Viramed Biotech AG – Borrelia B31 IgM ViraStripe®

A line blot serologic assay for the detection of IgM-specific antibodies against Borrelia burgdorferi in human serum

The Borrelia B31 IgM ViraStripe® uses the strain Borrelia burgdorferi sensu stricto (American strain Borrelia burgdorferi B31, low passage, tick isolate). Each membrane strip has an integrated control system with negative control band, serum control, three conjugate controls (IgG, IgA, IgM) and cut off control.

Viramed Biotech AG – Borrelia B31 IgM ViraStripe®

IgM Kit	**1 x 50 tests: Product-No.: V-BBSMUS** **10 x 50 tests: Product-No.: V-BBSMDS (Decakit)**
Specimen:	20µl serum
Time for testing:	90 minutes
Storage/Stability:	12 months at 2-8 °C

Materials Provided (Decakit)*:

1(10)* x 50	**Borrelia B31 ViraStripe® Antigen Strips (IgM),** strips of nitrocellulose, striped with purified antigens *of Borrelia burgdorferi sensu stricto* B31 and with bands for assay function and conjugate specificity; ready to use. **(Product No.: V-BBSMAS)**
1(10)* x 0.33ml	**Borrelia B31 ViraStripe® IgM Positive Control**, human serum, ready to use. **(Product No.: V-BBSMPK)**
1(10)* x 0.33ml	**Borrelia ViraStripe® IgG,A,M Negative Control**, human serum, ready to use. **(Product No.: V-BSSPNK)**
1(10)* x 4.5 ml	**AP- Anti-Human IgM Conjugate** concentrate. **(Product No.: V-UVNMKI45)** **to be used as 20x concentrate**.
1(10)* x 100 ml	**Diluent / Wash Buffer** 10x concentrate. **(Product No.: V-UVNUWP)**
1(10)* packets	**Diluent / Wash Powder** (5g). **(Product No.: V-UVNUMP)**
1(10)* x 90 ml	**Chromogen /Substrate Solution,** ready to use. **(Product No.: V-UVNUCS)**
1ea	**Instructions for Use** Viramed Biotech AG Borrelia B31 IgM ViraStripe® test kit
2(20)*ea	**Evaluation protocol** for Viramed Biotech AG Borrelia B31 IgM ViraStripe®

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 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 anytime after onset provided the EIA or IFA are positive or equivocal. It should also be used for follow-up when: 1) Only IgG antibodies were found positive in a line blot assay or Western blot, 2) IgM antibodies were found by line blot or Western blot but were not considered significant by the CDC criteria for a positive IgM Western blot, 3) previously tested seronegative individuals are shown to develop antibodies by an EIA or IFA test. 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.

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After this early period, infected patients are usually found to be Western blot positive for IgG. A positive IgM test alone is not recommended for use in determining active disease in persons with illness of longer than one month.

Summary and Explanation:

Borrelia burgdorferi is a spirochete that causes Lyme disease. The organism is transmitted by ticks of the genus *Ixodes*. In endemic areas, these ticks are commonly found on vegetation and animals such as deer, mice, dogs, horses, and birds (4).

B. burgdorferi infection shares features with other spirochetal infections (diseases caused by three genera in humans: *Treponema, Borrelia,* and *Leptospira*). Skin is the portal of entry for *B. burgdorferi* and the tick bite often causes a characteristic rash called *Erythema migrans* (EM) developed around the tick bite in 60% to 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids. Lyme disease occurs in stages, often with intervening latent periods and with different clinical manifestations (14).

In Lyme disease there are generally three stages of disease often with overlapping symptoms. Symptoms vary according to the sites affected by the infection such as joints, skin, central nervous system, heart, eye, bone, spleen, and kidney. Late disease is most often associated with arthritis or CNS syndromes. Asymptomatic subclinical infection is possible and infection may not become clinically evident until the later stages.

