recomBead Borrelia IgG 2.0
recomBead Borrelia IgM 2.0

[IVD]

Instructions for use (English)

1 Purpose
The recomBead Borrelia 2.0 is a qualitative in vitro test for the detection of IgG and IgM antibodies against Borrelia burgdorferi sensu stricto, B. garinii, B. afzelii, B. bavariensis (formerly B. garinii OspA type 4 = B. garinii 1) and B. spielmani in human serum, plasma or cerebrospinal fluid.

2 Field of application
The recomBead Borrelia 2.0 is used to confirm samples that were positive or uncertain in the screening test. In addition, it can be used to detect human IgG and IgM antibodies formed intrathecally in the cerebrospinal fluid [cerebrospinal fluid/serum pairs].

The recomBead Borrelia 2.0 enables reliable identification of specific antibodies against selected antigens of human-pathogenic B. burgdorferi s.l. species through the individual antigens immobilised on distinct microparticles. Antibodies against certain antigens can provide additional information on the stage of infection.

2.1 Licence information
On purchasing the IVD (in vitro diagnostic) recomBead Borrelia 2.0, based on fluorescing MagPlex® microparticles from the company LumineX®, the client agrees to the licence condition of LumineX®, that this test or its components are used exclusively in association with the LumineX100, LumineX200 or MAGPIX® analysis system.

3 Test principle
Highly purified, recombinant Borrelia burgdorferi s.l. antigens (OspA, OspC, p100, VlsE, p39, p58, p18 (= DpbA, Decorin binding protein A)) are applied separately to different microparticles (beads) with differing fluorescence codings. Antibodies against individual Borrelia burgdorferi s.l. antigens are recorded separately from each other in one solution. Detection of the antibodies corresponds to the Immunoblot test principle.

1. The antigen particles are incubated with the diluted serum/plasma or cerebrospinal fluid sample, where specific antibodies attach to the pathogen antigens on the microparticles.
2. Unbound antibodies are then washed away.
3. In a second step, the microparticles are incubated with anti-human immunoglobulin antibodies (IgG or IgM), which are coupled to R-Phycoerythrin (PE).
4. Unbound conjugate antibodies are then washed away.
5. Specifically bound antibodies are detected by the fluorescence of R-Phycoerythrin when excited by light. If an antigen-antibody reaction has occurred, the typical fluorescence appears on the surface of the microparticles and can be measured with the LumineX analysis system.

In addition to the antigen-coated microspheres four controlbeads are included in the mixture:
- An incubation control, which must display a reaction in every solution upon addition of serum or plasma.
- A conjugate control, which must display a reaction in every solution upon addition of conjugate.
- A conjugate control, which distinguishes IgG or IgM conjugate.
- A negative control, which must not exceed a defined threshold when the test is performed.

4 Reagents
4.1 Package contents
The reagents of one pack are sufficient for 96 assays. Each set of reagents contains:

8 DILUBUF ×5
100 ml wash and dilution buffer (six-fold concentration)
Contains Tris buffer, NaCl, detergent, preservative MIT (0.06%) and Oxypyrion (0.6%) and protein

1 LMP
2 96-well microtitre plates

1 TAPE
3 sealing films

1 INSTUT
1 instructions for use

1 LOTCERT
1 batch certificate with cut-off values

4.1.1 recomBead Borrelia IgG 2.0
In addition to the components listed under point 4.1, each set of reagents contains:

5.5 ml microparticle suspension, coated with recombinant B. burgdorferi antigens (ready-to-use, black sealing cap), contains preservative MIT (0.01%) and Oxypyrion (0.1%)

4.1.2 recomBead Borrelia IgM 2.0
In addition to the components listed under point 4.1, each set of reagents contains:

5.5 ml microparticle suspension, coated with recombinant B. burgdorferi antigens (ready-to-use, black sealing cap), contains preservative MIT (0.01%) and Oxypyrion (0.1%)

4.2 Additionally required reagents, materials and equipment
- Deionised water (high quality)
- Measuring cylinder
- ELISA washer with magnetic base plate
- Equipment for producing the serum dilutions (e.g. micro and multipipettes, 8-channel pipette 10 - 100 μl, Masterblock or reaction containers, vortexer, plate shaker)
- Incubator (37°C)
- LumineX100, LumineX200 or MAGPIX® analysis system
- recomQuant-Software from Mikrogen incl. parameter-specific template for IS- or xPONENT software
- Timer
- Disposable protective gloves
- Waste receptacle for biohazardous substances
- If required, positive and negative controls are available from MIKROGEN. These controls are not necessary for implementing and evaluating the test.

