LABORATORY • DIAGNOSTICS

Borrelia B31 ViraChip® IgM Test Kit

Intended Use:

The Viramed Biotech AG Borrelia B31 ViraChip® IgM Test Kit is an *in vitro* qualitative protein microarray assay for the detection of IgM antibodies to *Borrelia burgdorferi* in human serum. It is intended for use in the testing of human serum samples which have been found positive or equivocal using an EIA or IFA test procedure for *B. burgdorferi* antibodies. Positive results from this assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease.

The Viramed Biotech AG Borrelia B31 ViraChip® IgM Test must be used with a ViraChip® Reader and the ViraChip® Software.

Test Kit Information:

Order No.:	**V-BBCMOK**	Order No.:	**V-BBCMDK (Deca Kit)**
Kit size:	**96 wells**	Kit size:	**10x 96 wells**
Specimen:	**10µL serum**	Specimen:	**10µL serum**
Time for testing:	**approx. 130 minutes**	Time for testing:	**approx. 130 minutes**

For *In Vitro* Diagnostic Use
For prescription use only
CLIA: High Complexity Test

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 its infection 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.

The Viramed Biotech AG Borrelia B31 ViraChip® IgM Test should be used during the first four weeks after onset of symptoms provided the EIA or IFA are positive or equivocal. It should also be used for follow-up when previously tested sero-negative individuals are shown to develop antibodies by an EIA or IFA test.

Principle of the Assay:

The Viramed Biotech AG Borrelia B31 ViraChip® IgM is a protein microarray assay. A protein microarray can be considered as a modified solid-phase enzyme linked immunosorbent assay. Isolated antigens are bound to a solid phase nitrocellulose support membrane. Purified *B. burgdorferi* antigens with the following molecular weights are used: 41kD, 39kD, and 23kD. The antigens were immobilized as individual spots onto the nitrocellulose membrane. Positions of the spots are exactly defined and can be assigned to the antigen reliably. A negative control, two serum controls, four conjugate controls (two for IgG, two for IgM) and six calibrator controls are also applied to each microarray. One microarray is fixed on the bottom of each cavity of a standard microtiter plate (MTP). The cavities are single breakable wells on a strip in a holding frame with 96 positions.

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

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Biological Source of Antigens and Anti-Human Antibody:

Antigens used for the Borrelia B31 ViraChip® IgM are highly purified proteins derived from the strain *Borrelia burgdorferi sensu stricto* (American strain *Borrelia burgdorferi B31*). The AP-Anti-human IgM Conjugate is produced by conjugation of anti-human IgM antibodies from goat with bovine mucosal alkaline phosphatase.

Materials Provided:

1x or 10x 96 wells	**Borrelia B31 ViraChip® IgM Antigen Coated Wells** Wells with ViraChip® microarrays, ready to use, quarter green circle	(Prod. No.: V-BBCMAC)
1x or 10x 1.5mL	**ViraChip® AP-Anti-Human IgM Conjugate** Universal anti-human IgM conjugate for ViraChip® tests	(Order No.: V-UVNMKI15)
1x or 10x 100mL	**ViraChip® / ViraStripe® / ViraBlot® Diluent / Wash Buffer** Universal Diluent / Wash Buffer for ViraChip® and ViraStripe®	(Order No.: V-UVNUWP)
1x or 10x 5g	**ViraChip® / ViraStripe® / ViraBlot® Diluent / Wash Powder** Universal Diluent / Wash Powder for ViraChip® and ViraStripe®	(Order No.: V-UVNUMP)
1x or 10x 12mL	**ViraChip® Chromogen / Substrate Solution** Universal Chromogen / Substrate Solution for ViraChip® tests	(Order No.: V-UVCUCS)
0.11mL	**Borrelia B31 ViraChip® IgM Positive Control** Human, ready to use	(Order No.: V-BBCMPK)
0.11mL	**Borrelia B31 ViraChip® IgG,A,M Negative Control** Human, ready to use	(Order No.: V-BBCPNK)
1 ea	**Instructions for Use for Borrelia B31 ViraChip® IgM Test Kit**	

Materials Required but not Provided:

1. Washing steps will require a 500mL wash bottle or a microarray washer.
2. Assorted graduated cylinders: 20mL, 100mL and 1000mL.
3. Paper towels.
4. Pipettes and micropipettes capable of 10µL to 1000µL.
5. Appropriate pipette tips.
6. Distilled or deionized water.
7. A 0–60 minute laboratory timer of an accuracy of +/- one second.
8. A basin or disposal area containing a 0.5% sodium hypochlorite solution for disinfection.
9. Orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz.
10. A 2D barcode scanner.

