Borrelia B31 ViraChip® IgM Test Kit

CE

Chip-Immunoblot for the qualitative detection of **IgM** antibodies against specific *Borrelia species* antigens in human serum.

The Viramed Biotech AG **Borrelia B31 ViraChip® IgM Test Kit** is an immunoblot based on an enzyme-immunoassay in a microarray format, carrying highly purified specific antigens derived from the strain *Borrelia burgdorferi sensu stricto* (American strain *Borrelia burgdorferi B31*) at defined positions. The Borrelia B31 ViraChip® IgM Test Kit is manufactured according to the guidelines **98/79/EG** and **DIN 58967-40**.

Each membrane has an integrated control system with **serum controls, conjugate controls**, **calibrator controls** and a **negative control**. The analyte spots serve to detect *Borrelia* specific IgM antibodies against the following antigens **41, 39,** and **23.** Each well is coded by a colour system: The Borrelia B31 ViraChip® IgM is marked with a **quarter green circle**.

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**

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)
1 ea	**Instructions for Use for Borrelia B31 ViraChip® IgM Test Kit**	
0.33mL	**Borrelia B31 ViraChip® IgM Positive Control** Human, ready to use	(Order No.: V-BBCMPK)
0.33mL	**Borrelia B31 ViraChip® IgG,A,M Negative Control** Human, ready to use	(Order No.: V-BBCPNK)

Intended Use:

The Viramed Biotech AG Borrelia B31 ViraChip® IgM is an in vitro qualitative 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 protein microarray assay are supportive evidence of infection with *B. burgdorferi*, the causative agent for Lyme disease. The Viramed Biotech AG Borrelia B31 ViraChip® IgM can be used any time after onset provided the EIA or IFA are positive or equivocal. It should also be used for follow-up when: 1) Only IgG antibodies were found positive in a protein microarray assay, line blot assay or Western blot, 2) IgM antibodies were found by protein microarray assay, line blot or Western blot but were not considered significant by the CDC criteria for a positive IgM Western blot, 3) previously tested sero-negative individuals are shown to develop antibodies by an EIA or IFA test. The Viramed Biotech AG Borrelia B31 ViraChip® IgM must be used with a ViraChip® Reader and the ViraChip® Software. Altogether, this is an automated test system.

For In Vitro Diagnostic Use-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 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.

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. In vitro cultures of *Borrelia burgdorferi B31* spirochetes were harvested, concentrated, washed, and extracted to produce antigen fractions. Applying biotechnological purification methods purified antigens with the following molecular weights could be obtained: 41kD, 39kD, 23kD. The purified antigens were immobilized as individual 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 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 *Borrelia burgdorferi* specific antibodies in the patient specimen. Visualized spots from the reaction are compared for intensity with the integrated calibrator controls for evaluation.

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*). Antigens are purified by molecular size using biotechnological purification methods. The AP-Anti-human IgM Conjugate is produced by conjugation of anti-human IgM antibodies from goat with bovine mucosal alkaline phosphatase.

Materials Required but not Provided:

1. Washing steps will require a 500mL Wash bottle or a microarray assay processor containing a wash step and a rocking function.
2. Assorted graduated cylinders: 100mL and 1000mL.
3. Paper towels.
4. Pipettes and micropipettes capable of 20µL, 100µL and 1000µL.
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 empty microplate holder.
10. Empty microplate wells to fill gap positions.
11. 3D rocker with a rocking frequency of approx. 750 rpm or a 2D rocker with a rocking frequency of approx. 20 Hz.
12. 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. Do not eat, drink or smoke in the working area.
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. 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.
16. Dust and other contaminations in the wells of the MTP have to 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.

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 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.
4. A minimum of 10µl 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.
5. Prior 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 acclimatise 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 deionised 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*.** 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)
Controls:	Optionally 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.

*) Depending on processor performances, dilutions may be done in several steps.