5 Shelf life and handling
- Store reagents away from light at +2°C - +8°C before and after use. do not freeze.
- The same components (wash and dilution buffers, conjugate) from different recomBead 2.0 tests can be used across parameters and batches. In doing so, attention must be paid to the shelf life of these components.
- Mix the patient samples thoroughly before use.
- The container with the particle mixture (Beadmix) must be vortexed thoroughly immediately prior to each use (> 30 sec., max. rpm), in order to attain an even suspension.
- Conjugates and particle mixtures that are not needed remain in the tube and continue to be stored at +2°C - +8°C (reseal tube properly).
- The packages have an expiry date, after which no further guarantee of quality can be given.
- Keep the kit components away from direct sunlight throughout the test procedure.
- Unused wells can be covered with sealing film.
- The test must only be performed by trained and authorised qualified personnel.
- If substantial changes are made by the user to the product or the directions for use, usage may be beyond the intended purpose specified by Mikrogen.

6 Warnings and safety precautions
- Only use for in vitro diagnostics.
- All blood products must be treated as potentially infectious.
Suitable disposable protective gloves must be worn during the entire test procedure.

The conjugates contain Proclin (0.1%). The wash and dilution buffer and the particle mixtures contain MIT (Methylisothiazolzone) and Oxypryn. Avoid contact with the skin or mucous membranes.

The LumineX100, LumineX200 or MAGPIX® analysis system requires system fluid from a reservoir and discharges this as waste after the reading. Clear separation of supply and waste container must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly. All aspirated fluids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens. All reagents and materials, which come into contact with potentially infectious samples, must be treated with suitable disinfectants or be disposed of according to their hygiene requirements. The concentration specifications and incubation times of the manufacturer must be taken into consideration.

Individual wells of the microtitre plate must only be used once.

Do not replace or mix the reagents with reagents of other manufacturers.

Read through and carefully follow the entire instructions for use before performing the test. Deviations from the test protocol presented in the instructions for use can lead to false results.

7 Sampling and preparation of reagents

7.1 Sample material

The sample material can be serum, plasma (CPD, EDTA, citrate, heparin) or cerebrospinal fluid. Serum and plasma should be separated from the blood clot as quickly as possible after removal, to avoid haemolysis. Microbial contamination of the sample must absolutely be avoided. Non-soluble substances must be removed from the sample prior to incubation. Use of heat-inactivated, icteric, haemolytic, lipaemic or cloudy samples is not recommended.

Caution! If the tests are not carried out immediately, the sample material can be stored for up to 2 weeks at +2°C - +8°C. Longer storage of the samples is possible at -20°C or lower. Repeated freezing and thawing of the samples is not recommended due to the risk of false results.

7.2 Preparation of the ready-to-use wash and dilution buffer

This buffer is required for the washing steps and for the sample dilution. The buffer concentrate is diluted 1 + 5 with deionised water. 24 ml of ready-to-use buffer (4 ml concentrate + 20 ml deionised H₂O) are prepared for one 8-well microtitre plate strip. Ready-to-use wash and dilution buffer can be stored at +2°C - +8°C for four weeks.

8 Test procedure

<table>
<thead>
<tr>
<th>No.</th>
<th>Implementation</th>
<th>Note</th>
</tr>
</thead>
</table>
| 1   | Preparation of the samples (and, where applicable, controls) | Prior to use, the sample must be pre-diluted 1 + 50. Controls must be ordered separately, they are not necessary for the test evaluation.
|     | Prepare 500 µl buffer, add 10 µl non-diluted sample | Mix thoroughly! |
| 2   | Preparation of the particle mixture | Homogeneous particle suspension must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly.
|     | The particle mixture is mixed thoroughly (> 30 sec, max. rpm). | Add 50 µl of the reconstituted particle mixture to the equilibrated wells. At least one value is created for each control and patient sample. |
| 3   | Preparation of the microtitre plate | Vortex the particle mixture (Beadmix) before every use, to guarantee a homogeneous suspension. |
| a)  | The required strips of the microtitre plate are equilibrated with 50 µl buffer per cavity. | At least one value is created for each control and patient sample. |
| b)  | Empty the wells by aspiration. | Empty the wells by aspiration, but do not tap out! |
| 4   | Sample incubation | Empty the wells by aspiration, but do not tap out! |
| a)  | 50 µl of the resuspended particle mixture are placed in the equilibrated wells. | Homogeneous particle suspension must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly.
| b)  | 50 µl of the diluted samples are added per well. | Homogeneous particle suspension must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly. |
| c)  | The samples and the particle mixture are mixed briefly on a shaker. | Empty the wells by aspiration; do not tap out! |
| d)  | Incubate for 20 minutes at 37°C. | Perform wash steps 8.5b - 8.5c a total of five times. |
| 5   | Wash (5x) | Empty the wells by aspiration on a magnetic plate. |
| a)  | Empty the wells by aspiration on a magnetic plate. | Caution, the microtitre plate must not be tapped out! The microtitre plate must lie correctly on the magnetic plate! |
| b)  | Pipette 200 µl buffer into each well and incubate for at least 30 sec. | Perform wash steps 8.5b - 8.5c a total of five times. |
| c)  | Empty the wells by aspiration on a magnetic plate. | The microtitre plate must lie correctly on the magnetic plate! |