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.

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 according to safety precautions. While working with potentially infectious/hazardous materials, all national and international rules, regulations, guidelines and laws must be taken into account. This also applies to storage and disposal of chemicals and reagents being used.
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. Potentially contaminated materials must be decontaminated using established laboratory techniques, e.g. by autoclaving at 121.5°C for 20 minutes. Liquid disposals can be mixed with sodium hypochlorite to a final concentration of 1% sodium hypochlorite.
13. Please refer to material safety data sheets for detailed information on potential risks, first aid guidelines, accidental release measures, handling and storage recommendations, personal protective equipment, directions for disposal and indications to toxicology.
14. Dust and other contaminations in the wells of the microtiter plate (MTP) must be avoided, as this might lead to invalid results.

Storage and Stability:

1. Store kits at 2-8°C. The unopened test kit is usable until date of expiration.
2. ViraChip® microarrays: In closed bags stable until expiration date if stored at 2-8°C. Close bags with unused microarrays tightly.
3. Wash Buffer, 10x concentrate: Stable until expiration date if stored at 2-8°C.
4. Wash 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.
5. Chromogen/Substrate Solution: Stable until expiration date if stored at 2-8°C.
6. Conjugate, 10x concentrate: Stable until expiration date if stored at 2-8°C.
7. Conjugate Working dilution: Prepare freshly prior to each run. Do not store for further use.

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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 human 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. Specimens may be stored at -20°C (or below) for long term storage. (CLSI document M34-A, Vol. 20 No. 20).
4. A minimum of 10µL of serum is required to perform this test. It would be recommended to draw 50 to 100µL of serum if repeat testing is required.
5. Prior to test processing, specimens should have reached room temperature. Mix specimens carefully after thawing. Precipitates in specimens can be removed by centrifugation.
6. Avoid multiple freeze and thaw cycles.

Methods for Use:

Bring all reagents to room temperature (20-23°C) prior to use. Let the packed microtiter plate acclimatize for at least 30 min before opening. The test has to be performed at room temperature.

Diluent / Wash Buffer Working Dilution:	Dilute **Diluent / Wash Buffer Concentrate 1:10** with distilled or deionized water (100mL concentrate + 900mL water). Add Diluent / Wash Powder completely and stir well until all powder is dissolved. If needed, place onto a magnetic stirrer for 10-15 minutes.
Wells:	Carefully unpack the microtiter plate (MTP) and place the required number of wells in an empty holding frame for microtiter plates (see assay procedure, step 2). Use wells directly after removing from packing. Return unused test strips directly into the original packing, seal well and store at 2-8°C.
Patient samples:	Use 100µL patient serum diluted **1:76** per well, e.g. **10µL of patient serum** with **750µL Diluent / Wash Buffer Working Dilution**.
Controls:	Use 100µL of Positive Control and 100µL of Negative Control, both diluted **1:16**, e.g. **10µL of control serum** with **150µL Diluent / Wash Buffer Working Dilution***.
Conjugate Working Dilution:	Prepare **Conjugate Concentrate 1:10** with Diluent / Wash Buffer Working Dilution (see table 1). Prepare freshly prior to each test run. Do not store for further use.
Chromogen / Substrate Solution:	Ready to use.

Preparation of the Test Run Using the ViraChip® Software:

Basic processes are **assigning, assembling, processing, scanning** and **analyzing.**

After starting the ViraChip® Software:
- **Assign**: Test selection and input of sample data.
- **Assemble**: Template for preparing the MTP and entering lot specific factors. These are scanned using a 2D bar code scanner from the packaging label of the MTP. **Each MTP can carry only one lot number for each ViraChip® test type.**
- **Process**: Data transfer to processor.