Patients with early infection produce IgM antibodies during the first few weeks after onset of EM and produce IgG antibodies more slowly (16). Although IgM only may be detected during the first month after onset of illness, the majority of patients develop IgG antibodies within one month. Both IgG and IgM antibodies can remain detectable for years.

For In Vitro Diagnostic Use-High complexity test.

Caution: U.S. Federal Law restricts this device to sale by or on the order of a licensed practitioner.

Isolation of *B. burgdorferi* from skin biopsy, blood, and spinal fluid has been reported (13). However, these direct culture detection methods may not be practical in the routine diagnosis of Lyme borreliosis. Serological testing methods for antibodies to *B. burgdorferi* include indirect fluorescent antibody (IFA) staining, immunoblotting, and enzyme immunoassay (EIA).

B. burgdorferi is antigenically complex with strains that vary considerably. Early antibody responses often are to flagellin which has cross reactive components. Patients in early stages of infection may not produce detectable levels of antibody. Also, early antibiotic therapy after EM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus, serological tests for antibodies to *B. burgdorferi* are known to have low sensitivity and specificity and these tests cannot be relied upon solely for establishing a diagnosis of Lyme disease (15, 3).

In 1994, the Second National Conference on Serological Diagnosis of Lyme disease recommended a two-step testing system toward standardizing laboratory serologic testing for *B. burgdorferi* (5). Because EIA and IFA methods were not sufficiently specific to support clinical diagnosis, it was recommended that positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested, or supplemented, by using a standardized Western Blot method (second step) for detecting antibodies to *B. burgdorferi*. Two-step positive results provide supportive evidence of exposure to *B. burgdorferi*, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis.

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Principle of the Assay

The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is a line blot assay. A line blot can be considered as a modified solid-phase enzyme linked immunosorbent assay. Isolated antigens are bound to a solid phase nitrocellulose support membrane. In vitro cultures of *Borrelia burgdorferi B31* spirochetes were harvested, concentrated, washed, and extracted to produce antigen fractions. Applying biotechnological purification methods purified antigens with the following molecular weights could be obtained: 41kD, 39kD, 23kD. The purified antigens were immobilized as individual bands (lines) onto the nitrocellulose membrane. Positions of the lines are exactly defined and can be assigned to the antigen bands reliably. A negative control band, a serum control, three conjugate controls (IgG, IgA, IgM) and a cut off control are also applied to the membrane, the membrane is labeled and cut into individual line blot assay 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 the negative control band, the serum control, three conjugate controls (IgG, IgA, IgM) and the Cut-off control. Visualized bands from the reaction are compared for intensity with the integrated Cut-off control for evaluation. Any band found having a visual intensity equal to or greater than the Cut-off control intensity is considered as a significant band.

Biological Source of Antigens and Anti-Human Antibody:

Antigens used for the Borrelia B31 IgM ViraStripe® are from cultured B31 low passage tick isolated *Borrelia burgdorferi* spirochetes. Antigens are separated and purified by molecular size using biotechnological purification methods. The AP-Anti-human IgM Conjugate is produced by conjugation of anti-human IgM antibodies from goat with bovine mucosal alkaline phosphatase.

Materials Required But Not Provided:

1. Washing steps will require a 500mL Wash bottle or Western blot/ line blot assay processor containing a wash step function.
2. Assorted graduated cylinders, 100mL and 1000mL.
3. Paper towels.
4. Pipettes and micropipettes capable of 20µL, 100µL and 10.0mL.
5. Appropriate pipette tips.
6. Distilled or deionized water.
7. A 30-minute or greater laboratory timer of an accuracy of +/- one second.
8. A basin or disposal area containing a 1% sodium hypochlorite solution (100mL household bleach in 900mL water) for disinfection.
9. A line *blot strip well tray* designed to contain line blot strips and test samples with a minimum volume capacity of 1.5mL.
10. One line blot platform rocker with a rocking frequency of 40/minute.

Note: Use clean and dry glass or plastic ware designed for laboratory use.

