

***B. burgdorferi* (IgM) MARBLOT STRIP TEST SYSTEM**  
**Western Blot System for the detection of IgM antibody**  
**to *Borrelia burgdorferi***  
**Product # 40-2065M, 40-2065M/B, 40-2075M**  
**For In Vitro Diagnostic Use**

**Materials Provided for Kit Product # 40-2065M**

Product #	Description	Quantity
40-2060M	<i>B. burgdorferi</i> Marblot Strip	40ea.
40-2022L	<i>B. burgdorferi</i> WB IgM Serum Band Locator	1x250µL
40-2029L	<i>B. burgdorferi</i> WB Weakly Reactive IgM Control	1x100µL
40-2021L	<i>B. burgdorferi</i> WB Negative Control	1x100µL
40-2525M	Alkaline Phosphatase Conj., Anti-Human IgM	1x9mL
44-2017	Alkaline Phosphatase Developing Solution	1x90mL
40-2019C	10x Sample Diluent/Wash Solution	1x100mL
	Blot Banding Template	1ea.
	Result Log	2ea.

**Materials Provided for Kit Product # 40-2065M/B**

Product #	Description	Quantity
40-2060M	<i>B. burgdorferi</i> Marblot Strip	10x40ea.
40-2022L	<i>B. burgdorferi</i> WB IgM Serum Band Locator	5x250µL
40-2029L	<i>B. burgdorferi</i> WB Weakly Reactive IgM Control	5x100µL
40-2021L	<i>B. burgdorferi</i> WB Negative Control	5x100µL
40-2525M	Alkaline Phosphatase Conj., Anti-Human IgM	10x9mL
44-2017	Alkaline Phosphatase Developing Solution	10x90mL
40-2019C	10x Sample Diluent/Wash Solution	10x100mL
	Blot Banding Template	10ea.
	Result Log	10ea.

**Materials Provided for Kit Product # 40-2075M**

Product #	Description	Quantity
40-2070M	<i>B. burgdorferi</i> Marblot Strip	20ea.
40-2022L	<i>B. burgdorferi</i> WB IgM Serum Band Locator	1x250µL
40-2029L	<i>B. burgdorferi</i> WB Weakly Reactive IgM Control	1x100µL
40-2021L	<i>B. burgdorferi</i> WB Negative Control	1x100µL
40-2025M	Alkaline Phosphatase Conj., Anti-Human IgM	1x4.5mL
40-2017	Alkaline Phosphatase Developing Solution	1x45mL
40-2019C	10x Sample Diluent/Wash Solution	1x100mL
	Blot Banding Template	1ea.
	Result Log	1ea.

Store at +2 to +8°C

**INTENDED USE**

MarDx *B. burgdorferi* (IgM) Marblot Strip Test System is a Western blot assay for the qualitative in vitro detection of human IgM antibody to individual proteins of *Borrelia burgdorferi* in human serum. The MarDx *B. burgdorferi* (IgM) Marblot Strip Test System is intended for use in testing human serum samples which have been found positive or equivocal using an EIA or IFA test procedure to provide supportive evidence of infection with *B. burgdorferi*. High Complexity Test.

The MarDx *B. burgdorferi* (IgM) Marblot Strip Test System can be used during the acute phase (0-4 weeks of symptoms onset) of *B. burgdorferi* infection. Patients initially infected with *B. burgdorferi* are commonly found to have a positive IgM blot and a negative IgG blot, indicative of an acute disease stage. After this early period, infected patients are usually found to be Western blot positive IgG, confirming seroconversion.

Caution must be used in supporting a diagnosis of *B. burgdorferi* infection when sera are Western blot IgM positive and Western blot IgG negative after the initial 4 week period from onset. **Because the likelihood of a false-positive test result is high for these individuals, a positive IgM test alone is not recommended for use in determining active disease in persons with illness of longer than one month duration.** If symptoms persist, the patient should be

retested for seroconversion to IgG antibodies after an additional 2 to 4 weeks.

**I. INTRODUCTION**

Lyme disease is a multisystem disease caused by a spirochete, *Borrelia burgdorferi*<sup>3,4,6</sup>. The disease has been documented in Europe since early this century. It has been recently documented in the United States during an epidemic in 1975 among children in Old Lyme, Connecticut, who demonstrated arthritic symptoms. Steere et al. recognized the disease as a separate clinical entity<sup>19</sup>. Its symptoms may be nonspecific and confused with those of juvenile rheumatoid arthritis, lupus erythematosus, multiple sclerosis, rheumatic fever, Reiter's Syndrome, myocarditis and viral meningitis.

The organism is transmitted through an arthropod vector from an animal reservoir. *B. burgdorferi* was first isolated from *Ixodes dammini* ticks, which was shown to be the etiologic agent<sup>1,5,18</sup>. *Ixodes scapularis* ticks are principally responsible for transmission in the northeastern and mid-Atlantic regions, as well as in Minnesota and Wisconsin. *Ixodes pacificus* transmits *B. burgdorferi* in California coastal and mountain regions.

