EPITOGENX

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Epitogen Lyme VlsE IgM Test

For Research Use Only

REF **EGX96LD4**

1. SIGNIFICANCE AND BACKGROUND

The *Borrelia burgdorferi sensu lato* complex shows substantial genetic diversity, significantly affecting the clinical manifestations of Lyme disease across different regions. In North America, *B. burgdorferi sensu stricto* is predominant and often linked to arthritis. In Europe, *B. afzelii* and *B. garinii* are most common. Emerging species such as *B. mayonii* (North America) and *B. spielmanii* (Europe) further complicate the clinical picture with unique pathogenic profiles. Other species like *B. bissetiae*, *B. bavariensis*, and *B. valaisiana* exhibit distinct host associations and ecological adaptations. This genetic variation impacts diagnostic sensitivity, clinical outcomes, and complicates vaccine and test development.

Differentiating between *Borrelia* species is crucial for accurate diagnosis, prognosis, and treatment. Different species cause distinct clinical forms of Lyme disease: *B. garinii* often results in neurological involvement, *B. afzelii* in chronic skin disease, and *B. mayonii* in severe systemic illness with high spirochetemia. Standard serological tests, mostly based on *B. burgdorferi sensu stricto* antigens, can miss infections caused by other species, leading to false negatives, delayed treatment, and misdiagnosis. For instance, U.S.-designed tests may underperform in detecting *B. garinii* or *B. spielmanii* infections in Europe. Furthermore, *B. mayonii*'s distinct clinical presentation often goes unrecognized without species-specific testing.

In summary, recognizing the infecting *Borrelia* species is essential for optimizing clinical care, adapting diagnostic tools to regional epidemiology, guiding treatment decisions, and improving public health responses. Failure to do so risks misdiagnosis, inappropriate management, and gaps in Lyme disease surveillance.

2. VlsE (Variable Major Protein-Like Sequence, Expressed)

Accurate differentiation of *Borrelia* species for Lyme disease diagnosis requires careful selection of antigens that meet specific criteria: strong immunogenicity, ability to elicit persistent humoral responses across infection stages, significant variability among species and strains, and consistent expression throughout infection. While some antigens like Outer Surface Protein C (OspC) meet some of these criteria due to its immunogenicity and variability, its expression is limited to early infection stages, reducing its diagnostic value. Other antigens, such as Decorin-binding protein A (DbpA) and OspA/B, also show variability but fail to meet all criteria.

VlsE, a key antigen encoded by a 36 kDa outer membrane lipoprotein, is an optimal candidate. VlsE induces a strong, sustained antibody response, is highly variable both inter- and intraspecies, and remains expressed throughout infection. The VlsE gene includes N- and C-terminal domains and a central cassette with six variable regions (VR1-VR6) that undergo antigenic variation, while the invariant regions (IR1-IR6) remain stable. This variation is driven by gene conversion from silent vls cassettes, generating diverse VlsE variants. This dynamic antigenic variation makes VlsE an ideal target for species-specific serological assays, improving the accuracy and comprehensiveness of Lyme disease diagnostics.

3. PRINCIPAL OF THE EPITOGEN LYME VlsE IgM TEST

The Epitogen Lyme VlsE IgM assay comprises 27 full-length VlsE variants representing *Borrelia* pathogenic species — *B. burgdorferi* sensu stricto*, B. afzelii, B. garinii, B. mayonii. B. spielmanii. B. bissetiae, B. bavariensis* and *B. valaisiana*.

Powered by our unique Epitogen® scaffold, it ensures precise antigen orientation for reliable comparison and high assay performance. This standardisation and direct comparison can only be possible using the Epitogen® scaffold.

Epitogen Lyme VlsE IgM Assay: Advancing Lyme Disease Research
The Epitogen Lyme IgM assay offers an expansive VlsE variants.
✅ **Unmatched VlsE Antigen Coverage**
27 full-length VlsE variants antigen.
✅ **Comprehensive *Borrelia* Species Detection**
The VlsE included in this assay covers multiple Lyme-causing ***Borrelia* species**, capturing species' diversity.
✅ **Optimized Antigen Orientation:**
The scaffold system ensures antigens bind directionally to the ELISA surface enabling reliable comparison between the different VlsE variants.

