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LABORATORY · DIAGNOSTICS

Viramed Biotech AG – Borrelia B31 IgM Virablot®

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

The Borrelia B31 IgM ViraBlot® uses the strain Borrelia burgdorferi sensu stricto (American strain Borrelia burgdorferi B31, low passage, tick isolate). This antigen composition guarantees an optimal sensitivity and specificity of the assay. Each membrane strip has an integrated control system with controls for function and conjugate specificity.
Each kit contains one developed positive control strip (band locator).

Intended Use:
The Viramed Biotech Borrelia B31 IgM ViraBlot® is intended for use in the testing of human serum for the presence of human IgM antibodies to Borrelia burgdorferi as an aid in the diagnosis of Lyme borreliosis. For InVitro Diagnostic Use. High complexity test.

Viramed Biotech AG – Borrelia B31 IgM Virablot®

IgM Kit	**50 tests: Product-No.: V-BBBMUS**
Specimen:	20µl serum
Time for testing:	90 minutes
Storage/Stability:	12 months at 2-8 °C

Introduction:
The following introduction is taken from the Center for Disease Control Webpage: http://www.cdc.gov/ncidod/dvbid/lyme/, March 2005. Additional Lyme disease reading material may also be found on this Webpage. All CDC cited references are included for review purposes (11-35).
Lyme disease was named in 1977 when arthritis was observed in a cluster of children in and around Lyme, Connecticut. Other clinical symptoms and environmental conditions suggested that this was an infectious disease probably transmitted by an arthropod. Further investigation revealed that Lyme disease is caused by the bacterium, *Borrelia burgdorferi*.

Vector: Black-legged ticks (*Ixodes scapularis*) are responsible for transmitting Lyme disease bacteria to humans in the northeastern and north-central United States. On the Pacific Coast, the bacteria are transmitted to humans by the western black-legged tick (*Ixodes pacificus*). *Ixodes* ticks are much smaller than common dog and cattle ticks. In their larval and nymphal stages, they are no bigger than a pinhead. Ticks feed by inserting their mouths into the skin of a host and slowly take in blood. *Ixodes* ticks are most likely to transmit infection after feeding for two or more days.

Risk: In the United States, Lyme disease is mostly localized to states in the northeastern, mid-Atlantic, and upper north-central regions, and to several counties in northwestern California. In 2002, 23,763 cases of Lyme disease were reported to the Centers for Disease Control and Prevention (CDC) (MMWR 52(31):741-750). Ninety-five percent of these cases were from the states of Connecticut, Delaware, Rhode Island, Maine, Maryland, Massachusetts, Minnesota, New Jersey, New Hampshire, New York, Pennsylvania, and Wisconsin.

Individuals who live or work in residential areas surrounded by tick-infested woods or overgrown brush are at risk of getting Lyme disease. Persons who work or play in their yard, participate in recreational activities away from home such as hiking, camping, fishing and hunting, or engage in outdoor occupations, such as landscaping, brush clearing, forestry, and wildlife and parks management in endemic areas may also be at risk of getting Lyme disease.

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Clinical Description:

Lyme disease most often presents with a characteristic "bull's-eye" rash, erythema migrans, accompanied by nonspecific symptoms such as fever, malaise, fatigue, headache, muscle aches (myalgia), and joint aches (arthralgia).The incubation period from infection to onset of erythema migrans is typically 7 to 14 days but may be as short as 3 days and as long as 30 days.
Some infected individuals have no recognized illness (asymptomatic infection determined by serological testing), or manifest only non-specific symptoms such as fever, headache, fatigue, and myalgia.
Lyme disease spirochetes disseminate from the site of the tick bite by cutaneous, lymphatic and blood borne routes. The signs of early disseminated infection usually occur days to weeks after the appearance of a solitary erythema migrans lesion. In addition to multiple (secondary) erythema migrans lesions, early disseminated infection may be manifest as disease of the nervous system, the musculoskeletal system, or the heart. Early neurologic manifestations include lymphocytic meningitis, cranial neuropathy (especially facial nerve palsy), and radiculoneuritis. Musculoskeletal manifestations may include migratory joint and muscle pains with or without objective signs of joint swelling. Cardiac manifestations are rare but may include myocarditis and transient atrioventricular blocks of varying degree.
B. burgdorferi infection in the untreated or inadequately treated patient may progress to late disseminated disease weeks to months after infection. The most common objective manifestation of late disseminated Lyme disease is intermittent swelling and pain of one or a few joints, usually large, weight-bearing joints such as the knee. Some patients develop chronic axonal polyneuropathy, or encephalopathy, the latter usually manifested by cognitive disorders, sleep disturbance, fatigue, and personality changes. Infrequently, Lyme disease morbidity may be severe, chronic, and disabling. An ill-defined post-Lyme disease syndrome occurs in some persons following treatment for Lyme disease. Lyme disease is rarely, if ever, fatal