Product No.: V-BBCMOK / V-BBCMDK

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Preparation of Conjugate Working Dilution:

Number of wells	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final Volume	Number of wells	Diluent / Wash Buffer Working Dilution		Conjugate Concentrate	Final Volume
1	0.09 mL	+	0.01 mL	0.1 mL	51	4.59 mL	+	0.51 mL	5.1 mL
2	0.18 mL	+	0.02 mL	0.2 mL	52	4.68 mL	+	0.52 mL	5.2 mL
3	0.27 mL	+	0.03 mL	0.3 mL	53	4.77 mL	+	0.53 mL	5.3 mL
4	0.36 mL	+	0.04 mL	0.4 mL	54	4.86 mL	+	0.54 mL	5.4 mL
5	0.45 mL	+	0.05 mL	0.5 mL	55	4.95 mL	+	0.55 mL	5.5 mL
6	0.54 mL	+	0.06 mL	0.6 mL	56	5.04 mL	+	0.56 mL	5.6 mL
7	0.63 mL	+	0.07 mL	0.7 mL	57	5.13 mL	+	0.57 mL	5.7 mL
8	0.72 mL	+	0.08 mL	0.8 mL	58	5.22 mL	+	0.58 mL	5.8 mL
9	0.81 mL	+	0.09 mL	0.9 mL	59	5.31 mL	+	0.59 mL	5.9 mL
10	0.90 mL	+	0.10 mL	1.0 mL	60	5.40 mL	+	0.60 mL	6.0 mL
11	0.99 mL	+	0.11 mL	1.1 mL	61	5.49 mL	+	0.61 mL	6.1 mL
12	1.08 mL	+	0.12 mL	1.2 mL	62	5.58 mL	+	0.62 mL	6.2 mL
13	1.17 mL	+	0.13 mL	1.3 mL	63	5.67 mL	+	0.63 mL	6.3 mL
14	1.26 mL	+	0.14 mL	1.4 mL	64	5.76 mL	+	0.64 mL	6.4 mL
15	1.35 mL	+	0.15 mL	1.5 mL	65	5.85 mL	+	0.65 mL	6.5 mL
16	1.44 mL	+	0.16 mL	1.6 mL	66	5.94 mL	+	0.66 mL	6.6 mL
17	1.53 mL	+	0.17 mL	1.7 mL	67	6.03 mL	+	0.67 mL	6.7 mL
18	1.62 mL	+	0.18 mL	1.8 mL	68	6.12 mL	+	0.68 mL	6.8 mL
19	1.71 mL	+	0.19 mL	1.9 mL	69	6.21 mL	+	0.69 mL	6.9 mL
20	1.80 mL	+	0.20 mL	2.0 mL	70	6.30 mL	+	0.70 mL	7.0 mL
21	1.89 mL	+	0.21 mL	2.1 mL	71	6.39 mL	+	0.71 mL	7.1 mL
22	1.98 mL	+	0.22 mL	2.2 mL	72	6.48 mL	+	0.72 mL	7.2 mL
23	2.07 mL	+	0.23 mL	2.3 mL	73	6.57 mL	+	0.73 mL	7.3 mL
24	2.16 mL	+	0.24 mL	2.4 mL	74	6.66 mL	+	0.74 mL	7.4 mL
25	2.25 mL	+	0.25 mL	2.5 mL	75	6.75 mL	+	0.75 mL	7.5 mL
26	2.34 mL	+	0.26 mL	2.6 mL	76	6.84 mL	+	0.76 mL	7.6 mL
27	2.43 mL	+	0.27 mL	2.7 mL	77	6.93 mL	+	0.77 mL	7.7 mL
28	2.52 mL	+	0.28 mL	2.8 mL	78	7.02 mL	+	0.78 mL	7.8 mL
29	2.61 mL	+	0.29 mL	2.9 mL	79	7.11 mL	+	0.79 mL	7.9 mL
30	2.70 mL	+	0.30 mL	3.0 mL	80	7.20 mL	+	0.80 mL	8.0 mL
31	2.79 mL	+	0.31 mL	3.1 mL	81	7.29 mL	+	0.81 mL	8.1 mL
32	2.88 mL	+	0.32 mL	3.2 mL	82	7.38 mL	+	0.82 mL	8.2 mL
33	2.97 mL	+	0.33 mL	3.3 mL	83	7.47 mL	+	0.83 mL	8.3 mL
34	3.06 mL	+	0.34 mL	3.4 mL	84	7.56 mL	+	0.84 mL	8.4 mL
35	3.15 mL	+	0.35 mL	3.5 mL	85	7.65 mL	+	0.85 mL	8.5 mL
36	3.24 mL	+	0.36 mL	3.6 mL	86	7.74 mL	+	0.86 mL	8.6 mL
37	3.33 mL	+	0.37 mL	3.7 mL	87	7.83 mL	+	0.87 mL	8.7 mL
38	3.42 mL	+	0.38 mL	3.8 mL	88	7.92 mL	+	0.88 mL	8.8 mL
39	3.51 mL	+	0.39 mL	3.9 mL	89	8.01 mL	+	0.89 mL	8.9 mL
40	3.60 mL	+	0.40 mL	4.0 mL	90	8.10 mL	+	0.90 mL	9.0 mL
41	3.69 mL	+	0.41 mL	4.1 mL	91	8.19 mL	+	0.91 mL	9.1 mL
42	3.78 mL	+	0.42 mL	4.2 mL	92	8.28 mL	+	0.92 mL	9.2 mL
43	3.87 mL	+	0.43 mL	4.3 mL	93	8.37 mL	+	0.93 mL	9.3 mL
44	3.96 mL	+	0.44 mL	4.4 mL	94	8.46 mL	+	0.94 mL	9.4 mL
45	4.05 mL	+	0.45 mL	4.5 mL	95	8.55 mL	+	0.95 mL	9.5 mL
46	4.14 mL	+	0.46 mL	4.6 mL	96	8.64 mL	+	0.96 mL	9.6 mL
47	4.23 mL	+	0.47 mL	4.7 mL	97	8.73 mL	+	0.97 mL	9.7 mL
48	4.32 mL	+	0.48 mL	4.8 mL	98	8.82 mL	+	0.98 mL	9.8 mL
49	4.41 mL	+	0.49 mL	4.9 mL	99	8.91 mL	+	0.99 mL	9.9 mL
50	4.50 mL	+	0.50 mL	5.0 mL	100	9.00 mL	+	1.00 mL	10.0 mL