4. INTENDED USE - For Research Use Only

The Epitogen Lyme VlsE IgM Test uses indirect ELISA to qualitatively detect IgM antibodies against VlsE antigens in human serum/plasma.

The assay is designed for use in two key scenarios:
a) Strain Identification in Confirmed Lyme Disease Cases:
It provides additional insight into the specific *Borrelia* strain responsible for infection in patients who have already tested positive for Lyme disease. This information can support epidemiological tracking, inform treatment decisions, and enhance our understanding of regional strain prevalence.

b) Re-evaluation of Serology-Negative but Clinically Suspected Lyme Cases:
In patients presenting with clinical symptoms consistent with Lyme disease but who test negative on standard serological assays, this test offers an additional diagnostic tool. It can detect immune responses to less commonly targeted *Borrelia* species or variants, potentially improving diagnostic sensitivity and supporting earlier intervention.

Complementary Use with the Lyme VlsE IgG Assay:
When used in combination with the Lyme VlsE IgG Assay, this test provides deeper insights into the infection stage and immune response profile—offering enhanced diagnostic clarity and greater confidence in clinical decision-making.
Note: *A positive result may appear across several VlsE variants, with the strongest signal indicating the likely infecting Borrelia species. This assay also supports analysis of IgG subclasses, including IgG1, IgG2, IgG3, and IgG4.*

5. MATERIALS PROVIDED

Each test system contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label.
Note: The following components contain **ProClin™ 300** as a preservative at a concentration of <0.01% (w/v): positive and negative controls, sample diluent and secondary diluent.

Reagent	Description	Quantity
96-well plate	96-microplate coated with proprietary Epitogen Lyme antigens, ready for use	1
Positive control (POS)	(human), ready for use	1 x 0.5 mL (**#1**)
Conjugate	Peroxidase-labelled anti-human IgM antibody	1 x 120 µl (**#2**)
10X wash buffer	10X concentrate	1 x 100 mL (**#3**)
Sample diluent	ready for use	1 x 15mL (**#4**)

Secondary diluent	ready for use	1 x 12 mL (**#5**)
TMB substrate	TMB/H_2O_2, ready for use	1 x 12 mL (**#6**)
Protective seals		2 pieces
Test instructions	PDF via email	1 booklet

6. MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 1 - 200µL.
3. Multichannel pipettes capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Plate rocker for all incubation steps.
6. Wash bottle or microwell washing system.
7. Distilled or deionized water.
8. One litre graduated cylinder.
9. Serological pipettes.
10. Disposable pipette tips.
11. Paper towels.
12. Laboratory timer to monitor incubation steps.
13. Disposal basin and disinfectant (i.e. 10% household bleach - 0.5% Sodium Hypochlorite).
14. Sulfuric acid (H_2SO_4) 95-98% for preparation of stop solution.

7. PREPARATION AND STABILITY OF THE REAGENTS

Note: All reagents, including plate, must be brought to room temperature (+18 °C to 25°C) 30 minutes before use.

- **Coated Wells**: Ready for use. Tear open the protective wrapping of the plate immediately before use.
- **Wash Buffer (#3)**: The wash buffer is a 10X concentrate. Dilute 1 part reagent plus 9 parts deionized or distilled water. If crystallization occurs in the concentrated buffer, warm it to 37°C and mix well before diluting.
- **Sample diluent (#4)**: Ready for use. Mix thoroughly before use.
- **Secondary diluent (#5)**: Mix gently with 120 µL conjugate (**#2**) before use to prepare the **conjugate cocktail**.
- **Conjugate (#2)**: Add 120 µL to secondary diluent (**#5**) before use as above.
- **Controls (#1)**: Ready for use. Mix thoroughly before use.
- **TMB substrate (#6)**: Ready for use. Light sensitive. Close the bottle immediately after use. The TMB substrate solution must be clear on use. Do not use the solution if it is coloured blue.

