premier®

WB Lyme IgM

CLIA Complexity: High

REF **612130** IVD **Rx Only**

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
612130	Borrelia burgdorferi (whole cell antigens) plus recombinant p41, flagellin	IgM	Antigen coated membrane strips	30 x 01 (30)

Intended use: The Anti-Borrelia burgdorferi Premier WB Lyme IgM kit is a Westernblot assay intended for the qualitative determination of IgM class antibodies against Borrelia burgdorferi in human serum and plasma (K^+-EDTA, Li^+-heparin, Na^+-citrate) samples that have been found positive or equivocal/borderline using an enzyme immunoassay (EIA) or immunofluorescence assay (IFA) test procedure for Borrelia burgdorferi antibodies. Results can be read manually or automated utilizing EUROLineScan. This test is used as an aid in the diagnosis of infections with Borrelia burgdorferi and the associated diseases, in conjunction with other laboratory and clinical findings.

Summary and Explanation

Clinical significance: Lyme disease is a condition caused by Borrelia burgdorferi. A B. burgdorferi infection can manifest itself in the areas of dermatology, neurology, ophthalmology, rheumatology and internal medicine [1, 2, 3]. The clinical expression of borreliosis can be divided into three stages:

Stage I: The typical primary manifestation of a B. burgdorferi infection is erythema migrans (EM) (80%), a reddening of the skin which appears around the area of the tick bite and spreads in a circular manner. The erythema is accompanied by influenza-like general symptoms with fever, shaking chill, headaches and vomiting. Lymphadenopathies are observed in a few cases (lymphadenosis cutis benigna) [2, 3]. The leading clinical types of the erythema-free borreliosis (20%) are neurological, arthromyalgic, influenza-like, cardiovascular borreliosis and borreliosis with hepatitis, with regional lymphadenitis and mixtures of these [1, 2, 3].

IgM antibodies against B. burgdorferi can be detected serologically in 50% to 90% of patients during stage I. Humans produce specific antibodies against the outer surface protein C (OspC) shortly after infection with B. burgdorferi [4, 5, 6]. VlsE antigen from B. burgdorferi predominantly captures IgG anti-bodies, whereas OspC is suitable for IgM antibody detection [7, 8, 9].

Stage I can result in spontaneous healing or can develop into a generalized borreliosis. The transition phase into stage II disease is generally symptom-free.

Stage II: A variety of symptoms can develop several weeks to some months after receiving the tick bite. In the foreground of these are neurological manifestations: meningitis, encephalitis, asymmetric poly-neuritis, cranial nerve paresis, and Bannwarth's lymphocytic meningoradiculoneuritis. One of the most frequent neurological symptoms of borreliosis in children is the peripheral facial palsy. Usually the paresis of the facial nerve appears on one side and after several weeks on the opposite side. Also arthritis, particularly of the knee joints, and non-localized pains in the bones, joints and muscles are frequently found. Less frequently are cardiological manifestations such as myocarditis and pericarditis [1, 3]. Antibodies against B. burgdorferi can be detected in 50% to 90% of patients in stage II. In the early phase of this stage mainly IgM antibodies are found but in the late phase often only IgG antibodies occur. However, IgM antibodies can persist for a long time [3, 10]. VlsE based detection systems were found to be suitable for the detection of anti-B. burgdorferi IgG antibodies [7, 8, 11].

Stage III: The typical manifestations of a B. burgdorferi infection in stage III are chronic-relapsing erosive arthritis, acrodermatitis chronica atrophicans and progressive encephalomyelitis [1, 3, 12]. Without treat-ment, the tertiary stage can develop over a period of years to decades after the original infection. In this stage, IgG antibody titers are significantly increased in 90% to 100% of patients, while IgM antibodies are only rarely detectable [11, 12, 13].

Serological diagnosis should follow the principle of a two-step procedure: ELISA as a first step, which, if reactive, is followed by an immunoblot [11, 15, 16].

Antigens: The antigen source for the Anti-Borrelia burgdorferi Premier WB Lyme IgM is provided by B. burgdorferi strain B31. The proteins from the B. burgdorferi extract have been separated according to their molecular masses, using discontinuous polyacrylamide gel electrophoresis and were finally transferred onto nitrocellulose. An additional membrane chip coated with p41 flagellin is fixed to each nitrocellulose membrane.