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	0.090mL	+	0.010mL	0.10mL	51	4.590mL	+	0.510mL	5.10mL
2	0.180mL	+	0.020mL	0.20mL	52	4.680mL	+	0.520mL	5.20mL
3	0.270mL	+	0.030mL	0.30mL	53	4.770mL	+	0.530mL	5.30mL
4	0.360mL	+	0.040mL	0.40mL	54	4.860mL	+	0.540mL	5.40mL
5	0.450mL	+	0.050mL	0.50mL	55	4.950mL	+	0.550mL	5.50mL
6	0.540mL	+	0.060mL	0.60mL	56	5.040mL	+	0.560mL	5.60mL
7	0.630mL	+	0.070mL	0.70mL	57	5.130mL	+	0.570mL	5.70mL
8	0.720mL	+	0.080mL	0.80mL	58	5.220mL	+	0.580mL	5.80mL
9	0.810mL	+	0.090mL	0.90mL	59	5.310mL	+	0.590mL	5.90mL
10	0.900mL	+	0.100mL	1.00mL	60	5.400mL	+	0.600mL	6.00mL
11	0.990mL	+	0.110mL	1.10mL	61	5.490mL	+	0.610mL	6.10mL
12	1.080mL	+	0.120mL	1.20mL	62	5.580mL	+	0.620mL	6.20mL
13	1.170mL	+	0.130mL	1.30mL	63	5.670mL	+	0.630mL	6.30mL
14	1.260mL	+	0.140mL	1.40mL	64	5.760mL	+	0.640mL	6.40mL
15	1.350mL	+	0.150mL	1.50mL	65	5.850mL	+	0.650mL	6.50mL
16	1.440mL	+	0.160mL	1.60mL	66	5.940mL	+	0.660mL	6.60mL
17	1.530mL	+	0.170mL	1.70mL	67	6.030mL	+	0.670mL	6.70mL
18	1.620mL	+	0.180mL	1.80mL	68	6.120mL	+	0.680mL	6.80mL
19	1.710mL	+	0.190mL	1.90mL	69	6.210mL	+	0.690mL	6.90mL
20	1.800mL	+	0.200mL	2.00mL	70	6.300mL	+	0.700mL	7.00mL
21	1.890mL	+	0.210mL	2.10mL	71	6.390mL	+	0.710mL	7.10mL
22	1.980mL	+	0.220mL	2.20mL	72	6.480mL	+	0.720mL	7.20mL
23	2.070mL	+	0.230mL	2.30mL	73	6.570mL	+	0.730mL	7.30mL
24	2.160mL	+	0.240mL	2.40mL	74	6.660mL	+	0.740mL	7.40mL
25	2.250mL	+	0.250mL	2.50mL	75	6.750mL	+	0.750mL	7.50mL
26	2.340mL	+	0.260mL	2.60mL	76	6.840mL	+	0.760mL	7.60mL
27	2.430mL	+	0.270mL	2.70mL	77	6.930mL	+	0.770mL	7.70mL
28	2.520mL	+	0.280mL	2.80mL	78	7.020mL	+	0.780mL	7.80mL
29	2.610mL	+	0.290mL	2.90mL	79	7.110mL	+	0.790mL	7.90mL
30	2.700mL	+	0.300mL	3.00mL	80	7.200mL	+	0.800mL	8.00mL
31	2.790mL	+	0.310mL	3.10mL	81	7.290mL	+	0.810mL	8.10mL
32	2.880mL	+	0.320mL	3.20mL	82	7.380mL	+	0.820mL	8.20mL
33	2.970mL	+	0.330mL	3.30mL	83	7.470mL	+	0.830mL	8.30mL
34	3.060mL	+	0.340mL	3.40mL	84	7.560mL	+	0.840mL	8.40mL
35	3.150mL	+	0.350mL	3.50mL	85	7.650mL	+	0.850mL	8.50mL
36	3.240mL	+	0.360mL	3.60mL	86	7.740ml	+	0.860mL	8.60mL
37	3.330mL	+	0.370mL	3.70mL	87	7.830mL	+	0.870mL	8.70mL
38	3.420mL	+	0.380mL	3.80mL	88	7.920mL	+	0.880mL	8.80mL
39	3.510mL	+	0.390mL	3.90mL	89	8.010mL	+	0.890mL	8.90mL
40	3.600mL	+	0.400mL	4.00mL	90	8.100mL	+	0.900mL	9.00mL
41	3.690mL	+	0.410mL	4.10mL	91	8.190mL	+	0.910mL	9.10mL
42	3.780mL	+	0.420mL	4.20mL	92	8.280mL	+	0.920mL	9.20mL
43	3.870mL	+	0.430mL	4.30mL	93	8.370mL	+	0.930mL	9.30mL
44	3.960mL	+	0.440mL	4.40mL	94	8.460mL	+	0.940mL	9.40mL
45	4.050mL	+	0.450mL	4.50mL	95	8.550mL	+	0.950mL	9.50mL
46	4.140mL	+	0.460mL	4.60mL	96	8.640mL	+	0.960mL	9.60mL
47	4.230mL	+	0.470mL	4.70mL	97	8.730mL	+	0.970mL	9.70mL
48	4.320mL	+	0.480mL	4.80mL	98	8.820mL	+	0.980mL	9.80mL
49	4.410mL	+	0.490mL	4.90mL	99	8.910mL	+	0.990mL	9.90mL
50	4.500mL	+	0.500mL	5.00mL	100	9.000mL	+	1.000mL	10.00mL