Table 1: '**1:10**' dilution of conjugate concentrate with Diluent / Wash Buffer Working Dilution.

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Assay Procedure:

1. Place the needed amount of wells into the holding frame. Fill free positions of the last column in the holding frame with blank wells.	Place the wells into the hold frame according to the plate layout. Pay attention that no plastic particles fall into the wells while breaking the bars.
2. Add 300µL Diluent / Wash Buffer Working Dilution to each well and incubate by shaking for approx. 5 minutes, aspirate.	Make sure the bottoms of the wells are completely covered with liquid. Use an orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz. The aspiration needles must not touch the bottom of the wells.
3. Add 100µL of each diluted patient serum or 100µL of each diluted control serum.	Add diluted patient sera and diluted control sera directly into the wells.
4. Incubate by shaking for 30 minutes at room temperature (RT).	Make sure the bottoms of the wells are completely covered with liquid. Use an orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz. Avoid spilling of liquid.
5. Aspirate the liquid.	The aspiration needles must not touch the bottom of the wells.
6. Wash 3 times: **- add 300µL Diluent / Wash Buffer Working Dilution** **- incubate by shaking for 5 minutes at RT** **- aspirate the liquid**	Make sure the bottoms of the wells are not damaged while adding the Diluent / Wash Buffer Working Dilution. The aspiration needles must not touch the bottom of the wells.
7. Add 100µL Conjugate Working Dilution.	Make sure the bottoms of the wells are completely covered with liquid.
8. Incubate by shaking for 30 minutes at RT.	Use an orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz.
9. Aspirate the liquid.	The aspiration needles must not touch the bottom of the wells.
10. Wash 3 times: as in step 6.	Make sure the bottoms of the wells are not damaged while adding the Diluent / Wash Buffer Working Dilution. The aspiration needles must not touch the bottom of the wells.
11. Add 300µL distilled or deionized water and incubate by shaking for approx. 5 minutes at RT.	Make sure the bottoms of the wells are completely covered with liquid.
12. Aspirate the liquid.	The aspiration needles must not touch the bottom of the wells.
13. Add 100µL Chromogen / Substrate Solution.	Make sure the bottoms of the wells are completely covered with liquid.
14. Incubate by shaking for 15 minutes at RT.	Use an orbital shaker with a shaking frequency of approx. 750 rpm or a linear shaker with a shaking frequency of approx. 20 Hz.
15. Stop the reaction by aspirating the liquid.	The aspiration needles must not touch the bottom of the wells.
16. Wash 3 times: by adding 300µL distilled or deionized water each time.	Without incubation.
17. Dry wells.	Visually check for residual liquid. If residual liquid is visible, tab the microplate gently upside down on paper tissue. Dry the wells under continuous airflow for 30 minutes.
18. Measure and interpret wells.	Measurements of spot intensities have to be performed within 24 hours (meanwhile store MTP in a dark place) by the ViraChip® Reader. The subsequent interpretation is done by the ViraChip® Software.