Principles of the test: The test kit contains test strips with electrophoretically separated antigen extracts of B. burgdorferi. Each test strip contains a membrane chip coated with p41 flagellin antigen. The blot strips will be blocked and incubated in the first reaction step with diluted patient samples. In the case of positive samples, specific antibodies of the class IgM will bind to the antigens. To detect the bound anti-bodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (enzyme conjugate), catalyzing a color reaction.

Materials

Contents of the test kit:

Component	Format	Symbol
1. Test strips single strips with electrophoretically separated B. burgdorferi antigens plus p41 flagellin	30 x 1	STRIPS
2. Evaluation matrix with control strip 1 test strip incubated with a positive control serum	1 sheet	---
3. Enzyme conjugate alkaline-phosphatase labelled anti-human IgM (goat), 10x concentrate	2 x 3 mL	CONJ 10X
4. Universal buffer 10x concentrate	1 x 100 mL	BUF 10X
5. Substrate solution nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP), ready for use	1 x 50 mL	SUBS

Additional materials and equipment (not supplied):

- Pipettes with a range of 10 µL to 1000 µL with single-use tips
- Tweezers (for handling of the strips)
- Multistep pipette with top for dispensing up to 1.5 mL per pass
- Rocker
- Vortex mixer
- Distilled or deionized water for buffer preparation, stopping
- Glass/plastic tubes for dilution of samples and reagents
- Lint-free towelling
- Filter paper
- Black tray (*Please note, each channel within the tray is for single use only)*
  - For Visual Evaluation or use with use with automated sample processors, order tray only (catalog # 11768)
  - For Automated Evaluation, the tray is included in Automated Evaluation Accessory Pack (catalog # 610216A)

For **visual evaluation of the strips**, please use the required Premier Western Blot Reaction Control Card (catalog # 11764) and the Premier Western Blot Lyme IgM Evaluation Form (available on the Meridian Bioscience, Inc. website).

For **automated evaluation with EUROLineScan** only:

- For automation purposes, it is necessary to order the Premier Western Blot Automated Evaluation Accessory Pack (catalog # 610216A). This pack contains the following items:
  - Green paper (5 sheets)
  - Adhesive foil for approx 30 test strips (2 foils)
  - Black tray for 30 strips (1 tray). *Please note, each channel within the tray is for single use only*
- For the creation of work protocols and the evaluation of test strips using EUROLineScan:
  - Catalog # 610216: EUROLineScan scanner
  - Catalog # 610214: EUROLineScan software

Warnings and Precautions

For in vitro diagnostic use.

Adherence to the procedures within this manual is necessary for the successful use of this product. Failure to follow the instructions may produce aberrant results.
Do not use the evaluation matrix from one kit batch for band location with any other kit batch.

Warning: The control sera used for the evaluation matrix have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using FDA-cleared or European CE-approved test systems. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care.

Some of the reagents contain sodium azide at a concentration of ≤ 0.09%. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions. Rinse sink thoroughly with water after disposing of solutions containing azide. Avoid skin contact.

Waste disposal: Patient samples and incubated blot strips should be handled as infectious waste. Follow local, state and federal regulations regarding handling and disposal of hazardous waste, including potentially explosive materials such as accumulated or concentrated sodium azide.

Preparation and Stability of the Reagents

Storage and stability: The test kit has to be stored at a temperature between +2 C and +8 C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.
All reagents must be brought to room temperature (+18 C to +25 C) approx. 30 minutes before use. Unopened, the reagents are stable until the indicated expiry date when stored at +2 C to +8 C. After initial opening, the reagents are stable until the indicated expiry date, if stored at +2 C to +8 C and protected from contamination, unless stated otherwise below.

- **Coated test strips:** Ready for use. Do not open until the test strips have reached room temperature to prevent condensation on the strips. After removal of the strips the packing should be sealed tightly and stored at +2 C to +8 C. To ensure correct evaluation of results, the strip lot number must match the number on the evaluation matrix.

- **Enzyme conjugate:** The enzyme conjugate is supplied as a 10x concentrate. For the preparation of the working-strength enzyme conjugate the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with working-strength universal buffer.
  For example: For 1 test strip dilute 0.15 mL anti-human IgM concentrate with 1.35 mL of the working-strength universal buffer.
  The working-strength enzyme conjugate should be used at the same working day.

- **Universal buffer:** The universal buffer is supplied as a 10x concentrate. For the preparation of the working-strength universal buffer the amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionized or distilled water.
  For example: For the incubation of 1 test strip 1.5 mL buffer concentrate should be diluted with 13.5 mL deionized or distilled water.
  The working-strength buffer should be used at the same working day.