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

Preparation of the Test Run Using the ViraChip® Software:

Basic processes are **Assembling, Loading, Processing, Scanning** and **Analysing.**

After starting the ViraChip® Software you may:

- **Assemble**: Test selection and input of sample data.
- **Load**: 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.**
- **Process**: Data transfer to processor.

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 empty wells.	Place the wells into the holding frame accordingly to the 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 rocking for approx. 5 minutes, aspirate.	Make sure the bottoms of the wells are completely covered with liquid. Use a 3D rocker with a rocking frequency of approx. 750 rpm or a 2D rocker with a rocking 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 rocking for 30 minutes at RT.	Make sure the bottoms of the wells are completely covered with liquid. Use a 3D rocker with a rocking frequency of approx. 750 rpm or a 2D rocker with a rocking 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. 3 x washing: **- add 300µL Diluent / Wash Buffer Working Dilution** **- incubate by rocking 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 rocking for 30 minutes at RT.	Use a 3D rocker with a rocking frequency of approx. 750 rpm or a 2D rocker with a rocking frequency of approx. 20 Hz.
9. Aspirate the liquid.	The aspiration needles must not touch the bottom of the wells.
10. 3 x washing 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 deionised water and incubate by rocking 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 rocking for 15 minutes at RT.	Use a 3D rocker with a rocking frequency of approx. 750 rpm or a 2D rocker with a rocking 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. 3 x washing by adding 300µL distilled or deionised water each time.	Without incubation.
17. Dry wells.	Dry the wells under continuous airflow for 20 minutes. Alternatively just by exposure to air for at least 12 hours at RT.
18. Measure and interpret wells.	Measurement 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.

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 guide.#

By using the ViraChip® Software you are able to:

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

- **Analyse:** 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 spot layout is shown in Fig. 1. The spot assignment is performed by the ViraChip® Software automatically.

The visual verification of the spots being detected is done by the user. For implausible assignments or wrongly detected spots the QC selection field of 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 a or cut off control for each run is recommended. The calibrator controls of the Borrelia B31 ViraChip® IgM are integrated on the ViraChip® microarray. 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 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**.

5. Interpretation of patient spots.

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 (1,5,7). The significance of the spots is different in the IgG- and IgM-assay (5). Therefore there is a different interpretation of the spots for the IgG and IgM ViraChip®.

"If the pattern of reactive bands meets the specific conditions, the result is positive, i.e. the positive result of an EIA or another test of the first step is confirmed. If, despite the presence of specific diagnostic bands, the criteria for a positive result are not fulfilled, the result is considered equivocal. In such a case a follow-up control may be recommended."

The following antigens of the Borrelia B31 ViraChip® IgM are considered as **highly specific** for *Borrelia species* for the detection of IgM antibodies: **41** (specific)**, 39,** and **23**.

*) For automated processing the incubation times of single steps of the procedure may be adjusted to the respective processor type. Refer to section "Notes to Equipment and Software".

#) We gladly provide a ViraChip® Software user guide.

