PLATELIA™ LYME IgM

1 plate - ▲ 96

QUALITATIVE DETECTION OF IgM ANTIBODIES TO BORRELIA BURGDORFERI SENSU LATO IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSAY

IVD

CE

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1- INTENDED USE

Platelia™ Lyme IgM is an immunoassay using immunocapture format for qualitative detection of IgM antibodies to *Borrelia burgdorferi* sensu lato in human serum or plasma.

2- CLINICAL VALUE

Lyme borreliosis (or Lyme disease) is a non contagious infection caused by a bacterium from the spirochetaceae family, *Borrelia burgdorferi*, which is transmitted by ticks of the *Ixodes* genus (2). Various animals are serving as hosts to the bacteria and transmission to humans occurs through bites of infected ticks. Transmission risk is higher when the tick bite is longer.

Prevalence of Lyme disease is high in temperate or cold climates countries in the northern hemisphere, from China to Northern America, and from Scandinavia to Northern Africa. About 17,000 cases are estimated to be reported each year in the United States (3), and probably more than 50,000 in Europe, where a positive gradient exists from west to east (4).

It is now clearly demonstrated that *Borrelia burgdorferi* described in 1984 as a unique bacteria species, is in reality a complex of several species, among which five of them are pathogenic for humans: *Borrelia burgdorferi* sensu stricto (ss), *Borrelia garinii*, *Borrelia afzelii*, *Borrelia spielmanii* and *Borrelia bavariensis* (6, 8). Two other species are potentially pathogenic: *Borrelia valaisiana* and *Borrelia lusitaniae*.

Those seven species are circulating in Europe, while only *B. burgdorferi* sensu stricto is present in the United States.

Clinical symptoms of Lyme disease are diverse and sometimes difficult to recognize (7). Three stages can be distinguished during the clinical evolution of the disease. The early stage (Stage I) can be asymptomatic and is characterized by a flu-like syndrome. In 50 to 80% of cases, several days or weeks after the tick bite, there appears a typical localized cutaneous rash with centrifuge expansion named erythema migrans (EM). Without treatment, haematogenous dissemination of *Borrelia* will induce several weeks later inflammatory arthritis, neurological or meningeal disorders, and cutaneous or cardiac symptoms (Stage II). After several months or years, the disease may develop to a chronic stage associating at different levels acrodermatitis chronica atrophicans, encephalopathy, encephalomyelitis and chronic arthritis (Stage III) (1).

Each species of *Borrelia burgdorferi* has a particular tropism. Erythema migrans in stage I is indifferently associated with the three species. However, neurological complications are more frequently associated with *B. garinii* and arthritis are more frequently associated with *B. burgdorferi* ss, while acrodermatitis chronica atrophicans is specific to *B. afzelii*. 
The diagnosis of Lyme disease must not be confirmed without a careful investigation of patient’s medical history, clinical and biological criteria, and estimation of the risk of tick exposure. Since difficulties of the direct detection, the culture isolation or the molecular biology methods implementation, serology remains a key element in biological diagnosis of Lyme disease (1,5,9). IgM antibodies to *Borrelia burgdorferi* appear about 3 to 6 weeks after contamination and may persist during the development of the disease, while IgG antibodies appear later and reach a peak only after months, or even years. Even if serology is less useful during the early stage, it is however essential during the secondary or tertiary stages, particularly in the absence of erythema migrans. When serology is negative despite a suggestive clinical status, a new serology has to be performed 3 weeks after the initial test. Presence of specific IgM antibodies is not synonymous of recent infection. Similarly, presence of specific IgG antibodies is not always the signature of a past-infection (9,10,12).

Antigens and antibodies used in Platelia™ Lyme IgM (Ref. 72951) and Platelia™ Lyme IgG (Ref. 72952) assays have been selected to allow detection of respectively specific IgM and specific IgG antibodies toward the different American and European strains of *Borrelia burgdorferi sensu lato* (*B. burgdorferi ss*, *B. garinii*, *B. azfeli*).

### 3- **PRINCIPLE**

Platelia™ Lyme IgM is a qualitative test for detection of IgM antibodies to *Borrelia burgdorferi sensu lato* in human serum or plasma by enzyme immunoassay with capture of the IgM on the solid phase.

