EPITOGEN

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Epitogen Lyme IgM Test

For Research Use Only

REF **EGX96LD1**

1. SIGNIFICANCE AND BACKGROUND

Lyme disease is a tick-borne infection caused by *Borrelia burgdorferi* sensu lato (s.l.). The characteristic rash, *erythema migrans* (EM), appears in 60–80% of cases. The disease progresses in three stages, from localized to systemic infection, though some cases remain asymptomatic or present symptoms that overlap with other conditions, complicating diagnosis.

While *B. burgdorferi* can be isolated from skin, blood, and cerebrospinal fluid, culture-based detection is impractical for large-scale diagnosis. Serological testing, including indirect fluorescent antibody (IFA) staining, immunoblotting, and enzyme immunoassays (EIA), is the preferred diagnostic approach.

The *B. burgdorferi* s.l. complex includes 20 spirochete species, several of which infect humans, causing Lyme borreliosis (LB). The genospecies *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, and *B. mayonii* are most commonly associated with human infection. Due to antigenic complexity, serological tests have limitations in sensitivity and specificity, requiring careful interpretation. Therefore, a two-step serology testing approach is recommended to support the clinical diagnosis of Lyme disease.

2. PRINCIPAL OF THE EPITOGEN LYME IgM TEST

The Epitogen Lyme IgM Test overcomes serological test limitations by targeting diverse *B. burgdorferi* antigens across species and disease stages, accounting for population-wide immune variability. Employing Epitogen® scaffold technology, we multiplexed IgM-seropositive immunodominant epitopes from 37 *Borrelia* antigens covering prevalent pathogenic species — *B. burgdorferi* sensu stricto*, B. afzelii, B. garinii, B. mayonii. B. spielmanii. B. bissetiae, B. bavariensis* and *B. valaisiana*.

Epitogen Lyme IgM Assay: Advancing Lyme Serology with Precision

The Epitogen Lyme IgM assay is designed to overcome the key limitations of existing serology tests.

✅ **Unmatched Antigen Coverage**

Maximized sensitivity by incorporating **120 linear epitopes, 4 subunits,** and **20 full-length VlsE and OspC antigens.**

✅ **Comprehensive *Borrelia* Species Detection**

Designed to detect multiple Lyme-causing ***Borrelia* species**, capturing species' diversity.

✅ **Fine-tuned to address Immune Response Variability**

The test captures immune response diversity with epitopes from **37 *Borrelia* antigens**.

✅ **Optimized Antigen Orientation:**

The scaffold system ensures antigens bind directionally to the ELISA surface, optimizing epitope exposure and boosting sensitivity.

✅ **Enhanced Specificity**

Cross reactive epitopes are identified and eliminated.

3. INTENDED USE - For Research Use Only

The Epitogen Lyme IgM Test uses indirect ELISA to qualitatively detect IgM antibodies against *Borrelia* antigens in human serum/plasma, indicating early *B. burgdorferi* s.l. infection. Due to its high specificity, it can be used as a screening tool for the general population.

A positive result indicates exposure to *B. burgdorferi*, while a negative result does not rule out infection, as early-stage infections may not produce detectable antibodies. Early antibiotic treatment may also reduce antibody response. IgM antibodies appear during the early stages, while IgG antibodies develop later. To confirm infection, additional testing with the **Epitogen Lyme IgG ELISA Test** or re-testing after 2-4 weeks may be necessary.

False positives can occur due to cross-reactivity with pre-existing antibodies or other factors.

4. 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)
Negative control (NEG)	(human), ready for use	1 x 0.5 mL (#2)
Conjugate	Peroxidase-labelled anti-human IgM antibody	1 x 120 µl (#3)
10X wash buffer	10X concentrate	1 x 100 mL (#4)
Sample diluent	ready for use	1 x 15mL (#5)
Secondary diluent	ready for use	1 x 12 mL (#6)
TMB substrate	TMB/H_2O_2, ready for use	1 x 12 mL (#7)
Protective seals		2 pieces
Test instructions	PDF via email	1 booklet

5. 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.

6. 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 (#4)**: 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 (#5)**: Ready for use. Mix thoroughly before use.
- **Secondary diluent (#6)**: Mix gently with 120 µL conjugate (**#3**) before use to prepare the **conjugate cocktail**.
- **Conjugate (#3)**: Add 120 µL to secondary diluent (**#6**) before use as above.
- **Controls (#1 & 2)**: Ready for use. Mix thoroughly before use.
- **TMB substrate (#7)**: 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.

7. STORAGE CONDITIONS

2°C – 8°C	Unopened Test System, Positive Control, Negative Control, TMB, Sample Diluent, Secondary Diluent
	Conjugate – DO NOT FREEZE
2°C – 25°C	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

8. PREPARATION AND STABILITY OF SPECIMEN

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

Sample dilution: Samples are diluted 1:51 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.

