Cerebrospinal fluid / Serum analytics
**recomWell Borrelia IgG, IgM**
**recomBead Borrelia IgG, IgM 2.0**
Evaluation Software

[IVD]

Instructions for Use (English)

1 **Intended use**
In the case of clinically suspected neuroborreliosis, assaying for intrathecally produced antibodies should be performed. For this purpose, CSF/serum pairs have to be examined in parallel in the **recomWell Borrelia**. The results can subsequently be verified with the **recomBead Borrelia 2.0**, in line with the usual two-step serodiagnosis. For CSF diagnostics (Reiber’s hyperbolic formula), MIKROGEN software solutions can be used (**recomWell** and **recomQuant** CSF diagnostics evaluation software).

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</table>

3 **recomWell Borrelia IgG, IgM**

3.1 **Sampling and Preparation**

3.1.1 **Samples (serum and CSF)**
Serum (EDTA, citrate, heparin, CPD) and cerebrospinal fluid from the patient must be collected on the same day. The use of heat-inactivated, icteric, haemolytic, lipaemic or cloudy samples is not recommended. Haemolytic CSF, in particular, can produce incorrect results.

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

3.1.2 **Preparation of solutions**
The detection reagents are sufficient for 96 IgG and / or IgM analyses. The following quantities apply to the processing of a single microtitre plate strip with 8 wells. Where several microtitre plate strips are used, the specified quantities must be multiplied with the number of microtitre plate strips used. The device-specific dead volume must be taken into account. The dilution buffer, substrate and stop solution are ready-to-use.

3.1.2.1 **Preparation of ready-to-use wash buffer**
The wash buffer concentrate is diluted 1 + 9 with H2O (deionised water). For each microtitre plate strip with 8 wells, 5 mL concentrate is mixed with 45 mL H2O (deionised water). The ready-to-use wash buffer can be stored for four weeks at +2°C to +8°C or at room temperature for one week.

3.1.2.2 **Preparation of conjugate solution**
For each microtitre plate strip with 8 wells, 1 mL of dilution buffer and 10 µL of anti-human IgG peroxidase conjugate (red cap) or IgM peroxidase conjugate (green cap) are transferred to a clean container and mixed well (dilution 1 + 100). The conjugate solution must be prepared just before use. The ready-to-use conjugate solution cannot be stored.

3.2 **Test procedure**

3.2.1 **Screening of CSF/serum pairs in the recomWell Borrelia**
To prevent deviations from test to test, serum and CSF must always be measured at the same time in the same test run. To increase diagnostic safety, we recommend preparing duplicate patient serum and CSF samples.

3.2.2 **Standard sample dilution and dilution of controls**
Pre-dilution should be carried out in polypropylene tubes! To avoid a subsequent dilution of serum (see 8.2), a second assay with a 1:500 dilution of serum can be started in parallel to the first assay with the standard 1:101 dilution.

<table>
<thead>
<tr>
<th>No.</th>
<th>Execution</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Preparing samples and controls</td>
<td>Samples and controls must only be diluted immediately prior to the test. Controls should be run simultaneously with the sample to be tested.</td>
</tr>
<tr>
<td>2</td>
<td>Washing</td>
<td>It is recommended to use a corresponding ELISA wash device for this step.</td>
</tr>
<tr>
<td>3</td>
<td>Incubation of samples</td>
<td>Assign at least one value from the negative control, positive control and patient samples. The cut-off control must be assigned twice. Preferably, a cut-off control should be included at the beginning of the series and at the end of the series respectively. In manual processing, cover the microtitre plate tightly with new, unused cover film. Use the incubation chamber at +37 °C.</td>
</tr>
<tr>
<td>4</td>
<td>Substrate reaction</td>
<td>Masking of the plate is not required. Protect from direct sunlight. The time is calculated from pipetting the first well.</td>
</tr>
<tr>
<td>5</td>
<td>Stoping the reaction</td>
<td>Do not remove the substrate solution before adding the stop solution! Follow the same pipetting scheme as for the substrate solution.</td>
</tr>
<tr>
<td>6</td>
<td>Measurement of extinction values</td>
<td>Zero adjustment is made against air. The measurement must be made within 60 minutes of stopping the reaction.</td>
</tr>
</tbody>
</table>

Caution!
Incubation solutions must not spill into other wells. Splashing must be avoided, particularly when placing and removing the covering film.

3.2.4 **Evaluation**
Cut-off (limit) = the mean value is calculated from the extinction values of both cut-off controls (at the beginning and at the end of the series).

3.2.4.1 **Validation - Quality Control**
The test can be evaluated under the following conditions:
- The individual extinction values of the double analysis of the cut-off control do not deviate by more than 20% from their mean value.