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Assay Interpretation with the ViraChip® Software:

1. After measuring the spot intensities the interpretation of the ViraChip® microarrays is performed using the ViraChip® Software. A detailed description of each step can be found in the ViraChip® Software user manual.

By using the ViraChip® Software you are able to:

- **Scan:** Measurement of the single ViraChip® microarrays by the ViraChip® Reader.

- **Analyze:** Calculation of the total result from the data

2. Check validity of ViraChip® microarrays.

The validity check is performed by the ViraChip® Software automatically.

A test run is valid, if the following spots are detectable on each ViraChip® microarray:
- **Serum controls (sc)**
- **Conjugate controls IgM (ccM)**
- **Calibrator controls (cal)**

and if the following spot is **not** visible:
- **Negative control (nc)**

If these validation criteria are not fulfilled, the ViraChip® microarray is classified as invalid. ViraChip® microarrays that are invalid must not be interpreted and should be repeated.

If multiple conjugate controls are detectable, the strongest spots must indicate the conjugate class being used.

3. Check spot assignment.

The well layout is shown in Fig. 1. The spot assignment is performed by the ViraChip® Software automatically.

The visual **verification of the spots being assigned** is done by the **user**. For any wrongly detected spots the **QC selection field** in the ViraChip® Software **has to be changed** to **"invalid"**. **This sample should be repeated.**

4. Assessment of ViraChip® microarrays.

According to quality laboratory guidelines, the use of cut off controls is recommended. The Borrelia B31 ViraChip® IgM contains calibrator spots to calculate the cut off for each antigen per well. The assessment is performed by the ViraChip® Software automatically.

The measured mean intensity of the calibrator controls is multiplied by the lot specific factor for each antigen (spot triplet). The resultant value is used as a cut off for the assessment of the respective antigen.

A spot triplet is considered as **distinct** if its mean intensity is **equal** to or **higher** than the intensity of the respective cut off.
A spot triplet is not assessed if its mean intensity is **lower** than the intensity of the respective cut off or if it is **not present**.

Negative Control: Interpretation of the Negative Control well must be negative.

The **Borrelia B31 ViraChip® IgG,A,M Negative Control** must be used for each run.

Positive Control: Interpretation of the Positive Control well must be positive.

The **Borrelia B31 ViraChip® IgM Positive Control** must be used for each run.

5. Interpretation of patient spots.

The following antigens of the Borrelia B31 ViraChip® IgM are considered for *Borrelia burgdorferi* for the detection of IgM antibodies: **41, 39,** and **23**.

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Figure:

Antigens and Controls

Each *Borrelia* specific antigen (**41, 39,** and**, 23)** is printed three times with the same concentration as a spot triplet. Each spot triplet corresponds to one band on an immunoblot.

Other spots include, serum controls (sc), negative control (nc), conjugate controls (ccG, ccM) and calibrator controls (cal), as shown in figure 1.

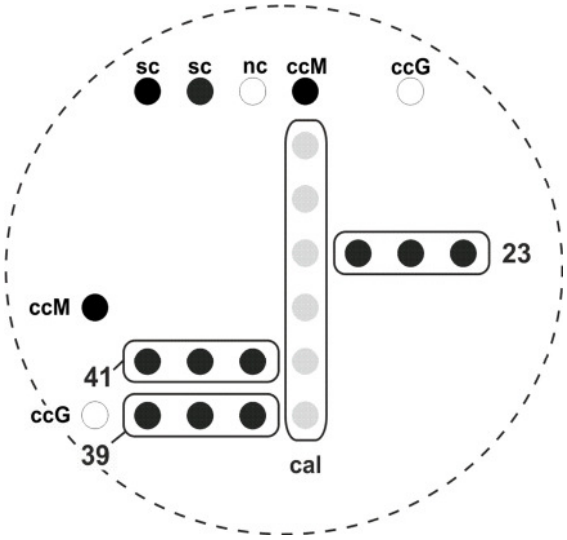

Figure 1: Schematic drawing of one well of the microtiter plate with the Borrelia B31 ViraChip® IgM microarray (magnified). Spot layout for antigens and integrated controls.