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Precautions:

1. For In Vitro Diagnostic Use Only.
2. All human serum components in this test kit have been tested and found to be negative for HIV 1,2 - and HCV-Antibodies and Hbs-Antigen. Nevertheless all human kit components and also the patient samples should be considered potentially infectious and carefully handled accordingly.
3. The CDC and the National Institutes of Health recommend that all potentially infectious material be handled at the Biosafety Level 2: CDC-NIH Manual, 1993. In: Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, U.S. Department of Health and Human Services, Public Health Service. pp 9-12.
4. Do not use test kit or components beyond published expiration dates.
5. Follow the test procedure; do not eliminate any recommended washing steps.
6. Do not mix components from different lot numbers.
7. Avoid cross-contamination of reagents by using dedicated labware and pipettes.
8. All reagents must be brought to room temperature (20- 23°C) before using. To prevent contamination, do not pour dispensed reagents back into original packaging.
9. Use only distilled water or deionized water for the test procedure.
10. Do not pipette by mouth.
11. Wear disposable gloves while working. Do not allow reagents or patient serum to come in contact with the skin, wash all contaminated areas with copious amounts of clean water.
12. The chromogen/substrate solution contains BCIP and NBT. Avoid contact with skin and eyes. In case of contact with skin and eyes wash with large quantities of water.
13. Specimen and all potentially contaminated materials have to be decontaminated using established laboratory techniques, e.g. by 20 minutes autoclaving at 121.5°C. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite.

Storage and Stability:

1. Store kits at 2-8°C. The unopened test kit is usable until date of expiration.
2. Antigen-Strips: Strips in closed bags are stable until expiration date if stored at 2 - 8°C. Close bags with unused strips tightly.
3. Conjugate, **20x concentrate**: Stable until expiration date if stored at 2-8°C. *Working dilution:* To be used in a single use.
4. Diluent/Wash Buffer: concentrate and powder: Stable until expiration date if stored at 2 - 8°C.
5. Buffer working dilution: 2 weeks usable if stored at 2 - 8°C. The buffer working dilution can be stored for 60 days in frozen aliquots.
6. Chromogen/Substrate Solution: Stable until expiration date if stored at 2 - 8°C.

Specimen Collection and Storage:

1. All blood and blood products should be handled as if infective; use safe laboratory methods for handling potentially infectious materials.
2. Use only freshly drawn serum for this test procedure; whole blood, lipaemic, hemolyzed, and icteric samples may have adverse effects on the performance of this product.
3. Store serum between 2 - 8°C for a period of no longer than 5 days. For long term storage, store sealed specimens between -20C° and -70C°. Do not expose specimens to multiple freeze / thaw cycles.
4. A minimum of 20µl of freshly drawn serum is required to perform this test. It would be recommended to draw 50 to 100µl of serum if repeat testing is required.

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Methods for Use:

Preparation of Reagents and Specimen:

1. Bring all components to room temperature (20-23°C) prior to use.
2. Antigen-strips: Carefully separate the required number of strips by use of forceps. Touch the strips with forceps only at the label.
3. Diluent/Wash Buffer working dilution: To prepare buffer working dilution, dilute the **Diluent** / **Wash Buffer** 10x concentrate with distilled water (100 ml concentrate + 900 ml dist. water) and add the **Diluent** / **Wash Powder** completely and mix until dissolved. Place the buffer working dilution for 10 to 15 minutes on a magnetic stir plate.
4. Conjugate working dilution: Dilute the needed amount of **20-fold concentrate** according to Table 1 prior to the first washing step (test procedure step 7).
5. Chromogen /Substrate Solution: Ready to use.
6. Controls: Use 100µl each of positive and negative control undiluted per test run.

Patient samples: Use 20µl patient serum undiluted per test.

Heat inactivation of serum may adversely affect the testing. The NCCLS provides recommendations for the storing of blood derived specimens (NCCLS Procedure M34-A, Vol. 20 No. 20, Western Blot Assay for Antibodies to *Borrelia burgdorferi;* Approved Guideline, 2000).

