510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K082329

B. Purpose for Submission:

To obtain a Substantial Equivalence determination for a new device

C. Measurand:

IgM antibodies to *Borrelia burgdorferi* (*B. burgdorferi*)

D. Type of Test:

Line blot assay

E. Applicant:

Viramed Biotech AG

F. Proprietary and Established Names:

Borrelia B31 IgM *ViraStripe*®

G. Regulatory Information:

1. Regulation section:

   21 CFR 866. 3830, Treponema pallidum treponemal test reagents

2. Classification:

   Class: II

3. Product code:

   LSR; Reagent, Borrelia Serological Reagent

4. Panel:

   83- Microbiology

H. Intended Use:

1. Intended use(s):

   The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is an in vitro qualitative assay for the detection of IgM antibodies against *Borrelia burgdorferi* in human serum. It is intended for use in the testing of human serum samples which have been found positive or equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies.

2. Indication(s) for use:

   The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is an in vitro qualitative assay for the detection of IgM antibodies against *Borrelia burgdorferi* in human serum. It is intended for use in the testing of human serum samples which have been found positive or equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies. Positive results from this line blot assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease. The Viramed Biotech AG Borrelia B31 IgM ViraStripe® can be used during the acute phase (0-4 weeks of symptoms onset) of *B. burgdorferi* infection. Patients who are positive by IgM but not IgG should have the test repeated a few weeks later if they remain ill. If they are still positive only by IgM and have been ill longer than one month, this is likely a false positive.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

   The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is a line blot assay to detect IgM to individual *B. burgdorferi* antigens. Isolated cultures of *B. burgdorferi* B31 spirochetes were harvested, concentrated, washed, and extracted to produce antigen fractions. Antigens were purified by electrophoretic and chromatographic methodologies and line striped on a nitrocellulose membrane. *B. burgdorferi* antigens are bound and fixed to the solid phase nitrocellulose membrane. The membrane is blocked, dried and cut into individual test strips.

   For each test to be performed, the line blot strip and diluted test serum is added to a line blot strip well. If specific antibodies that recognize an antigen are present, they will bind to the specific antigens on the strip. After incubation the line blot

strip is washed to remove unbound antibodies. Alkaline-phosphatase anti-human IgM conjugate is then added to each strip and incubated. If antibody is present, the conjugate will bind to the antibody attached to the specific antigens. The strip is washed to remove unbound conjugate and the substrate solution is added. If the enzyme/antibody complex is present, the substrate will undergo a precipitation and color change. After an incubation period, the reaction is stopped and the presence of precipitated substrate is visualized at specific locations on the strip. The presence of a colored precipitation at various locations on the line blot strip is an indirect measurement of *Borrelia burgdorferi* specific antibodies in the patient specimen. A uniform band locator is given on the evaluation protocol and used to locate and identify specific *Borrelia burgdorferi* B31 antibodies on the line blot test strip. Every strip has an integrated control system including function control and conjugate control. Visualized bands from the reaction are compared for intensity with a separate strip containing the Cut-off control band for evaluation. Any band found having a visual intensity equal to or greater than the Cut-off control band intensity is considered as a significant band.

Positivity: The criteria for a positive Western blot result defined by the CDC are followed. For *B. burgdorferi* IgM positivity, the blot should be positive for at least 2 of the 3 protein bands: p41, p39, p23kDa

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Viramed Biotech AG Borrelia B31 IgM ViraBlot

2. Predicate 510(k) number(s):

   K051169

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is an in vitro qualitative assay for the detection of IgM antibodies against *Borrelia burgdorferi* in human serum. It is intended for use in the testing of human serum samples which have been	The Viramed Biotech Borrelia B31 IgM ViraBlot® is an *in vitro* qualitative assay for the detection of IgM antibodies to *Borrelia burgdorferi* in human serum. It is intended for use in testing human serum samples which have been found positive or

	found positive or equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies.	equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies.
Assay	Line blot	Line blot
Specimen Type	Serum	Serum
Differences		
Item	**Device**	**Predicate**
Procedure	Qualitative; *B. burgdorferi* IgM antibodies to specific protein bands for the elution, purification and concentration of the membrane proteins	Qualitative; *B. burgdorferi* IgM antibodies to specific protein bands.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition and CLSI M34-A, Western Blot Assay for Antibodies to *Borrelia burgdorferi*, Approved Guidance

L. Test Principle:

Line blot

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Eight serum samples (low negative, high negative, low positive and moderate positive) were tested in duplicate over 5 working days (twice) by separate technicians at three laboratory sites using the same kit lot number. The serum panel specimens were selected to represent negative to high-positive immune-reactivity levels. For this study, two different readers within the three different laboratories assessed the band identification for each sample on the same blot. Six qualified technicians at the three sites assessed the Western blot bands from the same master lot kit number of Western blot kit. All samples were correctly interpreted by the 3 laboratories and 6 readers with 100% concordance for IgM.

