# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAY ONLY

## I Background Information:

### A 510(k) Number

K233367

### B Applicant

ID-FISH Technology, Inc.

### C Proprietary and Established Names

iDart Lyme IgG 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 IgG ImmunoBlot Kit for qualitative detection of IgG antibodies to *Borrelia burgdorferi* in human serum.

### B Measurand:

IgG antibodies to *Borrelia burgdorferi*.

### 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 IgG ImmunoBlot Kit is an immunoblot assay intended for the in vitro qualitative detection of IgG antibodies to *Borrelia burgdorferi* infection 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.

**C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

**D Special Instrument Requirements:**

Not applicable.

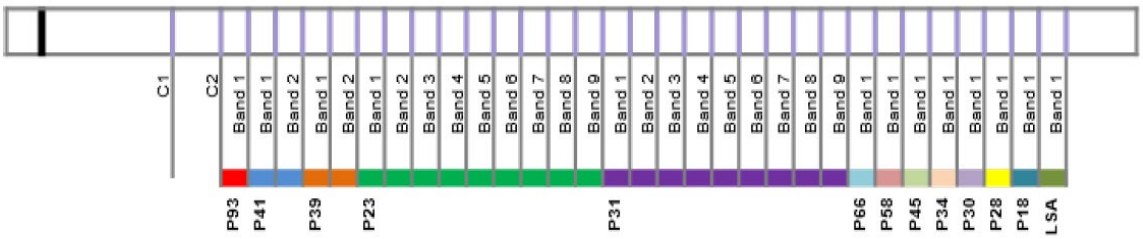
## IV Device/System Characteristics:

**A Device Description:**

The iDart Lyme IgG 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 then applied as discrete lines on a nitrocellulose membrane along with two control proteins.

The recombinant proteins are applied to the nitrocellulose membrane (test strip) of the iDart Lyme IgG ImmunoBlot Kit in the following order: Control 1 (C1: IgG/IgM), Control 2 (C2: Protein L), P93, P41, P39, P23, P31, P66, P58, P45, P34, P30, P28, P18 and LSA (a chimeric VlsE peptide termed the Lyme Screening Antigen).

**Figure 1: Schematic of the iDart Lyme IgG 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 IgG antibody for detection of IgG antibodies.

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 is stopped 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 (C1 and C2). Every test strip has two functional control bands. 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 IgG ImmunoBlot Kit.

**Table 1: iDart Lyme IgG ImmunoBlot Kit, 50 assays per kit**

| *Component* | *Packaging* | *Volume/ Quantity* | *Part. No.* |
|---|---|---|---|
| Lyme IgG ImmunoBlot strips | 15ml tube | 50 strips | LGIBS03 |
| IB Sample diluent | 60ml bottle | 55ml | IBSD03 |
| IB Wash Buffer | 60ml bottle | 60ml | IBWB03 |
| Milk powder | 0.5ml microcentrifuge tube | 0.75g | Milk03 |
| Lyme IgG IB Conjugate diluent | 60ml bottle | 60ml | LGIBCD03 |
| IB Phosphatase Substrate | 60ml bottle | 60ml | IBPS03 |
| LYME IgG IB Positive Kit Control Serum | 0.5ml microcentrifuge tube | 45µl | LGIBP03 |
| LYME IgG IB Negative Kit Control Serum | 0.5ml microcentrifuge tube | 45µl | LGIBN03 |
| LYME IgG IB Package Insert | Paper | 1 each | LGIBGPI |
| LYME IgG IB Reading Guide | Laminated paper | 1 each | LGIBGRG |

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
iv. Positive patient serum as positive control per run
v. Negative patient serum as negative control per run

**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. The positive and negative control strips of the run must be comparable to their previously established profiles due to subjectivity in reading.
2. 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.
3. If the negative control strip shows 2 or more reportable bands with intensity equal to or greater than 1+, the test must be repeated.
4. 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 preestablished 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 < calibration standard |
| + | A definite line or band intensity > or = to calibration standard |