The animals that may be infected: deer, wild mice, birds, raccoons, horses, dogs and cats. The ticks are commonly found on vegetation in epidemic areas especially in wooded areas common to the infected animals.

The incidence of human infection coincides with the tick season from May through September in most parts of the United States<sup>12,13</sup>.

Among the many symptoms of early onset of Lyme disease are:

1. A red lesion on or near the site of a tick bite. The lesion is called Erythema Migrans (EM).
2. Arthritic symptoms
3. Low grade "flu like" fever
4. Headaches
5. Dizziness
6. Stiff neck
7. Fatigue and general malaise
8. Muscular aches and pains
9. Abdominal pain
10. Irregular pulse and heart beat

EM develops in up to 60 - 70% of the cases within a few days to weeks following a tick bite<sup>14</sup>. The lesion typically starts at the site of the bite and radiates slowly in a circular pattern. It may reach 5-50 cm in diameter and generally clears centrally within a few weeks. The lesion, unfortunately, cannot be relied upon for the clinical diagnosis of Lyme disease. Additionally, because of the very small size of the nymphal tick (1-2 mm), as many as 80% of tick bites are unrecognized. During this period symptoms of headache, malaise, myalgia, fever, arthralgia, fatigue and lymphadenopathy are usually present.

In the later stages, symptoms can resemble a variety of different diseases.

Neurological, Cardiac and Musculoskeletal Involvement. Generally these symptoms may appear from weeks to months following initial infection. This stage is characterized by symptoms of dizziness, weakness and irregular heartbeat, meningitis, inflamed nerve roots in the neck and facial palsy. Other symptoms include: mood swings, loss of memory, inability to concentrate, poor motor coordination, and somnolence.

Arthritic Symptoms. Generally the large joints are affected with pain and swelling. The arthritic attacks may be recurrent.

## I. INTRODUCTION (continued)

Isolation of *B. burgdorferi* from skin biopsy, blood and spinal fluid has been reported and is definitive for establishing *B. burgdorferi* infection. However, these direct cultural methods cannot be practically relied upon for routine laboratory diagnosis of *B. burgdorferi* infection. Overgrowth by the competing microflora, complicated growth medium requirements, and the slow growth rate of the spirochete are all factors influencing the outcome of direct culture.

Serologic methods are the most commonly used for presumptive diagnosis of infection. Both enzyme immunoassay (EIA) and indirect immunofluorescence assays (IFA) have been employed. EIA is considered more sensitive and less subjective than IFA<sup>7,15,16</sup>. The use of Western Blot is useful for characterizing the specificity of the antibody response to *B. burgdorferi*. This discrimination is not possible with the IFA or EIA because these procedures measure the total antibody response only.

Steere et al. reported that patients with Lyme disease produce antibodies of the IgM class during the first few weeks after onset of EM and produce antibodies of the IgG class more slowly<sup>19</sup>. Both IgM and IgG titers can remain positive for many months or years<sup>7,9</sup>.

Persons with very early stages of Lyme disease treated with antibiotics may not develop titers or will develop only low antibody levels. Antibody cross reactions in Western blot and other serologic methods have been reported with other pathogenic spirochetes such as *Treponema pallidum*, the causative agent for syphilis, and *T. pertenuis*, the causative agent for yaws<sup>11</sup>. The VDRL (Venereal Disease Research Lab) or RPR (Rapid Plasma Reagin) tests may be useful for differentiating treponematoses (the VDRL or RPR are usually positive) from Lyme disease without treponemal exposure (the VDRL and RPR are negative).

## II. PRINCIPLES OF THE TEST

The MarDx *B. burgdorferi* (IgM) Strip Test System is a Western blot technique utilizing antigens of *B. burgdorferi* (Strain B 31) which are separated in the presence of sodium dodecyl sulfate (SDS) by polyacrylamide gel electrophoresis. The resolved protein bands are then transferred by electrophoresis to a nitrocellulose membrane. The membrane is dried, cut into strips, and packaged.

Serum is incubated with individual Marblot *B. burgdorferi* strips. If specific antibodies to individual proteins are present, they will bind to the corresponding *B. burgdorferi* antigen bands. After washing the unbound serum from the strip, the bound *B. burgdorferi* specific antibody is reacted with alkaline phosphatase conjugated anti-human IgM. The strip is then washed to remove the unbound conjugated antibody, and the strip is finally reacted with a precipitating color developing solution which deposits a purple precipitate on antibody reacted antigen bands. Bands are visualized, scored for intensity relative to the 41kDa band of the Weakly Reactive Control and recorded. Strips which have 2 of the 3 significant bands are considered positive for specific IgM antibody to *B. burgdorferi*.

## III. WARNINGS AND PRECAUTIONS

### WARNINGS

#### FOR IN VITRO DIAGNOSTIC USE

- Handle samples, assay strips, immunoblot controls and serum locator as if capable of transmitting an infectious agent (Biosafety Level 2).
- The preservatives used in the reagents may be toxic if ingested.
- 10X Sample Wash Diluent/Wash Solution contains <1% Sodium Azide. Each control and conjugate component contains less than 0.1% sodium azide. Sodium azide is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.