Anti-human µ-chains antibodies are coated on the solid phase (wells of the microplate). A mixture of the *Borrelia* native antigen and the monoclonal anti-*Borrelia* flagellar antigen antibody labeled with peroxydase is used as the conjugate. The test uses the following steps:

- **Step 1**

Patients samples and controls are diluted 1/101 and then distributed in the wells of the microplate. During this incubation of one hour at 37°C, IgM antibodies to *Borrelia burgdorferi* present in the sample bind to the anti-µ antibodies coated on the microplate wells. After incubation, unbound antibodies and other serum proteins are removed by washings.
• **Step 2**
The conjugate (mixture of *Borrelia* antigen and anti-*Borrelia* antibody labeled with peroxydase) is added to the microplate wells. During this incubation of one hour at 37°C, the conjugate binds to the specific IgM anti-*Borrelia* antibodies. The unbound conjugate is removed by washings at the end of the incubation.

• **Step 3**
The presence of immun-complexes (Anti-human µ-chains / IgM anti-*Borrelia* / *Borrelia* Antigen / anti-*Borrelia* monoclonal antibody labeled with peroxydase) is demonstrated by the addition in each well of an enzymatic development solution.

• **Step 4**
After incubation at room temperature (+18-30°C), the enzymatic reaction is stopped by addition of 1N sulfuric acid solution. The optical density reading obtained with a spectrophotometer set at 450/620 nm is proportional to the amount of IgM antibodies to *Borrelia burgdorferi* present in the sample.

### 4- PRODUCT INFORMATION

Supplied quantities of reagents have been calculated to allow 96 tests in a maximum of 6 runs. All reagents are exclusively for *in vitro* diagnostic use.

<table>
<thead>
<tr>
<th>Label</th>
<th>Reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td><strong>Microplate</strong> (Ready-to-use):Microplate: 12 strips with 8 breakable wells, coated with anti-human µ chains</td>
<td>1 microplate</td>
</tr>
<tr>
<td>R2</td>
<td><strong>Concentrated Washing Solution (20x):</strong> Tampon TRIS-NaCl (pH 7.4), 2% Tween® 20 Preservative : 0.04% ProClin™ 300</td>
<td>1 x 70 ml</td>
</tr>
<tr>
<td>R3</td>
<td><strong>Negative Control:</strong> Negative human serum for IgM antibodies to <em>Borrelia burgdorferi</em> sensu lato, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative : 0,15% ProClin™ 300</td>
<td>1 x 0.75 ml</td>
</tr>
<tr>
<td>R4</td>
<td><strong>Cut-off Control:</strong> Human serum reactive for IgM antibodies to <em>Borrelia burgdorferi</em> sensu lato, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative : 0,15% ProClin™ 300</td>
<td>1 x 0.75 ml</td>
</tr>
</tbody>
</table>
For storage conditions and expiration date, please refer to the indications mentioned on the box.

### 5- WARNINGS AND PRECAUTIONS

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- Do not use expired reagents.
- Do not mix or associate within a given run reagents from different lots.

**REMARK:** For Washing Solution (R2, label identification: 20x colored green), Chromogen (R9, label identification: TMB colored turquoise) and Stopping Solution (R10, label identification: 1N colored red), it is possible to use other lots than those contained in the kit, provided these reagents are strictly equivalent and the same lot is used within a given test run.