Figure:

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

Controls
The following integrated controls are implemented on the Borrelia B31 ViraChip® IgM:
Serum controls (sc), negative control (nc), conjugate controls (ccG, ccM) and **calibrator controls (cal).**

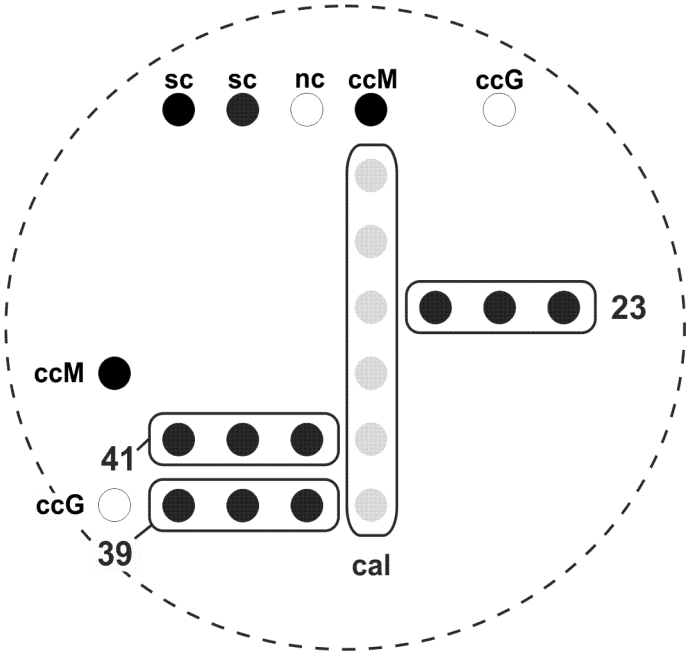

Figure 1: Schematic drawing of one well of the microtiter plate with the Borrelia B31 ViraChip® IgM microarray (magnified). Spot layout for antigens and integrated controls.

Interpretation Criteria:

Distinct *Borrelia* specific spot triplets are calculated in relation to the calibrator control and the lot specific factors by the ViraChip® Software. Calibrator controls are implemented on each ViraChip® microarray.

Identified spot triplet	Result	Interpretation
At least **two distinct** spot triplets from: **41, 39, 23**	**Positive**	IgM-antibodies against *Borrelia species* detectable. Presumptive evidence of *B. burgdorferi* infection.
No spot triplets or **less than two distinct** spot triplets	**Negative**	No specific antibodies against *Borrelia species* detectable. In case of a clinically based suspicion of an infection with *Borrelia species*: check additionally for IgG-antibodies and possibly check a second sample for IgG- and IgM-antibodies after 2-3 weeks.

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 (6,14,17). 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 (6,14,17).
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. Borrelia B31 ViraChip® IgM spot 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 IgM specific spots 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 IgM antibodies to *B. burgdorferi* antigenic proteins used in the Borrelia B31 ViraChip® IgM are shown in Table 2. The 41kD flagellar protein is most often seen in both Lyme and blood donor populations. The incidence of specific spots 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 spot at the 41kD flagellar protein. Disease sera from patients diagnosed with *Ehrlichia*, *Babesia* can have other *Borrelia* specific spots, possibly from co-infection with *Borrelia burgdorferi*.

Borrelia B31 ViraChip® IgM spots	41	39	23
Early Lyme Disease	78%	24%	60%
Disseminated Lyme Disease	82%	55%	91%
Late Lyme Disease	69%	19%	56%
Non-Endemic Blood Donors	35%	0%	1%
Endemic Blood Donors	40%	1%	3%

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

Limitations of Use:

1. Test results are valid only if the test procedure is strictly followed.
2. To ensure reliable results follow the “Good Laboratory Practice” guidelines.
3. 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 (9,10,11). Cross-reactions are also described in cases of autoimmune diseases, MS, ALS, and Influenza.
4. 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 enterica 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.
5. 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.
6. 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.
7. 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.
8. Do not use heat-inactivated sera.
9. Antibiotic therapy given to Lyme disease patients in early stages of the disease can suppress the development of specific *Borrelia* antibodies (2).
10. If comparison with other methodologies is required, simultaneous testing should be performed.
11. 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.
12. 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.
13. 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.
14. A positive result is based on elevated specific antibody titres and should be considered as a symptom. The correlation to a disease is only conditionally possible.
15. A negative result does not exclude a contact with the pathogen or the presence of a disease.
16. Adequately trained personnel only should perform the assay procedure.
17. The detection of specific antibodies can vary within different assays from different manufacturers and can lead to different results due to different sensitivity, specificity and assay methodologies.
18. ViraChip® microarrays showing a high background level should not be interpreted, especially if spot intensities are lighter than the background level.
19. In vitro diagnostics must not be used beyond expiration date as reliable results may not be possible.
20. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