- **Substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light  .

Preparation and Stability of the Patient Samples

Samples: Human serum or plasma (K^+-EDTA, Li^+-heparin, Na^+-citrate). Samples are to be obtained by venipuncture, following CLSI (formerly NCCLS) document H03-A6.
Use of hemolyzed, hyperlipemic, hemolytic, heat-treated or contaminated samples should be avoided.

Stability: The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2 C to +8 C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20 C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should be incubated within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.

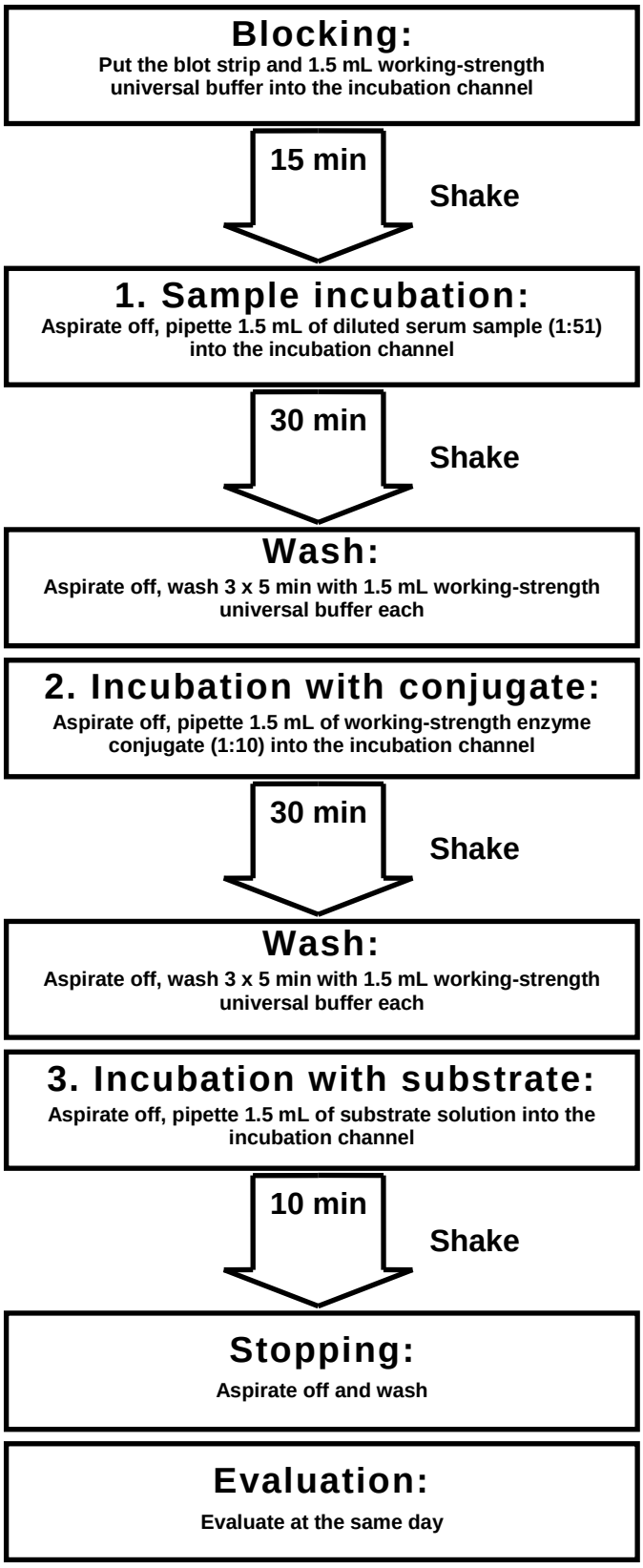
Sample dilution: The **patient samples** to be investigated are diluted **1:51** in ready for use diluted universal buffer. For example: Add 30 µL of sample to 1.5 mL ready for use diluted universal buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