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• Negative control extinction value ≤ 0.150
• Cut-off control extinction value - Negative control extinction value ≥ 0.050
  (E_{cut} - E_{neg. contr.} ≥ 0.050)
• Positive control extinction value - Cut-off control extinction value ≥ 0.300
  (E_{pos. contr.} - E_{cut} ≥ 0.300)

3.2.4.2 Threshold values
In the case of the qualitative evaluation, the threshold for the evaluation of sera and CSF is calculated as follows:

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>Dilution buffer</th>
<th>CSF dilutions</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>50 µl</td>
<td>50 µl</td>
<td>Mix directly in well</td>
</tr>
<tr>
<td>1:4</td>
<td>75 µl</td>
<td>20 µl</td>
<td></td>
</tr>
<tr>
<td>1:5</td>
<td>90 µl</td>
<td>20 µl</td>
<td></td>
</tr>
<tr>
<td>1:10</td>
<td>90 µl</td>
<td>10 µl</td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td>95 µl</td>
<td>5 µl</td>
<td></td>
</tr>
<tr>
<td>1:40</td>
<td>300 µl</td>
<td>10 µl</td>
<td>Mix in tube</td>
</tr>
<tr>
<td>1:200</td>
<td>400 µl</td>
<td>100 µl from 1:40</td>
<td></td>
</tr>
<tr>
<td>1:1000</td>
<td>400 µl</td>
<td>100 µl from 1:200</td>
<td></td>
</tr>
</tbody>
</table>

With a number of suitable OD values for serum and CSF, the intrathecal antibody index (AI) can be quantified by transferring extinctions and clinical chemistry values (albumin, total Igg and/or IgM) to the evaluation programme (see CSF/serum analytics evaluation programme). Mikrogen can provide you with a calculation template if you prefer to calculate the AI manually.

With a serum value of <0.100 and CSF results above the threshold, an extinction of 0.100 is assumed for the unit calculation of the serum.

3.2.5 Determination of Borrelia-specific antibody concentrations in serum and CSF

For the conversion of the extinctions of CSF and serum into units, extinction values between 0.100 and 2.000 are required (linear part of a dilution curve). It is important to ensure that the OD values of serum and CSF are not too far apart (difference <1). If the extinction values for serum and CSF exceed 2.000 or are outside the measuring range, the CSF/serum pair must be assayed again together in a higher dilution. We recommend the following dilutions:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>dition)</td>
<td>_tion for other dilutions</td>
<td>dition)</td>
<td>_tion for other dilutions</td>
<td></td>
</tr>
<tr>
<td>≤ 2.000</td>
<td>No other dilutions</td>
<td>≤ 2.000</td>
<td>No other dilutions</td>
<td></td>
</tr>
<tr>
<td>&gt; 2.000 - 3.000</td>
<td>1:500</td>
<td>&gt; 2.000 - 3.000</td>
<td>1:1000, 1:2000, 1:4000</td>
<td></td>
</tr>
<tr>
<td>&gt; 3.000</td>
<td>1:1000, 1:2000, 1:4000</td>
<td>&gt; 3.000</td>
<td>1:8000, 1:16000</td>
<td></td>
</tr>
</tbody>
</table>

Mikrogen recommends the following pipetting schedules:

<table>
<thead>
<tr>
<th>Serum dilutions</th>
<th>Dilution buffer</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:101</td>
<td>1000 µl</td>
<td>10 µl</td>
</tr>
<tr>
<td>1:500</td>
<td>800 µl</td>
<td>200 µl from 1:101</td>
</tr>
<tr>
<td>1:1000</td>
<td>900 µl</td>
<td>100 µl from 1:101</td>
</tr>
<tr>
<td>1:2000</td>
<td>500 µl</td>
<td>500 µl from 1:1000</td>
</tr>
<tr>
<td>1:4000</td>
<td>500 µl</td>
<td>500 µl from 1:2000</td>
</tr>
<tr>
<td>1:8000</td>
<td>500 µl</td>
<td>500 µl from 1:4000</td>
</tr>
<tr>
<td>1:16000</td>
<td>500 µl</td>
<td>500 µl from 1:8000</td>
</tr>
</tbody>
</table>

Antibody index values between 0.6 ≤ AI ≤ 1.3 are within the normal range. An intrathecal synthesis of Borrelia antibodies is unlikely. The detected Borrelia-specific antibodies have entered the subarachnoid space by passive transfer from the serum via the blood-CSF barrier. Antibody index values of > 0.6 indicate an error in the analysis (e.g. clinical chemistry). It is recommended to verify whether the serum and CSF were sampled on the same day and/or whether the samples got mixed up. Where necessary, the determination of clinical chemistry and/or Borrelia-specific antibodies may be repeated.