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R5</td>
<td>Positive Control</td>
<td>1 x 0.75 ml</td>
</tr>
<tr>
<td></td>
<td>Positive Control:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human serum reactive for IgM antibodies to <em>Borrelia burgdorferi</em> sensu lato, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: 0.15% ProClin™ 300</td>
<td></td>
</tr>
<tr>
<td>R6a</td>
<td>Antigen</td>
<td>2 x qs 8.0 ml</td>
</tr>
<tr>
<td></td>
<td>Antigen (lyophilized):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bovine serum albumin, <em>Borrelia burgdorferi</em> sensu stricto native whole antigen.</td>
<td></td>
</tr>
<tr>
<td>R6b</td>
<td>Conjugate (51x)</td>
<td>1 x 0.4 ml</td>
</tr>
<tr>
<td></td>
<td>Conjugate (51x):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Murine monoclonal antibody anti-<em>Borrelia</em> labeled with peroxydase Preservative: 0.16% ProClin™ 300</td>
<td></td>
</tr>
<tr>
<td>R7</td>
<td>Diluent</td>
<td>2 x 65 ml</td>
</tr>
<tr>
<td></td>
<td>Diluent for samples and conjugate (Ready-to-use):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tris-NaCl (pH 7.7), fetal calf serum, 0.1% Tween® 20 and phenol red Preservative: 0.15% ProClin™ 300</td>
<td></td>
</tr>
<tr>
<td>R9</td>
<td>Chromogen TMB</td>
<td>1 x 28 ml</td>
</tr>
<tr>
<td></td>
<td>Chromogen (Ready-to-use):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3’.5.5’ tetramethylbenzidine (&lt; 0.1%), H₂O₂ (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>R10</td>
<td>Stopping Solution</td>
<td>1 x 28 ml</td>
</tr>
<tr>
<td></td>
<td>Stopping Solution (Ready-to-use):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1N sulfuric acid solution.</td>
<td></td>
</tr>
</tbody>
</table>
• Before use, wait for 30 minutes to allow reagents to reach room temperature (+18-30°C).
• Carefully reconstitute or dilute the reagents avoiding any contamination.
• Do not carry out the test in the presence of reactive vapors (acid, alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
• Use glassware thoroughly washed and rinsed with deionized water or, preferably disposable material.
• Washing the microplate is a critical step in the procedure: follow the recommended number of washings cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washings may lead to inaccurate results.
• Do not allow the microplate to dry between the end of the washings operation and the reagent distribution.
• Never use the same container to distribute the conjugate and the development solution.
• The enzymatic reaction is very sensitive to metal or metal ions. Consequently, do not allow any metal element to come into contact with the various solutions containing the conjugate or the chromogen.
• Chromogen solution (R9) should be colorless. The appearance of a blue color indicates that the reagent cannot be used and must be replaced.
• Use a new pipette tip for each sample.
• Check the pipettes and other equipments for accuracy and correct operations.

HEALTH AND SAFETY INSTRUCTIONS

Human origin material used in the preparation of reagents has been tested and founded non-reactive for hepatitis B surface antigen (HBs Ag), antibodies for hepatitis C virus (anti-HCV), and to human immunodeficiency virus (anti-HIV1 et anti-HIV2). Because no method can absolutely guarantee the absence of infectious agents, handle reagents of human origin and patient samples as potentially capable of transmitting infectious diseases.
• Any material, including washings solutions, that comes directly in contact with samples and reagents containing materials of human origin should be considered capable of transmitting infectious diseases.
• Wear disposable gloves when handling samples and reagents.
• Do not pipette by mouth.
• Avoid spilling samples or solutions containing samples. Spills must be rinsed with bleach diluted to 10%. In the event of a spill with an acid, it must be first neutralized with sodium bicarbonate, and then cleaned with bleach diluted to 10% and dried with adsorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
• Patient samples, reagents containing human origin material, as well as contaminated material and products should be discarded after decontamination only:
  - either by immersion in bleach at the final concentration of 5% of sodium hypochloride during 30 minutes,
  - or by autoclaving at 121°C for 2 hours at the minimum.

CAUTION: do not introduce solutions containing sodium hypochloride into the autoclave

• Avoid any contact of the Chromogen and Stopping Solution with skin and mucosa.
• Chemical and biological residues must be handled and disposed off in accordance with Good Laboratories Practices.
• All reagents in the kit are exclusively for in vitro diagnostic use.
• For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

6- SPECIMEN COLLECTION, PREPARATION AND STORAGE
1. Serum and plasma (EDTA, heparin or citrate) are the recommended sample types.
2. Observe the following recommendations for handling, processing and storage of blood samples:
   • Collect all blood samples observing routine precaution for venipuncture.
   • For serum, allow samples to clot completely before centrifugation.
• Keep tubes stoppered at all times.
• After centrifugation, separate the serum or plasma from the clot or red cells in a tightly stoppered storage tube.
• The specimens can be stored at +2-8°C if test is performed within 7 days.
• If test will not be completed within 7 days, or for shipment, freeze the samples at -20°C or colder.
• Do not use samples that have been thawed more than five times. Previously frozen specimens should be thoroughly mixed (Vortex) after thawing prior to testing.

3. Samples containing 90 g/l of albumin or 100 mg/l of unconjugated bilirubin, lipemic samples containing the equivalent of 36 g/l of triolein (triglyceride), and hemolysed samples containing up to 10 g/l of hemoglobin do not affect the results.