Procedure

Blocking:	According to the number of serum samples to be tested, fill each channel of the incubation tray with 1.5 mL ready for use diluted universal buffer. Remove the required amount of blot strips from the packing using a pair of tweezers. Insert the blot strip in the incubation tray such that the number on the test strip is visible. Incubate for **15 minutes** at room temperature (+18 C to +25 C) on a rocking shaker. Afterwards aspirate off all the liquid.
Sample incubation: (1st step)	Fill each channel with 1.5 mL of the diluted serum samples. One serum sample per channel. Incubate at room temperature (+18 C to +25 C) for **30 minutes** on a rocking shaker.
Washing:	Aspirate off the liquid from each channel and wash **3 x 5 minutes** each with 1.5 mL working-strength universal buffer on a rocking shaker.
Conjugate incubation: (2nd step)	Pipette 1.5 mL ready for use diluted enzyme conjugate (alkaline phosphatase conjugated anti-human IgM) into each channel. Incubate for **30 minutes** at room temperature (+18 C to +25 C) on a rocking shaker.
Washing:	Aspirate off the liquid from each channel. Wash as described above.
Substrate incubation: (3rd step)	Pipette 1.5 mL substrate solution into the channels of the incubation tray. Incubate for **10 minutes** at room temperature (+18 C to +25 C) on a rocking shaker.
Stopping:	Aspirate off the liquid from each channel and wash each strip **3 x 1 minute** with deionized or distilled water.
Evaluation:	Results should be read once strips have dried. Results can be read either by manual/visual interpretation or automated using EUROLineScan. It is recommended to evaluate the strips at the day of incubation. See Evaluation of Results section below.

Anti-Borrelia burgdorferi Premier-WB Lyme IgM

Incubation Protocol



Evaluation of Results

After stopping the reaction using deionized or distilled water, apply adhesive foil to the Lyme IgM Evaluation Form. (Note: for Automated Evaluation, print an evaluation protocol on green paper using the EUROLineScan software; refer to EUROLineScan user manual). Place the incubated blot strips onto the corresponding field of the Evaluation Form using a pair of tweezers. The position of the blot strips can be corrected while they are wet. As soon as all blot strips have been placed onto the Evaluation Form, they should be blotted using filter paper to remove excess moisture. After they have dried for 1 hour, the blot strips will be fixed to the adhesive foil.

The test strip lot-specific evaluation matrix included in the kit is fitted with a blot strip originating from the same gel as the other blot strips in the test kit. This blot strip has been incubated with a positive control serum and indicates the exact position of the relevant bands. The blot strips are labelled with a specific number. This number refers to the strip lot and is also printed on the corresponding evaluation matrix. **The strip lot number on the evaluation matrix must match the strip lot number on the blot strip.**

For evaluation, put the evaluation matrix against the blot strip and align the black band above the strip lot number with the alignment bar of the evaluation matrix.

Band	**Antigen**
41 kDa	Flagellin, p41 (membrane chip, recombinant)
39 kDa	Borrelia membrane protein A, BmpA, p39
25 kDa	Outer surface protein C, OspC, p25

Visual evaluation with reaction control card: Hold the reaction control card (catalog # 11764) next to the incubated strip and compare the band intensity with the two color bars of the card. Band intensities weaker than the positive color bar are evaluated as negative. Band intensities stronger than the positive color bar are evaluated as positive. Band intensities stronger than the negative color bar and weaker than the positive color bar are evaluated as borderline.

Note: When blot strips are evaluated visually with the reaction control card, staining could occasionally completely or partially mask the bands making them unreadable. Masking may appear as heavy back-ground staining, heavy speckling or blotching. Due to variations in test performance and the uncertainty associated with unreadable Western blots, it is recommended that all unreadable blots be retested, using the original specimen. If the original specimen repeatedly yields unreadable blots, and symptoms persist, a fresh specimen should be tested in 2 to 4 weeks. By utilizing EUROLineScan for automated evaluation of the strips, the software calculates and considers the background intensity to avoid misinterpretation.

Automated evaluation with EUROLineScan: The evaluation protocol (printed on green paper) with the test strips is scanned using a flatbed scanner (catalog # 610216) and evaluated with EUROLineScan software (catalog # 610214). For use of the EUROLineScan program please refer to the EUROLineScan user manual. The code for entering the test into EUROLineScan is **B. burg US IgM**.

Blot reader correlation: Comparison of EUROLineScan (automated) and visual (manual) reading was investigated using 111 characterized samples with three different human readers and the correlation observed was ≥ 99%.

Quality control: Each blot strip contains a control band. This must show an intense staining after the incubation, in order for the results to be valid.
Additional controls may be tested in accordance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. It is recommended that the user refer to CLSI document C24-A and 42 CFR 493.1256 for guidance on appropriate quality control practices.