Performance Characteristics:

185 sera were obtained from patients that were clinically defined and culture confirmed with Lyme Borreliosis; of these 185 sera, 158 were paired (79 acute and 79 convalescent) sera from patients diagnosed with Erythema migrans (EM), 11 with early-disseminated Lyme Disease / Carditis / Acute Neuroborreliosis and 16 with late stage Lyme arthritis. The Borrelia B31 ViraChip® IgM results are presented in table 3a. Comparisons to the predicate Borrelia B31 IgM ViraStripe® are found in tables 3b and 3c.

Stage	Borrelia B31 ViraChip® IgM				
	Total	Positive	Negative	% Sensitivity	95% Confidence Intervals
Acute EM 8-10 days from onset	79	38	41	48.1% (38/79)	(37.4% - 58.9%)
Convalescent EM 4 weeks after onset	79	57	22	72.2% (57/79)	(61.4% - 80.8%)
Early Neurologic	11	9	2	81.8% (9/11)	(52.3% - 94.9%)
Late Arthritis	16	9	7	56.3% (9/16)	(33.2% - 76.9%)
Total	185	113	72	-	-

Table 3a: Clinically-defined Lyme disease samples.

Borrelia B31 IgM ViraStripe®	Borrelia B31 ViraChip® IgM		
	Positive	Negative	Total
Positive	95	4	99
Negative	18	68	86
Total	113	72	185

Table 3b: Concordance with predicate device.

	% Agreement	95% Confidence Intervals
Positive	96.0% (95/99)	(90.1% - 98.4%)
Negative	79.1% (68/86)	(69.3% - 86.3%)
Overall	88.1% (163/185)	(82.7% - 92.0%)

Table 3c: Percent agreement with predicate device.

CDC Serum Panel:

A Lyme Disease Clinical 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 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.

	Borrelia B31 ViraChip® IgM				
CDC Reported Results	Positive	Negative	Total	% Agreement	95% Confidence Intervals
Positive	13	2	15	86.7% (13/15)	(62.1% - 96.3%)
Negative	6	23	29	79.3% (23/29)	(61.6% - 90.1%)
Total	19	25	44	-	-
% Clinical Sensitivity / Specificity	68.4% (13/19)	92.0% (23/25)	-	-	-
95% Confidence Intervals	(46.0% - 84.6%)	(75.0% - 97.8%)	-	-	-

Table 4: CDC National Lyme Disease Panel.

Prospective Study:

Three independent clinical laboratories were requested to parallel immunoblot testing of routinely submitted specimens with established immunoblot procedures. Tables 5a and 5b display the results for all three laboratories. Site 1 is a clinical laboratory in Minnesota / Site 2 is a clinical laboratory in Massachusetts / Site 3 is clinical laboratory in California. All three laboratories use the Borrelia B31 IgM ViraStripe® for routine testing. Samples were tested with the Borrelia B31 ViraChip® IgM and the Borrelia B31 IgM ViraStripe®.

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

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

	% Agreement	95% Confidence Intervals
Positive	97.1% (33/34)	(85.1% - 99.5%)
Negative	94.7% (89/94)	(88.1% - 97.7%)
Overall	95.3% (122/128)	(90.2% - 97.8%)

Table 5b: Percent agreement with predicate device.

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 - table 6:

	Total	Negative	Positive	% Positive	% Specificity	95% Confidence Intervals
Endemic	100	97	3	3%	97.0% (97/100)	(91.5% - 99.0%)
Non-endemic	99	98	1	1%	99.0% (98/99)	(94.5% - 99.8%)
Total	199	195	4	2%	98.0% (195/199)	(94.9% - 99.2%)

Table 6: Specificity studies.

