Optiplex Borrelia IgM Screening Test
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Multiplex Bead Assay (MBA) for Screening of IgM-Antibodies based on Semi-Quantitative Measurements of Borrelia Antigens in Human Serum and Cerebrospinal Fluid (CSF)

Cat.No: IN0501
Contents: 96 Tests
Store at: 2–8 °C

Instruction sheet / Gebrauchsanweisung / Carnet d’instruction / instrucciones de uso / gebruiksaanwijzing / istruzioni per l’uso / σελίδα οδηγιών / bruksanvisning / modo de emprego

1. Introduction

Lyme borreliosis is the most common tick-borne disease in the northern hemisphere. The prevalence of antibodies to Borrelia sp. in blood donors is 5-19 % with regional differences. Serological detection of IgM antibodies is usually not possible before 3 weeks after infection (2) and thus often only after subsidence of an erythema migrans (EM). With beginning generalization of the infection after approximately 6 weeks, IgG antibodies to Borrelia antigens are increasingly detectable (4,5) whereas IgM response slowly subsides (seroconversion). Late stages of Lyme borreliosis are characterized by high specific IgG titres in combination with low or not detectable IgM titres (3,6).

2. Principle of the Test

The MBA is designed to detect IgM class antibodies in properly diluted human sera to 3 Borrelia antigens. The MBA consists of optically distinguishable sets of beads labeled with different combinations of two fluorescent dyes. Each bead set is coated with a different antigen.

The test procedure involves two incubation steps. The diluted sera are first incubated with the multiplexed mixture of the bead suspension. If present in the diluted patient specimen, specific antibodies will bind to the antigens of a bead set of the MBA. The various sets of beads are coated with the following antigens isolated from various Borrelia species (1):

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Species</th>
<th>Antigen type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysat MMS</td>
<td>Borrelia afzelii</td>
<td>native</td>
</tr>
<tr>
<td>OspC PBi</td>
<td>Borrelia garinii</td>
<td>recombinant</td>
</tr>
<tr>
<td>OspC PKo</td>
<td>Borrelia afzelii</td>
<td>recombinant</td>
</tr>
</tbody>
</table>

In the second incubation step phycoerythrin-conjugated goat anti-human IgM reporter molecules („conjugate“) are added to detect IgM antibodies bound to the antigens on the bead surface. The beads are identified by the Luminex analyzer based on the bead set-specific dye combination and the amount of bound phycoerythrin-labelled reporter molecules (anti-IgM), which are indicative of anti-Borrelia antigen-specific antibodies. Conjugate binding to free IgM in the sample is not detected by the analyzer. Thus, there is no need for a washing step to remove unbound antibodies. A fourth control bead set of the assay CR1 serves for monitoring the IgM level of the specimen and as a control of the reporter reaction of the conjugate.

3. Test Kit Components

DIL Assay buffer, 1 x 100 ml. Ready to use!
NEG M Negative control (human serum)*. Ready to use, 300 µl vial. The serum does not contain detectable Borrelia-specific IgM antibodies.
REF M Reference control (human serum)*. Ready to use, 300 µl vial. The serum contains Borrelia-specific IgM antibodies.
POS M Positive control (human serum)*. Ready to use, 300 µl vial. The serum contains Borrelia-specific IgM antibodies.
BMM Bead suspension („Bead Mix“). Ready to use, 2.6 ml vial. The suspension contains 4 antigen-specific bead sets.
CON M Phycoerythrin-conjugated goat anti-human IgM. Ready to use, 6 ml vial.
MTP One 96-well polystyrene microtitre plate with break-apart-strips.

Instruction sheet, batch certificate, adhesive foil

4. Notes for the User

For professional use only.

End-User Licence
By purchasing this kit with fluorescent dye coated beads, which are authorized by LuminexTM, the customer acquires the right to use this product only in combination with the Luminex analyzer. The use of Luminex beads is covered by US patents.