- CAUTION: all blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
- Wear suitable protective clothing, gloves and eye/face protection throughout the test procedure. Thoroughly wash hands after handling test reagents.
- If substances come into contact with eyes or skin, wash immediately with plenty of water.
- Wipe spills immediately with a laboratory approved disinfectant.
- Dispose of all specimens and materials used in the MarDx Marblot procedure as biohazardous waste.

## PRECAUTIONS

- Do not dilute the conjugate beyond the stated concentration.
- Do not deviate from the specified temperature and timing requirements as listed in the package insert for both incubation and washing steps. Deviations will significantly alter the results of this test.
- All reagents must be brought to +20 to +25°C before performing this test procedure. Temperatures above or below the recommended range will result in substantial variation of the test results.
- Do not interchange kit components from one kit lot with another kit lot.
- Do not interchange strips from one vial to another.
- Dry Marblot strips are extremely fragile, handle with care.
- Do not use kit beyond its expiration date. The date is printed on the kit box.
- Do not cut strips. Narrower strips could lead to misinterpretation.
- Artifacts in the reactive zones could be mistaken for positive bands or may prevent recognition of positive bands.
- Use distilled or deionized water to dilute Wash/Diluent Concentrate (10X) in order to avoid substances which may interfere with the assay.
- Adherence to the procedures within this manual is necessary for the successful use of this product.
- Do not interpret results obtained with grossly contaminated specimens or strips.
- DO NOT USE THE *B. burgdorferi* WB IgM SERUM BAND LOCATOR STRIP FROM THIS KIT FOR BAND LOCATION WITH ANY OTHER KIT LOT NUMBER. EACH ASSAYED CONTROL IS A VIAL SPECIFIC AND MUST BE USED WITH THAT VIAL ONLY.

## IV. REAGENTS

Description	Active Ingredient
1. Marblot Strips	<i>B. burgdorferi</i> antigen, B-31, Low passage
2. WB IgM Serum Band Locator	<i>B. burgdorferi</i> antibody*
3. WB Weakly Reactive IgM Control	<i>B. burgdorferi</i> antibody*
4. WB Negative Control	Negative for detectable <i>B. burgdorferi</i> antibody*
5. IgM Alkaline Phosphatase Conjugate.	Alkaline Phosphatase Conj., Anti-Human IgM*
6. Color Developing Solution	BCIP/NBT**
7. Sample Diluent/Wash Solution	Phosphate Buffered Saline†

\*These are human sourced controls

\*\*5-Bromo-4-chloro-3-indoyl phosphate/Nitroblue Tetrazolium

†Contains Sodium Azide (NaN<sub>3</sub>)

#### IV. REAGENTS (continued)

##### STORAGE AND STABILITY

1. Store kit at +2 to +8°C.
2. Bring all required components to room temperature prior to use.
3. Refer to the expiration date on all reagents. Do not use beyond expiration.
4. The diluted, 1X working conjugate is good the day of preparation only.
5. The diluted, 1X Sample Diluent/Wash Solution is good the day of preparation only.

##### INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Changes in physical appearance of the reagents may indicate instability or deterioration of these materials.

Note: The Wash/Diluent working solution contains a blocking agent and should appear opaque with a white or beige sediment at the bottom of the bottle. Mix thoroughly before use; do not filter.

##### REAGENT PREPARATION

###### 1. Preparation of 1X Sample Diluent/Wash Solution

On removal of the 10X Sample Diluent/Wash Solution from refrigeration, undissolved salts and a white or beige sediment may be present at the bottom of the bottle. Allow the reagent to reach room temperature and shake the bottle to dissolve the solids.

Prepare the 1X Sample Diluent/Wash Solution by adding 1 part of the 10X Sample Diluent/Wash Solution to 9 parts of distilled or deionized water in a clean bottle. Mix solution until dissolved.

**Prepare only the needed amount for that day run of 1X Sample Diluent/Wash Solution with some extra. Diluted (1X) Sample Diluent/ Wash Solution cannot be stored for future use.**

###### 2. Preparation of 1X Alkaline Phosphatase Anti-human IgM Conjugate

To prepare the Alkaline Phosphatase Anti-human IgM Conjugate dilute 1 part to 9 parts with prepared 1X Diluent/Wash Solution. 2.0 mL of diluted conjugate will be required for each specimen or control. Do not prepare more conjugate than needed for any specific test run. THIS DILUTED CONJUGATE CAN NOT BE STORED FOR FUTURE USE.

**Please refer to the chart Page 9 for help in preparation of 1X Sample Diluent/Wash Solution and 1X Conjugate.**

#### V. SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Avoid hemolysis by prompt separation of the serum from the clot. Serum should be stored at +2 to +8°C if it is to be analyzed within 4-7 days. Serum may be held for 3 to 6 months by storage at -20°C or lower. All lipemic and hemolysed sera should be avoided. If specimens are shipped, they should be shipped in accordance with requirements for transporting etiological agents. All specimens should be shipped frozen to avoid degradation of antibodies.

#### VI. PREPARATION OF CONTROLS

Controls are ready to use and do not require dilution before addition to the strip incubation tray.

