LABORATORY • DIAGNOSTICS

Viramed Biotech AG – Borrelia B31 IgG ViraStripe®

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

The Borrelia B31 IgG 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 IgG ViraStripe®

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

Materials Provided (Decakit)*:

1(10)* x 50	**Membrane-Strips,** strips of nitrocellulose, striped with purified antigens *of Borrelia burgdorferi* strips *sensu stricto* B31 and with bands for assay function and conjugate specificity; ready to use. **(Product No.: V-BBSGAS)**
1(10)* x 0.33ml	Borrelia burgdorferi B31 ViraStripe® **IgG Positive Control**, human serum, ready to use. **(Product No.: V-BBSGPK)**
1(10)* x 0.33ml	Borrelia burgdorferi ViraStripe® **Negative Control**, human serum, ready to use. **(Product No.: V-BSSPNK)**
1(10)* x 9.0 ml	**AP- Anti-Human IgG Conjugate** concentrate. **(Product No.: V-UVNGKI)**
1(10)* x 100 ml	**Sample Diluent** / **Wash Buffer** 10x concentrate. **(Product No.: V-UVNUWP)**
1(10)* packets	**Sample 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 IgG ViraStripe® test kit
2(20)*ea	**Evaluation protocol** for Viramed Biotech AG Borrelia B31 IgG ViraStripe®

Intended Use:

The Viramed Biotech AG Borrelia B31 IgG ViraStripe® is an in vitro qualitative assay for the detection of IgG 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 line blot assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease. The Viramed Biotech AG Borrelia B31 IgG 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 IgM antibodies were found positive in an line blot assay or Western blot, 2) IgG antibodies were found by line blot or Western blot but were not considered significant by the CDC criteria for a positive IgG Western blot, 3) previously tested sero-negative individuals are shown to develop antibodies by an EIA or IFA test.

For In Vitro Diagnostic Use-High complexity test

Product No.: V-BBSGUS *: V-BBSGDS (Decakit) Issued January, 2017
1768_Borrelia_B31_IgG_ViraStripe_AL_Rev_H.doc
Manufactured by Viramed Biotech AG, Behringstrasse 11, D-82152 Planegg/Steinkirchen, Germany

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.

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 IgG 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: 93kD, 66kD, 58kD, 45kD, 41kD, 39kD, 30kD, 28kD, 23kD, 18kD. 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 IgG (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 IgG 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 IgG Conjugate is produced by conjugation of anti-human IgG 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, 100 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 (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. 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.

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

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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 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. 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, **10x 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, 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.
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 **10-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 (10 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	1.35 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

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Assay Procedure:

1. Rinse the incubation tray with diluent/wash buffer and decant solution.	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 blunt tipped forceps.
3. Fill each channel with 1.5 ml buffer and 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. Incubate for 1 min by rocking 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 on the rocker platform: 5 – 15 min.	**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. Reacted strips may be mounted and stored in a dry and dark location for up to a year.

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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 IgG 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: Bands must be present and 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 integrated on each strip as follows:

Band intensity	Score
not visible	**-**
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 five significant (+) bands from: 93, 66, 58, 45, 41, 39, 30, 28, 23, 18.	IgG-antibodies against *Borrelia species* detectable. Presumptive evidence of *B. burgdorferi* infection.
Negative	No bands or less than five significant bands.	No IgG-antibodies against *Borrelia species* detectable. In case of a clinically based suspicion of an infection with *Borrelia*: check additionally for IgM-antibodies 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 (6, 14, and 17). 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 (6, 14, and 17).
2. IgM antibodies usually appear 2-3 weeks after beginning of the disease (4, 7 and 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. Borrelia B31 IgG ViraStripe® band patterns will differ from sample to sample due to differences in patient immune responses and the stage to which the disease has progressed. A general rule is that antibody types and the amount of Borrelia B31 IgG specific bands will increase with the continuance of the disease (1).
4. Antibiotic therapy given to Lyme disease patients in early stages of the disease can suppress the development of specific Borrelia antibodies (2).
5. The incidence of IgG antibodies to *B. burgdorferi* antigenic proteins used in the Borrelia IgG ViraStripe® are shown in Table 2. The 41kD flagellar protein is most often seen in both Lyme and blood donor populations. The incidence of specific bands increases in later stages of 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. At a much lower frequency the 66kD Hsp (heat shock protein) can be seen. Disease sera from patients diagnosed with *Ehrlichia*, *Babesia* can have other *Borrelia* specific bands, possibly from co-infection with *Borrelia burgdorferi*.

Table 2: Expected Values for the Viramed Biotech AG Borrelia B31 IgG ViraStripe®

Bands in kD	93	66	58	45	41	39	30	28	23	18
Early Lyme Disease	8%	28%	17%	45%	**67%**	52%	5%	13%	62%	62%
Late Lyme Disease	85%	90%	73%	85%	**88%**	93%	80%	90%	80%	95%
Non-Endemic Blood Donors	1%	1%	2%	3%	**12%**	1%	0%	1%	4%	8%
Endemic Blood Donors	2%	0%	2%	0%	**9%**	0%	0%	2%	3%	12%

Limitations 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, Salmonell enteric*a serovar *Typhimurium, Shigella flexneri*, *Legionella micdadei,* and *Rickettsia rickettsii 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, lipemic, or icteric sera should not be used for testing. In addition, sera with elevated bilirubin, and triglycerides were not tested.
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. 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.
7. Do not use heat-inactivated sera.
8. Antibiotic therapy given to Lyme disease patients in early stages of the disease can suppress the development of specific Borrelia antibodies (2).
9. If comparison with other methodologies is required, simultaneous testing should be performed.
10. 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.
11. The Viramed Biotech AG Borrelia B31 IgG 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.
12. This test is not intended for the determination of immune status but is only for the detection of IgG antibody to *Borrelia burgdorferi* B31 antigens.