Table 1: Dilution of Conjugate Working Dilution (20 fold dilution)

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.425 ml	+	0.075 ml	1.5 ml	26	37.050 ml	+	1.950 ml	39.0 ml
2	2.850 ml	+	0.150 ml	3.0 ml	27	38.475 ml	+	2.025 ml	40.5 ml
3	4.275 ml	+	0.225 ml	4.5 ml	28	39.900 ml	+	2.100 ml	42.0 ml
4	5.700 ml	+	0.300 ml	6.0 ml	29	41.325 ml	+	2.175 ml	43.5 ml
5	7.125 ml	+	0.375 ml	7.5 ml	30	42.750 ml	+	2.250 ml	45.0 ml
6	8.550 ml	+	0.450 ml	9.0 ml	31	44.175 ml	+	2.325 ml	46.5 ml
7	9.975 ml	+	0.525 ml	10.5 ml	32	45.600 ml	+	2.400 ml	48.0 ml
8	11.400 ml	+	0.600 ml	12.0 ml	33	47.025 ml	+	2.475 ml	49.5 ml
9	12.825 ml	+	0.675 ml	13.5 ml	34	48.450 ml	+	2.550 ml	51.0 ml
10	14.250 ml	+	0.750 ml	15.0 ml	35	49.875 ml	+	2.625 ml	52.5 ml
11	15.675 ml	+	0.825 ml	16.5 ml	36	51.300 ml	+	2.700 ml	54.0 ml
12	17.100 ml	+	0.900 ml	18.0 ml	37	52.725 ml	+	2.775 ml	55.5 ml
13	18.525 ml	+	0.975 ml	19.5 ml	38	54.150 ml	+	2.850 ml	57.0 ml
14	19.950 ml	+	1.050 ml	21.0 ml	39	55.575 ml	+	2.925 ml	58.5 ml
15	21.375 ml	+	1.125 ml	22.5 ml	40	57.000 ml	+	3.000 ml	60.0 ml
16	22.800 ml	+	1.200 ml	24.0 ml	41	58.425 ml	+	3.075 ml	61.5 ml
17	24.225 ml	+	1.275 ml	25.5 ml	42	59.850 ml	+	3.150 ml	63.0 ml
18	25.650 ml	+	1.350 ml	27.0 ml	43	61.275 ml	+	3.225 ml	64.5 ml
19	27.075 ml	+	1.425 ml	28.5 ml	44	62.700 ml	+	3.300 ml	66.0 ml
20	28.500 ml	+	1.500 ml	30.0 ml	45	64.125 ml	+	3.375 ml	67.5 ml
21	29.925 ml	+	1.575 ml	31.5 ml	46	65.550 ml	+	3.450 ml	69.0 ml
22	31.350 ml	+	1.650 ml	33.0 ml	47	66.975 ml	+	3.525 ml	70.5 ml
23	32.775 ml	+	1.725 ml	34.5 ml	48	68.400 ml	+	3.600 ml	72.0 ml
24	34.200 ml	+	1.800 ml	36.0 ml	49	69.825 ml	+	3.675 ml	73.5 ml
25	35.625 ml	+	1.875 ml	37.5 ml	50	71.250 ml	+	3.750 ml	75.0 ml

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Assay Procedure:

1. Rinse the incubation tray with diluent/wash buffer and decant the liquid.	Mark the trays for identification. Rinse with buffer before using.
2. Place one strip per test into the channels.	Place the strips in channels of the incubation tray, one strip per sample and controls with the number facing up. Use forceps.
3. Fill each channel with 1.5 ml buffer and incubate by rocking for 5 min at room temperature.	Visually check to make sure strips are completely wet and not partially floating on top of the buffer. Use a platform rocker with a rocking frequency of approximately 40/min.
4. Add 20 µL of each patient sample or 100 µl of each control.	Pipette the controls and samples directly onto the number end of the strips while the rocking platform is stopped with numbered end of the strips in the full down position.
5. Incubate by rocking for 30 min at room temperature.	Visually check to make sure buffer remains in channel during rocking. Adjust rocker speed down if buffer is spilling out of the channel.
6. Decant the liquid.	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper. Strips adhere to the incubation tray when fluid is decanted.
7. 3 x 5 minutes washing: Add 1.5 ml diluent/wash buffer, incubate by rocking for 5 min at room temperature, decant the liquid completely.	Wash on the platform rocker. While washing prepare conjugate working dilution according to the conjugate dilution table. Tap the incubation tray on absorbent paper to remove remaining liquid.
8. Pipette 1.5 ml of conjugate working dilution into each channel.	Visually check to make sure strips are completely wet and not partially floating on top of the buffer.
9. Incubate by rocking for 15 min at room temperature.	
10. Decant the liquid.	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
11. 3 times washing as in step 7.	
12. Add 1.5 ml distilled water. Incubate by rocking for 1 min at room temperature.	
13. Decant the liquid.	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
14. Add 1.5 ml chromogen/substrate solution.	Visually check to make sure strips are completely wet and not partially floating on top of the chromogen/substrate solution.
15. Incubate by rocking for 5 – 15 min at room temperature.	**Stop the reaction when the Cut-off control is clearly visible.**
16. Stop the reaction by decanting the liquid.	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
17. Washing 3 times with 1.5 ml distilled water.	Wash without incubation time.
18. Dry the strips for interpretation.	Remove the wet strips from the channels by using forceps. Allow strips to air dry before interpretation of the data. **Do Not** read the results when the strips are wet. Read the results within the same working day after drying.

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Quality Control:

1. **Prepare evaluation protocol:** Record the data in the evaluation protocol. Glue (using a glue stick) the strips onto the protocol. Place the green separation line exactly onto the printed separation line in the protocol.
2. **Validity of the Test: Each individual test strip is valid when:** the bands for the function control, the conjugate control of the conjugate class being used and the Cut-off control **are clearly visible for that strip** and the negative control band **is not visible.** If a single test strip or strips are not valid, that individual test(s) must be repeated under exact observance of the working instructions. All valid test strips for the test run may be interpreted for results. Do not assess invalid test strips.
3. **Assignment of patient samples:** The provided Borrelia B31 IgM ViraStripe® evaluation protocol is used for band location and identification. Align the separation lines of the patient strips with the template strip. The band positions are shown on the template strip. Assign the bands on the patient strips and note them on the protocol.
4. **Controls:** For the results of the assay to be considered valid, the following conditions must be met:
   Negative Control: Interpretation of the Negative Control strip must be negative.
   Positive Control: Interpretation of the Positive Control strip must be positive.

Interpretation of results:

1. **Do Not** read the results when the strips are wet. Read the results **when dry** within the same working day.
2. **Score relative intensity** of bands present on patient specimen strips by comparing to the Cut-off control band (OspC) on the Cut-off control strips as follows:

Band intensity	Score
not visible	**-**
less than the Cut-off control	**+/-**
at least as intensely reactive as the Cut-off control = significant band	**+**

3. **For a final clinical diagnosis** all results from this and other tests must be correlated with clinical history, epidemiological data and other data available to the attending physician. Antibodies to different antigens are developed in the case of an infection with *Borrelia* species. These antibodies have different specificity and are typical for certain stages of the disease (1, 5, and 7). The significance of the bands is different in the IgG- and IgM-assay (5). Therefore there is a different interpretation of the bands for the IgG- and IgM ViraStripe®.

Result	Bands (kD)	Interpretation (5)
Positive	At least two significant (+) bands from: 41, 39, 23.	IgM-antibodies against *Borrelia species* detectable. Presumptive evidence of *B. burgdorferi* infection.
Negative	No bands or less than two significant bands.	No IgM-antibodies against *Borrelia species* detectable. In case of a clinically based suspicion of an infection with *Borrelia*: check additionally for IgG-antibodies and possibly check a second sample for IgG- and IgM-antibodies after 2-3 weeks.