Summary and explanation of the test

The Viramed Borrelia B31 IgM Virablot® is a Western blot procedure specific for human IgM antibodies against B31 low passage tick isolated Borrelia burgdorferi spirochetes. The Western blot is part of a two-tiered methodology using sensitivity IgM or polyclonal enzyme immunoassay (EIA) screening method for initial testing. Following a positive or equivocal EIA result, the Borrelia burgdorferi Western blot is suggested for confirmation of the EIA test result.
Principle of the Assay
The Viramed Borrelia B31 IgM Virablot® is a Western blot assay utilizing the principles of polyacrylamide gel electrophoresis migration and transblot electrophoresis transfer. InVitro cultures of Borrelia burgdorferi B31 spirochetes are harvested, concentrated, washed, and sonicated to produce a Borrelia burgdorferi B31 antigen mixture. The antigen mixture is treated and applied to the top of a polyacrylamide gel for vertical flow electrophoresis. During the electrophoresis process, the specific Borrelia burgdorferi B31 antigens are separated and distributed by molecular size in the poly-acrylamide gel. The antigen containing gel is then placed on a nitrocellulose sheet for transblotting. During the transblotting procedure, the Borrelia burgdorferi B31 antigens are transferred from the gel to the nitrocellulose by transblot electrophoresis. The nitrocellulose membrane is then washed, treated, and inspected before a final drying procedure. After the application of function and conjugate controls to the membrane, the membrane is labeled and cut into individual Western blot strips.
A Western blot can be considered a modified solid-phase enzyme linked immunosorbent assay. Electrophoretically isolated antigens are bound to a solid phase nitrocellulose support membrane called an immunoblot strip. For each test to be performed, the immunoblot strip and diluted test sera is added to a *immunoblot strip well* designed to containing the immunoblot strip and sample. If specific antibodies that recognize an antigen are present, they will bind to the specific antigens on the strip. After incubation the immunoblot strip is washed to remove unbound antibody. An enzyme labeled anti-human IgM 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 a 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 Virastripe strip is an indirect measurement of Borrelia burgdorferi specific antibody in the patient specimen. A lot specific band locator (reacted immunoblot strip) is supplied and used to locate and identify specific Borrelia burgdorferi B31 antibodies on the test strip.

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The criteria for a positive Western blot result has been defined by the CDC: MMWR Weekly August 11, 1995/44(31); 590-591 and is found in the *Interpretation* section of this procedure. Every strip has an integrated control system including function control and conjugate control. Visualized bands from the reaction are compared for intensity with the integrated control system cut off for a positive or negative interpretation. Any band found having a visual intensity equal to or greater than the cut-off intensity is considered positive.

Biological Source of Antigens and anti-human antibody

All antigens used for the Borrelia B31 IgM Virablot® are from InVitro cultured B31 low passage tick isolated Borrelia burgdorferi spirochetes. Antigens are separated by molecular size using methods of electrophoretic isolation. The anti-human IgM antibody is produced in goat and purified by immunospecifc isolation prior to conjugation with bovine mucosal alkaline phosphatase.

Materials Supplied

50 strips		**Membrane-Strips,** strips of nitrocellulose, blotted with electrophoretically separated antigens *of Borrelia burgdorferi sensu stricto* B31 with bands for assay function and conjugate specificity; ready to use.	
0.33	ml	Borrelia burgdorferi **IgM Positive Control**, human serum, ready to use	
0.33	ml	Borrelia burgdorferi **IgM Cut-off-Control**, human serum, ready to use	
0.33	ml	Borrelia burgdorferi **Negative Control**, human serum, ready to use	
9.0	ml	IgM specific **AP –Anti-Human Conjugate,**	10x concentrate
100	ml	**Sample Diluent / Wash Buffer,**	10x concentrate
1 packet		**Sample Diluent / Wash Powder**	(5g)
90	ml	**Chromogen-/Substrate Solution,**	ready to use
1ea		Instructions for Use Viramed Borrelia B31 IgM Virablot® test kit	
1ea		Viramed Borrelia B31 IgM Virablot® lot specific Band Locator	
2ea		Viramed Borrelia B31 IgM Virablot® evaluation protocol	

Diluent/Wash Buffer, Diluent/Wash Powder and Chromagen/Substrate are interchangeable between different Virablot® and Virastrip® assays.