4. Do not heat the samples.

7- ASSAY PROCEDURE

7.1. MATERIALS REQUIRED BUT NOT PROVIDED
• Vortex mixer.
• Microplate reader equipped with 450 nm and 620 nm filters (*).
• Microplate incubator thermostatically set at 37±1°C (*).
• Automatic, semi-automatic or manual microplate washer (*).
• Sterile distilled or deionized water.
• Disposable gloves.
• Goggles or safety glasses.
• Adsorbent paper.
• Automatic or semi-automatic, adjustable or preset, pipettes or multi-pipettes, to measure and dispense 10 µl to 1000 µl, and 1 ml, 2 ml and 10 ml.
• Graduated cylinders of 25 ml, 50 ml, 100 ml and 1000 ml capacity.
• Sodium hypochloride (bleach) and sodium bicarbonate.
• Container for biohazard waste.
• Disposable tubes.

(*) Consult our technical department for detailed information about the recommended equipment.
7.2. REAGENTS RECONSTITUTION

- **R1**: Allow 30 minutes at room temperature (+18-30°C) before opening the bag. Take out the carrier tray, return unused strips in the bag immediately and check the presence of desiccant. Carefully reseal the bag and store it at +2-8°C.

- **R2**: Dilute 1/20 the washing solution R2 in distilled water: for example 50 ml of R2 and 950 ml of distilled water to get the ready-to-use washing solution. Prepare 350 ml of diluted washing solution for one plate of 12 strips if washing manually.

- **R3, R4, R5**: Dilute 1/10 in Diluent (R7) (example: 10 µl of R3 + 1 ml of R7).

- **R6a**: For one plate, reconstitute the lyophilized antigen by adding 8 ml of Diluent (R7) to each vial. Mix thoroughly. Waiting for 15 minutes before mixing with the Conjugate (R6b) allows a homogenous rehydration of the antigen.

- **R6b**: Prepare the necessary quantity of conjugate for one run by adding one volume of concentrated conjugate (51x) to 50 volumes of Diluant (R7). For one plate, mix 0.3 ml of R6b and 15 ml of R7.

- **R6 (R6a+R6b)**: Mix equal volumes of reconstituted reagents R6a and R6b. For one plate, mix 12 ml of the reconstituted solution R6a and 12 ml of the reconstituted solution R6b. Wait for 45 minutes before use.

7.3. STORAGE AND VALIDITY OF OPENED AND / OR RECONSTITUTED REAGENTS

The kit must be stored at +2-8°C. When the kit is stored at +2-8°C before opening, each component can be used until the expiration date indicated on the outer label of the kit.

- **R1**: Once opened, the strips remain stable for up to one month if stored at +2-8°C in the same carefully closed bag (check the presence of desiccant).

- **R2**: Once diluted, the Washing Solution can be kept for 2 weeks at +2-30°C. Once opened, the concentrated Washing Solution stored at +2-30°C, in absence of contamination, is stable until the expiration date indicated on the label.

- **R3, R4, R5, R6b, R7**: Once opened and without any contamination, the reagents stored at +2-8°C are stable for up to one month.

- **R6a+R7**: Once reconstituted, the antigen is stable 15 days at +2-8°C or frozen at -20°C. The antigen reconstituted and stored frozen must not be thawed more than three times.

- **R6b+R7**: Once reconstituted, the conjugate solution is stable for 8 hours at room temperature (+18-30°C) or 24 hours at +2-8°C.
• **R6 (R6a+R6b):** Once reconstituted, the conjugate working solution is stable 8 hours at room temperature (+18-30°C).

• **R9:** Once opened and without any contamination, the reagent stored at +2-8°C is stable for up to 2 months.

• **R10:** Once opened and without any contamination, the reagent stored at +2-8°C is stable until the expiration date indicated on the label.

### 7.4. PROCEDURE

Strictly follow the assay procedure and Good Laboratory Practices.

Before use, allow reagents to reach room temperature (+18-30°C).

Use the negative, cut-off and positive controls with each run to validate the assay results.

1. Carefully establish the distribution and identification plan for controls and patients samples.

2. Prepare the diluted Washing Solution (R2) [*Refer to Section 7.2*].

3. Take the carrier tray and the strips (R1) out of the protective pouch [*Refer to Section 7.2*].

4. Reconstitute the antigen R6a vial by adding 8 ml of Diluent (R7). Mix thoroughly.

5. Dilute controls R3, R4, R5 and patients samples (S1, S2…) in Diluent (R7) to give a 1/101 dilution: 10 µl of sample and 1.0 ml of Diluent (R7). Vortex diluted samples.