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Expected Values

1. IgM antibodies usually appear 2-3 weeks after beginning of the disease (4, 7, 8). The antibody-titers often decrease some weeks to months after convalescence. It is also possible that antibody-titers remain constant up to some years (4, 5).
2. IgG-antibodies appear some weeks to months after an infection. In the early stage of the infection they often are not yet detectable (4, 7, 8). IgM should be checked in case of a suspected recent infection. In this case a second sample should be checked some time later. Patients in the 2nd or 3rd stage of the disease are usually positive for IgG-antibodies. The antibody-titers steadily decrease in convalescence (4, 7, 8).
3. The immune response and therefore the band pattern differ from patient to patient. A general rule is the increase of antibody types and the amount of specific bands with the progression of the disease (1).
4. An early antibiotic therapy can suppress the development of antibodies (2).
5. The incident if IgM antibodies found to various *B. burgdorferi* antigenic proteins with the Viramed Biotech B31 IgM ViraStripe are shown in Table 2. The 41kD flagellar protein is most often seen in both Lyme Borreliosis and blood donor populations. The incident of specific bands 39kD and 23kD increases in later stages of Lyme Borreliosis but is infrequent in the blood donor populations.
6. Specimens from potential cross-reactive diseases are frequently found to have a band at the 41kD flagellar protein. Disease sera from patients diagnosed with Ehrlichia, Babesia can have Borrelia specific bands from co-infection with Borrelia burgdorferi.

Table 2: Expected Values

Bands in kD	41	39	23
Early Lyme Disease	57%	17%	57%
Late Lyme Disease	38%	31%	56%
Non-Endemic Blood Donors	9%	1%	1%
Endemic Blood Donors	16%	0%	0%

Limitation of Use

1. Test results are valid only if the test procedure is strictly followed.
2. Serum from normal individuals or patients with other spirochetal infections may have cross-reactive antibodies present. Cross-reactions with antigens of *Borrelia* are described in infections with *Treponema*, *Leptospira* and other bacteria (9, 10, and 11). Cross-reactions are also described in cases of autoimmune diseases, MS, ALS, and Influenza.
3. Potential cross-reactivity due to circulating antibodies from infections with *Treponema phagedenis, Neisseria meningitidis, Haemophilus influenza, Yersinia enterocolitica, Campylobacter jejuni, Listeria 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.
4. Freshly drawn clear serum is required for the performance of this assay system. Haemolysed, lipaemic, or icteric sera should not be used for testing in addition sera with elevated bilirubin, and triglycerides were not tested. Also we do not recommend testing sera from patients with any immune-deficient diseases such as HIV, HTLV, etc.. Also we do not recommend testing sera from patients that have had immune-suppressive therapy with drugs or medications. Do not use heat-inactivated sera.
5. Reproducible results are dependent on good laboratory practices. Careful observation of all testing parameters, incubation timing and incubation temperature, preparation and washing between steps is required.
6. An early antibiotic therapy can suppress the development of antibodies (2).
7. If comparison with other methodologies is required, simultaneous testing should be performed.
8. The detection of specific antibodies for *Borrelia burgdorferi* in any given specimen can vary with assays from different manufacturers due to reagent specificity, assay methodology.
9. The Viramed Biotech AG Borrelia B31 IgM ViraStripe® is intended to be an aid to diagnosis only. It is to be performed on samples that are found to be positive or equivocal in an EIA or IFA test. Results must be used in conjunction with symptoms, patient's history, and other clinical findings.
10. This test is not intended for the determination of immune status but is only for the detection of IgM antibody to *Borrelia burgdorferi* B31 antigens.

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Performance Characteristics

Agreement

One hundred and eighty five (185) 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. The Borrelia B31 IgM ViraStripe® results are presented in Tables 3a,b,c.