Interpretation of Results

Band cut-off determination
The cut-off of the Anti-Borrelia burgdorferi Premier WB Lyme IgM test system is defined as the lowest limit of a clearly visible band. Since visual examination by the operator might be subjective, the reaction control card was developed to standardize strip evaluation. Alternatively, the EUROLineScan software was established to allow for automated evaluation.

Results

Positive: According to the CDC criteria, the IgM Blot is considered as positive if any 2 of the following 3 bands are positive: p25 (OspC) p39 (BmpA) and p41.

Negative: The result is negative if one or no bands are found positive. If a negative result is obtained and infection with B. burgdorferi is clinically suspected, a new sample should be tested 3 to 4 weeks later.

Note: Borderline results are considered as negative. It is known that B. burgdorferi-infected individuals who have recently seroconverted may display incomplete patterns, but may develop increased reactivity (both in intensity and number) when followed for a period of four to six months.

Limitations of the Procedure

1. This kit is used as an aid in diagnosis only. A positive result should be interpreted together with clinical findings and other serological tests. The results obtained from this assay are not diagnostic proof of the presence or absence of a disease.
2. Negative results do not exclude the possibility of infection with B. burgdorferi.
3. Interference from albumin, intralipids and cholesterol with the assay performance has not been investigated.
4. In the serological investigation of a B. burgdorferi infection the determination of antibodies of class IgM often yields unclear results. IgM antibodies can sometimes be found in serum years after an infection or following antibiotic treatment. Therefore, the detection of IgM antibodies does not necessarily indicate a fresh infection. A negative IgM result does not exclude a fresh infection. With a second infection, only antibodies of class IgG and not IgM can be formed. In the late stage of borreliosis a positive IgM result does not provide any additional information, due to the antibody persistence mentioned above [17, 18, 19].
5. Insufficient washing (e.g. too small wash buffer volumes, or shortened reaction times) can lead to incorrect results.
6. The test is only to be performed by laboratory professionals in a clinical laboratory setting. Strictly adhere to the test procedure. Deviations from the indicated volumes, times etc. can lead to incorrect results.
7. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that its automated procedure yields test results within acceptable limits.

Expected Values

The incidence of IgM antibodies and positivity in different populations as tested by the Anti-Borrelia burgdorferi Premier WB Lyme IgM are presented below with available patient demo-graphics.

Population		n	Gender	Mean Age & Range	p25	p39	p41	No. Pos. (%)
Sensitivity study	< 1 mth	11	4 men 7 women	45 yrs 16-70 yrs 1 unknown	8	0	8	7 (63.6)
	> 1-3 mths	23	4 men 19 women	48 yrs 21-80 yrs	11	4	16	12 (52.2)
	> 3-12 mths	38	17 men 17 women 4 unknown	46 yrs 18-87 yrs 4 unknown	14	1	20	15 (39.5)
	> yr(s)	29	10 men 15 women 4 unknown	42 yrs 18-63 yrs 4 unknown	11	2	17	11 (37.9)
	Total	101	35 men 58 women 8 unknown	45 yrs 16-87 yrs 11 unknown	44	7	61	45 (44.6)
Prospective study	Site 1	142	88 men 52 women 2 unknown	51 yrs 4-88 yrs 4 unknown	45	38	112	56 (39.4)
	Site 2	97	51 men 46 women	57 yrs 20-75 yrs	33	5	53	29 (29.9)
	Site 3	65	30 men 35 women	60 yrs 19-86 yrs	13	4	26	11 (16.9)
	Total	304	169 men 133 women 2 unknown	56 yrs 4-88 yrs 4 unknown	91	47	191	96 (31.6)
Normal population study	Endemic	98	89 men 9 women	34 yrs 18-56 yrs	3	4	84	5 (5.1)
	Non-endemic	100	82 men 18 women	38 yrs 3-62 yrs	2	2	87	4 (4.0)
	Total	198	171 men 27 women	36 yrs 3-62 yrs	5	6	171	9 (4.5)

Note: It is recommended that each laboratory determine its own normal range based on the population and the equipment used.

Performance Characteristics

Clinical/diagnostic sensitivity study: A study consisting of 101 clinically characterized Lyme disease specimens was conducted at the manufacturer's site with the Anti-Borrelia burgdorferi Premier WB Lyme IgM test device. These specimens contain samples from early, early disseminated and late phases of the disease. The panel consisted of 35 men, 58 women and 8 unknowns. The age ranged from 16 to 87 years with a mean age of 45 years.

