Anti-Borrelia IgM ELISA Assay Kit

Catalog Number: ABM31-K01

Enzyme immunoassay (ELISA) for the determination of IgM antibodies to Borrelia burgdorferi in human serum, joint fluid and CSF

INTENDED USE

The Eagle Biosciences Anti-Borrelia IgM ELISA Assay Kit is used for the qualitative and quantitative determination of IgM antibodies to Borrelia burgdorferi antigens in human serum, joint fluid and cerebrospinal fluid (CSF).

The spirochete bacterium Borrelia burgdorferi is the causative agent of the systemic infectious disease called Borreliosis or Lyme disease. Borrelia burgdorferi is solely transferred to humans by ticks (Ixodes ricinus). Consequently, Borreliosis is endemic in areas where ticks are found (e.g., Austria, Italy, several parts of Germany). Clinical illness brought about by an infection with Borrelia burgdorferi is diverse and demonstrates three distinct stages:

I Early stage (4 - 8 weeks) Erythema migans
II Generalization (1 - 12 months) meningitis, meningopolyneuritis, myalgia, Lymphadenitis cutis benigna, carditis
III Late Stage (months to years) Acrodermatitis chronica atrophicans, arthritis, neuro-Borreliosis

The possible severe outcome of an infection with Borrelia burgdorferi and the complicated treatment of late stages of this infection demand diagnosis as early as possible.

The determination of IgM and IgG antibodies to Borrelia burgdorferi by enzyme immunoassay provides the first important step towards a serological diagnosis of Borreliosis. Anti-Borrelia burgdorferi IgM antibodies are detected mainly during the first stage of infection. Following the course of infection anti-Borrelia burgdorferi IgG antibodies occur, whereas the specific IgM antibodies disappear steadily.

Positive results should be confirmed by western blot analysis. Results obtained by in vitro diagnostics are to be interpreted in context with the clinical signs of the infection. For the diagnosis of neuro-Borreliosis we suggest the determination of the antibody index using both serum and CSF samples. On request an instruction manual will be provided.

- Kaiser R Lücking CH; Intrathecal synthesis of specific antibodies in neuroborreliosis: Comparison of different ELISA techniques and calculation methods. J Neurol Sciences, 1993, 118:64-72

PRINCIPLE of the TEST

The Eagle Biosciences Anti-Borrelia IgM ELISA Assay kit is an enzyme immunoassay for the qualitative or semi-quantitative determination of IgM antibodies to Borrelia burgdorferi. Alternatively a calibrator set (3301) can be ordered to run the assay quantitatively.

The antibodies of the controls and diluted patient samples react with purified Borrelia afzelii antigens enriched with OspC and synthetic VlsE antigen immobilized on the solid phase of microtiter plates. Purified antigens of an European isolate of Borrelia afzelii coated on the microtiter plate guarantees the specific binding of Borrelia burgdorferi IgM antibodies of the specimen under investigation. Following an incubation period of 30 min at 37 °C, unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgM-antibodies conjugated to horse radish peroxidase (HRP). Following an incubation period of 30 min at 37 °C, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H₂SO₄) into the wells after 15 min at 37°C turning the solution from blue to yellow. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound.
Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C. Lipemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1,0 ml sample diluent (C), prior to assay. (controls of the kit are ready for use, prediluted accordingly)