Safety Precautions
*The NEG M, REF M and POS M controls have been tested for HIV-1 and HIV-2, HbS Ag and antibodies to HVC and found to be negative by approved methods. However, controls and samples should be considered to be potentially infectious. Recommendation: Treat the waste bottle with a suitable disinfectant, autoclave solid waste at 121 °C for 30 minutes. Follow the rules of good laboratory practice!
Liquid components contain proclin 0.05% as a preservative. Avoid contact with eyes, skin or mucous membrane.

Waste Management
When managing the waste generated by the use of the test adhere to all applicable regulations. Chemicals and mixtures containing chemicals and items contaminated by serum are, as a rule, considered hazardous and biohazardous waste. Special notes for waste disposal are listed in the safety data sheet.

Damage in Transit
If a test kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerably damaged components for a test procedure. Keep damaged components or kits until the claim has been settled. Thereafter, they should be disposed of in compliance with existing regulations.

5. Materials Required but not Provided
- Tubes (1.5 ml) for dilution of samples
- Precision pipettes (5 µl-1000 µl)
- Vortex mixer
- Orbital shaker
- 37 °C incubator
- Luminex analyzer
- Timer
- Gloves

6. Reagent Stability
The unopened kit should be stored at 2-8 °C and used before expiration date indicated on the label. After opening the reagents are stable for 6 weeks.
Do not freeze reagents! Beads are light sensitive! Avoid direct light exposure!
Except assay buffer, do not mix reagents from different lots.

7. Sample Material
Use only fresh human serum samples. However, if stored below –20 °C, samples can also be used after several months of storage. Avoid multiple freeze/thaw cycles. Hemolyzed, lipemic, icteric or contaminated samples, as well as precipitated or viscous samples should not be analyzed, since they may generate erroneous results.

8. Preparation of Test
Set-up the Luminex analyzer according to the instructions in the manual. The parameters required for the creation of a template are listed in the appendix. Select template suitable for the test. Enter name of test run. Enter names of sample according to pipetting scheme (see below).
Allow all components to warm up to room temperature (20-25 °C) before use in the assay.

Preparation of bead suspension (BMM)
Resuspend Bead Mix IgM (BMM) just prior to use by thorough vortexing. Avoid direct light exposure, beads are light sensitive!

Preparation of samples
Serum samples
Dilute serum samples 1:201 with ready-to-use assay buffer. Ensure thorough mixing of dilutions!
1+200 5 µl serum  + 1000 µl buffer

9. Test Performance
The instructions provided in this sheet must be strictly observed to ensure optimal function of detection. Incubation times have tolerances of ± 5 minutes, incubation temperatures have tolerances of ± 2 °C. To ensure that the reagents are properly mixed and to minimize the measuring time per sample we recommend to proceed as follows: agitate the plate for 15-30 seconds with an orbital shaker operated with 600-800 rpm before each incubation step or right before reading the measured value. If the results cannot be read immediately after the last incubation step, the plate can be stored temporarily for a maximum of 30 minutes at 2-8 °C.
1. Add 25 µl of negative control (NEG M) to well A1.
2. Add 25 µl of positive control (POS M) to well B1.
3. Add 25 µl of reference control (REF M) to well C1.
4. Add 25 µl of diluted serum sample to the micro-wells required.
5. Add 25 µl of bead mix (BMM) to each well.
6. Seal microtitre plate with adhesive foil.
7. Incubate for 60 minutes at 37 °C in the dark.
8. Add 50 µl of conjugate (CON M) to each well.
9. Seal microtitre plate with adhesive foil.
10. Incute for for 60 minutes at 37 °C in the dark
11. Read plate on the Luminex analyzer immediately.

10. Quality Control
The values of the positive, reference and negative controls serve as quality control parameters for the performance of the whole assay. For a reliable evaluation, the ratio of the MFI-values of the reference and positive control (quotient) must be within the range indicated in the batch certificate. The negative control must be negative for all antigens.
The fluorescence signal of the control bead set CR1 serves as a validity criterion for the testing of individual samples in all wells. Addition and ratio of diluted serum sample/conjugate is controlled by CR1. If properly performed, the measured result must be within the range indicated in the batch certificate.