Interval	n	Anti-Borrelia burgdorferi Premier WB Lyme IgM			Predicate IgM WB		
		Positive	%	95% C.I.	Positive	%	95% C.I.
< 1 month	11	7	63.6%	30.8 - 89.1%	4	36.4%	10.9 - 69.2%
> 1-3 months	23	12	52.2%	30.6 - 73.2%	9	39.1%	19.7 - 61.5%
> 3-12 months	38	15	39.5%	24.0 - 56.6%	8	21.1%	9.6 - 37.3%
> 12 months	29	11	37.9%	20.7 - 57.7%	4	13.8%	3.9 - 31.7%
Overall	101	45	44.6%	34.7 - 54.8%	25	24.8%	16.7 - 34.3%

Prospective study: A prospective study was performed at 3 different sites in the U.S. Samples were tested as per the CDC two-tiered testing for Lyme disease algorithm. All samples that were positive and equivocal by the first-tier EIA were tested with the Anti-Borrelia burgdorferi Premier WB Lyme IgM and the predicate IgM Western blot. Results are interpreted by the CDC criteria.

n = 304		**Predicate IgM WB**	
		Positive	Negative
Anti-Borrelia burgdorferi Premier WB Lyme IgM	Positive	95	1
	Negative	3	205

Positive agreement	95 / 98 =	96.9%	95% C.I.:	91.3% -	99.4%
Negative agreement	205 / 206 =	99.5%	95% C.I.:	97.3% -	100.0%

CDC panel results: 34 sera of patients with clinically characterized borreliosis in different disease stages and 5 normals, obtained from the Centers for Disease Control and Prevention, Atlanta, GA, USA, were tested with the Anti-Borrelia burgdorferi Premier WB Lyme IgM in parallel with the predicate device. The results stratified by time after onset of infection are shown below.

Interval	**n**	**Anti-Borrelia burgdorferi Premier WB Lyme IgM**			**Predicate IgM WB**		
		Negative	**%**	**95% C.I.**	**Negative**	**%**	**95% C.I.**
Normals	5	5	100.0%	47.8 - 100.0%	5	100.0%	47.8 - 100.0%

Interval	**n**	**Anti-Borrelia burgdorferi Premier WB Lyme IgM**			**Predicate IgM WB**		
		Positive	**%**	**95% C.I.**	**Positive**	**%**	**95% C.I.**
< 1 month	6	3	50.0%	11.8 - 88.2%	3	50.0%	11.8 - 88.2%
> 1-3 months	10	7	70.0%	34.8 - 93.3%	7	70.0%	34.8 - 93.3%
> 3-12 months	12	2	16.7%	2.1 - 48.4%	2	16.7%	2.1 - 48.4%
> 12 months	6	0	0.0%	0.0 - 45.9%	0	0.0%	0.0 - 45.9%
Overall	34	12	35.3%	19.7 - 53.5%	12	35.3%	19.7 - 53.5%

Note: The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

Normal population study: Testing of samples from asymptomatic population in both endemic and non-endemic regions was performed. The presence of anti-B. burgdorferi antibodies was analyzed with the Anti-Borrelia burgdorferi Premier WB Lyme IgM in a panel of 98 samples from an endemic region (Pennsylvania, 89 men and 9 women with an average age of 34 y, age range: 18 to 56 y) and in a panel of 100 samples from a non-endemic region (Tennessee, 82 men and 18 women with an average age of 38 y, age range: 3 to 62 y). 5.1% of the samples from the endemic region and 4.0% of the samples from the non-endemic region were found positive for anti-B. burgdorferi (IgM).

Panel	n	**Anti-Borrelia burgdorferi Premier WB Lyme IgM**	
		Positive (%)	**Negative (%)**
Endemic	98	5 (5.1%)	93 (94.9%)
Non-endemic	100	4 (4.0%)	96 (96.0%)

Cross-reactivity: Cross-reactivity was investigated using characterized samples from the following groups as shown in the table below. All the samples tested were found negative with the Anti-Borrelia burgdorferi Premier WB Lyme IgM per the CDC criteria of results interpretation.