TEST COMPONENTS for 96 determinations

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Microtiter plate, 12 breakable strips per 8 wells (total 96 individual wells) coated with Borrelia afzelii antigens enriched with purified OspC and synthetic VlsE</td>
</tr>
<tr>
<td>B</td>
<td>Concentrated wash buffer sufficient for 1000 ml solution each</td>
</tr>
<tr>
<td>C</td>
<td>Sample diluent 100 ml ready for use capped white</td>
</tr>
<tr>
<td>D</td>
<td>Conjugate containing polyclonal anti-human-IgG (Fab), (sheep) coupled with HRP</td>
</tr>
<tr>
<td>E</td>
<td>Substrate 3,3',5,5' tetramethylbenzidine in citrate buffer containing hydrogen peroxide</td>
</tr>
<tr>
<td>F</td>
<td>Stop solution 0.25 M sulfuric acid</td>
</tr>
<tr>
<td>G</td>
<td>Calibrators (human serum diluted) conc.: see leaflet enclosed 1 ml each ready for use capped white</td>
</tr>
<tr>
<td>H</td>
<td>Positive Control (human serum diluted) conc.: see leaflet enclosed 1 ml ready for use capped red</td>
</tr>
<tr>
<td>I</td>
<td>Negative Control (human serum diluted) ready for use capped green</td>
</tr>
</tbody>
</table>

For the quantitative determination of anti-Borrelia IgM antibodies in serum and liquor specimens to calculate antibody indices in the diagnosis of neuroborreliosis 4 anti-Borrelia IgM calibrators are separately.

Materials required:
- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- incubator (37 °C)
- microplate reader with optical filters for 450 nm and 620 or 690 nm
- graduated cylinders 100 ml
- distilled or de-ionized water

Size and storage

Anti-Borrelia IgM ELISA Assay kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete Anti-Borrelia IgM ELISA Assay kit on the box label.

Upon receipt, all components of the Anti-Borrelia IgM ELISA Assay kit have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all Anti-Borrelia IgM ELISA Assay kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components of the Anti-Borrelia IgM ELISA Assay kit to reach room temperature prior to use in the assay.

The microtiter plate is vacuum sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days. Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the substrate to light.

ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1,0 ml sample diluent (C).

- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18 - 25°C) before use. Mix gently without causing foam.
2. Dispense 100 µl controls (P, N) or calibrators 1 - 4 100 µl diluted patient samples into the respective wells.
3. Seal plate, incubate 30 min at 37 °C.
4. Decant, then wash each well five times using 300 µl wash solution (made of B).
5. Add 100 µl of conjugate (D) solution to each well.
6. Seal plate, incubate 30 min at 37 °C.
7. Decant, then wash each well five times using 300 µl wash solution (made of B).
8. Add 100 µl of substrate (E) to each well.
9. Incubate 15 min in the dark at 37°C.
10. Add 100 µl of stop solution (F) to each well and mix gently.
11. Read the optical density at 450 nm versus 620 or 690 nm within 15 min after adding the stop solution.
1. QUALITATIVE/SEMI-QUANTITATIVE EVALUATION

Cut-off determination

The cut-off for qualitative evaluation of the test is calculated according to the following criterion:

OD of the negative control + 0.45

Alternatively the results can be interpreted by calculating the binding index (BI = ratio between OD of the sample and OD of cut-off) using the following formula:

\[ BI = \frac{OD_{\text{sample}}}{(OD_{\text{negative control}} + 0.45)} \]

REFERENCE VALUES

<table>
<thead>
<tr>
<th>Anti-Borrelia IgM</th>
<th>OD</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>&lt; cut-off x 0.9</td>
<td>&lt; 0.9</td>
</tr>
<tr>
<td>positive</td>
<td>&gt; cut-off</td>
<td>&gt; 1.0</td>
</tr>
</tbody>
</table>

Specimens with concentrations detected in the grey zone (0.9 x cut-off < OD sample < cut-off, 0.9 < BI sample < 1.0) should be tested again. Alternatively, the patient is to be re-examined within 1 to 2 weeks.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-Borrelia burgdorferi levels, as usually done for other diagnostic parameters, too.

Test validity

The test run is valid if:

- the mean OD of the positive control is \( \geq 0.80 \)
- the mean OD of the negative control is \( \leq 0.15 \)

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

2. QUANTITATIVE EVALUATION

For the calculation of antibody indices regarding the diagnosis of neuroborreliosis quantitative determination of IgM antibodies to Borrelia burgdorferi is recommended. An instruction manual for the calculation of antibody indices can be ordered from Generic Assays.