Table 3a: 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	

Table 3b:

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

Table 3c:

	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%

CDC Serum Panel

A Lyme Disease Clinical panel containing 44 clinically defined positives and negative samples was obtained from the Centers for Disease Control and Prevention, Fort Collins, Colorado. The Borrelia B31 IgM ViraStripe® results for these specimens are summarized in Table 4. The results are presented as a means to convey further information on the performance of this assay with a masked characterized serum panel from the CDC. This does not imply an endorsement of the assay by the CDC.

Table 4: CDC National Lyme Disease Panel

		Borrelia B31 IgM ViraStripe®		
Time after Onset	Total	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%

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Prospective samples

A total of 436 which were prospectively collected and sent to laboratories in California, Wisconsin, and Minnesota for Lyme disease testing. Samples were tested with the Viramed Biotech AG Borrelia B31 ViraBlot® IgM Western blot and the Viramed Biotech AG Borrelia B31 IgM ViraStripe®. Results are presented in Tables 5a,b,c:

Table 5a: Subjects Sent to the Laboratory for Lyme Disease Testing

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

Table 5b:

Percent Agreement		Exact 95% Confidence Intervals
Positive	88.7% (55/62)	(81.6% – 97.2%)
Negative	98.9% (370/374)	(97.3% - 99.7%)
Overall	97.5% (425/436)	(96.1% - 99.0%)

Below in Table 5c are the Western Blot results of the 59 samples that were positive by the Borrelia B31 IgM ViraStripe® assay in the 436 prospective sample study. Of the 59 samples that were Borrelia B31 IgM ViraStripe® positive, 55 were IgM positive by Western blot. The Lyme EIA results were positive for all prospective samples.

Table 5c:

Result	Total	Borrelia B31 ViraBlot® IgM	
Borrelia B31 IgM ViraStripe®		Positive	Negative
Positive	59	55	4

Analytical Specificity Studies

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® - Table 6:

Table 6: Specificity

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

Specificity (197/200) = 98.5%
95% Confidence Interval = 95.5% – 99.5%[8]

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Precision Study

Assay precision was established at Viramed Biotech AG following a protocol outlined in CLSI document, EP5-A2. Eight (8) serum samples and one lot of Viramed Biotech AG Borrelia B31 IgM ViraStripe® test kits were tested in duplicate over 5 working days (twice) by separate technicians. The serum panel specimens were selected to represent negative to high-positive immune-reactivity levels. The sample aliquots were stored frozen prior to testing. The assay was performed according to the Instructions for Use, See Assay Procedure. The results (as "bands determined") are listed below.

Table 7:

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%

Cross Reactivity

Seventy-five sera determined to contain antibodies to other infectious disease agents are presented in Table 8. 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 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. See Limitations for list of untested, potentially cross-reactive organisms.

Table 8:

Disease State Sera	Number	Borrelia B31 IgM ViraStripe® Positive	Percent cross-reactivity
Ehrlichia chafeensis	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

Haemolysed, lipaemic, or icteric sera should not be used for testing in addition sera with elevated bilirubin, and triglycerides were not tested. Also we do not recommend testing sera from patients with any immune-deficient diseases such as HIV, HTLV, etc. Also we do not recommend testing sera from patients that have had immune-suppressive therapy with drugs or medications. Do not use heat-inactivated sera.

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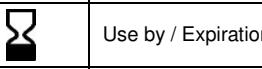
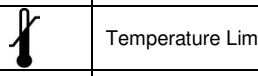
Symbols used

	Manufacturer	REF	Order Number
	Refer to Instructions for Use		Use by / Expiration Date
IVD	*In-Vitro* Diagnostic Medical Device		Temperature Limitation (Storage)
LOT	Test Kit Lot Number	CONTROL +	Positive Serum Control
	Sufficient for 50 Tests	CONTROL -	Negative Serum Control
	Room Temperature in °C	CONTROL	Control
	User	DATE	Date
#	Serum Number		Chromogen/Substrate Incubation Time in Minutes
PROTOCOL	Evaluation Protocol	No	Protocol Number

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