Interpretation of Results:

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

Identified spot triplet	Result	Interpretation
At least two **distinct** spot triplets from: **41, 39, 23**	**Positive**	IgM antibodies against *Borrelia burgdorferi* detectable. Evidence of past or present *Borrelia burgdorferi* infection.
No spot triplets or **less than two distinct** spot triplets	**Negative**	No IgM specific antibodies against *Borrelia burgdorferi* detectable. If an infection with *Borrelia burgdorferi* is suspected, check additionally for IgG antibodies and possibly check a second sample for IgG and IgM antibodies after 2-3 weeks.

Limitations of Use:

1. Trained personnel only should perform the assay procedure. Test results are valid only if the test procedure is strictly followed. To ensure reliable results follow the "Good Laboratory Practice" guidelines.
2. Drying of processed ViraChip® microarrays is essential prior to scanning for consistent results.
3. Serum from individuals with other spirochetal and tick-borne infections may have cross-reactive antibodies present to *B. burgdorferi* proteins (9,10,11). Refer to the cross-reactivity section for specific examples.
4. Do not use heat-inactivated sera. Hemolyzed, lipemic, icteric or microbially contaminated sera should not be used for testing. The effect of elevated bilirubin and triglycerides in sera was not tested with Borrelia B31 ViraChip® IgM Test.
5. 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.
6. Antibiotic therapy given to Lyme disease patients in early stages of the disease can suppress the development of specific *Borrelia* antibodies (2).
7. The detection of specific antibodies for *Borrelia burgdorferi* in any given specimen can vary with assays from different manufacturers due to reagent specificity and assay methodology. If comparison with other methodologies is required, simultaneous testing should be performed.
8. The Borrelia B31 ViraChip® IgM 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.
9. 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. A positive result indicates detectable IgM antibody titers and indicates exposure to *B. burgdorferi*. A correlation to Lyme disease is possible conditionally. A negative result does not exclude an infection with the pathogen or the presence of the disease.

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Expected Values:

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

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

Table 2: Expected Values for the Borrelia B31 ViraChip® IgM.

Performance Characteristics:

Sensitivity Study: 185 sera were obtained from patients that were clinically defined and/or culture confirmed with Lyme Borreliosis; of these 185 sera, 158 were paired (79 acute and 79 convalescent) sera from patients diagnosed with erythema migrans (EM), 11 with early-disseminated Lyme Disease / Carditis / Acute Neuroborreliosis and 16 with late stage Lyme arthritis. The Borrelia B31 ViraChip® IgM results are presented in table 3 in comparison to the predicate device.

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

Table 3: Case confirmed Lyme disease samples.

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

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Prospective Study:

Three independent clinical laboratories located in Minnesota, Massachusetts, and California performed comparative testing of routinely submitted specimens for *B. burgdorferi* infection. The specimens testing positive or equivocal on a FDA cleared first-step EIA were tested with Borrelia B31 ViraChip® IgM Test and an FDA cleared immunoblot. Interpretation of immunoblot results followed the recommended criteria described by CDC (5). The results are summarized in Tables 4a and 4b.

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

Table 4a: Samples sent to the Laboratory for Lyme Disease Testing.

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

Table 4b: Percent agreement with predicate device.

CDC Serum Panel:

A Lyme disease panel containing 44 clinically defined positive and negative samples was obtained from the Centers for Disease Control and Prevention, Fort Collins, Colorado. The Borrelia B31 ViraChip® IgM results for these specimens are summarized in table 5. The results are presented as a means to convey further information on the performance of this assay with a characterized serum panel from the CDC. This does not imply an endorsement of the assay by the CDC.

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

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

Table 5: Testing of CDC Lyme Disease Panel.

Analytical Specificity Study:

For determination of analytical specificity, 199 sera from normal blood donor individuals representing endemic and non-endemic geographic regions of the United States were tested for IgM *Borrelia burgdorferi* antibodies by the Borrelia B31 ViraChip® IgM as shown in table 6.

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

Table 6: Specificity studies.

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Cross-Reactivity Study:

A total of 215 potentially cross-reactive specimens from individuals with infectious conditions or autoimmune disorders were tested with Borrelia B31 ViraChip IgM Test. The results are shown in Table 7.

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

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

Table 7: Cross-reactivity study.