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (separately available – Order No. 3301) on the ordinate, y-axis, (lin. scale) versus their respective anti-Borrelia-concentrations on the abscissa, x-axis, (log. scale).

The anti-Borrelia IgM concentrations of the unknown samples are directly read off in U/ml against the respective OD values. The reference values for the decision positive-negative in quantitative evaluation are stated on the leaflet enclosed to the separately available calibrators.

Example of typical assay results

<table>
<thead>
<tr>
<th>Well</th>
<th>OD 450/620 nm</th>
<th>U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 1</td>
<td>0.579</td>
<td>45</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>1.011</td>
<td>90</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>1.586</td>
<td>200</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>2.133</td>
<td>500</td>
</tr>
<tr>
<td>Patient 1</td>
<td>1.102</td>
<td>102</td>
</tr>
</tbody>
</table>

Limits of the Method

The early immune response of patients infected with Borrelia burgdorferi is directed towards a protein of the flagella of these spirochete bacteria, called flagellin. However, the flagellin of B. burgdorferi shows sequence homologies at the C- and N-terminus with flagellin proteins of other spirochete species (e.g. Treponema pallidum). Consequently, an infection with other spirochete bacteria may trigger cross-reacting antibodies. These antibodies will lead to false-positive results if produced abundantly.

The in vitro results should be interpreted always in context with the clinical status of the patient. Repeated testing over several weeks is recommended in order to discriminate an active infection from long term persistent antibody titers without clinical implication.

Positive anti-Borrelia IgM antibody sera should be confirmed by Western blot (3500) analysis.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference material the Anti-Borrelia IgM is calibrated in arbitrary units (U/ml) or interpreted qualitatively.

Linearity

Dilutions of specimens in anti-Borrelia burgdorferi-IgM antibody free human serum are determined according to the expected theoretical values with Anti-Borrelia IgM.

Diagnostic sensitivity and specificity

Specificity data of the Anti-Borrelia IgM have been determined by examining more than 1000 patients with no clinical signs of an infection with Borrelia burgdorferi and 70 patients with the clinical diagnosis of Borreliosis.

Specificity: 97 %
Sensitivity: 90 %

Precision

<table>
<thead>
<tr>
<th>Intraassay</th>
<th>Interassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD (mean)</td>
<td>CV %</td>
</tr>
<tr>
<td>0.85</td>
<td>3.6</td>
</tr>
<tr>
<td>1.22</td>
<td>5.1</td>
</tr>
<tr>
<td>1.79</td>
<td>4.6</td>
</tr>
</tbody>
</table>
### Incubation Scheme

**Anti-Borrelia IgM ELISA Assay kit**

| Dilute patient sera | 10 µl serum + 1.0 ml sample diluent (C), sample diluent contains rheumatoid factor absorbent reagent |

<table>
<thead>
<tr>
<th></th>
<th>Positive control (P)</th>
<th>Negative control (N)</th>
<th>patient sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Pipette Positive control (P) Negative control (N) 1 + 100 diluted sera</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>3</td>
<td>Seal plate and incubate</td>
<td>30 min, 37 °C</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td>Decant, 5 x 300 µl (made of B)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Pipette conjugate (D)</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>6</td>
<td>Seal plate and incubate</td>
<td>30 min, 37 °C</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Wash</td>
<td>Decant, 5 x 300 µl (made of B)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pipette substrate (E)</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>9</td>
<td>Incubate in the dark</td>
<td>15 min, 37°C</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Pipette stop solution (F)</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>11</td>
<td>Read at 450 nm against 690 (620) nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Safety Precautions

- **This kit is for Research Use Only.** Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates for the Anti-Borrelia IgM ELISA Assay kit stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents of the Anti-Borrelia IgM ELISA Assay kit from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents of the Anti-Borrelia IgM ELISA Assay kit should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents of the Anti-Borrelia IgM ELISA Assay kit contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the Anti-Borrelia IgM ELISA Assay kit contains potentially hazardous materials the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-Borrelia levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned data only provide a guide to values which might be expected.
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.