Storage and stability: Store kit between +2°C and +8°C. Do not freeze. Unopened, all the test components are stable until the indicated expiry date.

Waste Disposal: Patient samples, controls and microplates should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

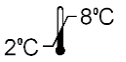
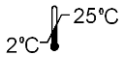
Preparation of stop solution (not supplied): Sulfuric acid 95-98% (~18 M).

a. Wear Safety goggles, acid resistant gloves, lab coat and work in a fume hood or well-ventilated area.
b. Add around 80 mL of distilled water first in glass flask (never add acid first!).
c. Using a graduated pipette or cylinder, carefully measure 11.2 mL of sulfuric acid.
d. Slowly pour the acid into the water while stirring continuously with a glass rod.
e. Heat will be generated, so allow the solution to cool if necessary, or place glass flask in ice bath.
f. Once mixed and cooled, add more distilled water to bring the total volume to 100 mL.
g. Mix well to ensure uniform dilution.

Safety Reminders

- ☑ Always add acid to water, never water to acid!
- ☑ Work in a ventilated area.
- ☑ If spilled, neutralize with sodium bicarbonate before cleaning.
- ☑ If contact occurs, rinse immediately with plenty of water and seek medical help if needed.

8. STORAGE CONDITIONS

	Unopened Test System, Positive Control, Negative Control, TMB, Sample Diluent, Secondary Diluent
	Conjugate – DO NOT FREEZE
	Stop Solution: +2 – 25°C Wash Buffer (1X): +20 - 25°C for up to 7 days, +2 - 8°C for 30 days. Wash Buffer (10X): +2 - 25°C

9. PREPARATION AND STABILITY OF SPECIMEN

Sample material: Human serum or EDTA, heparin or citrate plasma.

Sample dilution: Samples are diluted 1:101-1001 in diluent buffer.

Stability: Samples to be investigated must be properly stored. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and provide erroneous results. Do not use if there are any other added anticoagulants/preservatives. Avoid using haemolyzed, lipemic, or bacterially contaminated sera.

10. PLATE LAYOUT AND ASSAY PROCEDURE

Antigen coating

1-27 VlsE *Borrelia* variants.

B Empty well.

N Scaffold control.

P Lyme antigen.

Borrelia Hermssi antigens

Borrelia miyamotoi antigens

Differential VlsE Layout

	Sample 1				Sample 2				Sample 3			
	1	2	3	4	5	6	7	8	9	10	11	12
A	VlsE 1	VlsE 2	VlsE 3	VlsE 4	VlsE 1	VlsE 2	VlsE 3	VlsE 4	VlsE 1	VlsE 2	VlsE 3	VlsE 4
B	VlsE 5	VlsE 6	VlsE 7	VlsE 8	VlsE 5	VlsE 6	VlsE 7	VlsE 8	VlsE 5	VlsE 6	VlsE 7	VlsE 8
C	VlsE 9	VlsE 10	VlsE 11	VlsE 12	VlsE 9	VlsE 10	VlsE 11	VlsE 12	VlsE 9	VlsE 10	VlsE 11	VlsE 12
D	VlsE 13	VlsE 14	VlsE 15	VlsE 16	VlsE 13	VlsE 14	VlsE 15	VlsE 16	VlsE 13	VlsE 14	VlsE 15	VlsE 16
E	VlsE 17	VlsE 18	VlsE 19	VlsE 20	VlsE 17	VlsE 18	VlsE 19	VlsE 20	VlsE 17	VlsE 18	VlsE 19	VlsE 20
F	VlsE 21	VlsE 22	VlsE 23	VlsE 24	VlsE 21	VlsE 22	VlsE 23	VlsE 24	VlsE 21	VlsE 22	VlsE 23	VlsE 24
G	VlsE 25	VlsE 26	VlsE 27	B	VlsE 25	VlsE 26	VlsE 27	B	VlsE 25	VlsE 26	VlsE 27	B
H	N	P	Hermsii	Miyamotoi	N	P	Hermsii	Miyamotoi	N	P	Hermsii	Miyamotoi

Test Sample (3 samples/plate) – apply Lyme positive sample.
Note: VlsE identifiers and their corresponding species of origin will be provided in the analysis sheet.