**Table 3: Interpretation of results for iDart Lyme IgG ImmunoBlot test**

| Test Result | Interpretation |
|---|---|
| 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. |

## V Substantial Equivalence Information:

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

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

## C Comparison with Predicate(s):

| **Device & Predicate Device(s):** | K233367 | K220016 |
|---|---|---|
| Device Trade Name | iDart Lyme IgG ImmunoBlot Kit | Viramed Borrelia All-In-One ViraChip Test Kit |
| **General Device Characteristic Similarities** | | |
| Intended Use/Indications For Use | The iDart Lyme IgG ImmunoBlot Kit is an immunoblot assay intended for the in vitro qualitative detection of IgG antibodies to *Borrelia burgdorferi* infection 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.<br>Test results are to be used in conjunction with information obtained from the patient's clinical evaluation and other diagnostic procedures | The Viramed Biotech AG Borrelia All-In-One ViraChip is an in vitro qualitative microarrayassay 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 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 ViraChipReader and the ViraChip Software |
| Sample Type | Serum | Serum |
| Controls | Positive Control serum, Negative Control Serum | Positive Control serum, Negative Control Serum |
| Assay Type | Qualitative | Qualitative |
| **General Device Characteristic Differences** | | |
| Antibodies Detected | IgG | IgM and IgG |
| Assay Technology | Immunoblot | Antigen Coated wells (Microarrays) |

| | | |
|---|---|---|
| Antigens | P93, P41, P39, P23, P31, P66, P58, P45, P34, P30, P28, P18 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* |
| Procedure | Wash between sample and conjugate incubation steps, incubate with substrate. | Wash after Sample and Conjugate Step |
| Sample volume | 10-20µL neat serum in 1000µL sample diluent | Samples diluted 1:76 and 100 µL added per well |
| Reagents | Sample diluent, Wash Buffer, Milk powder, Conjugate Buffer, Substrate solution | 10X Wash Buffer, Sample Buffer, Sample Buffer, Chromogen/ Substrate Solution |
| Instrumentation | None, Manual | Automated with ViraChip Reader |
| Result Generation | Visually read (Color development) | 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 IgG ImmunoBlot Kit was evaluated in a study that included six anti-*Borrelia* IgG human serum samples at different analyte levels: high positive, moderate positive, low positive, high negative, and two 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 with expected results on all bands on all runs, all days and with all operators at all three sites.

   **Table 4: iDart Lyme IgG ImmunoBlot Kit Reproducibility study results (all sites, six operators).**

| 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% |
| GF | Low 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% |

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

*Cross-reactivity*
Potential cross-reactivity of the iDart Lyme IgG ImmunoBlot Kit was evaluated in a study that tested left-over patient sera containing antibodies to potentially cross-reacting conditions (viral and bacterial infection 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. No cross-reactivity was observed with any of the tested samples.

**Table 5: Cross-Reactivity study results for iDart Lyme IgG ImmunoBlot Kit**

| Source | Disease State | N (376) | iDart Lyme IgG ImmunoBlot | | | % Cross-reactivity |
|---|---|---|---|---|---|---|
| | | | LSA | 2+ Bands | IgG Result | |
| 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% |
| | Rickettsiosiis | 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, Hydrabad (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%** | |

*Endogenous Interference*
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:

**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 |

All samples were tested in singlicate. No interference effect was observed in the tested samples.

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values:

   *Fresh versus Frozen Sample Study*. 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 IgG ImmunoBlot Kit when using fresh and frozen samples, a total of 72 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 9 days, and not more than 44 days. All positive samples (N=33) remained positive, and all negative samples (N=39) remained negative when tested fresh and after storage at -20°C.

6. Detection Limit:

   Not applicable

7. Assay Analytical Specificity:

   Not Applicable

## B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable

2. Matrix Comparison:

   Not Applicable

## C Clinical Studies:

1. Clinical Sensitivity:

   The purpose of this study was to determine the clinical positive percent agreement and negative percent agreement of the iDart Lyme IgG ImmunoBlot kit by comparison to an FDA-cleared EIA and immunoblot for detection of antibodies to *B. burgdorferi* as part of a standard two-tier test methodology (STTT).