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Performance Characteristics

One hundred (100) sera were obtained from patients that were clinically defined and culture confirmed with Lyme borreliosis; of these 100 sera, 40 were paired (20 acute and 20 convalescent) sera from patients diagnosed with Erythema migrans (EM), 20 with early-disseminated Lyme Disease/Carditis/Acute Neuroborreliosis and 40 with late stage Lyme arthritis. The Borrelia B31 IgG ViraStripe® results are presented in Tables 3a. Comparisons to the predicate Borrelia B31 IgG Western blot are found in Tables 3b and 3c.

Table 3a: Clinically-defined Lyme disease samples

Stage	Borrelia B31 IgG ViraStripe®			
	Total	Positive	Negative	Sensitivity (95% Confidence Intervals)
Acute EM 1-21 days from Onset	20	5	15	25% (8.6% – 49.1%)
Convalescent EM 4 weeks after Onset	20	5	15	25% (8.6% – 49.1%)
Early Neurologic	20	13	7	65% (40.8% – 84.6%)
Late Arthritis	40	37	3	92.5% (79.6% – 98.4%)
Total	100	60	40	

Table 3b: Concordance with predicate device

Borrelia B31 ViraBlot® IgG	Borrelia B31 IgG ViraStripe®		
	Positive	Negative	Total
Positive	59	1	60
Negative	1	39	40
Total	60	40	100

Table 3c: Percent agreement with predicate device

	Percent Agreement	95% Confidence Intervals
Positive	98.3% (59/60)	91.1% - 100%
Negative	97.5% (39/40)	86.8% - 99.9%
Overall	98.0% (98/100)	93.0% - 99.8%

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 IgG 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 IgG ViraStripe®		
Time after Onset	Total	Positive	Negative	% Agreement
Normals	5	0	5	100%
Clinically Undefined	3	3	0	100%
Early Localized	27	6	21	93%
Disseminated Disease	9	8	1	89%
Total	44	17	27	93%

Product No.: V-BBSGUS *: V-BBSGDS (Decakit) Issued January, 2017
1768_Borrelia_B31_IgG_ViraStripe_AL_Rev_H.doc
Manufactured by Viramed Biotech AG, Behringstrasse 11, D-82152 Planegg/Steinkirchen, Germany

Prospective samples

A total of 435 samples that 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 ViraBlot® IgG Western blot and the Viramed Biotech AG Borrelia B31 IgG ViraStripe®. Results are presented in Tables 5a,b:

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

Borrelia B31 ViraBlot® IgG	Borrelia B31 IgG ViraStripe®		Total
	Positive	Negative	
Positive	58	3	61
Negative	1	373	374
Total	59	376	435

Table 5b:

Percent Agreement		Exact 95% Confidence Intervals
Positive	95.1% (58/61)	(86.3%-99.0%)
Negative	99.7% (373/374)	(98.5% - 100.0%)
Overall	99.1% (431/435)	(97.7% - 99.7%)

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 IgG *Borrelia burgdorferi* antibodies by the Viramed Biotech AG Borrelia B31 IgG ViraStripe® - Table 6:

Table 6: Specificity

	N	Negative	Positive	% Positive
Endemic	100	100	0	0%
Non-endemic	100	100	0	0%

Precision/ Reproducibility Study

Assay precision/reproducibility 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 IgG 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)	41,23,18	41,23,18	41,23,18	41,23,18	100%
High negative (2)	41,23,18	41,23,18	41,23,18	41,23,18	100%
Low Positive (1)	66,41,39,28,23,18	66,41,39,28,23,18	66,41,39,28,23,18	66,41,39,28,23,18	100%
Low Positive (2)	93,45,41,28,18	93,45,41,28,18	93,45,41,28,18	93,45,41,28,18	100%
Low Positive (3)	66,58,45,41,39,23,18	66,58,45,41,39,23,18	66,58,45,41,39,23,18	66,58,45,41,39,23,18	100%
Moderate Positive (1)	93,66,58,45,41,39,30, 28,23,18	93,66,58,45,41,39,30, 28,23,18	93,66,58,45,41,39,30, 28,23,18	93,66,58,45,41,39,30, 28,23,18	100%
Moderate Positive (2)	93,66,58,41,39,30, 28,18	93,66,58,41,39,30, 28,18	93,66,58,41,39,30, 28,18	93,66,58,41,39,30, 28,18	100%

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1768_Borrelia_B31_IgG_ViraStripe_AL_Rev_H.doc
Manufactured by Viramed Biotech AG, Behringstrasse 11, D-82152 Planegg/Steinkirchen, Germany

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 where these 12* clinical specimens were obtained. See Limitations for Use list of untested, potentially cross-reactive organisms.

Table 8:

Disease State Sera	Number	Borrelia B31 IgG ViraStripe® Positive	Percent cross-reactivity
*Ehrlichia chafeensis**	7	1	14%
*Babesia microti**	5	1	20%
Borrelia hermsii	6	0	0%
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%

†Extractable Nuclear Antigens (ENA) is not an infectious disease but can produce autoimmune antibodies with varied known and unknown specificity.

Interfering Substances

Haemolysed, lipemic, or icteric sera should not be used for testing, in addition sera with elevated bilirubin, and triglycerides were not tested. 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.

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Product No.: V-BBSGUS *: V-BBSGDS (Decakit) Issued January, 2017

1768_Borrelia_B31_IgG_ViraStripe_AL_Rev_H.doc

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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	№	Protocol Number

Product No.: V-BBSGUS *: V-BBSGDS (Decakit) Issued January, 2017
1768_Borrelia_B31_IgG_ViraStripe_AL_Rev_H.doc
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