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Precision Study:

A panel of six specimens was tested by Borrelia B31 ViraChip IgM, at one site in 2 replicates, two operators per day over 12 days for a total of 48 tests for each specimen. Results were read by one ViraChip® Reader. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative samples, one high negative sample, two low positive samples and one moderate positive sample. Final positive (≥2 spots) or negative (≤1 spot) agreement was 100% for all specimens. The results of the 3 significant *B. burgdorferi* antigen spots are shown in the table 8.

				Antigens		
Sample	**ELISA**	**Reactivity**	**Test Results**	**p41**	**p39**	**p23**
VM4281	*2.85*	Pos test results	**48**			
	Mod. Pos	Neg test results	0			
		Distinct signals		**48**	**48**	**9**
		% distinct signals		**100%**	**100%**	**19%**
VM4321	*1.28*	Pos test results	**48**			
	Low Pos	Neg test results	0			
		Distinct signals		**48**	**0**	**48**
		% distinct signals		**100%**	**0%**	**100%**
VM3065	*1.17*	Pos test results	**48**			
	Low Pos	Neg test results	0			
		Distinct signals		**48**	**0**	**48**
		% distinct signals		**100%**	**0%**	**100%**
VM3843	*0.89*	Pos test results	**0**			
	High Neg	Neg test results	48			
		Distinct signals		**6**	**0**	**0**
		% distinct signals		**13%**	**0%**	**0%**
VM3032	*0.41*	Pos test results	**0**			
	Low Neg	Neg test results	48			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**
VM2701		Pos test results	**0**			
	Low Neg	Neg test results	48			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**

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

Table 8: Precision study.

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Reproducibility Study:

A panel of six specimens was tested with the Borrelia B31 ViraChip IgM at three sites, on 5 days, by two operators, in 3 replicates, equaling a total of 90 tests per specimen. Samples were selected based on FDA cleared *B. burgdorferi* ELISA results, including 2 low negative, one high negative, two low positive, and one moderate positive sample.
Final positive (≥2 spots) or negative (≤1 spot) agreement was 100% for all specimens. The results of the 3 significant *B. burgdorferi* antigen spots are shown in table 9.

				Antigens		
Sample	**ELISA**	**Reactivity**	**Test Results**	**p41**	**p39**	**p23**
VM4281	*2.85*	Pos test results	**90**			
	Mod. Pos	Neg test results	0			
		Distinct signals		**90**	**90**	**0**
		% distinct signals		**100%**	**100%**	**0%**
VM4321	*1.28*	Pos test results	**90**			
	Low Pos	Neg test results	0			
		Distinct signals		**90**	**0**	**90**
		% distinct signals		**100%**	**0%**	**100%**
VM3065	*1.17*	Pos test results	**90**			
	Low Pos	Neg test results	0			
		Distinct signals		**90**	**0**	**90**
		% distinct signals		**100%**	**0%**	**100%**
VM3843	*0.89*	Pos test results	**0**			
	High Neg	Neg test results	90			
		Distinct signals		**54**	**0**	**0**
		% distinct signals		**60%**	**0%**	**0%**
VM3032	*0.41*	Pos test results	**0**			
	Low Neg	Neg test results	90			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**
VM2701	*0.26*	Pos test results	**0**			
	Low Neg	Neg test results	90			
		Distinct signals		**0**	**0**	**0**
		% distinct signals		**0%**	**0%**	**0%**

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

Table 9: Reproducibility study.

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Notes to Equipment and Software:

1. Automated plate processing requires the use of ViraChip® assay specific parameters and procedures i.e., incubations and washing steps, according to the instructions by Viramed Biotech AG (see ViraChip® Methods for Use and Assay Procedure sections).
2. Usage of processor specific consumables (i.e. reagents) must be according to the manufacturer's instruction by Viramed Biotech AG (see ViraChip® Methods for Use section).
3. The equipment and software configuration provided by Viramed Biotech AG must not be changed.
4. Assay interpretation of ViraChip® microarrays must be performed using the ViraChip® Software. A manually/visually interpretation is not possible.

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Symbols Used:

	Manufacturer		Order Number
	Refer to Instructions for Use		Use by / Expiration Date
	*In-Vitro* Diagnostic Medical Device		Temperature Limitation (Storage)
	Lot Number		Positive serum control
	Sufficient for 96 tests		Negative serum control

Product No.: V-BBCMOK / V-BBCMDK

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