8.1 Cerebrospinal fluid/serum analysis

Separate instructions and a patient record are available for the detection of human IgG and IgM antibodies formed intrathecally in the cerebrospinal fluid (cerebrospinal fluid/serum pairs). You can request or download the current versions from Mikrogen (+49 89 54801-0) (www.mikrogen.de ➔ Downloads).

9 Results

Caution: Do not use the automated interpretation without taking note of the advice on the interpretation described below.

9.1 Validation – Quality control

The test can only be evaluated when the following criteria are fulfilled:

1. The incubation control must be positive, in the event of insufficient signal strength, an error message occurs in the evaluation program recomQuant ("no serum").
2. The conjugate control must be positive, in the event of insufficient signal strength, an error message occurs in the evaluation program recomQuant ("no conjugate").
3. The negative control must be negative, if a defined signal strengtch is exceeded an error message occurs in the evaluation program recomQuant ("insufficient beads").
4. A defined number of particles must have been evaluated from all used particle regions (corresponding to the attached antigens). If fewer particles were determined in the prescribed time interval, an error message occurs in the evaluation program recomQuant ("insufficient beads").

If one of these error messages occurs, testing of the sample is not valid and the sample is not evaluated.

9.2 Evaluation of the antigen reactivities

The averaged fluorescence intensities of the antigen reactivities of each sample are compared with the reactivities of the incubation control and a Cutoff-Index (COI) is calculated via a batch-dependent limit value. Conversion of the raw data of the Luminex analysis system is made with the calculation formula provided in the evaluation program recomQuant.

Evaluation of the individual antigen reactivities is summarised in the following table.

<table>
<thead>
<tr>
<th>Signal strength</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below the limit value (COI &lt; 0.67)</td>
<td>negative</td>
</tr>
<tr>
<td>equal to or above the limit value (COI ≥ 1.00)</td>
<td>positive</td>
</tr>
<tr>
<td>very clearly above the limit value (COI is no longer calculated)</td>
<td>highly positive</td>
</tr>
</tbody>
</table>

Table 1: Evaluation of fluorescence intensity (signal strength) in relation to the limit value

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9.3 Interpretation of the test results
For reliable and simple conversion of the analysis specifications from MIQ "Lyme borreliosis" and DIN 58969-44, a point evaluation of the Borrelia burgdorferi antigens was established in the recomBead Borrelia 2.0.

The test result is obtained by adding the corresponding point values in the case of borderline and positive results of individual antigens. Antigens rated as positive enter the summation with their full point count, borderline antigens in each case contribute only 1 point to the point evaluation.

It must be noted that for reaction of the OspC and p18 antigens, the point value is only calculated once, regardless of which and how many of the OspCs and/or p18 react.

10 Limits of the method, restrictions
- Serological test results must always be viewed in context with the clinical picture. Therapeutic consequences of the serological finding must be connected with the clinical data.
- A negative recomBead Borrelia 2.0 test result cannot rule out infection with Borrelia burgdorferi. Especially in the early phase of infection, antibodies may not yet be present or may be present in undetectable amounts. Antibiotic treatment in the early stage can prevent formation of detectable antibodies. In case of clinical suspicion of Lyme borreliosis and negative or unclear serum results, sampling and testing should be carried out again after three weeks.
- A positive result in the recomBead Borrelia IgG and/or IgM 2.0 does not mean that active Lyme borreliosis is present in every case. As IgG antibodies and occasionally also IgM antibodies persist over a longer period, antibodies of a recent infection may have been detected.
- In case of clinical suspicion of neuroborreliosis, detection of intrathecally formed antibodies should be performed. For this, it is necessary to determine cerebrospinal fluid/serum pairs in parallel in a suitable test (e.g. recomBead Borrelia). Instructions from MiKROGEN can be obtained for these determinations (Cerebrospinal fluid diagnostics Borrelia burgdorferi).
- A reaction with OspC is very characteristic for the early immune response (IgM). With sera from late stages of infection (IgG), there is usually a strong reaction with the following antigens p100, VlsE, p58, p39 and p18. By contrast, antibodies against OspA are found less commonly. VlsE is a very early marker of the IgG response, but also frequently accompanies the immune response in late manifestations and then arises alongside p100 and or p18.
- Selective use of the recombinant Borrelia burgdorferi antigens extensively rules out any cross-reaction with antibodies, which are induced through infection with the causative agent of Syphilis (Treponema pallidum). Syphilis infection should be ruled out in the case of unclear Borrelia serology.
- Polyclonal stimulation of B-lymphocytes can occur in the case of infectious mononucleosis (glandular fever, EBV infection). This can lead to non-specific reactions when detecting IgM class antibodies. It is recommended to rule out EBV infection by differential diagnosis in the case of unclear anamnesis and presence of a weak IgM response.