#### VII. PREPARATION OF SPECIMENS

Specimens do not require any prior dilution before addition to strip incubation tray.

#### VIII. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Platform rocker. Capable of rocking at a maximum platform angle of  $7^{\circ} \pm 2^{\circ}$  at 40 to 45 cycles per minute.
2. Blunt tipped forceps.
3. Pipettes capable of delivering 20  $\mu$ L, 80 $\mu$ L, 2.0 mL.
4. Disposable pipette tips.
5. Graduated serological pipette capable of delivering 10.0 mL.
6. 100 mL and 1.0 L graduated cylinder.
7. Laboratory timer.
8. Trinity Strip Incubation Tray or equivalent.

#### IX. TEST PROCEDURE

##### PROCEDURAL NOTES

1. All procedural steps should be followed as written. Failure to do so may result in aberrant test results.
2. Record the Kit Lot number and expiration date on the record sheet. Use strips from each kit in consecutive, numerical order.
3. Always use forceps when handling the strips.
4. Assay trays should be rocked at a maximum platform angle of  $7^{\circ} \pm 2^{\circ}$  at 40 to 45 cycles per minute. Position the tray so that reagents mix lengthwise along the strips.

CAUTION: Proper rocking of assay trays is essential. Use of devices that do not provide rocking motion, such as orbital shakers, may adversely affect test sensitivity and result in aberrant test results.

5. Fluid in the channels must be completely decanted prior to the next rinse or reagent addition.
6. Keep the tray level when adding reagents and specimens to the channel. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing the control directly onto the strip.
7. Add samples, conjugate, and substrate to the ends of the channels, not directly onto the strips. This will prevent dark spots and strike lines on the strips.

CAUTION: It is extremely important to prevent cross-contamination between channels. Exercise care when dispensing, rocking and decanting fluids.

8. The Weakly Reactive IgM Control and Negative Control provided must be run in each reaction tray.
9. The IgM Serum Band Locator is required for band location. The locator needs only to be run once for each strip vial.
10. If reusable acrylic trays are used for processing the specimens, thorough cleaning of the trays is essential.

DAILY - Rinse tray(s) thoroughly with distilled water AFTER USE.

WEEKLY - Soak tray(s) in sodium hypochlorite\* (household bleach) solution overnight. Rinse tray(s) **thoroughly** with tap then distilled water. ANY RESIDUAL BLEACH WILL ALTER THE REACTIVITY OF THE ASSAY. \*Dilute to a 10% solution with distilled or deionized water.

**IX. TEST PROCEDURE (continued)****TEST PROCEDURE**

1. Carefully remove the required number of Marblot strips from the strip vial with blunt forceps. **DO NOT TOUCH STRIPS WITH YOUR HANDS.** Place the strips in channels of a strip incubation tray, one strip per channel, numbers facing up. Use strips in consecutive numerical order.
2. For each control, IgM Serum Band Locator, or sample to be tested, fill a channel with exactly 2.0mL Sample Diluent/Wash Solution. Add Sample Diluent slowly and directly to the space above the strip number. Visually check to make sure strip is completely wet and not partially floating on top of the buffer.
3. Allow the strips to soak for a minimum of five minutes while rocking on an appropriate platform rocker.
4. Remove the incubation tray from the platform rocker. Do not decant the 1X Wash Diluent/Wash Solution.
5. Add 80uL of the IgM Serum Band Locator to channel 1 in the space above the strip number. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing the control directly onto the strip. Make sure the strip is fully wet and is not floating on top of the diluent.
6. Add 20uL of Weakly Reactive Control to channel 2 and add 20uL of the Negative Control to channel 3 in the space above the strip number. If necessary carefully tip the tray toward you to position the strips at the end of the channel to avoid dispensing the control directly onto the strip.
7. Add 20uL of each sample to the appropriately marked channel in the space above the strip number using the same technique stated in step 6.
8. Place the Western Blot strip incubation tray on the platform rocker and incubate by rocking for 30 minutes.
9. Remove the strip incubation tray from the platform rocker after incubation.
10. Decant the diluted specimens by carefully tipping the strip incubation tray. Treat decanted fluid as potentially biohazardous.
11. Add 2.0mL of Sample Diluent/Wash Solution to each channel of the strip incubation tray and incubate for 5 minutes while rocking on the platform rocker.
12. Completely decant the Sample Diluent/Wash Solution. *Repeat this process (steps 10 and 11) two additional times to ensure thorough rinsing of the unbound specimen from the membrane strips.* Do not use squeeze bottle to wash strips.
13. Pipette 2.0mL of the previously prepared Alkaline Phosphatase conjugated Anti- human IgM (See section VI-2) into each channel of the strip incubation tray.
14. Incubate the strip incubation tray while rocking on the platform rocker for 15 minutes.
15. Remove the strip incubation tray from the platform rocker after incubation and decant the conjugate by carefully tipping the strip incubation tray.
16. Add 2.0mL of Sample Diluent/Wash Solution to each channel of the strip incubation tray and incubate for 5 minutes while rocking on the platform rocker.
17. Completely decant the Sample Diluent/Wash Solution. *Repeat this process (steps 15 and 16) two additional times to ensure thorough rinsing of the unbound conjugate from the membrane strips.*
18. Add 2.0mL of distilled or deionized water to each channel of the strip incubation tray. Allow strips to rock one minute.
19. Decant and completely drain the distilled or deionized water from the strip incubation tray.
20. Add 2.0mL of Color Developing Solution to each channel of the strip incubation tray.
21. Incubate the strip incubation tray on the platform rocker until the Weakly Reactive Control produces a very faint but visible band at 41kD. Other bands may be visible but are not used to determine the endpoint of the Color Developing Solution reaction. At this level of development the IgM Serum Band Locator should produce bands which are easily read and may be used to identify unknown bands on patient strips. Color Developing Solution reaction time may vary between 4 and 12 minutes. The reaction should not be stopped prior to 4 minutes and should not exceed 12 minutes. The actual length of incubation in the Color Developing Solution will vary according to the individual laboratory conditions. Laboratory temperature, platform rocker speed, water quality, and cleanliness of laboratory glassware and incubation trays etc. will all alter the Color Developing Solution reaction time. If reacted properly the Weakly Reactive Control should produce a very faint band. If the Weakly Reactive Control is over reacted it will produce a darker more visible band. Please note that bands on patient strips must not be called if they are less intense than the 41kD band of the Weakly Reactive Control.
22. Remove the strip incubation tray from the platform rocker after incubation and decant the substrate by carefully tipping the strip incubation tray.
23. Add 2.0mL of distilled or deionized water to each channel of the strip incubation tray. Rock the strip incubation tray by hand 3-4 times. Repeat this step two (2) additional times.
24. Remove the strips from the channels while wet using blunt forceps. Place the wet strips on paper towel and allow to air dry before interpretation of the data.
25. Developed Western blot strips are a permanent record which can be read anytime. Glue or tape developed sample and Negative Control strips to the Result Log provided. Insert mounted strips into a plastic protector and store in a cool, dry, dark place.
26. Glue or tape developed IgM Serum Band Locator and Weakly Reactive IgM Control strips to the locations provided on the Blot Banding Template. Identify significant bands and their relative location by comparing the IgM Serum Band Locator strip with the printed image on the Blot Banding Template. Compare the patient sample strips to the IgM Serum Band Locator and Weakly Reactive Control reacted strips. Refer to XIII. Interpretation of Results for the interpretation criteria.

