Borrelia IgM Blot

Immunoblot for the confirmation of Borrelia burgdorferi sensu lato specific IgM antibodies in human serum

Product Number: LYM110 (10 strips)
**Intended use**

Borrelia Blot is a qualitative assay for the confirmation of Borrelia burgdorferi sensu lato specific IgM antibodies in human serum.

**Summary**

Lyme disease is a tick-borne multistage disease which is caused by spirochetal bacteria, Borrelia burgdorferi, Borrelia garinii and Borrelia afzelii (1). Following the bite of an infected tick, the spirochetes undergo a rapid hematogenous dissemination and can be found in many of the major organ systems. The first stage and hallmark of Lyme disease is a distinctive skin rash, erythema migrans (EM), that frequently appears at the site of the tick bite (2).

Unfortunately, EM is present in or recalled by 50 to 70% of patients and the other signs of infection can be variable and nonspecific, e.g. headache, fever, myalgia, arthralgia and lymphadenopathy (3).

Without recognizing and medical treatment the diseases progresses towards secondary and tertiary stages. In Europe, neurological symptoms appear in 30% of untreated patients and musculoskeletal symptoms in 20% of patients.

Late Lyme disease is frequently characterized by arthritis, acrodermatitis chronica atrophicans or by neurological symptoms, depending upon the involved Borrelia species. It is generally considered that Borrelia burgdorferi sensu stricto is more classically associated with arthritis, Borrelia garinii with neurological symptoms and Borrelia afzelii with acrodermatitis chronica atrophicans (4).

Due to its variable symptomatology, borreliosis has been often misdiagnosed and confused with multiple sclerosis, rheumatoid fever or viral meningitis.

Culture of Borrelia burgdorferi from clinical specimens, direct detection (PCR) or direct examination of tissue for spirochetes is difficult and impractical for the clinical laboratory (5).

Today, the laboratory workup of Borrelia disease relies on serological tests. The standard approach involves screening the patient's sample using a technology such as ELISA or IFA. Unfortunately, false-positive or false-negative results are not rare (6) and a confirmatory test such as a Western Blot is mandatory to report a positive result.

The serological response to a Borrelia infection is complex as the delay in the development of detectable antibodies may vary in different individuals. Cross-reactivity of some Borrelia proteins with antigens from other bacteria are also well-known (7). At the early stage of Borrelia disease, the immune response, as detected in the laboratory, is not highly specific of the infecting strain. As the disease progresses towards advanced stages, the serological pattern becomes more specific and, in such cases, it is advisable to use target antigens prepared with the suspected infecting strain. While in North America, most infections are caused by Borrelia burgdorferi sensu stricto, in Western Europe, Borrelia garinii is the dominant strain, followed by Borrelia burgdorferi and Borrelia afzelii.

The Western Blot technology is extremely useful in dissecting the immune response to Borrelia infections which develops gradually over a period of weeks to years and which involves the appearance of IgM and IgG antibodies directed against a number of Borrelia-associated proteins (8).

**PRINCIPLE OF THE TEST**

Proteins derived from Borrelia garinii are electrophoretically separated according to molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoresis, the separated protein bands are transferred to a PVDF membrane. Antigens are blocked before cutting the membrane into ready-to-use strips.

Diluted patient sera are incubated with the antigen strips. Borrelia garinii-specific antibodies, if present, will bind to their target antigens. After washing, rabbit anti-human IgM conjugated with horseradish peroxidase is added. At the end of a second incubation, unbound conjugate is removed by washing and aspiration. The bound conjugate is visualized by the addition of a chromogenic substrate. Using the template supplied with the kit, the position of the stained bands can be correlated with defined Borrelia antigens.
MATERIALS SUPPLIED

No. LYG110, Borrelia- Blot (10 strips)

Kit contains:

1. **STRIP** 1 tube of 10 strips
2. **CONJ** Anti-IgM conjugate: 1 bottle containing 20 ml of peroxidase labelled rabbit antibody to human IgM; coloured red, ready to use; black cap. Contains: 0.2% Bronidox L
3. **CONTROL** IgM positive control: 1 bottle containing 1 ml; coloured yellow; ready to use; red cap. Contains: 0.01% Kathon
4. **DIL** Sample Diluent, 1 bottle containing 50 ml of ready to use buffer for sample dilution, ph 7.2 ± 0.2, coloured yellow, white cap. Contains: 0.01% Kathon
5. **WASHBUF** Wash Buffer 2 bottles containing 50 ml each, ph 7.2 ± 0.2 for washing the strips, ready to use, white cap. Contains: 0.01% Kathon
6. **TEMP** Predeveloped control strip and template
7. **TMB** Chromogenic Substrate-Membrane, 1 bottle (15 ml) containing 3, 3', 5, 5'-tetra-methylbenzidine (TMB), ready to use, blue cap.

- **Materials required but not provided**
  - Distilled or deionized water.
  - Shaking platform producing 10-200 rpm.
  - Micropipettes, 10 and 1000 µl.
  - Sample test tubes, polystyrene 12 x 75 mm.
  - Plastic or stainless steel forceps for handling antigen strips.
  - Vacuum apparatus.
  - Timer.
  - Filter paper.

- **Precautions**
  - For in vitro diagnostic use.
  - Template **TEMP** and strips **STRIP** are matched for optimal performance; do not interchange.
  - Avoid cutting, bending or touching the antigen strips when opening the strip package.
  - Do not mix reagents from different lots.
  - Do not use kit components beyond the expiration date.
  - Reagents, including the conjugate **CONJ**, the chromogenic substrate **TMB**, and the antigen strips **STRIP**, should not be exposed to strong light during storage or incubation.
  - Always use forceps or tweezers to handle antigen strips.
  - Do not pipette by mouth.
  - When opening and removing aliquots from the primary vials, avoid microbial or cross-contamination of reagents.
  - Proceed through the different steps of the test protocol without interruption and comply with the prescribed incubation times.
  - During incubations and washing, the antigen strips must remain completely covered with fluid and the numbered side of the strips must face up.
  - Comply with the number of wash cycles.
  - Check that the chromogenic substrate is colorless before addition.
  - Strips with high levels of multiple antibodies should be viewed carefully during the substrate incubation. It may be necessary to stop the reaction early to reduce the risk of overreaction and the obliteration of minor bands.
**Warning:** potential biohazardous material

This kit contains some reagents made with human serum which have been tested and found to be non-reactive for the presence of HBsAg and for the presence of antibodies to hepatitis C and to HIV. Since no known test method can offer complete assurance that infectious agents are absent, the positive control and patient samples should therefore be handled as though capable of transmitting infection.

**Serum sample dilution**

Dilute each patient serum sample:
- add 10 µl of serum to 1 ml of diluent [DIL].
Mix thoroughly prior to use.

**Reagent stability and storage**

All kit components should be stored at 2º-8º C and are stable to the labeled expiration date. **Do not freeze kit components.**

**Specimens collection and storage**

This kit has been validated with serum. If specimens are to be stored, they should be refrigerated at 2-8º C or frozen at -20º C or lower.

**Reagent preparations**

All reagents are ready to use

**Assay procedure**

1. Place the kit at room temperature for 30 minutes.

2. Transfer 1 ml of diluted patient serum or 1 ml of **undiluted** positive control [CONTROL] to the appropriate channel containing an antigen strip. A new pipette tip must be used with each separate sample.

3. Using tweezers or forceps, carefully remove the number of requested strips [STRIP]. Check that all antigen strips are completely **immersed** and, if needed, gently shake the tray or push delicately the strips into the solution with a clean pipette tip. Place the strips, with the **numbered side face up**, in the channels of the incubation tray.

4. Incubate the strips at room temperature (20-25ºC) for **1 hour** on a shaking platform.

5. **Wash procedure**
   - Carefully aspirate the contents of each channel, add 1 ml of Borrelia wash buffer [WASHBUF].
   - Place the incubation tray on the shaking platform for 5 minutes at room temperature
   - Aspirate the contents of each channel and repeat the Wash procedure two more times.

6. Add 1 ml of Borrelia IgM conjugate [CONJ] to the appropriate channels and incubate at room temperature for 30 min. on the shaking platform.

