510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K163695

B. Purpose for Submission: To obtain a substantial equivalence determination and FDA clearance for a new device

C. Measurand: Anti-*Borrelia burgdorferi* (IgM) antibodies

D. Type of Test: Enzyme Immunoassay

E. Applicant: Viramed Biotech AG

F. Proprietary and Established Names: Borrelia B31 ViraChip lgM Test Kit

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: Microbiology (83)

H. Intended Use:

1. Intended use(s): The Viramed Biotech AG Borrelia B31 ViraChip IgM Test Kit is an *in vitro* qualitative protein microarray assay for the detection of IgM antibodies to *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 assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease.
The Viramed Biotech AG Borrelia B31 ViraChip IgM Test Kit must be used with a

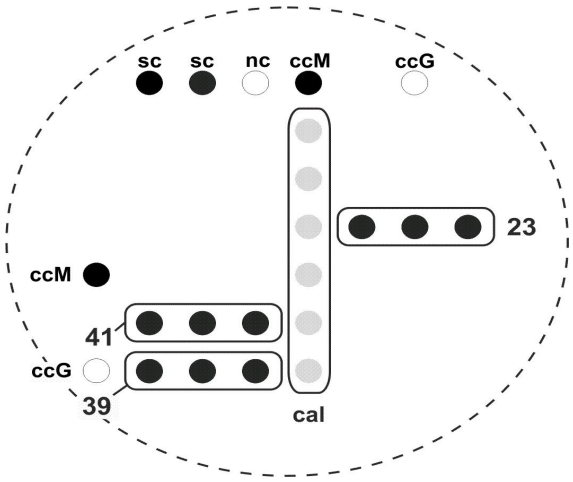
ViraChip Reader and the ViraChip Software.

2. Indication(s) for use: Same as Intended Use

3. Special conditions for use statement(s): For prescription use only

4. Special instrument requirements: ViraChip Reader and the ViraChip Software (Reviewed under K163504)

I. Device Description: The Viramed Biotech AG Borrelia B31 ViraChip IgM Test Kit is a protein microarray assay. A protein microarray can be considered as a modified solid-phase enzyme linked immunosorbent assay. Purified *B*. *burgdorferi* antigens (41kD, 39kD, and 23kD) were immobilized as individual spots onto a nitrocellulose membrane. A negative control (nc), two serum controls (sc), four conjugate controls, two for IgG and two for IgM (ccG and ccM), and six calibrator controls (cal) are also applied to each microarray. One microarray is fixed on the bottom of each cavity of a standard microtiter plate. The cavities are single breakable wells on a strip in a holding frame with 96 positions. Each *Borrelia* specific antigen is printed three times with the same concentration as a spot triplet. Each spot triplet corresponds to one band on an immunoblot. A schematic of the Borrelia B31 ViraChip IgM microarray is shown below.



For each test to be performed, the diluted test serum is added to one microarray. If specific antibodies that recognize an antigen are present, they will bind to the specific antigens on the microarray. After incubation the microarray is washed to remove unbound antibodies. Alkaline- phosphatase anti-human IgM (conjugate) is then added to each microarray and incubated. If antibodies are present, the conjugate will bind to the antibodies attached to the specific antigens. The microarray 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 microarray. The presence of a colored precipitation at various locations on the microarray is an indirect measurement of *B. burgdorferi* specific antibodies in the patient specimen. Visualized spots from the reaction are compared for intensity with the integrated calibrator controls for evaluation.

J. Substantial Equivalence Information:

1. Predicate device name(s): Viramed Borrelia B31 IgM ViraStripe

2. Predicate 510(k) number(s): K082329

3. Comparison with predicate:

Device	Predicate (K082329)
Similarities	
Reagent Comparison	
Nitro Cellulose Membrane Antigen Support	Nitro Cellulose Membrane Antigen Support
10X Wash Sample Diluent / Wash Buffer	10X Wash Sample Diluent / Wash Buffer
Chromogen/Substrate Solution RTU	Chromogen/Substrate Solution RTU
IgM Positive Control	IgM Positive Control
Negative Control	Negative Control
Procedure Comparison	
Dilute Samples 1:76 and use 100 µl dilution/well	Dilute Samples 20µl to 1.5mL
Dilute Controls 1:16 and use 100 µl dilution/well	Dilute Controls 100µL to 1.5mL
Dilute Wash Buffer 1:10	Dilute Wash Buffer 1:10
Wash with Recon buffer after Sample and Conjugate step.	Wash with Recon buffer after Sample and Conjugate step.
Dry Membrane/wells before reading	Dry Membrane/strips before reading
Differences	
Reading by use of the ViraChip Reader and ViraChip Software	Manual reading