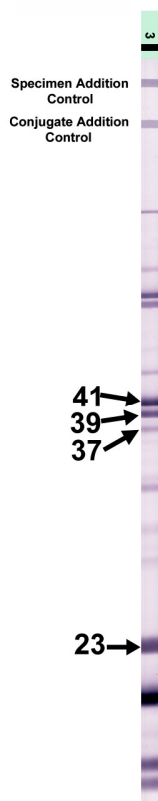
**X. QUALITY CONTROL**

**The Weakly Reactive IgM Control and Negative Control must be included in each reaction tray. The IgM Serum Band Locator needs only to be run once per strip vial.** A strip assayed with the IgM Serum Band Locator must be compared with the image on the Blot Banding Template provided to identify the relative positions of reactive bands for the kit. These reagents fulfill a function of calibration. An additional *B. burgdorferi* reactive serum may be used as a positive test control, however, it should be tested in accordance with guidelines of appropriate accrediting organizations and the monitoring programs within each individual laboratory.

## X. Quality Control (continued)

1. IgM Serum Band Locator: This control must show reactivities with bands at positions: Serum Addition Control (**SAC**), Conjugate Addition Control (**CAC**), **41, 39 and 23 kDa**. Reactions may be also seen at other positions.
2. Weakly Reactive IgM Control: This control must produce a bands at positions: Serum Addition Control (**SAC**), Conjugate Addition Control (**CAC**) and at **41 kDa**. Other bands may be present but should not be used as the reading standard or to determine when to stop the reaction. Reference to the Blot Banding Template for location of the band.
3. The Negative Control should not show any significant bands. Any non-significant bands should be weakly visible if present at all. It must show reactivity with Serum Addition Control (**SAC**), Conjugate Addition Control (**CAC**)
4. If the Serum band locator, Weakly Reactive or Negative Control do not perform as indicated, the run is invalid and patient results may not be reported.
5. Patient Strips run must show reactivity with both the Serum Addition Control (**SAC**) and Conjugate Addition Control (**CAC**) bands. If there is no reactivity with either or both of these bands, the patient results cannot be reported.

Figure 1



\* The 30 kD band may be diffuse or have multiple thin bands.

\*\* The 28kD(s) band can be seen with either one of the two bands or both bands.

## XI. INTERPRETATION OF RESULTS

Score the relative intensity of the bands as follows:

<u>Intensity of Band</u>	<u>Reactivity Score</u>
○ Absent	Neg.
○ Less than the intensity of the 41 kDa band on the Weakly Reactive Control strip	Neg.
○ At least as intensely reactive as 41 kDa band on the Weakly Reactive Control strip.	Pos.