Panel	n	Anti-Borrelia burgdorferi Premier WB Lyme IgM
		Negative (%)
EBV	15	15 (100.0%)
HSV	14	14 (100.0%)
Influenza viruses	15	15 (100.0%)
H. pylori	11	11 (100.0%)
Measles	15	15 (100.0%)
Parvovirus B19	12	12 (100.0%)
Rubella	14	14 (100.0%)
Treponema	10	10 (100.0%)
CMV	10	10 (100.0%)
Babesiosis	3	3 (100.0%)
Anaplasmosis (Ehrlichiosis)	10	10 (100.0%)
Rickettsial diseases	4	4 (100.0%)
Rheumatoid arthritis	22	22 (100.0%)
Total	155	155 (100.0%)

Note: The results obtained with babesiosis (3) and rickettsial diseases (4) samples are not conclusive as the number of tested samples was insufficient.

Interferences: Hemolytic, lipemic and icteric samples showed no influence on the result up to a concentration of 500 mg/dl for hemoglobin, 2000 mg/dl for triglycerides and 40 mg/dl for bilirubin in this test system. Interferences with albumin, intralipids and cholesterol have not been investigated.

Precision/repeatability: Repeatability was investigated by repeated determinations of 7 characterized samples. The samples were tested on 4 different days with 2 runs per day, 2 replicates per run according to the package insert. Repeatability was found to be sufficient as no positive sample was found negative and vice versa.

n = 16	Anti-Borrelia burgdorferi Premier WB Lyme IgM						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Characterization	negative	negative	negative	negative	negative	positive	positive
% negative	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
% positive	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%

Band(s)	Anti-Borrelia burgdorferi Premier WB Lyme IgM		
	p25	p39	p41
Sample 1	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	100.0%
Sample 3	0.0%	0.0%	100.0%
Sample 4	0.0%	0.0%	100.0%
Sample 5	0.0%	0.0%	100.0%
Sample 6	100.0%	100.0%	100.0%
Sample 7	0.0%	100.0%	100.0%

Reproducibility: Reproducibility was investigated by repeated determinations of 7 characterized samples. The samples were tested on 4 different days with 2 runs per day, 2 replicates per run at 3 different sites according to the package insert. Reproducibility was found to be sufficient as no positive sample was found negative and vice versa.

n = 48	Anti-Borrelia burgdorferi Premier WB Lyme IgM						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Characterization	negative	negative	negative	negative	negative	positive	positive
% negative	100.0%	100.0%	100.0%	100.0%	100.0%	0.0%	0.0%
% positive	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	100.0%

Band(s)	Anti-Borrelia burgdorferi Premier WB Lyme IgM		
	p25	p39	p41
Sample 1	0.0%	0.0%	0.0%
Sample 2	0.0%	0.0%	100.0%
Sample 3	0.0%	0.0%	100.0%
Sample 4	0.0%	0.0%	100.0%
Sample 5	0.0%	0.0%	100.0%
Sample 6	100.0%	100.0%	100.0%
Sample 7	0.0%	100.0%	100.0%

Serum/plasma comparison: The use of K^+-EDTA, Li^+-heparin, Na^+-citrate plasma samples was confirmed by a correlation of 10 sample sets of serum and corresponding plasma. The sample sets were selected to cover both negative and positive results. The results of the plasma samples and the corresponding serum samples were compared and found to be sufficient as no positive sample was found negative and vice versa.

Literature References

1. Dryden MS, Saeed K, Ogborn S, Swales P. Lyme borreliosis in southern United Kingdom and a case for a new syndrome, chronic arthropod-borne neuropathy. Epidemiol Infect 143 (2015)561-572.
2. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet 379 (2012) 461-473.
3. Wright WF, Riedel DJ, Talwani R, Gilliam BL. Diagnosis and Management of Lyme Disease. Am Fam Physician 85 (2012) 1086-1093.
4. Fung BP, McHugh GL, Leong JM, Steere AC. Humoral immune response to outer surface protein C of Borrelia burgdorferi in Lyme disease: role of the immunoglobulin M response in the serodiagnosis of early infection. Infect Immun 62 (1994) 3213-3221.
5. Padula SJ, Sampieri A, Dias F, Szczepanski A, Ryan RW. Molecular characterization and expression of p23 (OspC) from a North American strain of Borrelia burgdorferi. Infect Immun 61 (1993) 5097-5105.
6. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of lyme borreliosis. Clin Microbiol Rev 18 (2005) 484-509.
7. Norris SJ. How do lyme borrelia organisms cause disease? The quest for virulence deter-minants. Open Neurol J 6 (2012) 119-123.
8. Wang D, Botkin DJ, Norris SJ. Characterization of the vls antigenic variation loci of the Lyme disease spirochaetes Borrelia garinii Ip90 and Borrelia afzelii ACAI. Mol Microbiol 47 (2003) 1407-1417.
9. Lawrenz MB, Hardham JM, Owens RT, Nowakowski J, Steere AC, Wormser GP, Norris SJ. Human antibody responses to VlsE antigenic variation protein of Borrelia burgdorferi. J Clin Microbiol 37 (1999) 3997-4004.