1. Remove the individual components from storage and allow them to warm to room temperature (+18 – 25°C).
2. Prepare the **conjugate cocktail**: pipette 120 µL conjugate (**#2**) into the secondary diluent (**#5**) and mix gently.
3. Each plate can test 3 samples. For each sample, allow for 3 wells, Positive Control, Negative and Blank (see plate layout).
4. Prepare a 1:101-1001 dilution (e.g.: 4 µL-40 µL of sample + 4 ML of sample diluent (**#4**) for each patient sample. Positive control (**#1**) is pre-diluted and ready for use.
5. Wash the microwell plate once with 300 µL/well Wash Buffer. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains.
6. Apply 100 µL to Lyme VlsE variants and Blank (B), Negative (N), *Borrelia Hermssi and Borrelia miyamotoi;* apply 100 µL Positive Control (**#1**) to Positive (P) well (see above layout).

For example, after diluting Sample 1 in 4 mL of sample diluent, dispense 100 µL into wells A1–A4, B1–B4, C1–C4, D1–D4, E1–E4, F1–F4, G1–G4, and H1, H3, H4. Add 100 µL of Positive Control (#1) to well H2.
Ensure that the samples are properly mixed. Use a different pipette tip for each patient sample.

7. Cover the plate with the protective seal and incubate the plate on a plate rocker at room temperature (+18 - 25°C) for 60 ± 1 minutes.
8. Wash the microplate 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with 300 µL Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps **1** and **2** for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no

residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

b. **Automated Wash Procedure**:

If using an automated microwell wash system, set the dispensing volume to 300 µL/well. Set the wash cycle for 5 washes with (10 seconds incubation between washes). If necessary, the microwell plate may be removed from the washer, inverted over a paper towel, and tapped firmly to remove any residual wash solution from the microwells.

9. Add 100 µL of the **conjugate cocktail** to each well, including the sample Blank wells, at the same rate and in the same order as the specimens.
10. Cover the plate with the protective seal and incubate the plate on a plate rocker at room temperature (+18 - 25°C) for 30 ± 1 minutes.
11. Wash the microplate 5 times following the same procedure as described in step **7**.
12. Add 100 µL of TMB to each well, including the sample Blank wells, at the same rate and in the same order as the specimens.
13. Incubate the plate at room temperature (+18 - 25°C) for 5 minutes.
14. After 5 minutes:

a. **If 2M stop solution (H_2SO_4) is not available**: immediately read the plate at a wavelength of 650nm.

b. **If 2M stop solution (H_2SO_4) is available**:

- Stop the reaction by adding 100 µL of Stop Solution to each well, including the sample Blank wells, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow.
- Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD). Read the plate within 10 minutes after the addition of the Stop Solution.

11. QUALITY CONTROL

Each time the assay is performed, the Blank sample, Negative Control and Positive Control must also be included.
The OD value for the Negative Control and Positive Control should fall within the following ranges:

	OD Range
Positive Control	≥ 0.5

***Note**: If the above conditions are not met the test should be considered invalid.*

12. INTERPRETATION OF RESULTS

Calculations:
Signal to noise ratio (S/N): The result of each well is calculated as the signal to noise (S/N) ratio of the OD readout from the Lyme VlsE antigen coated well, subtracted from the scaffold coated control (N) of the same sample, then divided by the absorbance of the Blank sample well (B):

$$S/N = (OD_{VlsE} - OD_N) / OD_B$$

Example: The measured absorbance at 450 nm with a serum or plasma sample in the VlsE coated well is 1.00 and the same sample in the N coated well is 0.10, and the Blank (B) readout is 0.09.

Therefore, the S/N = (1.00 – 0.10) / 0.09 = 10.

Interpretations: The S/N Ratios are interpreted as follows.