11. Test Evaluation
The Luminex system analyses the fluorescence signal of 70 beads of each individual bead set per well. The fluorescence signals are evaluated by the software of the Luminex analyzer and are saved in a folder named
with the test run in the “Output.csv” file. The fluorescence intensities of each sample are listed in tabular form under "Data Type: Median". The cut-off value specific for this test run is calculated from the result of the reference control in well C1 for each bead set by means of the cut-off range indicated in the batch certificate as follows:

\[
\text{Measured value}_{\text{REF.M.}A_{\text{REF}}} = \text{Cut-off}_{A_{\text{REF}}}
\]

The fluorescence intensities of the beads incubated with the serum samples ("measured values") are divided by the cut-off of the corresponding bead sets to calculate the cut-off index:

\[
\text{Measured value}_{\text{y,x sample.}A_{\text{sample}}} = \frac{\text{Cut-off}_{A_{\text{sample}}}}{\text{index}_{\text{sample}}}
\]

The cut-off index indicates by which factor the fluorescence of the respective sample determined on this bead set is below or above the calculated test-specific cut-off value.

The single cut-off indices of the samples are summed up, whereas only the higher OspC-value contributes to the evaluation:

\[
\sum \text{index}_{\text{sample}} = \text{Cut-off} - \text{index}_{\text{sample}}
\]

- **Negative**: Cut-off index < 2.5
  - No antibodies to antigens of these bead sets detected.
- **Weakly positive**: 2.5 ≤ Cut-off index < 3.0
  - Antibodies to antigens of these bead sets suspected.
- **Positive**: Cut-off index ≥ 3.0
  - Antibodies to antigens of these bead sets detected.

12. Interpretation of Results

Based on the detection of specific antibodies to different Borrelia antigens and their combination, the following evaluation scheme can be applied:

1. **Cut-off index < 2.5**
   - No suspicion of Lyme borreliosis immunoserologically
2. 2.5 ≤ Cut-off index < 3.0
   - Lyme borreliosis not reliably excluded immunoserologically
3. **Cut-off index ≥ 3.0**
   - Strong suspicion of Lyme borreliosis immunoserologically

In the case of 2 and 3 the sample should be tested using Optiplex Borrelia IgG / IgM Test.

Upon request, DiaMex GmbH will provide free of charge a software for automatic control of quality parameters and evaluation of the measured values.

13. Test Characteristics

Intra-assay precision was determined from 12 assays of sera and CSF reactive to all antigens used in Optiplex Borrelia IgM Screening Test.

- Inter-assay coefficient of variation: CV < 8.0 %

Inter-assay precision was determined from 5 independent tests of sera reactive to all antigens used in Optiplex Borrelia IgM Screening Test.

- Inter-assay coefficient of variation: CV < 6 %

14. Limitations

The results obtained with the Optiplex Borrelia IgM Screening Test should be interpreted only in combination with the clinical diagnosis. In the serological characterization of patients with a clinical suspicion of Lyme borreliosis the results of both the Optiplex Borrelia IgM Test and the Optiplex Borrelia IgG Test should be included.

Antibiotics therapy applied in the early stage of the infection can suppress an immunological response to Borrelia spec.. Polyclonal B-cell activation (e.g. by EBV infections), autoimmune diseases or immunodeficiencies can lead to erroneous results (8, 10). Such results are to be excluded by differential diagnosis.

Flagellin antigens (p41) are also common with other genera of spirochaetes (Treponema spec., Leptospira spec.) and may induce respective cross-reactivities (7, 8, 9).

Plasmid-coded antigens (e.g. OspC) may be laterally transferred also to other species (11). Cross-reactions can occur with similar antigens of other pathogenic bacteria (e.g. Yersinia and Chlamydia).