10. Kalish RA, McHugh G, Granquist J, Shea B, Ruthazer R, Steere AC. Persistence of immuno-globulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease. Clin Infect Dis 33 (2001) 780-785.
11. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 49 (2007) 13-21.
12. EUROIMMUN: Steinhagen K, Schlumberger W, Stöcker W. Nachweis einer spezifischen intrathekalen Antikörpersynthese mit modernen ELISA-Testsystemen: Hohe Trefferquote bei Multipler Sklerose und Neuroborreliose. J Lab Med 25 (2001) 135-149.
13. EUROIMMUN: Meyer W, Janssen A, Scheper T, Schlumberger W, Stöcker W. Lyme borreliosis: Prevalence of antibodies against non-proteinic (lipid) antigens. Int J Med Microbiol 298S2 (Suppl. 45) (2008) 39.
14. EUROIMMUN: Probst C, Ott A, Scheper T, Meyer W, Stöcker W, Komorowski L. N-Terminal disulfide-bridging of Borrelia outer surface protein C increases its diagnostic and vaccine potentials. Ticks and Tick-Borne Diseases 3 (2012) 1-7.
15. Wormser GP, Tang AT, Schimmoeller NR, Bittker S, Cooper D, Visintainer P, Aguero-Rosenfeld ME, Ogrinc K, Strle F, Stanek G. Utility of serodiagnostics designed for use in the United States for detection of Lyme borreliosis acquired in Europe and vice versa. Med Microbiol Immunol 203 (2014) 65-71.
16. Christova I. Enzyme-linked immunosorbent assay, immunofluorescent assay, and recombinant immunoblotting in the serodiagnosis of early Lyme borreliosis. Int J Immunopathol Pharmacol 16 (2003) 261-268.
17. Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J Clin Microbiol 34 (1996) 1-9.
18. Sivak SL, Aguero-Rosenfeld ME, Nowakowski J, Nadelman RB, Wormser GP. Accuracy of IgM immunoblotting to confirm the clinical diagnosis of early Lyme disease. Arch Intern Med 156 (1996) 2105-2109.
19. Porwancher R. A reanalysis of IgM Western blot criteria for the diagnosis of early Lyme disease. J Infect Dis 179 (1999) 1021-1024.

Manufactured For

Meridian Bioscience, Inc.
Corporate Office
3471 River Hills Drive
Cincinnati, Ohio 45244 USA
Telephone: 513.271.3700
Orders/Customer Service: 800.543.1980
Technical Support Center: 800.343.3858
Information Fax: 513.272.5432
Ordering Fax: 513.271.0124

SYMBOL USAGE
You may see one or more of these symbols on the labeling/packing of this product.
Key Guide to symbols

	Use By	CONTROL +	Positive control
LOT	Batch Code	CONTROL −	Negative control
IVD	In vitro diagnostic medical device	FOIL	Protective foil
REF	Catalogue number		Do not freeze
	Consult Instructions for Use	CAL	Calibrator
	Manufacturer	STRIPS	Test Strips
	Contains sufficient for <n> tests	SOLN STOP	Stopping Solution
Rx Only	Prescription Use Only	CONJ ENZ	Ezyme Conjugate
	Temperature limitation		Potential Biohazard
SN	Serial number	CONJ 10X	Conjugate Concentration 10X
TEST	Test Device	REAG	Reagent
	Date of manufacture	BUF WASH	Wash Buffer
BUF	Buffer		Warning
CONJ	Conjugate	DIL SPE	Specimen Diuent (or Sample Diluent)
SUBS	Substrate	BUF 10X	Buffer Concentration 10X
	Keep away from sunlight	SMP BUF	Sample Buffer

For technical assistance, call Technical Support Services at (800) 343-3858 between the hours of 8AM and 6PM, USA Eastern Standard Time. To place an order, call Customer Service Department at (800) 543-1980.

 EUROIMMUN US 1 Bloomfield Ave, Mountain Lakes, New Jersey 07046 USA 973.656.1000

612130
REV. 03/19