11 Performance characteristics

11.1 Diagnostic sensitivity

<table>
<thead>
<tr>
<th>Number</th>
<th>IgG-positive</th>
<th>IgM-positive</th>
<th>IgG/IgM-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyme arthritis</td>
<td>27 (100%)</td>
<td>6 (30%)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>ACA*</td>
<td>11 (100%)</td>
<td>3 (27%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Neuroborreliosis</td>
<td>30 (93%)</td>
<td>16 (53%)</td>
<td>29 (97%)</td>
</tr>
<tr>
<td>Erythema migrans</td>
<td>38 (74%)</td>
<td>27 (71%)</td>
<td>34 (89%)</td>
</tr>
</tbody>
</table>

*Acrodernatitis chronica atrophicans

11.2 Diagnostic specificity

<table>
<thead>
<tr>
<th>Two comparison tests negative</th>
<th>recomBead Borrelia 2.0</th>
<th>Number</th>
<th>IgG (n=173)</th>
<th>IgM (n=190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>166</td>
<td>179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclear</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>94%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11.3 Prevalence rate

<table>
<thead>
<tr>
<th>Blood donor sera*</th>
<th>Number</th>
<th>IgG-positive</th>
<th>IgM-positive</th>
<th>IgG/IgM-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>53 (13.3%)</td>
<td>28 (6.5%)</td>
<td>74 (18.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*From Southern Germany

11.4 Analytical specificity
The analytical specificity is defined as the suitability of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) Interferences: Control studies on potentially interfering factors have shown that the test performance is not influenced by anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis or lipaemia of the sample. False positive results occurred in cases of icteric sera.

b) Cross-reactions: Potential interference of antibodies against other organisms (Treponema) were investigated in control studies. In addition, conditions were tested, which are attributable to atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, pregnancy, EBV infection, CMV infection). No cross-reactions were detected.

12 Literature


Upon request, we are pleased to send you additional literature on the diagnosis of Lyme borreliosis.

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### 13 Explanation of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>∑</td>
<td>Contents are sufficient for &lt;n&gt; formulations</td>
</tr>
<tr>
<td>DILUBF</td>
<td>Wash and dilution buffer (six-fold concentration)</td>
</tr>
<tr>
<td>MTP</td>
<td>96-well microtitre plates</td>
</tr>
<tr>
<td>TAPE</td>
<td>Sealing film</td>
</tr>
<tr>
<td>INSTRU</td>
<td>Instructions for use</td>
</tr>
<tr>
<td>LOTCERT</td>
<td>Batch certificate</td>
</tr>
<tr>
<td>BEADMIX</td>
<td>Microparticle suspension</td>
</tr>
<tr>
<td>CONJ IgG</td>
<td>Anti-human IgG conjugate</td>
</tr>
<tr>
<td>CONJ IgM</td>
<td>Anti-human IgM conjugate</td>
</tr>
<tr>
<td></td>
<td>Take note of the instructions for use</td>
</tr>
<tr>
<td>CONV</td>
<td>Contents, contains</td>
</tr>
<tr>
<td>INV</td>
<td>In vitro diagnostic</td>
</tr>
<tr>
<td>BLOT</td>
<td>Batch number</td>
</tr>
<tr>
<td>REFR</td>
<td>Order number</td>
</tr>
<tr>
<td></td>
<td>Do not freeze</td>
</tr>
<tr>
<td></td>
<td>Use by Expiry date</td>
</tr>
<tr>
<td></td>
<td>Storage at x°C to y°C</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
</tbody>
</table>

### 14 Manufacture and version dates

| recomBead Borrelia IgG 2.0 | Article no. 4254 |
| recomBead Borrelia IgM 2.0 | Article no. 4255 |

**Instructions for use valid from**

| recomBead Borrelia IgG 2.0 | Article no. 4254 |
| recomBead Borrelia IgM 2.0 | Article no. 4255 |

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GARXBB009EN 2016-06

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