Study Summary	Day 1- 5				
Sample ID	Tech 1		Tech 2		All Technicians
	Rep 1	Rep 2	Rep 1	Rep 2	Agreement
Low negative	-	-	-	-	100%
High negative (1)	39	39	39	39	100%
High negative (2)	23	23	23	23	100%
Low Positive (1)	41,23	41,23	41,23	41,23	100%
Low Positive (2)	41,39,23	41,39,23	41,39,23	41,39,23	100%
Low Positive (3)	39,23	39,23	39,23	39,23	100%
Moderate Positive (1)	41,23	41,23	41,23	41,23	100%
Moderate Positive (2)	41,23	41,23	41,23	41,23	100%

The Reproducibility study was satisfactory for this type of assay.

b. *Linearity/assay reportable range:*

Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Not applicable

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Cross-Reactivity

Seventy-five sera determined to contain antibodies to other infectious disease agents were tested. Cross-reactivity data for *Ehrlichia chafeensis* and *Babesia microti* may represent an actual co-infection with *B. burgdorferi*. All three tick borne organisms have been found to reside in the geographic location from which these 15 clinical specimens were obtained. Both of the specimens found positive in the Viramed Biotech AG Borrelia B31 IgM ViraStripe® were also found to be positive in a commercially available Lyme Western blot test system. Potential cross-reactivity due to circulating antibodies from infections with *Treponema phagedenis, Neisseria meningitidis, Haemophilus influenza, Yersinia enterocolitica, Campylobacter jejuni, Listeria*

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monocytogenes, Pseudomonas aeruginosa, E. coli, Salmonella enterica serovar *typhimurium, Shigella flexneri*, and *Legionella micdadei* have not been challenged, therefore the performance of this device is unknown if the specimen contains any of these circulating antibodies. See the Limitations section of the package insert for a list of untested, potentially cross-reactive organisms.

Disease State Sera	Number	Borrelia B31 IgM ViraStripe® Positive	Percent cross-reactivity
Ehrlichia chaffeensis	7	0	0%
Babesia microti	5	0	0%
Borrelia hermsii	6	2	33%
Leptospira interrogans	10	0	0%
Helicobacter pylori	10	0	0%
Epstein Barr Virus	6	0	0%
ENA Autoimmune	16	0	0%
Treponema pallidum	15	0	0%

INTERFERING SUBSTANCES

The following substances had an effect on test results when present in sera: hemolyzed, lipemic, or icteric sera and should not be used for testing. Because the firm did not test any sera from patients with immune-deficient diseases, elevated bilirubin, triglycerides, and heat-inactivated sera, the following statements will be added to the package insert: "The performance of this assay when testing sera from patients with any immune-deficient diseases such as HIV, HTLV, etc. and sera from patients that have had immune-suppressive therapy with drugs or medications is not known because no studies were conducted to assess the performance. Do not use heat-inactivated sera. Sera with elevated bilirubin, and triglycerides were not tested.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Correlations to the CDC Lyme Disease Panel

A Lyme Disease Clinical panel containing 44 clinically defined positives and negative samples was obtained from the Center for Disease Control and Prevention, Fort Collins, Colorado.

Time after Onset	Total	Borrelia B31 IgM ViraStripe®		
		Positive	Negative	% Agreement
Normals	5	0	5	100%
Clinically Undefined	3	1	2	100%
Early Localized	27	13	14	85%
Disseminated Disease	9	1	8	89%
Total	44	15	29	89%

College of American Pathologists 2003 Tick-borne Disease Proficiency Panel

The CAP Tick-borne Proficiency samples for the year 2003 were tested.

Samples tested: Positive 2   Negative 13
Correlation to Published CAP results proficiency = 93% (14/15)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Sensitivity:*

A total of 436 samples were prospectively collected and found to be EIA positive were sent to laboratories in California, Wisconsin, and Minnesota for Lyme disease testing. Samples were tested with the Viramed Biotech AG Borrelia B31 IgM ViraStripe® and the predicate. Results are presented in the tables below.

Subjects Sent to the Laboratory for Lyme Disease Testing

Predicate	Borrelia B31 IgM ViraStripe®		Total
	Positive	Negative	
Positive	55	7	62
Negative	4	370	374
Total	59	377	436

Percent Agreement			Exact 95% Confidence Intervals
Positive	88.7%	(55/62)	(78.1% – 95.3%)
Negative	98.9%	(370/374)	(97.3% - 99.7%)
Overall	97.5%	(425/436)	(95.5% - 98.7%)

One hundred and eighty five sera were obtained from patients that were clinically defined (culture confirmed) with Lyme borreliosis. Of these 185 sera, 158 were paired (79 acute and 79 convalescent) sera from patients diagnosed with Erythema migrans (EM), 11 with early-disseminated Lyme Disease/Carditis/Acute Neuroborreliosis and 16 with late stage Lyme arthritis. These samples were tested with the Borrelia B31 IgM ViraStripe® and the predicated. Results are presented in the tables below.

Clinically-defined Lyme disease samples

Stage	Borrelia B31 IgM ViraStripe®			
	Total	Positive	Negative	Sensitivity (95% Confidence Intervals)
Acute EM 1-21 days from Onset	79	30	49	38% (27.3% – 49.6%)
Convalescent EM 4 weeks after Onset	79	53	26	67% (55.6% – 77.3%)
Early Neurologic	11	9	2	82% (48.2% – 97.7%)
Late Arthritis	16	6	10	38% (15.2% – 64.6%)
Total	185	98	87	

Borrelia B31 ViraBlot® IgM	Borrelia B31 IgM ViraStripe®		
	Positive	Negative	Total
Positive	91	15	106
Negative	7	72	79
Total	98	87	185

	Percent Agreement	95% Confidence Intervals
Positive	92.8% (91/98)	85.8% - 97.1%
Negative	82.7% (72/87)	73.2% - 90.0%
Overall	88.1% (163/185)	83.4% - 92.8%

b. *Clinical specificity:*

For determination of analytical specificity, two hundred of the sera from normal blood donor individuals representing endemic and non-endemic geographic regions of the United States were tested for IgM *Borrelia burgdorferi* antibodies by the Viramed Biotech AG Borrelia B31 IgM ViraStripe® - see table below:

Analytical Specificity

	N	Negative	Positive	% Positive
Endemic	100	98	2	2%
Non-endemic	100	99	1	1%

    c. Other clinical supportive data (when a. and b. are not applicable):

    Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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