Materials Required But Not Provided

1. Washing steps will required a 500mL Wash bottle or Western blot/Immunostrip assay processor containing a wash step function.
2. Assorted graduated cylinders, 100 and 1000mL
3. Paper towels
4. Pipettes and micropipettes capable of 10µL, 100µL and 10.0mL
5. Appropriate pipette tips
6. Distilled or deionized water
7. A 30-minute or greater (0 – 60 minute) laboratory timer of an accuracy of +/- one second
8. A basin or disposal area containing a 0.5% sodium hypochlorite solution (50mL household bleach in 950mL water) for disinfection
9. An *immunoblot strip well tray* designed to containing Western blot or immunostripe strips and test samples with a minimum volume capacity of 3.0mL.
10. One Western blot or immunostrip platform rocker with a rocking frequency of 40/minute

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

Storage and Stability

1. Store unopened kits at 2-8°C. The 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, 10x concentrate: stable until expiration date if stored at 2-8°C. *Working dilution:* To be used in a single day.
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.

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Precautions

1. For InVitro Diagnostic Use Only.
2. All human serum components in this test kit have been tested and found to be negative for HIV- 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 form from different lot numbers except for Diluent/Wash Buffer and Chromogen/Substrate Solution.
7. Avoid cross-contamination of reagents by using dedicated labware and pipettes.
8. All reagents must be brought to room temperature (21- 25°C) before using. To prevent contamination, do not pour dispensed reagents back into original packaging.
9. Use only distilled water or de-ionized 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. Conjugates contain 0.1% sodium azide, controls and diluent/wash buffer contain 0,02% thimerosal. Attention: injurious to health. Wash with substantial amount of water after contact with skin or eyes. Poisonous when swallowed!
13. 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.
14. 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.
15. 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.
16. **Do Not** read the results when the strips are wet. Read the results when dry **within the same working day.**
17. Each **individual test strip is valid when:** the bands for the function control and the conjugate control **are clearly visible for that strip.** *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.*

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, lipemic, 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. 50 to 100µl of serum is recommended should repeat testing be required.
5. Heat inactivation of serum may adversely effect the testing. Do not use heat inactivated serum. The NCCLS provides recommendations for the storing of blood derived specimens (NCCLS Procedure H18-A, 1990).

Methods for Use

Preparation of Reagents and Specimen:

1. **Bring all components to room temperature (20-25°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 to15 minutes on a magnetic stir plate.
4. Conjugate working dilution: Dilute the needed amount of 10-fold concentrate according to Table 1 prior to the first washing step (test procedure step 7).

Other reagents: ready to use
Patient samples: Use 20µl patient serum undiluted per test
Controls: Use 100µl each of positive and negative control undiluted per test run.

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Assay Procedure:

	Step	Comment
1.	**Rinse the incubation tray with diluent/wash buffer and decant solution**	Mark the trays for identification. Rinsing 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 blunt tipped forceps.
3.	**Fill each channel with 1.5 ml buffer incubate 5 min at room temperature on a platform rocker**	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 for 5 min., decant 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 for 15 min by rocking 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**	
13.	**Incubate for 1 min by rocking at room temperature**	
14.	**Decant the liquid**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
15.	**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.
16.	**Incubate on the rocker platform:** **5 – 15 min**	**Stop the reaction when the cut off control is clearly visible.**
17.	**Stop the reaction by decanting the liquid.**	Remove the remaining liquid by carefully tapping the incubation tray on absorbent paper.
18.	**Washing 3 times with 1.5 ml distilled water.**	Wash without incubation time.
19.	**Dry the strips for interpretation**	Remove the wet strips from the channels by using forceps. Place the wet strips on absorbent paper towel and allow to air dry before interpretation of the data. **Do Not** read the results when the strips are wet. Remove the wet strips from the channels by using forceps. Place the wet strips on absorbent paper towel and allow 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. Reacted strips may be mounted and stored in a dry and dark location for up to a year.