6. Prepare the Conjugate working solution (R6): dilute 1/51 the necessary quantity of Conjugate (R6b) with the Diluent (R7). Then mix equal volumes of reconstituted R6a et R6b reagents [*Refer to Section 7.2*]. The Conjugate working solution must be prepared at least 45 minutes before distribution into the plate. Mix thoroughly.

7. Strictly following the indicated sequence below, distribute in each well with 200µl of diluted controls and patient samples:
8. Cover the microplate with an adhesive plate sealer, then press firmly onto the plate to ensure a tight seal. Incubate the microplate immediately in a thermostat controlled water bath or in a dry incubator for 1 hour ± 5 minutes at 37°C ± 1°C.

9. At the end of the first incubation period, remove the adhesive plate sealer. Aspirate the content of all wells into a container for biohazard waste (containing sodium hypochloride). Wash microplate 4 times with 350 µl of the Washing Solution (R2). Invert the microplate and gently tap on adsorbent paper to remove remaining liquid.

10. Distribute 200 µl of the conjugate working solution (R6) in all wells. The solution must be shaken gently before use.

11. Cover the microplate with an adhesive plate sealer, then press firmly onto the plate to ensure a tight seal. Incubate the microplate immediately in a thermostat controlled water bath or in a dry incubator for 1 hour ± 5 minutes at 37°C ± 1°C.

12. At the end of the second incubation period, remove the adhesive plate sealer. Aspirate the content of all wells into a container for biohazard waste (containing sodium hypochloride). Wash microplate 4 times with 350 µl of the Washing Solution (R2). Invert the microplate and gently tap on adsorbent paper to remove remaining liquid.

13. Quickly distribute into each well and away from light 200 µl of Chromogen solution (R9). Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (+18-30°C). Do no use adhesive plate sealer during this incubation.
14. Stop the enzymatic reaction by adding 100 µl of Stopping Solution (R10) in each well. Use the same sequence and rate of distribution as for the development solution.
15. Carefully wipe the plate bottom. Read the optical density at 450/620 nm using a plate reader within 30 minutes after stopping the reaction. The strips must always be kept away from light before reading.
16. Before reporting results, check for agreement between the reading and the distribution plan of plate and samples.

8- CALCULATION AND INTERPRETATION OF RESULTS

8.1. CALCULATION OF THE CUT-OFF VALUE (CO)
The Cut-Off value (CO) corresponds to the mean value of the optical densities (OD) of the cut-off Control duplicates (R4):

\[ \text{CO} = \text{mean of OD R4} \]

8.2. CALCULATION OF THE SAMPLE RATIO
Sample result is expressed by Ratio using the following formula:

\[ \text{Sample Ratio} = \frac{\text{Sample OD}}{\text{CO}} \]

8.3. QUALITY CONTROL
Include all the controls for each microplate and for each run, and analyse the obtained results. For validation of the assay, the following criteria must be met:

- **Optical density values**:
  - \( \text{CO} > 0,2 \)
  - \( 0.80 \times \text{CO} < \text{OD R4 Replicate 1} < 1.20 \times \text{CO} \)
  - \( 0.80 \times \text{CO} < \text{OD R4 Replicate 2} < 1.20 \times \text{CO} \)

(Individual OD of each replicate of the Cut-Off control (R4) must not differ more than 20% of the CO value).

- **Optical density ratios**:
  - \( \text{Ratio R3 (OD R3 / CO)} < 0,5 \)
  - \( \text{Ratio R5 (OD R5 / CO)} > 2,0 \)

If those quality control criteria are not met, the test run should be repeated.
8.4. INTERPRETATION OF RESULTS

If an infection is suspected, complementary serological tests like the detection of IgG anti-*Borrelia* antibodies can be useful to confirm the diagnosis. If the serology is positive or equivocal, it is recommended to test the sample with a confirmation method like a Western-Blot (9).

### 8.5. TROUBLE SHOOTING GUIDE

Non validated or non repeatable reactions are often caused by:
- Inadequate microplate washings.
- Contamination of negative samples by serum or plasma with a high antibody titer.
- Contamination of the development solution by chemical oxidizing agents (bleach, metal ions...).
- Contamination of the Stopping Solution.