   A total of 768 serum samples were procured from two vendors and tested at three US sites. The table below summarizes the distribution of samples per testing site and source of samples.

   **Table 7: 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. |

   The samples tested at Site 1 (N=290) were prospectively collected from Lyme disease patients in endemic areas, (states of Wisconsin, New York, and Massachusetts) during years 2014 through 2018.

   Samples tested at sites 2 and 3 were unselected, leftover decoded serum samples from patients with Lyme-like symptoms, that were collected in Lyme endemic regions. Specifically, Cohort 2 (n=248) samples were submitted for routine Lyme Disease testing between January 2022 to April 2023. Cohort 3 (n=230) samples were received for routine Lyme disease testing between November 2023 and January 2024.

   Additionally, a well characterized panel containing 280 samples were received from CDC for testing. 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. All CDC samples were tested at ID-FISH Technology Inc.

*Study Results*

Clinical samples

The table below summarizes the iDart Lyme IgG ImmunoBlot Kit results for samples tested in the three clinical sites stratified by cohorts. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) together with the 95% Confidence Interval (CI).

**Table 8: Prospectively Banked Cohort 1: iDart Lyme IgG ImmunoBlot Kit clinical performance summary (N=290).**

| | | **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 9: Cohort 2: iDart Lyme IgG ImmunoBlot Kit clinical performance summary (N=248).**

| | | **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 10: Cohort 3: iDart Lyme IgG ImmunoBlot Kit clinical performance summary (N=230).**

| | | **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%) | |

CDC Reference Panel

The table below summarizes the iDart Lyme IgG ImmunoBlot Kit results for samples in the CDC reference panel.

**Table 11: CDC Reference Panel Testing results for iDart Lyme IgG ImmunoBlot Kit**

| | **Stage I (N=60)** | | **Stage II (N=10)** | | **Stage III (N=20)** | | **Healthy Controls (N=100)** | | **Disease Controls (N=90)** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Results** | ID-FISH | STTT | ID-FISH | STTT | ID-FISH | STTT | ID-FISH | STTT | ID-FISH | STTT |
| **Positive** | 35 | 18 | 9 | 9 | 20 | 20 | 0 | 0 | 0 | 0 |
| **Negative** | 25 | 42 | 1 | 1 | 0 | 0 | 100 | 100 | 90 | 90 |
| **Sensitivity** | 58.33% | 30.00% | 90.00% | 90.00% | 100% | 100% | | | | |
| **Agreement** | | | | | | | 100% | 100% | 100% | 100% |

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 IgG ImmunoBlot Kit following the instructions. Tables below summarize the performance of the device when testing samples from endemic and non-endemic areas respectively.

**Table 12: iDart Lyme IgG ImmunoBlot positive results for samples collected from healthy individuals in endemic areas.**

| **Source** | **N (313)** | **iDart Lyme IgG ImmunoBlot Results** | | |
|---|---|---|---|---|
| | | **LSA** | **2+ bands** | **IgG Positive** |
| **CDC** | **62** | 0 | 3 | 0 |
| **BAY AREA FOUNDATION (NY, MA, WI)** | **251** | 6 | 25 | 2 |
| **TOTAL** | **313** | 6 | 28 | 2 |
| **% Positive** | | | | 0.64% |

**Table 13: iDart Lyme IgG ImmunoBlot results for samples collected from healthy individuals in non-endemic areas.**

| Source | N (112) | iDART Lyme IgG ImmunoBlot Results | | |
|---|---|---|---|---|
| | | LSAr | 2+ bands | IgG Positive |
| **CDC** | **62** | 0 | 2 | 0 |
| **CA** | **50** | 1 | 3 | 0 |
| **TOTAL** | **112** | 1 | 5 | 0 |
| **% Positive** | | | | 0.00% |

## VIII Proposed Labeling:

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

## IX Conclusion:

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