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Table 1: Dilution of conjugate working dilution

Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate	Number of Tests	Diluted Working Buffer	+	Conjugate Concentrate	Final volume, Conjugate
1	1.35 ml	+	0.15 ml	1.5 ml	26	35.10 ml	+	3.90 ml	39.0 ml
2	2.70 ml	+	0.30 ml	3.0 ml	27	36.45 ml	+	4.05 ml	40.5 ml
3	4.05 ml	+	0.45 ml	4.5 ml	28	37.80 ml	+	4.20 ml	42.0 ml
4	5.40 ml	+	0.60 ml	6.0 ml	29	39.15 ml	+	4.35 ml	43.5 ml
5	6.75 ml	+	0.75 ml	7.5 ml	30	40.50 ml	+	4.50 ml	45.0 ml
6	8.10 ml	+	0.90 ml	9.0 ml	31	41.85 ml	+	4.65 ml	46.5 ml
7	9.45 ml	+	1.05 ml	10.5 ml	32	43.20 ml	+	4.80 ml	48.0 ml
8	10.80 ml	+	1.20 ml	12.0 ml	33	44.55 ml	+	4.95 ml	49.5 ml
9	12.15 ml	+	1.35 ml	13.5 ml	34	45.90 ml	+	5.10 ml	51.0 ml
10	13.50 ml	+	1.50 ml	15.0 ml	35	47.25 ml	+	5.25 ml	52.5 ml
11	14.85 ml	+	1.65 ml	16.5 ml	36	48.60 ml	+	5.40 ml	54.0 ml
12	16.20 ml	+	1.80 ml	18.0 ml	37	49.95 ml	+	5.55 ml	55.5 ml
13	17.55 ml	+	1.95 ml	19.5 ml	38	51.30 ml	+	5.70 ml	57.0 ml
14	18.90 ml	+	2.10 ml	21.0 ml	39	52.65 ml	+	5.85 ml	58.5 ml
15	20.25 ml	+	2.25 ml	22.5 ml	40	54.00 ml	+	6.00 ml	60.0 ml
16	21.60 ml	+	2.40 ml	24.0 ml	41	55.35 ml	+	6.15 ml	61.5 ml
17	22.95 ml	+	2.55 ml	25.5 ml	42	56.70 ml	+	6.30 ml	63.0 ml
18	24.30 ml	+	2.70 ml	27.0 ml	43	58.05 ml	+	6.45 ml	64.5 ml
19	25.65 ml	+	2.85 ml	28.5 ml	44	59.40 ml	+	6.60 ml	66.0 ml
20	27.00 ml	+	3.00 ml	30.0 ml	45	60.75 ml	+	6.75 ml	67.5 ml
21	28.35 ml	+	3.15 ml	31.5 ml	46	62.10 ml	+	6.90 ml	69.0 ml
22	29.70 ml	+	3.30 ml	33.0 ml	47	63.45 ml	+	7.05 ml	70.5 ml
23	31.05 ml	+	3.45 ml	34.5 ml	48	64.80 ml	+	7.20 ml	72.0 ml
24	32.40 ml	+	3.60 ml	36.0 ml	49	66.15 ml	+	7.35 ml	73.5 ml
25	33.75 ml	+	3.75 ml	37.5 ml	50	67.50 ml	+	7.50 ml	75.0 ml

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 and the conjugate control **are clearly visible for that strip.** *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 Virablot® lot specific Band Locator is used for band location and identification. Align the separation lines of the patient strips with the band locator strip. The band position is shown on the band locator. Assign the bands on the patient strips and note them on the protocol.

Interpretation of results

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. Theses antibodies have different specificity and are typical for certain stages of the disease (1, 3, and 5).
The significance of the bands is different in the IgG- and IgM-assay (3). Therefore there is a different interpretation of the bands for the IgG- and IgM ViraBlot®.

Meaning	Positive bands (kD)	Interpretation (3)
Positive	At least two significant bands from: 41, 39, 23kDa	IgM-antibodies against *Borrelia species* detectable. Recent borreliosis is suspected.
Negative	No bands or less than two significant bands.	No IgM-antibodies against *Borrelia species* detectable. No serological indication of borreliosis. In case of suspicion of an early infection with *Borrelia* use PCR and possibly check a second sample for IgG- and IgM-antibodies after 2-3 weeks.

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Expected Values

1. 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).
2. 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).
3. IgA-antibodies are detectable in many patients with an early stage of borreliosis. In some cases they are detectable before the appearance of IgM antibodies.
4. 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 continuance of the disease (1).
5. An early antibiotic therapy can suppress the development of antibodies (2).
6. Cross-reactions with antigens of Borrelia are described in infections with Treponema, Leptospira and other bacteria (6, 9, and 10).
7. Cross-reactions are also described in cases of autoimmune diseases, MS, ALS, and influenza.

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.
3. Freshly drawn clear serum is required for the performance of this assay system. Hemolyzed, lipemic, or icteric sera should not be used for testing. Do not use heat-inactivated sera.
4. 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.
5. If comparison with other methodologies is required, simultaneous testing should be performed.