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

N/A

L. Test Principle:

Enzyme Immunoassay

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision Study: A panel of six specimens was tested by Borrelia B31 ViraChip IgM at one site in 2 replicates, two operators per day over 12 days for a total of 48 tests for each specimen. Results were read by one ViraChip Reader. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative samples, one high negative sample, two low positive samples and one moderate positive sample. Final positive (≥2 spots) or negative (≤1 spot) agreement was 100% for all specimens. The results of the 3 significant *B. burgdorferi* antigen spots are shown in the table below:

Table 1: Precision Study

				Antigens		
Sample	**ELISA**	**Reactivity**	**Test Results**	**p41**	**p39**	**p23**
VM4281	*2.85*	Pos test results	**48**			
	Mod. Pos	Neg test results	0			
		Distinct signals		**48**	**48**	**9**
		% distinct signals		**100%**	**100%**	**19%**
VM4321	*1.28*	Pos test results	**48**			
	Low Pos	Neg test results	0			
		Distinct signals		**48**	**0**	**48**
		% distinct signals		**100%**	**0%**	**100%**
VM3065	*1.17*	Pos test results	**48**			
	Low Pos	Neg test results	0			
		Distinct signals		**48**	**0**	**48**
		% distinct signals		**100%**	**0%**	**100%**

VM3843	*0.89*	Pos test results	**0**			
	High Neg	Neg test results	48			
		Distinct signals		**6**	**0**	**0**
		% distinct signals		**13%**	**0%**	**0%**
VM3032	*0.41*	Pos test results	**0**			
	Low Neg	Neg test results	48			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**
VM2701		Pos test results	**0**			
	Low Neg	Neg test results	48			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**

Pos = positive; Mod = moderate; Neg = negative; Distinct signals = positive spots

Reproducibility Study: A panel of six specimens was tested with the Borrelia B31 ViraChip IgM at three sites, on 5 days, by two operators, in 3 replicates, equaling a total of 90 tests per specimen. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative, one high negative, two low positive, and one moderate positive sample. Final positive (≥2 spots) or negative (≤1 spot) agreement was 100% for all specimens. The results of the 3 significant *B. burgdorferi* antigen spots are shown in the table below:

Table 2: Reproducibility Study

				Antigens		
Sample	**ELISA**	**Reactivity**	**Test Results**	**p41**	**p39**	**p23**
VM4281	*2.85*	Pos test results	**90**			
	Mod. Pos	Neg test results	0			
		Distinct signals		**90**	**90**	**0**
		% distinct signals		**100%**	**100%**	**0%**
VM4321	*1.28*	Pos test results	**90**			
	Low Pos	Neg test results	0			
		Distinct signals		**90**	**0**	**90**
		% distinct signals		**100%**	**0%**	**100%**

VM3065	*1.17*	Pos test results	**90**			
	Low Pos	Neg test results	0			
		Distinct signals		**90**	**0**	**90**
		% distinct signals		**100%**	**0%**	**100%**
VM3843	*0.89*	Pos test results	**0**			
	High Neg	Neg test results	90			
		Distinct signals		**54**	**0**	**0**
		% distinct signals		**60%**	**0%**	**0%**
VM3032	*0.41*	Pos test results	**0**			
	Low Neg	Neg test results	90			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**
VM2701	*0.26*	Pos test results	**0**			
	Low Neg	Neg test results	90			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**

Pos = positive; Mod = moderate; Neg = negative; Distinct signals = positive spots

b. Linearity/assay reportable range: N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods): N/A

d. Detection limit: N/A

e. Analytical specificity:

Analytical Specificity Study: For determination of analytical specificity, 199 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 Borrelia B31 ViraChip IgM Test Kit.

Table 3: Analytical Specificity Study

	Total	**Negative**	**Positive**	**% Positive**	**% Negative**
Endemic	100	97	3	3%	97%
Non-endemic	99	98	1	1%	99%

Cross-Reactivity Study: A total of 215 potentially cross-reactive specimens from individuals with infectious conditions or autoimmune disorders were tested with Borrelia B31 ViraChip IgM Test. The results are shown in the table below:

Table 4: Cross-Reactivity Study

Disease State Sera	Total	Borrelia B31 ViraChip IgM Positive*	% Cross-reactivity
ENA autoimmune	16	0	0%
Babesia microti	10	1 (1)	10%
Borrelia hermsii	6	4 (2)	66.7%
Celiac disease	10	0	0%
Chlamydia trachomatis	10	0	0%
Cytomegalovirus	10	4 (3)	40%
Epstein–Barr virus	10	0	0%
Ehrlichia chaffeensis	10	1	10%
Fibromyalgia	10	2	20%
Helicobacter pylori	10	0	0%
Herpes simplex virus	10	0	0%
Influenza	10	0	0%
Leptospira interrogans	10	2	20%
Lupus	10	0	0%
Parvovirus B19	10	1 (1)	10%
Rheumatoid arthritis	10	0	0%
Rickettsia spp.	10	0	0%
Rubella virus	10	0	0%
Toxoplasma gondii	10	3 (3)	30%
Treponema pallidum	13	1	7.7%
Varicella zoster virus	10	0	0%

*Samples found positive by consensus testing with three FDA-cleared Western blot IgM assays are shown in parenthesis

Interfering Substances: Hemolyzed, lipemic, icteric, or microbially contaminated sera should not be used for testing by the Borrelia B31 ViraChip IgM Test. In addition the effect of elevated bilirubin and triglycerides on test results is not established.

f. Assay cut-off: Known positives of different levels and known negative samples were tested to determine the cut-off values for each antigen spot. Spot triplets are calculated in relation to the cut off by the ViraChip Software.