### 8.6. CALCULATION EXAMPLE

**Note:** The following data are given as an example and should not be used instead of the results of the user.

<table>
<thead>
<tr>
<th>Controls and Patients samples</th>
<th>OD (450/620 nm)</th>
<th>Ratio</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R3</em></td>
<td>0.155</td>
<td>0.22</td>
<td>Negative</td>
</tr>
<tr>
<td><em>R4</em></td>
<td>0.709</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><em>R4</em></td>
<td>0.697</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td><em>R5</em></td>
<td>2.280</td>
<td>3.24</td>
<td>Positive</td>
</tr>
<tr>
<td><em>Sample 1</em></td>
<td>1.181</td>
<td>1.67</td>
<td>Positive</td>
</tr>
<tr>
<td><em>Sample 2, etc...</em></td>
<td>0.597</td>
<td>0.85</td>
<td>Positive</td>
</tr>
</tbody>
</table>
• Cut-Off value:
  - CO = (0.709+0.697)/2 = 0.703

• Optical density values:
  - OD CO = 0.703 (N > 0.200)
  - OD R4 Replicate 1 = 0.709 (0.80 x OD CO < OD R4 Replicate 1 < 1.20 x OD CO)
  - OD R4 Replicate 2 = 0.697 (0.80 x OD CO < OD R4 Replicate 2 < 1.20 x OD CO)

• Optical density ratios:
  - Ratio R3 = 0.22 (N < 0.5)
  - Ratio R5 = 3.24 (N > 2.0)

• Quality control : Accepted

9- PERFORMANCES

9.1. PREVALENCE
Prevalence determination of IgM antibodies to *Borrelia burgdorferi* sensu lato in human serum was estimated using a panel of 296 samples obtained from blood donors from the northern part of France. The following results were obtained: 286 negative, 8 equivocal and 2 positive sera. Prevalence using the Platelia™ Lyme IgM assay is established at 0.68% (2/296).

9.2. SPECIFICITY

9.2.1 Specificity in non endemic area
Specificity was estimated on a panel of 286 sera obtained from healthy blood donors living in a non endemic area in the northern part of France. Samples were selected on negative results obtained with a commercialized EIA assay used in Europe (CE marked) and considered as a reference.

9.2.2 Specificity in endemic area
Specificity was also determined on a panel of 197 sera obtained from healthy blood donors living in endemic area in the eastern part of France, which none of which were presenting criteria related to Lyme disease (no clinical symptoms of borreliosis, neither history of tick bites). Samples were selected on negative results obtained with a commercialized EIA assay used in Europe (CE marked) and considered as a reference.
Obtained results are presented in the following table:

<table>
<thead>
<tr>
<th>Panel of sera</th>
<th>Negative</th>
<th>Equivocal (¹)</th>
<th>Positive (²)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non endemic area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(n=286)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>5</td>
<td>1</td>
<td><strong>99.6%</strong></td>
<td><em>(280/281)</em></td>
</tr>
<tr>
<td>[98.0% - 100.0%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endemic area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(n=197)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>191</td>
<td>4</td>
<td>2</td>
<td><strong>99.0%</strong></td>
<td><em>(191/193)</em></td>
</tr>
<tr>
<td>[96.3% - 99.9%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(¹) Equivocal results were excluded for calculation of specificity.
(²) One of the three positive samples with Platelia™ Lyme IgM assay was also found positive by Western-blot.
(³) IC 95%: 95% confidence interval.

### 9.3. SENSITIVITY

Sensitivity of the Platelia™ Lyme IgM assay was calculated and included with sensitivity results from Platelia™ Lyme IgG assay (Ref. 72952) on a panel of 70 samples from patients presenting various forms of Lyme disease at different clinical stages. Results are presented in the following table and were compared with sensitivities obtained with commercialized EIA assays used in Europe (CE marked) and considered as reference:

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Number of sera</th>
<th>Platelia™ Lyme (¹)</th>
<th>Reference Europe (¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Erythema migrans (Stage I)</strong></td>
<td><strong>17</strong></td>
<td>58.8% [32.9%-81.6%]</td>
<td>66.7% [38.4%-88.2%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neuroborreliosis (Stage II)</strong></td>
<td><strong>33</strong></td>
<td>36.7% [19.9%-56.1%]</td>
<td>96.9% [83.4%-99.9%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acrodermatitis chronica atrophicans (Stage III)</strong></td>
<td><strong>5</strong></td>
<td>20.0% [0.0%-71.6%]</td>
<td>100.0% [54.9%-100.0%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lyme Arthritis (Stage III)</strong></td>
<td><strong>15</strong></td>
<td>0.0% [0.0%-18.1%]</td>
<td>100.0% [81.9%-100.0%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(¹) Equivocal results were excluded for calculation of sensitivity.
Sensitivity of the Platelia™ Lyme IgM assay was also demonstrated on a documented panel of samples supplied by the CDC (Center for Disease Control), and were compared with sensitivities obtained with commercialized EIA assays used in Europe (CE marked) and USA (FDA approved) and considered as reference. Results are presented in the following table:

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Number of sera</th>
<th>Platelia™ Lyme ((^n))</th>
<th>Reference Europe ((^n))</th>
<th>Reference USA ((^n))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM + IgG</td>
</tr>
<tr>
<td>Erythema migrans</td>
<td>28</td>
<td>73.7% [48.8%-90.9%]</td>
<td>38.5% [20.2%-59.5%]</td>
<td>86.4% [65.1%-97.1%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM + IgG</td>
</tr>
<tr>
<td>Arthritis / Arthralgia</td>
<td>6</td>
<td>40.0% [5.3%-85.3%]</td>
<td>100.0% [60.7%-100.0%]</td>
<td>100.0% [60.7%-100.0%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM + IgG</td>
</tr>
<tr>
<td>Unknown stage</td>
<td>4</td>
<td>100.0% [0.05%-100.0%]</td>
<td>100.0% [36.8%-100.0%]</td>
<td>100.0% [47.3%-100.0%]</td>
</tr>
<tr>
<td>[IC 95%]</td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td>IgM + IgG</td>
</tr>
</tbody>
</table>

\(^n\) Equivocal results were excluded for calculation of sensitivity.

### 9.4. PRECISION

- **Within-run precision (repeatability):**
  In order to evaluate intra-assay repeatability, one negative and three positive samples were tested 30 times during the same run. The ratio (Sample OD / CO) was determined for each sample. Mean Ratio, Standard Deviation (SD) and Coefficient of Variation (%CV) for each of the four specimens are listed in the table below:

**Within-run precision (repeatability)**

<table>
<thead>
<tr>
<th>N=30</th>
<th>Negative Sample</th>
<th>Low Positive Sample</th>
<th>Positive Sample</th>
<th>High Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.24</td>
<td>1.17</td>
<td>1.88</td>
<td>4.23</td>
</tr>
<tr>
<td>SD</td>
<td>0.040</td>
<td>0.052</td>
<td>0.070</td>
<td>0.104</td>
</tr>
<tr>
<td>% CV</td>
<td>15.2%</td>
<td>4.4%</td>
<td>3.7%</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

- **Between-run precision (reproducibility):**
  In order to evaluate inter-assay reproducibility, each of four specimens (one negative and three positive samples) was tested in duplicate in two runs per day over a 20 days period. The ratio (Sample OD / CO) was determined for each sample. Mean Ratio, Standard Deviation (SD) and
Coefficient of Variation (%CV) for each of the four specimens are listed in the table below:

**Between-run precision (reproducibility)**

<table>
<thead>
<tr>
<th></th>
<th>Negative Sample</th>
<th>Low Positive Sample</th>
<th>Positive Sample</th>
<th>High Positive Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N=80</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ratio (Sample OD / Cut-Off value)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.24</td>
<td>1.21</td>
<td>1.76</td>
<td>4.18</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.029</td>
<td>0.067</td>
<td>0.104</td>
<td>0.153</td>
</tr>
<tr>
<td><strong>% CV</strong></td>
<td>12.0%</td>
<td>5.5%</td>
<td>5.9%</td>
<td>3.7%</td>
</tr>
</tbody>
</table>

9.5. CROSS REACTIVITY

338 samples with characteristics which could potentially result in non specific reactions were tested with the Platelia™ Lyme IgM assay. Results are presented in the following table:

<table>
<thead>
<tr>
<th>Panel</th>
<th>Number of samples</th>
<th>Equivocal (1)</th>
<th>Positive</th>
<th>Cross-reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>83</td>
<td>0</td>
<td>1</td>
<td>1.2% (2)</td>
</tr>
<tr>
<td>CMV</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>3.3% (2)</td>
</tr>
<tr>
<td>EBV</td>
<td>17</td>
<td>1</td>
<td>5</td>
<td>29.4% (3)</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>13.3% (3)</td>
</tr>
<tr>
<td>Malaria</td>
<td>20</td>
<td>0</td>
<td>6</td>
<td>30.0% (3)</td>
</tr>
<tr>
<td>Anti-nuclear antibodies (ANA)</td>
<td>22</td>
<td>1</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Heterophile antibodies (HAMA)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>HSV</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>38</td>
<td>0</td>
<td>1</td>
<td>2.6% (2)</td>
</tr>
<tr>
<td>Rubella</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Measles</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Mumps</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>HIV</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>VZV</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>338</strong></td>
<td><strong>4</strong></td>
<td><strong>16</strong></td>
<td><strong>4.8%</strong></td>
</tr>
</tbody>
</table>

(1) Equivocal results were excluded for calculation of cross-reactivity.
(2) Not significantly different from prevalence in endemic area (Fisher test, p>0.05).
(3) Significantly different from prevalence in endemic area (Fisher test, p<0.05).
The 16 samples found positive with Platelia™ Lyme IgM assay were also tested with a commercialized EIA assays used in Europe (CE marked) and with a Western-Blot method. Results are presented in the following table:

<table>
<thead>
<tr>
<th>Sample</th>
<th>EIA Assay</th>
<th>Western blot (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>CMV</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>EBV</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>EBV</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>EBV</td>
<td>Positive</td>
<td>NT</td>
</tr>
<tr>
<td>EBV</td>
<td>Positive</td>
<td>Equivocal</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

10- LIMITATIONS OF THE PROCEDURE

Diagnosis of *Borrelia burgdorferi* infection can only be established on the basis of a combination of clinical and biological data. The result of a single test of detection of IgM anti-*Borrelia burgdorferi* antibodies does not constitute a sufficient proof for the diagnosis of infection by *Borrelia burgdorferi* sensu lato. If the serology is positive or equivocal, it is recommended to test the sample with a confirmation method like a Western-Blot (9).

Particularly, it has been reported that patients with malaria or patients infected with Epstein Barr virus or other spirochetes species (leptospirosis, etc…) could potentially present false positive reactions with diagnostic assays for antibodies to *Borrelia burgdorferi*. Those situations should be considered when interpreting results obtained with such tests.

11- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within Bio-Rad.

NT: not tested due to insufficient volume of sample.
12- REFERENCES


Този продукт съдържа човешки или животински компоненти. Бъдете внимателни при работа с него.
Danger  
Causes severe skin burns and eye damage. May cause an allergic skin reaction.  
Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.
Llevar guantes que aíslen del frío/gafas/máscara.

EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos.

Quitar las lentes de contacto, si lleva y resulta fácil.

Seguir aclaramiento. EN CASO DE INGESTION: Enjuagarse la boca. NO provocar el vómito. EN CASO DE CONTACTO CON LA PIEL (o el pelo): Quitarse inmediatamente las prendas contaminadas.

Aclararse la piel con agua o ducharse. En caso de irritación o erupción cutánea: Consultar a un médico.

Eliminar el contenido o el recipiente conforme a la reglamentación local/regional/nacional/internacional.

(FI)
Vaara
Vomakkaasti ihoa syövyttää ja silmiä vaurioittava. Voi aiheuttaa allergisen ihoreaktion.


(PT)
Luz do corpo
Vomacamente de queima e lesão ocular grave. Pode originar uma reação alérgica cutânea.


Lavagem apropriada da pele com água/serviço de duche. Em caso de irritação ou erupção cutânea: Consultar um médico. Manter o conteúdo/container de acordo com as regulamentações locais/regionais/nacionais/Internacionais.

(HR)
Opasnost
Uzrokuje teške opekline kože i ozljede oka. Mogu izazvati alergijsku reakciju na koži.

Pavojinga

Smarkiai nudegina odą ir pažeidžia akis. Gali sukelti alerginę odos reakciją.

Mūvėti apsaugines pirštines/dėvėti apsauginius drabužius/naudoti akių (veido) apsaugos priemones.

PATEKUS Į AKIS: Kelias minutes atsargiai plauti vandeniu. Išimti kontaktinius lęšius, jeigu jie yra ir jeigu lengvai galima tai padaryti. Toliau plauti akis.

PRARIJUS: išskalauti burną. NESKATINTI vėmimo.

Nevarno
Povzroča hude opekline kože in poškodbe oči.
Lahko povzroči alergijski odziv kože.

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