**Carefully compare the intensity of bands detected on patient strips with the intensity of the 41kDa band on the Weakly Reactive Control. The importance of the Weakly Reactive Control is two fold. It provides a reading standard by which patient bands can be graded and it provides an indication of the assay reproducibility. Bands which have intensity equal to or greater than the Weakly Reactive Control will be reproducible from kit to kit and lot to lot. Bands which have intensity less than that of the Weakly Reactive Control should be considered negative and are not reportable.**

The image on the Blot Banding Template is used to locate and identify bands on the strip reacted with the IgM Serum Band Locator. This strip is then used to identify bands present on strips used to test specific samples.

Using the IgM Serum Band Locator as a reference for position and the Weakly Reactive Control as a reference for intensity, each band on a strip should be assigned a reactivity score. When analyzing test specimens it is helpful to place the control strips side by side with unknown strips to facilitate the identification of bands. The result is then interpreted as Negative or Positive based on the interpretive criteria below.

Use the following criteria for interpretations <sup>17</sup>.

### POSITIVE

Any 2 of the following 3 bands:  
23, 39, 41kDa

### REPORT

IgM antibodies to significant *B. burgdorferi* proteins detected; presumptive evidence of probable exposure.

### NEGATIVE

Patterns other than positive

IgM antibodies to less than 2 of the 3 significant *B. burgdorferi* proteins detected; or no IgM antibodies to significant *B. burgdorferi* proteins detected. Additional specimens should be submitted in 2-4 weeks if *B. burgdorferi* exposure has not been ruled out.

### UNREADABLE

Unable to interpret western blot results; resubmit fresh specimen.

It is inappropriate to assign a POSITIVE interpretation to strips which display bands but have intensities less than the Weakly Reactive Control. 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.

## XI. INTERPRETATION OF RESULTS (continued)

**UNREADABLE BLOTS** are those blots in which staining has completely or partially masked the bands thereby making them difficult to read. Masking may appear as heavy background 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 2-4 weeks.

## XII. LIMITATIONS OF PROCEDURE

1. The assay must be performed in strict accordance with these instructions to obtain reproducible results.
2. The MarDx *B. burgdorferi* (IgM) Marblot Strip Test System should only be used to test human serum samples which have been found positive or equivocal using an EIA or IFA test procedure.
3. Individuals with POSITIVE Western blot for antibodies to *B. burgdorferi* should be referred for medical evaluation which may include additional testing. The diagnosis of Lyme Disease must include careful clinical evaluation and should not be based only on detection of antibodies to *B. burgdorferi*.
4. If a specimen repeatedly yields unreadable blots and symptoms persist, a fresh specimen should be tested in 2-4 weeks.
5. A negative western blot does not exclude the possibility of infection with *B. burgdorferi*.
6. Sera from individuals with other pathogenic spirochetal diseases such as syphilis, yaws, pinta, leptospirosis, relapsing fever and periodontal disease may give false positive results. Individuals with connective tissue autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, and individuals with anti-nuclear antibody may also give false positive results. Individuals with other bacterial and viral infections such as Rocky Mountain Spotted Fever, Epstein-Barr Virus, and cytomegalovirus may also have antibodies which cross-react with *B. burgdorferi*.
7. The continued presence or absence of antibodies to *B. burgdorferi* cannot be used to determine the success or failure of therapy.
8. Western blot testing should not be performed as a screening procedure.
9. A positive *B. burgdorferi* IgM Western Blot result only indicates probable immunologic exposure, however, the presence of an Immunologic response has not been correlated with active infection.
10. When testing specimens from patients during early *B. burgdorferi* infection, IgM tests are generally sensitive within the first 2 months after onset of symptoms. A suitable IgG Western blot test can be used at any time after onset but is most sensitive during the later stages of the disease.
11. Because the likelihood of a false-positive test result is high for these individuals, a positive IgM test alone is not recommended for use in supporting the diagnosis of Lyme disease in persons with illness of longer than one month duration.
12. Studies have demonstrated that antibiotic therapy may or may not affect the seroconversion from IgM to IgG during the course of the disease<sup>1</sup>.

## XIII. EXPECTED VALUES

The immune response to *B. burgdorferi* infection appears to follow a classic response pattern. Serum IgM can be detected in some patients within days after disease onset. At about four weeks after onset, the IgM response has attained maximum serum concentration and complexity of Western blot banding patterns. Serum IgG is detected as early as two weeks after onset. Significant concentrations of antibody and Western blot banding patterns for *B. burgdorferi* can be found years after onset<sup>9</sup>.

The performance of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System was evaluated in clinical studies using sera obtained from the following: 1) patients meeting a case definition for Lyme disease based on physician diagnosis, *B. burgdorferi* culture, and other laboratory tests<sup>13</sup>; 2) patients with potentially cross-reactive conditions or infections; and 3) apparently healthy individuals. Specimens from documented cases of Lyme disease included sera provided by the Centers for Disease Control and Prevention (CDC), and other research centers. Potentially cross-reactive conditions included syphilis, tick-borne relapsing fever, periodontal disease, leptospirosis, tularemia, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). Specimens from apparently healthy individuals included sera collected from healthy blood donors in endemic and non-endemic areas.