2. Comparison studies:

a. Method comparison with predicate device:

Prospective Study: Three independent clinical laboratories located in Minnesota, Massachusetts, and California performed comparative testing of routinely submitted

specimens for *B. burgdorferi* infection. The specimens testing positive or equivocal on a FDA cleared first-step EIA were tested with Borrelia B31 ViraChip IgM Test and an FDA cleared immunoblot. Interpretation of immunoblot results followed the recommended criteria described by CDC. The percent agreement with predicate device is given below.

Table 5: Percent Agreement with Predicate Device

Borrelia B31 ViraChip IgM	**Predicate Western Blot IgM**		
	Positive	**Negative**	**Total**
Positive	33	1	34
Negative	5	89	94
Total	38	90	128

	% Agreement	**95% Confidence Intervals**
Positive	86.8% (33/38)	(72.7% - 94.3%)
Negative	98.9% (89/90)	(94.0% - 99.8%)

b. Matrix comparison: N/A

3. Clinical studies:

a. Clinical Sensitivity:

Sensitivity Study: One hundred eighty five (185) sera were obtained from patients that were clinically defined and 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. The Borrelia B31 ViraChip IgM results are presented in comparison to the predicate device.

Table 6: Case Confirmed Lyme Disease Samples

Stage of Lyme Disease	**Borrelia B31 ViraChip IgM**			**Predicate Western Blot IgM**	
	Total	Positive	% Sensitivity	Positive	% Sensitivity
Acute EM 8-10 days from onset	79	38	41.1% (38/79)	30	37.9% (30/79)
Convalescent EM 4 weeks after onset	79	57	72.2% (57/79)	53	67.0% (53/79)
Early Neurologic	11	9	81.8% (9/11)	9	81.8% (9/11)
Late Arthritis	16	9	56.3% (9/16)	7	43.7% (7/16)
Total	185	113	61.1% (113/185)	99	53.5% (99/185)

Sensitivity Comparison:
Borrelia B31 ViraChip IgM: 61.1% (113/185) (CI: 53.9%-67.8%)
Predicate device: 53.5% (99/185) (CI: 46.3%-60.5%)
Difference in proportion: (14/185) 7.6%

CDC Serum Panel: A Lyme disease panel containing 44 clinically defined positive and negative samples was obtained from the Centers for Disease Control and Prevention, Fort Collins, Colorado. The Borrelia B31 ViraChip IgM results for these specimens are summarized in the table below:

Table 7: Testing of CDC Lyme Disease Panel

	Borrelia B31 ViraChip IgM				
CDC Reported Results	**Positive**	**Negative**	**Total**	**% Agreement**	**95% Confidence Intervals**
Positive	13	6*	19	68.4% (13/19)	(46.0% - 84.6%)
Negative	2	23	25	92.0% (23/25)	(75.0% - 97.8%)
Total	15	29	44	-	-

* Four of the six (4/6) samples were found to be negative by the predicate.

Note: The results are presented as a means to convey further information on the performance of this assay with a characterized serum panel from the CDC. This does not imply an endorsement of the assay by the CDC.

b. Clinical specificity: N/A

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

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

Expected Values: The incidence of IgM antibodies to *B. burgdorferi* antigenic proteins in different patient populations tested by the Borrelia B31 ViraChip IgM Test are shown in the table below. Lyme disease specimens were obtained from patients from Wakefield/Rhode Island and Lyme/Connecticut. For the prospective studies specimens originated from areas in Massachusetts, Minnesota, and California. Non-endemic blood donor samples were collected in Texas and endemic blood donor samples in Pennsylvania.

Table 8: Expected Values for the Borrelia B31 ViraChip IgM Test

	Antigens (% incidence)		
Borrelia B31 ViraChip IgM Spots	**p41**	**p39**	**p23**
Early Lyme Disease (n=39)	77%	24%	60%
Disseminated Lyme Disease (n=20)	82%	55%	91%
Late Lyme Disease (n=39)	69%	19%	56%
Non-Endemic Blood Donors (n=100)	35%	0%	1%
Endemic Blood Donors (n=100)	40%	1%	3%

Q. Proposed Labeling:

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

R. Conclusion:

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