In rare cases sera may bind unspecifically to the bead sets. Unspecific binding is often indicated by low cut-off indices on the early Borreliosis marker antigens, whereas the later phase markers typical for an IgM-response show higher indices. Such sera should be analyzed by alternative technologies.
15. Trouble Shooting

Test results of the controls are outside of the cut-off range
→ Check the pipetting volume of the controls and of the conjugate;
→ Repeat the test of the controls.
If the problem should persist, please contact the local distributor.

CR1 results of serum samples are outside of the cut-off range
→ Check the pipetting volume of the serum samples and the serum dilution;
→ Repeat test with freshly diluted sera.
If the problem should persist, please contact the local distributor.
Rarely, single sera (especially sera with high- or low-antibody titre) may lead to test results outside the ranges. In these cases, determination of the antibody titre and, if necessary, an individually adapted dilution are recommended.
Considerably scattered CR1 results within a test run indicate of an inhomogeneous dilution or poor mixing.

16. References

**Protocol Summary for Optiplex Borrelia IgM Screening Test**

Prepare serum dilution, 1:201:
1+200: 5 µl serum + 1000 µl buffer

<table>
<thead>
<tr>
<th>Samples:</th>
<th>25 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG M, REF M, POS M, (undiluted), Serum samples (diluted), see pipetting scheme</td>
<td></td>
</tr>
</tbody>
</table>

Add **BMM** to each well (mix just prior to use!)  

Add **CON M** to each well  

Agitate for 15 - 30 sec with 600 - 800 rpm, incubate for 60 minutes at 37 °C in the dark

Agitate for 15 - 30 sec with 600 - 800 rpm, incubate for 60 minutes at 37 °C in the dark

Agitate for 15 - 30 sec with 600 - 800 rpm
Read immediately

### Pipetting scheme

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<tbody>
<tr>
<td>A</td>
<td>NEG M</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>POS M</td>
<td></td>
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</tbody>
</table>
Settings for the Creation of Templates for Optiplex Borrelia IgM Screening Test
with the Luminex IS Software (version 2.2 SP1 and higher)

Entries - page 1:

Template Name: DM_BorScreen_IgM
Version No.: 1.0
Template Type: Data collection only
Sample Vol. (µl): 50
Sample Timeout (sec): 70
Doublet Discriminator Gate Low Limit: 6800
High Limit: 16000

Entries - page 2:

Tests: 4

<table>
<thead>
<tr>
<th>Name</th>
<th>Units</th>
<th>Description</th>
<th>Bead ID</th>
<th>MinBeads</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>MFI</td>
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<td>62</td>
<td>70</td>
</tr>
<tr>
<td>LysMMS</td>
<td>MFI</td>
<td></td>
<td>17</td>
<td>70</td>
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<tr>
<td>OspCPBi</td>
<td>MFI</td>
<td></td>
<td>54</td>
<td>70</td>
</tr>
<tr>
<td>OspCPKo</td>
<td>MFI</td>
<td></td>
<td>52</td>
<td>70</td>
</tr>
</tbody>
</table>

When using the evaluation software provided by DiaMex GmbH, the sequence and spelling of the bead sets have to be strictly observed.

Entries - page 3:

Template Commands
- Wash from reservoir
- Acquire Test specimen

For back-up of the templates: Save + Export

Index of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>⎯</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>⎯</td>
<td>Tests per kit</td>
</tr>
<tr>
<td>⎯</td>
<td>LOT</td>
</tr>
<tr>
<td>⎯</td>
<td>Catalog Number</td>
</tr>
<tr>
<td>⎯</td>
<td>Store at</td>
</tr>
<tr>
<td>⎯</td>
<td>For in vitro diagnostic use only</td>
</tr>
<tr>
<td>⎯</td>
<td>Use by Manufacturer</td>
</tr>
<tr>
<td>⎯</td>
<td>European Conformity</td>
</tr>
</tbody>
</table>

When using the evaluation software provided by DiaMex GmbH, the sequence and spelling of the bead sets have to be strictly observed.