7. Repeat step 5.

8. Set a timer for 12 minutes; add 1 ml of chromogenic substrate-membrane [TMB].

9. Allow the Substrate to react at room temperature (20-25ºC) for **12 minutes** (maximum: 15 minutes) on the shaking platform.
   **CAUTION:** DO NOT OVERDEVELOP. To avoid background, stop the reaction when the bands become noticeable. This will take about 10 - 12 minutes under normal conditions.

10. Stop the reaction by aspirating the contents of each channel and rinse each strip with 1 ml of deionized water. Replace the incubation tray on the shaking platform for 5 minutes. Repeat the 1 ml - 5 minute rinse.

Remove the strips [STRIP] from the incubation tray and place them on filter paper to dry (approximately 30 minutes at room temperature).

**IMPORTANT:** to prevent fading, the strips should be protected from light during the drying process. Use a dark box or a drawer. Once dried, the bands remain visible for years (provided that they have been correctly rinsed).
Summary of Assay Procedure

<table>
<thead>
<tr>
<th>Borrelia IgM Blot</th>
</tr>
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<tbody>
<tr>
<td><strong>Dilute</strong> samples: 10 µl serum + 1 ml diluent DIL</td>
</tr>
</tbody>
</table>

| Pipette 1 ml diluted sample/ 1 ml undiluted control CONTROL |

| ⇓⇓ ⇓⇓ |
| Incubate 60 minutes (RT) |

| Wash 3 times (3 x 1ml WASHBUF - 5 minutes) |
| Add conjugate CONJ : 1 ml |

| ⇓⇓ ⇓⇓ |
| Incubate 30 minutes (RT) |

| Wash 3 times (3 x 1ml WASHBUF - 5 minutes) |
| Add substrate TMB : 1 ml |

| ⇓⇓ ⇓⇓ |
| Incubate 12 minutes (RT) |

| Rinse 2 times (2 x 1ml - 5 minutes) |

| Dry and read |

**Results**

Once dried, attach the strips to the result sheet included in the kit using clear tape.

- The Borrelia IgM Blot utilises a pre-qualified template [TEMP] consisting of a developed antigen strip cut from the membrane used to prepare the antigen strips [STRIP] from the kit. This strip has been exposed to the kit positive control [CONTROL] in order to exhibit bands representative of a Lyme infection. Protein bands are listed on the right side of the template.

**Important note:** the kit positive control does not exhibit all the bands that may appear during an infection. Visible bands are referenced by solid lines; the position of other bands is referenced by dotted lines.

- To determine the specific antibody reactivity’s in a patient sample, the horizontal line at the bottom of the strip must be aligned with the index line near the bottom of the control strip.

- Identification of the reactive bands is based upon comparison with the exposed bands on the template [TEMP].

- The interpretation of the blot is based upon qualitative and quantitative components of the immune response to Borrelia garinii.

- **Attribute:**
  - 1 point to faint bands
  - 2 points to weak bands
  - 3 points to dark bands

- For band scoring, refer to the developed Antigen Strip from the template:
  - band at 60 kD: 1 point
  - band at 41 kD (flagellin): 2 points
  - band at 22-23 kD (OspC): 3 points

- Using the above described scoring scale, determine the reactivity of each patient specimen for the following bands: 93 kD, 41 kD, 39 kD, 32.5 kD, 22-23 kD
• Sum up the points for each patient specimen and interpret as follows:

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>&lt; 4 points</td>
<td>negative</td>
</tr>
</tbody>
</table>
| ≥ 4 points       | Positive if OspC and flagellin are present

An EIA/IFA positive or equivocal test followed by a positive Western Blot should be reported as positive.

An EIA/IFA positive or equivocal test followed by a negative Western Blot should be reported as negative