Table 1 summarizes the prevalence of *B. burgdorferi* specific bands in specimens from documented cases of Lyme disease. Specimens have been grouped by the time of specimen collection after onset of symptoms: less than one month; one to two months; three to 12 months; and years after onset. Table 2 summarizes the prevalence of each *B. burgdorferi* specific band in specimens meeting the interpretive criteria for a positive test.

**Table 1**

Band Frequency on *B. burgdorferi* IgM Marblots of All Specimens from Lyme Disease Cases

Drawn From Date of Onset	Number		Number of Marblots with Given Bands Present		
	Studied	With Any Band	41kD	39kD	23kD
<1 month	127	64	45	27	53
1-2 months	35	34	33	13	30
3-12 months	40	33	20	7	14
years	33	28	10	4	7

**Table 2**

Band Frequency on Positive *B. burgdorferi* IgM Marblots of Specimens from Lyme Disease Cases

Drawn From Date of Onset	Number of Marblots with Given Bands Present			
	No. Testing Positive	41kD	39kD	23kD
<1 month	46	41	30	41
1-2 months	31	31	13	30
3-12 months	15	12	5	13
years	5	3	3	5

The frequency of *B. burgdorferi* specific bands in sera from patients with clinical tests that were positive for potentially cross-reactive conditions and from apparently healthy individuals ("normals") is shown in Table 3. Some of the blots in Table 3 and Table 5 were interpreted as positive, but it is important to note that these specimens would not have usually been tested with an IgM assay because they did not meet the criteria for IgM testing: (1) patient exhibits symptoms of Lyme Disease, (2) specimen collected within the first few weeks of onset of symptoms and (3) a positive or equivocal EIA or IFA for *B. burgdorferi* antibodies. However, presentations of other syndromes can be confused with that of Lyme disease, especially in the absence of the characteristic EM rash. The presence of low level IgM antibodies in sera from some apparently healthy individuals or from patients found positive by tests other than that for *B. burgdorferi* does not rule out previous or sub-clinical infection with *B. burgdorferi*.

## XIV.Expected Values (continued)

Table 3

Frequency of Bands on *B. burgdorferi* IgM Marblots  
Using Sera Found Positive on Other Laboratory Tests and Sera  
from Apparently Healthy (Normal) Individuals

	Number Studied	Number Positive	No. with Bands	Band Distribution		
				41kD	39kD	23kD
Syphilis	25	1	10	10	1	0
ALS	9	0	5	5	1	0
MS	15	0	1	1	0	0
Influenza	25	0	3	2	1	0
RA	15	1	4	4	1	0
SLE	10	1	4	3	1	1
Normal	291	11	69	61	8	25

## XIV. PERFORMANCE CHARACTERISTICS

## SENSITIVITY AND SPECIFICITY

The sensitivity of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System was assessed relative to a case definition of Lyme disease based on physician diagnosis, *B. burgdorferi* culture, and other laboratory tests. Results for 427 specimens have been grouped according to the time of specimen collection after onset of symptoms (Table 4).

Table 4

Sensitivity of the *B. burgdorferi* IgM Marblot (MB) Relative to Lyme Disease Case Definition (CD)<sup>a</sup>

Drawn from Date of Onset	Number	MB+/CD+	MB-/CD+	Sensitivity	95%CI
< month	229	113/229	116/229	49%	42.9 - 55.8
1-2 months	44	39/44	5/44	89%	75.4 - 96.2
3-12 months	96	48/96	48/96	50%	39.6 - 60.4
years	58	25/58	33/58	43%	30.2 - 56.8

<sup>a</sup>Physician diagnosis, *B. burgdorferi* culture, and other laboratory test results

The specificity of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System was assessed in 228 specimens from patients with clinical tests that were positive for potentially cross-reactive conditions and from 514 apparently healthy individuals ("normals"). The results, summarized in Table 5, include the results of sera shown in Table 3, and additional specimens from patients with potentially cross-reactive diseases and normal (apparently healthy) individuals. There was some cross-reactivity with specimens from patients with tick-borne relapsing fever (14% or 1 of 7 specimens), leptospirosis (14% or 3 of 22 specimens), ALS (11% or 1 of 9 specimens), SLE (10% or 4 of 39 specimens), syphilis (8% or 4 of 52 specimens), and RA (2 of 40 specimens). In the apparently healthy population, 27 of 514 (5%) of the specimens had blots that were interpreted as positive. These specimens were obtained from individuals who would not usually be selected for *B. burgdorferi* IgM testing. The results demonstrate the specificity of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System to be 94.3% (700/742) with a 95% confidence interval of 92.4 - 95.9%.