The Viramed Borrelia B31IgM Virablot® is intended to be an aid to diagnosis only. Results must be used in conjunction with symptom's, patient's history, and other clinical findings. 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.

Performance Characteristics

Clinical Sensitivity Studies

One hundred sera were obtained from patients that were clinically diagnosed with Lyme borreliosis; Of these 100 sera, forty were paired (20 acute and 20 convalescent) sera from patients diagnosed with erythema migrans, 20 with early-disseminated neuroborreliosis and 40 with late stage Lyme arthritis. The Virablot® IgM Western blot results are presented in Table 2.

Table 2 Clinical Sensitivity

	Clinical Stage
Viramed Borrelia B31 IgM Western blot	***Acute/Erythema Migrans (Sampled 8-10 days after onset)***
Positive	14
Negative	6
	Clinical Sensitivity at 8-10 days after onset =**70%**
Viramed Borrelia B31 IgM Western blot	***Convalescent/Erythema Migrans (Sampled 4 weeks after onset)***
Positive	19
Negative	1
	Clinical Sensitivity at 4 weeks after onset =**95%**
Viramed Borrelia B31 IgM Western blot	***Early dissiminated neuroborreliosis***
Positive	19
Negative	1
	Clinical Sensitivity for early disseminated neuroborreliosis = **95%**
Viramed Borrelia B31 IgM Western blot	***Late Stage Lyme Arthritis***
Positive	14
Negative	26
	Clinical Sensitivity Late Stage Lyme Arthritis = **35%**

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Correlations to the Center for Disease Control National Lyme Disease Panel

A Lyme Disease Clinical panel containing 44 clinical defined positives and negative samples was obtained from the Center for Disease Control and Prevention, Fort Collins, Colo. The Virablot® results for these specimens are summarized in Table 3.

Table 3

Viramed Borrelia B31 IgM Western blot	*CDC National Lyme Disease Panel*
Positive	15/17
Negative	23/27
	Correlation = 86%

College of American Pathologists 2003 Tick-borne Disease Proficiency Panel

The CAP Tick-borne Proficiency for the year of 2003 was tested prospectively. The original proficiency panel was released as LY-A, LY-B, and LY-C over the course of 2003. Testing laboratories originally reported blind results for tabulation. The results in Table 4 summarized the performance of the Viramed Borrelia B31 IgM Western blot for these specimens.

Table 4

	CAP Published		*Viramed Borrelia B31 blot*	
	IgM Pos	IgM Neg	IgM Pos	IgM Neg
LY-A 2003 TTD01-05	1	4	0	5
LY-B 2003 TTD06-10	1	4	2	3
LY-C 2003 TTD11-15	1	4	1	4
Total	3	12	3	12
Correlation to Published CAP results for 2003 proficiency = 86%				

Specificity Studies

For determination of specificity, two hundred of the sera were from normal individuals representing endemic and non-endemic geographic regions of the United States that should not have antibodies to B. burgdorferi antigens were tested for IgM Borrelia burgdorferi antibodies by the Viramed Borrelia B31 IgM Virablot® - Table 5

Table 5

		Positive	Total
Viramed Borrelia	Positive	6	
B31 IgM Virablot®			
	Negative	194	
	Total		200

Specificity (194/200) = 96%

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Cross Reactivity

Seventy-nine sera determined to contain antibodies to other infectious disease agents are presented in Table 6. Cross-reactivity data for Erlichia 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 Borrelia B31 IgM Virablot® and a commercially available Lyme Western blot test system.

Table 6

Disease State Sera	Number	Virablot® Positive	Percent cross-reactivity
*Ehrlichia chafeensis**	7	0	0%*
*Babesia microti**	5	3	60%*
Borrelia hermsii	6	3	50%
Leptospira interrogans	10	2	0%
Helicobacter pylori	10	0	0%
Epstein Barr Virus	7	0	0%
ENA Autoimmune	16	0	0%
Treponema palidum	15	1	6%

Prospective Studies

At two commercial clinical testing laboratories, 134 specimens (Site 1) and 107 specimens (Site 2) were tested in parallel with the existing laboratory Western blot methodology. Laboratories collected Virablot® data from samples that were found to contain Borrelia burgdorferi banding patterns on their existing Western blot methodology, but not necessarily positive by the CDC Lyme Western blot testing criteria.

Commercial Clinical Site 1 Lyme Western blot testing correlation: 125/132=95%

Commercial Clinical Site 2 Lyme Western blot testing correlation: 101/109=93%

Product No.: V-BBBMUS Issued April, 2005 Rev E

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