9. PLATE LAYOUT AND ASSAY PROCEDURE

Legend – Antigen coating

Lyme-antigen coating: A cocktail of full-length and composite specific epitope antigens

Scaffold-control coating: The Epitogen® scaffold protein (control).

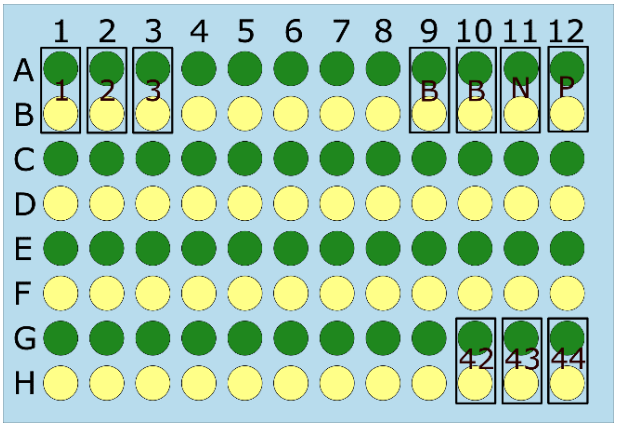
1 **Test Sample (1 – 44) –** 44 single tests.

B **Blank (B) –** Sample diluent buffer.

N **Negative serum (N) –** Lyme disease negative sera.

P **Positive serum (P) –** Lyme disease positive sera.

Epitogen Lyme IgM Test Layout



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 (**#3**) into the secondary diluent (**#6**) and mix gently.
3. Each plate can test 44 samples. Allow for four wells, two for the Positive Control and two for the Negative. Also, allow for four Blanks per plate (see plate layout).
4. Prepare a 1:51 dilution (e.g.: 5 µL of test sample + 250 µL of sample diluent (**#5**)) for each patient sample. Controls are 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. To a pair set of Lyme-antigen and scaffold-control wells (see above layout), add 100 µL to each well of diluted patient specimen, positive and negative controls (provided). Add sample diluent to the Blank wells. 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.

10. 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
Negative Control	≤ 0.15
Positive Control	≥ 0.5

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

11. INTERPRETATION OF RESULTS

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

$$S/N = (OD_{Ag} - OD_{Ctl}) / OD_{B}$$

Example: The measured absorbance at 450 nm with a serum or plasma sample in the Ag coated well is 1.00 and the same sample in the Ctl coated well is 0.10, and the mean of the four Blanks (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	< 2.5	No significant amount of IgM antibodies to *B. burgdorferi* s.l. detected.
Borderline	≥ 2.5 to < 3.0	Antibodies specific to *B. burgdorferi* s.l. were detected. This indicates presumptive evidence of probable exposure. The specimen should be tested by a second step IgM and/or IgM western blot, or by testing a fresh sample 7 days later and re-tested in parallel with the first sample.
Positive	≥ 3.0	Antibodies specific to *B. burgdorferi* s.l. were detected - supportive evidence of probable exposure.

12. TEST CHARACTERISTICS

Antigen: A cocktail of full-length and composite specific epitope antigens **including** OspC, OspA, flagellin and VlsE covering the major pathogenic 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%.

Cross reactivity: The quality of the antigen used ensures high specificity of the ELISA. Serum samples from healthy individuals and patients with infections caused by agents known to have cross-reactivity with Lyme serology tests were investigated using the Epitogen Lyme IgM Test

Table 1. Specificity of the Epitogen Lyme IgM Test.

Sample type & size	Number of non-reactive	Specificity (%)
Cytomegalovirus (n = 20)	20	100
Epstein–Barr virus (n = 20)	19	95
Syphilis (n = 30)	29	97
Rheumatoid arthritis (n = 17)	17	100
Multiple sclerosis (n = 11)	9	82
Healthy (n = 100)	98	98
Total (n = 198)	192	**97%**

Table 2. Sensitivity of the Epitogen Lyme IgM Test. 252 clinically characterised patient samples were examined with the Epitogen Lyme IgM Test.

Sample type & size	Number of non-reactive	Sensitivity (%)
Lyme neuroborreliosis (n = 30)	23	**76.7**
ACA (n = 7)	4	**57.1**
Lyme arthritis (n = 24)	11	**45**
Erythema migrans (n = 191)	103	**53.9**
↳ Acute EM (n = 98)	54	**79.4**
↳ Early EM (n = 57)	34	**59.6**
↳ Late EM (n = 36)	15	**41.7**
Total (n = 252)	141	**56%**

[a] Acrodermatitis chronica atrophicans (ACA)

13. 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. 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.
4. The performance characteristics of the Epitogen Lyme IgM Test have not been established with samples from individuals vaccinated with *B. Burgdorferi* antigens.

14. 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 (**#3**) to secondary diluent (**#6**) and mix thoroughly (**conjugate cocktail**).
3. Dilute samples 1:51 (e.g.: 5 µL of sample + 250 µL of sample diluent)
4. Wash plate once then add 100 µL of each diluted sample and controls to each paired 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 – 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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