Table 5

Percent Cross Reactivity of *B. burgdorferi* IgM Marblot  
Using Sera From Other Clinically Defined Diseases  
and from Apparently Healthy (Normal) Individuals

Potentially Cross Reactive Diseases and Normal Individuals	Number Studied	Number Positive	Cross Reactivity
Syphilis	52	4	8%
ALS	9	1	11%
MS	15	0	0%
Influenza	25	0	0%
RA	40	2	5%
SLE	39	4	10%
Leptospirosis	22	3	14%
Periodontal Disease	9	0	0%
Tularemia	8	0	0%
Felty's Syndrome	2	0	0%
Tick-borne Relapsing fever	7	1	14%
Normals	514	27	5%

## REPRODUCIBILITY

The reproducibility of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System was evaluated in a study in which six coded specimens of varying reactivities were tested in duplicate on each of three separate days in three clinical laboratories. All three sites used the same lot of material. One technologist at each site performed the assays and scored the presence or absence of bands. The number of times that each band was scored as present is shown in Table 6.

Table 6

Reproducibility of Bands on *B. burgdorferi* IgM Marblots

Specimen	Reactivity	Number of Bands		
		41Kd	39kD	23kD
1	++	18	18	18
2	++	18	6	18
3	+	16	2	8
4	+	14	0	0
5	-	0	0	0
6	-	18	0	0

These results demonstrate that the reproducibility of the Marblot assay is high for strongly reactive specimens and negative specimens. The weakly reactive specimen, Number 3, was less than 100% reproducible for less intense or serum with fewer significant bands.

The reproducibility of the MarDx *B. burgdorferi* (IgM) Marblot Strip Test System was also evaluated in a study in which 15 coded specimens of varying reactivities were tested once each in three clinical laboratories. One technologist at each site performed the assays and scored the presence or absence of bands. The number of times that each band was scored as present is shown in Table 7. Percent agreement with expected results was calculated to determine if expected bands were scored correctly as present or absent in these three laboratories (Table 8).

**XIV.Expected Values (continued)****Table 7**Reproducibility of Bands on *B. burgdorferi* IgM Marblots

Specimen	Interpretation	Number of Bands		
		41kD	39kD	23kD
1	POS	3	0	3
2	POS	3	3	3
3	POS	3	0	3
4	POS	3	0	3
5	POS	3	0	3
6	POS	3	0	3
7	POS	3	3	2
8	NEG	1	0	1
9	POS	3	3	3
10	POS	3	1	3
11	NEG	2	0	0
12	NEG	0	0	3
13	NEG	0	0	1
14	NEG	2	0	0
15	NEG	0	0	0

**Table 8**

Agreement with Expected Results Across Three Laboratories

	Specific Bands		Final Interpretation (positive/negative)	
	Number of Correct Bands	Percentage of Correct Bands	Correct No. of Interpretations	Percent (%)
Laboratory 1	23/25	92%	15/15	100
Laboratory 2	23/25	92%	14/15	93
Laboratory 3	24/25	96%	15/15	100
Overall	70/75	93%	44/45	98

These results demonstrate that the bands on the Marblot strips can be correctly identified in separate laboratory settings.

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## Chart for Section VI. Reagent Preparation
















Volume of 1X Working Sample Diluent / Wash Solution  
Necessary

Number of Strips to be Assayed	4	6	9	12	15	18	24	30	36	40
Quantity of 10X Sample Diluent / Wash Buffer	7.5ml	12.5ml	17.5ml	22.5ml	30.0ml	35.0ml	45.0ml	57.5ml	70.0ml	75.0ml
Quantity of Distilled Water	67.5ml	112.5ml	157.5ml	202.5ml	270.0ml	315.0ml	405.0ml	517.5ml	630.0ml	675.0ml
Total Volume Necessary	75.0ml	125.0ml	175.0ml	225.0ml	300.0ml	350.0ml	450.0ml	575.0ml	700.0ml	750.0ml

## Volume of 1X Working Conjugate Necessary

Number of Strips to be Assayed	4	6	9	12	15	18	24	30	36	40
Quantity of 10X Alkaline Phosphatase Conjugate	1.0ml	1.4ml	2.0ml	2.6ml	3.2ml	3.8ml	5.0ml	6.2ml	7.4ml	8.8ml
Quantity of 1X Working Sample Diluent Wash Solution	9.0ml	12.6ml	18.0ml	23.4ml	28.8ml	34.2ml	45.0ml	55.8ml	66.6ml	79.2ml
Total Volume Necessary (Plus 2.0ml Additional)	10.0ml	14.0ml	20.0ml	26.0ml	32.0ml	38.0ml	50.0ml	62.0ml	74.0ml	88.0ml

## Key guide to symbols

Symbol	
	Product Number
	Lot Number
	<i>In Vitro</i> Diagnostic Medical Device
	Authorized Representative in the European Community
	Use By
	Caution, consult accompanying documents
	Temperature limitation
	Manufacturer
	Negative Control
	Weakly Reactive Control
	Serum Band Locator
	Conjugate
	WB Color Developer
	10X Sample Diluent/Wash Solution
	Western Blot Strips



AR-Trinity Biotech plc.  
IDA Business Park  
Bray, County Wicklow, Ireland  
Phone: +353-1-276-9800  
Fax: +353-1-276-9888  
Web: [www.trinitybiotech.com](http://www.trinitybiotech.com)

Manufactured By  
MarDx Diagnostics, Inc.  
A Trinity Biotech Company  
5919 Farnsworth Court  
Carlsbad, CA 92008  
Phone: 760-929-0500  
Fax: 760-929-0124