	S/N Ratio	Interpretation
Negative	< 2.00	No significant amount of IgM antibodies to VlsE antigens detected.
Borderline	≥ 2.00 to < 2.5	Antibodies targeting VlsE antigens were detected, providing presumptive evidence of likely exposure to a corresponding *Borrelia* species. It is recommended to perform additional testing using a Lyme VlsE IgM assay or to collect a new sample >7 days later and retest it alongside the original specimen using the Lyme VlsE IgM assay.
Positive	≥ 2.5	Antibodies specific to VlsE variants were detected with the highest signal supportive of *Borrelia* species.

13. TEST CHARACTERISTICS

Antigen: 27 full-length VlsE variants covering the major *Borrelia* species.

Detection Limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample.

Precision: The reproducibility of the test was investigated by determining the intra- and inter- assay coefficients of variation (CV) using three sera. The Inter- and intra- assay CVs were less than 10%.

14. LIMITATIONS OF THE ASSAY

1. Serum samples from patients with other spirochetal diseases (syphilis, yaws, pinta, leptospirosis, and relapsing fever), or infectious mononucleosis or systemic lupus erythematosus may give false positive results. In cases where false positive reactions are observed, extensive clinical epidemiologic and laboratory workups should be carried out to determine the specific diagnosis.
2. False negative results may be obtained if serum samples are drawn too early after the onset of the disease, that is, if taken before antibody levels reached significant levels. Also, early antibiotic therapy may abort an antibody response to the spirochetes.
3. A negative result may occur despite a positive Lyme diagnosis if the patient has not mounted a detectable antibody response to the VlsE antigen.
4. A negative result may also occur if the infecting *Borrelia* species expresses a VlsE variant not represented in this assay.
5. All data must be interpreted in conjunction with the clinical symptoms of the disease, epidemiologic data, exposure in endemic areas, and results of other laboratory tests.

15. PRECAUTIONS

1. For Research Use only.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water then seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection.
3. All materials of human origin should be handled as being potentially infectious. Handle these products at the **Biosafety Level 2** as recommended for any potentially infectious human serum or blood specimen.
4. Adherence to the specified time and temperature of incubations is essential for accurate results.
5. Improper washing may cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution before adding Conjugate or Substrate and do not allow the wells to dry out between incubations.

6. The Stop Solution is TOXIC, if inhaled, comes into contact with skin or if swallowed. It can also cause burns. If there is an accident or someone feels ill, immediately seek medical advice.
7. The TMB Solution is HARMFUL. It is an irritant to the eyes, the respiratory system, and the skin.
8. The Wash Buffer concentrate is also an irritant to the eyes, the respiratory system, and the skin.
9. Wipe the bottom of the plate free of any residual liquid and/or fingerprints that can alter optical density (OD) readings.
10. Do not use reagents from any other sources or manufacturers.
11. The TMB solution should be colourless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change colour prematurely. **Do not use** the TMB if it is noticeably blue in colour.
12. Never pipette via the mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
13. Cross contamination of reagents and/or samples could cause erroneous results.
14. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
15. Do not expose reagents to strong light during storage or incubation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant. Avoid exposure of reagents to bleach fumes.
17. **Caution**: Neutralize any liquid waste of an acidic pH before adding a bleach solution.
18. Do not use an ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
19. Do not use reagents after the expiry date (6 months after the manufacture date).

Abbreviated Test Procedure

1. Remove plate from sealed bag.
2. Add 120 µL conjugate (**#2**) to secondary diluent (**#5**) and mix thoroughly (**conjugate cocktail**).
3. Dilute samples 1:101-1001 (e.g.: 4 µL – 40 µL of sample + 4 ML of sample diluent)
4. Wash plate once then add 100 µL of each diluted sample and control to the designated microwells.
5. Seal plate and incubate at RT for 60 ± 1 minutes.
6. Wash 5 times – 300 µL/well.
7. Add Conjugate – 100 µL/well.
8. Seal plate and incubate at RT for 30 ± 1 minutes.
9. Wash 5 times – 300 µL/well.
10. Add TMB (**#6**) – 100 µL/well.
11. Incubate for 5 minutes at RT.
12. Either:
    a. Read the plate OD immediately at a wavelength of 650nm.
    or
    b. Add 100 µL/well 2M stop solution (H_2SO_4) then read the plate OD at a wavelength of 450nm within 10 minutes.

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