

BORRELIA VIRCLIA® IgM MONOTEST

For *in vitro* diagnostic use

VCM010: Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against *Borrelia burgdorferi* in human serum/plasma. 24 tests.

INTRODUCTION:

Lyme borreliosis is a tick-transmitted bacterial infection caused by some members of the spirochete group *Borrelia burgdorferi* sensu lato. The *B. burgdorferi* complex comprises at least 15 genospecies worldwide. *Borrelia burgdorferi* sensu lato (sl) is the leading organism responsible for Lyme disease in Asia, Europe and in the United States.

The strains present in Europe are *Borrelia burgdorferi* sensu lato which groups *B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, *B. spielmanii*, *B. bavariensis* are pathogenic species. Potentially pathogenic species are *B. valaisiana* and *B. lusitaniae* and, only in the United States, *B. burgdorferi* sensu stricto.

They can cause neurological and arthritic complications. Transmission of *B. burgdorferi* to humans occurs through a bite from an infected tick of the genus Ixodes.

The disease phases include an early localized stage (3 to 30 days post-tick bite), characterized by a red, expanding rash called erythema migrans (70-80% of infected patients); fatigue, chills, fever, headache, muscle and joint aches, and swollen lymph nodes can also be present. In the early disseminated stage (days to weeks post-tick bite) the infection may spread from the site of the bite to other parts of the body, producing an array of specific symptoms: additional rashes on other areas of the body, facial or Bell's palsy, neuroborreliosis, borreliac lymphocytoma, intermittent arthritis and carditis. Finally, in late disseminated stage (months to years post-tick bite) untreated patients may begin to have intermittent bouts of arthritis with severe joint pain and swelling; Up to 5% of untreated patients may develop chronic neurological complaints. Approximately 10-20% of patients with Lyme disease have symptoms that last months to years after treatment with antibiotics.

Laboratory tests are necessary to confirm a diagnosis of later stage infection. Antibodies to *B. burgdorferi* are usually detectable within 4–8 weeks of infection. Patients with late-stage infection are rarely seronegative and usually have very strongly positive antibody tests. However, the occurrence of false-positive tests in patients with other infections or conditions, such as autoimmune diseases, can lead to misdiagnosis and inappropriate treatment. A two-step testing approach is frequently advised, with an enzyme immunoassay for the first step, and Western blot for the second. Assays based on highly purified antigens are used to achieve maximal specificity.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (ChemiLuminescent ImmunoAssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use. Serum dilution solution and conjugate are coloured to help in the performance of the technique. Sample predilution is not necessary. Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® BORRELIA IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with a combination of recombinant antigens from pathogenic species of *Borrelia afzelii* (OspC), *Borrelia garinii* (OspC, VlsE), *Borrelia burgdorferi sensu stricto* (OspC), *Borrelia burgdorferi sensu lato* (p41i, p39, p17 and OspE) and *Borrelia spielmanii* (OspC).

Well D: Conjugate: orange; containing anti-human IgM peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers, anti-human IgG and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8°C and check expiration date.

Materials required but not supplied:

- VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
- Precision micropipettes 5 and 100 µl.
- Eight channel micropipette 100 µl.
- Adapted microplate washer.
- Thermostated incubator/water bath.
- Microplate luminometer.
- Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored, closed and at 2-8°C.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day



STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents supplied are ready to use.

Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE:**• AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).

2. Follow the Operator's Manual of the Automated Processor.

• MANUAL

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.

The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:

Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.

Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.

Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.



7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.

8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

• SENSITIVITY AND SPECIFICITY:

141 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

	Samples No.	Sensitivity (%)	Specificity (%)
IgM	141	90	94
95% C.I.		81-95	85-98

C.I. Confidence intervals

Indeterminate values were omitted from the final calculations.

• INTRA-ASSAY PRECISION:

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.

The results were as follows:

Serum	N	% C.V.
Sample +	10	7
CAL	10	9
CN	10	13

C.V. Coefficient of variation

• INTER-ASSAY PRECISION:

3 sera were individually run on 5 consecutive days in 2 different automatic processors.

The results were as follows:

Serum	N	% C.V.
Sample +	10	17
CAL	10	18
CN	10	30

C.V. Coefficient of variation

Clinical evaluation:

86 clinical sera samples were analyzed and the results were as follows:

Stage	Clinical presentation	Number of patients	IgM Positive VirClia
STAGE I	Lyme after tick bite	37	23
STAGE II	Neurologic lyme disease	29	4
STAGE III	Disseminated lyme disease	20	12

Seroprevalence: 90 blood donors (aged 18 to 65 years) from Spain were analysed. 2 positive results were obtained giving a seroprevalence of 2%.

• CROSS REACTIVITY AND INTERFERENCES:









22 samples known to be positive for other microorganisms (Leptospira, syphilis and Epstein-Barr virus VCA) were assayed. 23 samples known to be positive for rheumatoid factor and antinuclear antibodies were assayed.

The results of the test demonstrate the specific reaction of the IgM assay with no cross-reactivity to Leptospira (4 samples tested) and syphilis (8 samples tested). Cross reactivity with Epstein-Barr virus VCA (2 out of 10 tested samples) was found.

The results of the test demonstrate the specific reaction of the assay with no cross-reactivity to rheumatoid factor (13 samples tested). The results of the test demonstrate the specific reaction of the assay with no cross-reactivity to antinuclear antibodies (10 samples tested).

No interferences were observed in 3 samples tested with haemolytic (max 8.5 g / L), lipemic (max 2 g / L of triglycerides), hypercholesterolemic (max 4 g / L) or bilirubin-containing (max 3 g / L) sera or plasma.

SYMBOLS USED IN LABELS:

	In vitro diagnostic medical device
	Use by (expiration date)
	Store at x-y°C
	Contains sufficient for <n> test
	Batch code
	Catalogue number
	Consult instructions for use
	<X> wells

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