Precision Study:

As a measure of kit membrane and kit lot precision, 6 blind coded specimens (2 strongly positive, 2 weakly positive, and 2 negative) were tested at three laboratory sites using the same kit lot number, three assay runs. Table 7 displays the Borrelia B31 ViraChip® IgM summary for data review, compilation and summary. The table includes spots that were found as "distinct" spots that were greater to or equal to the cut off control. The concordance is 100% (90/90) for distinct spots* and 100% (54/54) for negative spots.

	IgM spots identified			
	41	39	23	Results
VM4283 Strongly Positive 1	9	9	9	Positive – 3 distinct spots
VM4282 Strongly Positive 2	9	**5***	9	Positive – 3 distinct spots and 1 spot around cut off control
VM3254 Weakly Positive 1	9	0	9	Positive – 2 distinct spots
VM4321 Weakly Positive 2	9	0	9	Positive – 2 distinct spots
VM4050 Negative	**2***	0	0	Negative – 1 spot around cut off control
VM3576 Negative	9	0	0	Negative – 1 distinct spot

*) Spot 39 in serum VM4282 and spot 41 in serum VM4050 are expected around cut off control. Therefore they are excluded in the concordance calculation.

Table 7: Precision study.

Reproducibility Study:

The reproducibility data is presented in Table 8 for 6 sera assayed two times each on three different lots. The data indicates the Borrelia B31 ViraChip® IgM has a high degree of reproducibility both within and between assays. The reproducibility is 100%.

Sample	Lot: BBCMAC6001		Lot: BBCMAC6004		Lot: BBCMAC6005	
	Assay 1	Assay 2	Assay 1	Assay2	Assay 1	Assay 2
VM4283 Strongly Positive 1	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos
VM4282 Strongly Positive 2	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos
VM3254 Weakly Positive 1	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos
VM4321 Weakly Positive 2	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos	#/Pos
VM4050 Negative	#/Neg	#/Neg	#/Neg	#/Neg	#/Neg	#/Neg
VM3576 Negative	#/Neg	#/Neg	#/Neg	#/Neg	#/Neg	#/Neg

Table 8: Reproducibility study.

Cross-Reactivity Study:

73 sera determined to contain antibodies to other infectious disease agents are presented in Table 9. Cross-reactivity data for *Ehrlichia chafeensis*, *Babesia microti,* and *Borrelia hermsii* 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 18 clinical specimens were obtained. See Limitations for Use list of untested, potentially cross-reactive organisms.

Disease State Sera	Total	Borrelia B31 ViraChip® IgM Positive	% Cross-reactivity
*Ehrlichia chafeensis**	7	1	14.3%
*Babesia microti**	5	1	20.0%
*Borrelia hermsii**	6	4	66.7%
Leptospira interrogans	10	2	20.0%
Helicobacter pylori	10	0	0.0%
Epstein Barr Virus	6	0	0.0%
ENA Autoimmune[#]	16	0	0.0%
Treponema pallidum	13	1	7.7%

*) Possible co-infection with *B. burgdorferi*.

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

Table 9: Cross-reactivity study.

Interfering Substances:

Haemolysed, lipemic, icteric, or microbially contaminated 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.

Notes to Equipment and Software:

1. Automated processing requires usage of processor type specific test procedures (assays) which are programmed and validated by Viramed Biotech AG.
2. Usage of processor specific consumables requires approval of the respective configurations according to manufacturer's instruction by Viramed Biotech AG.
3. The equipment and software configuration provided by Viramed Biotech AG must not be changed. Any alteration can lead to false results.
4. Only equipment specific software must be used. Changes of configuration data may just be done by Viramed Biotech AG.
5. Only measurement devices approved by Viramed Biotech AG are allowed to be used.
6. Assay interpretation of ViraChip® microarrays has to be performed using the ViraChip® Software. A manually / visually interpretation is not possible.
7. *In vitro* diagnostics must not be used beyond expiration date as reliable results may not be possible.
8. Efficient washing after each incubation step is essential for consistent results; insufficient washing may lead to false results.

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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