burgdorferi*, the causative agent for Lyme disease. Negative results do not preclude infection with *B. burgdorferi*. Test

	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.	results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures as an aid in diagnosis of Lyme disease. The Viramed Biotech AG Borrelia All-In-One ViraChip Test must be used with a ViraChip Reader and the ViraChip Software.
Sample Type	Same	Serum
Controls	Same	Positive Control Serum, Negative Control Serum
Assay Type	Same	Qualitative
General Device Characteristic Differences		
Antibodies Detected	IgM	IgM and IgG
Antigens	P41, P39, P23, P31, P34, and LSA (a chimeric VlsE peptide)	VlsE, 93 kD, 58 kD, 45kD, 39 kD, 30 kD, 23kD, 21 kD, 19 kD, 18kD, and 17 kD antigens of *B. burgdorferi*
Assay Technology	ImmunoAssay	Antigen coated wells (Microarrays)
Reagents	Sample diluent, Wash Buffer, Milk powder, Conjugate Buffer, Substrate solution	10X Wash Buffer, Sample Buffer, Chromogen/Substrate Solution
Sample volume	20 µL neat serum in 1000 ml sample diluent	Samples diluted 1:76 and 100 µL added per well
Instrumentation	Manual	Automated with ViraChip Reader

VI Standards/Guidance Documents Referenced:

Establishing the Performance Characteristics of in Vitro Diagnostic Devices for the Detection of Antibodies to *Borrelia burgdorferi*. Guidance for Industry and Food and Drug Administration Staff

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility: The reproducibility of the iDart Lyme IgM ImmunoBlot Kit was evaluated in a study that included six anti-*Borrelia* IgM human serum samples at different analyte levels: high positive, two moderate positive, one low positive, one high negative, and one negative samples. The study was conducted across three sites, each with two operators over five non-consecutive days. On each of the five days, each operator performed one run, and each run included three replicates per sample. This generated a total of 90 replicates per sample (3 sites x 2 operators x 3 replicates x 5 days). There was 100% agreement on all bands on all runs, days, and operators at all three sites.

Table 4. iDart Lyme IgM ImmunoBlot Kit Reproducibility Study Results (all sites)

Sample #	Sample Type	IgM	# of Samples (+)	Expected Result	% agreement with 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. Linearity:
   Not Applicable

3. Analytical Specificity/Interference:

   Cross-Reactivity: Potential cross-reactivity of the iDart Lyme IgM ImmunoBlot Kit was evaluated in a study that tested left-over patient sera containing antibodies to potentially cross-reacting conditions (e.g., viral and bacterial infections as well as autoimmune disorders). The table below summarizes the potential cross-reactant and the number of samples per cross-reactant included in the study. Cross-reactivity was observed with samples containing antibodies to *Leptospira* (2/10), mononucleosis (1/15), *H. pylori* (1/9), and parvovirus-19 (1/10).

Table 5. Cross-Reactivity Study Results for iDart Lyme IgM ImmunoBlot Kit.

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,	Pregnant women	11	0	0%
Hyderabad (India)	*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.

Endogenous Interference: The potential interfering effect of endogenous substances in patient samples when using the iDart Lyme IgM ImmunoBlot was evaluated using one high positive, one moderate positive, and one negative *Borrelia* IgM samples. Samples were spiked with the endogenous substances at the final concentrations listed in the table below. No interference effect was observed in the tested samples.

Table 6. Endogenous Interference substances included in the study.

Endogenous Substance	Final Concentration
Bilirubin	1 mg/dL
	15 mg/dL
Albumin	3.5 g/dL
	5 g/dL
Cholesterol	150 mg/dL
	250 mg/dL
Triglycerides	150 mg/dL
	500 mg/dL
Hemoglobin	10 g/dL
	20 g/dL

4. Assay Reportable Range:
   Not Applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   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 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.

6. Detection Limit:
   Not Applicable

7. Assay Cut-Off:
   Not Applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:
   The purpose of this study was to determine the clinical performance of the iDart Lyme IgM ImmunoBlot kit when compared to an FDA-cleared EIA and immunoblot for detection of antibodies to *B. burgdorferi* following as part of the standard two-tier test (STTT) methodology. All samples were tested using the iDart Lyme IgM ImmunoBlot Kit and the STTT comparator.

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

Table 7: Sample distribution by clinical site and cohort.

Testing Site	Number of Samples	Sample Type	Vendors Providing Samples
Site 1	304	Prospective	IGeneX Inc.
Site 2	357	Prospective	
Site 3	336	Prospective	
Total	**997**		

Additionally, the CDC Premarket panel containing 258 samples was also evaluated. This panel contains samples collected 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 both endemic and non-endemic regions of Lyme disease.

Study Results

Clinical samples

All 997 samples were included in the performance calculations. The iDart Lyme IgM ImmunoBlot Kit Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) together with the 95% Confidence Interval (CI) were calculated against the comparator STTT. The table below summarize the iDart Lyme IgM ImmunoBlot Kit results.

Table 8. iDart Lyme IgM ImmunoBlot Kit Performance (N=997)

		STTT	
		Positive (+)	Negative (-)
iDart Lyme IgG ImmunoBlot	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%)	

CDC Premarket Panel

The table below summarizes the results of the iDart Lyme IgM ImmunoBlot Kit when testing samples from the CDC Premarket panel.

Table 9. CDC Premarket Panel: iDart Lyme IgM ImmunoBlot Kit Performance in Lyme Disease Samples.

Disease	**Stage I**		**Stage II**		**Stage III**		**Overall**	
N	**50**		**9**		**20**		**79**	
Test	**iDart**	**STTT**	**iDart**	**STTT**	**iDart**	**STTT**	**iDart**	**STTT**
Positive	33	28	8	7	11	9	52	44
Negative	17	22	1	2	9	11	27	35
Agreement	66.0%	56.0%	88.9%	77.8%	55.0%	45.0%	65.8%	55.7%

Table 10: CDC Premarket Panel: iDart Lyme IgM ImmunoBlot Kit performance in control samples.

	Healthy Controls		**Disease Controls**	
N	**95**		**84**	
Test	**iDart**	**STTT**	**iDart**	**STTT**
Positive	2	1	1	3
Negative	93	94	83	81
Agreement	97.9%	98.9%	98.8%	96.4%

2. Matrix Comparison:
   Not Applicable

C Clinical Studies:

1. Clinical Sensitivity:
   Not Applicable

2. Clinical Specificity:
   Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

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. Samples were provided by the CDC and the Bay Area Lyme foundation. Tables below summarize the performance of the device when testing samples from endemic and non-endemic areas respectively. One sample out of 177 (0.56%) tested samples from endemic areas was positive for IgM, using the iDart Lyme IgM ImmunoBlot Kit. Additionally, one sample out of 127 (0.79%) tested samples from non-endemic areas was positive for IgM using the iDart Lyme IgM ImmunoBlot Kit.

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

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 12: iDart Lyme IgM ImmunoBlot results for samples collected from healthy individuals in non-endemic areas.

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

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.