### Band specificity

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>93 kD</td>
<td>chromosomal protein highly specific of the Borrelia genus; the antibody response is mostly IgM and appears in the course of chronic infections; bands at 93 kD are strongly associated with advanced stages.</td>
</tr>
<tr>
<td>62-72 kD</td>
<td>heat shock proteins; these bands are not specifically associated with a Borrelia infection and are found in several bacterial infections.</td>
</tr>
<tr>
<td>60 kD</td>
<td>common bacterial antigen; non-specific.</td>
</tr>
<tr>
<td>41 kD</td>
<td>flagellin; this protein is not specific to the Borrelia genus but is useful in the test interpretation; cross-reaction with other spirochetes are common; a flagellin positive reaction may occur at early as well at late stages.</td>
</tr>
<tr>
<td>39 kD</td>
<td>p39 is a protein highly specific of the Borrelia genus.</td>
</tr>
<tr>
<td>32.5 kD</td>
<td>OspA: surface protein highly specific of Borrelia garinii.</td>
</tr>
<tr>
<td>28 kD</td>
<td>surface protein.</td>
</tr>
<tr>
<td>22-23 kD</td>
<td>OspC: surface protein highly specific of Borrelia garinii and a marker for early infections.</td>
</tr>
<tr>
<td>18 kD</td>
<td>the specificity of this protein has been recently established; excellent marker for late infections.</td>
</tr>
</tbody>
</table>

Patient sera may exhibit other bands than those above mentioned. Such bands should not be considered when interpreting the results.

### Quality control

It is not necessary, but suggested, to include a positive control serum or the kit positive control in each run.

### Limitations

• The presence (or absence) of antibodies reactive with Borrelia antigens on the Borrelia IgM Blot should not be used as the sole criterion on which a diagnosis is established. These reactivities are an aid to diagnosis only, and their significance must be interpreted by a qualified medical authority in relationship to the clinical presentation of the patient and the results of other tests.

• If a serum of a patient with a suspicion of early infection exhibits a weak reactivity (e.g. 2 points), a new specimen should be obtained in 14 - 21 days and tests should be repeated.

• The use of this kit should preferably be restricted to specimens collected within one month after the onset of symptoms.
Parallel testing for IgG and IgM antibodies is essential for the detection of early infections presenting with EM and/or neurologic symptoms (mainly facial palsy). It is now established that combined IgG and IgM testing has a sensitivity close to 100% while IgG testing alone may miss confirmed cases of neuroborreliosis (9).

Parallel testing for IgG and IgM antibodies is not recommended for specimens from patients with a suspicion of late infection as IgM seroreactivity is normally absent.

**Performance characteristics**

Clinical trials were conducted with the Borrelia Blot IgM and the Borrelia IgG Blot.

**Blood donors**

Serum samples were randomly selected in 3 transfusion centers in Switzerland, one in a rural area (center A), one in an urban area (center B) and one in a region where Lyme disease is endemic (center C), and were tested for Borrelia IgM antibodies.

<table>
<thead>
<tr>
<th>Center</th>
<th>n</th>
<th>% positive (IgM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>80</td>
<td>3.7</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

These results are in agreement with the literature (10).

**Specificity**

Serum samples from syphilitic patients (Group A), from patients with an history of Epstein-Barr infection (Group B) or with circulating autoimmune markers (Group C1: rheumatoid factor, Group C2: ANA, Group C3: anti-DNA antibodies) were tested for IgM antibodies. The percentages of positivity found in these groups were not significantly different from these found in normal blood donors.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% positive</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>11.1</td>
<td>89</td>
</tr>
<tr>
<td>C1</td>
<td>11</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C2</td>
<td>8</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>C3</td>
<td>10</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Sensitivity**

Serum samples from patients with a clinical diagnosis of Lyme disease were tested with the Borrelia Blot IgM Results differ depending upon the stage of the disease but as a rule, IgM seroreactivity is normally absent in advanced stages.

<table>
<thead>
<tr>
<th>Disease stage</th>
<th>n</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>Acrodermatitis chronica atrophicans</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Erythema migrans with seroconversion</td>
<td>35</td>
<td>71</td>
</tr>
<tr>
<td>Neurological symptoms</td>
<td>33</td>
<td>79</td>
</tr>
</tbody>
</table>

**Important notes:**

- neurological symptoms: the kit sensitivity is significantly increased, up to 97% by using a combined determination of IgG and IgM antibodies by immunoblotting.
- erythema migrans: combined IgG and IgM testing will provide a sensitivity of 92%; nevertheless, cutaneous symptoms may precede antibody production and some patients may remain seronegative during several months. Paired sera collected at 1-2 month intervals will usually clarify the diagnosis.
- arthritis and acrodermatitis: IgM testing is of little interest in these late infection
References