Antibody index values between 1.3 and 1.5 are borderline. Retesting of the CSF/serum pair is recommended. In the case of clinical issues, testing of a new CSF/serum pair over the course of the disease is recommended.

Antibody index values ≥1.5 are considered to be pathological. They indicate the presence of an intrathecal synthesis of Borrelia-specific antibodies, like those found in patients with neuroborreliosis.

We recommend examining pathological or borderline results using a confirmatory test (see CSF/Serum analytics recomBead Borrelia IgG, IgM).

3.3 Limitations of the method - restrictions

• Serological test results must always be viewed in the context of the patient's clinical picture. Any treatment resulting from the serological findings must always be based on the clinical data.

• A normal result does not exclude CSF infection with Borrelia burgdorferi. Particularly in the early stages of infection, antibodies may not be present or not present in a detectable quantity. In the early stages, antibiotic treatment may prevent the development of detectable antibodies. With clinically suspected neuroborreliosis and negative and/or inconclusive serum results, further sampling after three weeks and testing should be carried out.

• A pathological antibody index in IgG and/or IgM results is not always an indication of active neuroborreliosis. Since intrathecal antibodies have a long-term persistence, the presence of antibodies is evidence of a previous infection.

• Intrathecal synthesis of IgM antibodies is no indication of a recent infection.

• Cross-reactions with antibodies, produced by infection with spilth pathogens (Treponema pallidum), recurrent fever (Borrelia recurrentis, Borrelia duttonii, Borrelia hermsii) or leptospirosis (Leptospira sp.) can be largely excluded due to the selective use of recombinant Borrelia burgdorferi antigens. Isolated antibody activities against the antigen p41 were found in an active Lues infection and/or a Lues serum scar. With inconclusive Borrelia serology, a Lues infection should be excluded.

• An infectious mononucleosis (Pfeiffer’s gland fever, EBV infection) may result in polyclonal stimulation of B lymphocytes. This can lead to unspecific reactions during the detection of antibodies of the IgM class. If the medical history is unclear and in the presence of a weak IgM response, it is recommended to exclude an EBV infection by differential diagnosis.
3.4 Test performance
A total of 38 CSF/serum pairs were examined with recomWell Borrelia. Pathological and borderline antibody index values were retested with recomBead Borrelia. The samples were divided into groups, as described in Table 1.

<table>
<thead>
<tr>
<th>n</th>
<th>Known clinical data</th>
<th>Anti-B. antibodies in serum</th>
<th>IgG AI pathol.</th>
<th>IgM AI pathol.</th>
<th>IgG: Confirmation by recomBead*</th>
<th>IgM: Confirmation by recomBead*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Neuroborreliosis</td>
<td>Yes</td>
<td>10 (100%)</td>
<td>7 (70%)</td>
<td>10/10 (100%)</td>
<td>5/7 (71%)</td>
</tr>
</tbody>
</table>

Cell count >5/µl and impaired blood-CSF barrier (in at least one, where necessary, several samples from a patient) and intrathecal Ig synthesis (in at least one, where necessary, several samples from a patient), specific anti-Bb antibodies in serum.

<table>
<thead>
<tr>
<th>n</th>
<th>Known clinical data</th>
<th>Anti-B. antibodies in serum</th>
<th>IgG AI normal</th>
<th>IgM AI normal</th>
<th>IgG: Confirmation by recomBead*</th>
<th>IgM: Confirmation by recomBead*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Serological positive</td>
<td>yes</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>10/10 (100%)</td>
<td>10/10 (100%)</td>
</tr>
</tbody>
</table>

Cell count <5/µl, intact blood-CSF barrier, no intrathecal Ig synthesis, no specific anti-Bb antibodies in serum.

<table>
<thead>
<tr>
<th>n</th>
<th>Known clinical data</th>
<th>Anti-B. antibodies in serum</th>
<th>IgG AI normal</th>
<th>IgM AI normal</th>
<th>IgG: Confirmation by recomBead*</th>
<th>IgM: Confirmation by recomBead*</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Negative</td>
<td>no</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Cell count <5/µl, intact blood-CSF barrier, no intrathecal Ig synthesis, no specific anti-Bb antibodies in serum.

*Verified by at least one antigen with pathologic antigen specific AI.
4 recomBead Borrelia IgG/IgM 2.0

If by screening ELISA a pathological or borderline antibody index (AI) was detected for CSF and serum, intrathecal synthesis of Borrelia-specific antibodies can be confirmed with the recomBead Borrelia IgG/IgM 2.0 in two different ways:

a) Calculation of antigen specific antibody indices (according to Reiber) via a standardized dilution.

b) Calculation of antigen specific antibody indices (according to Reiber) after serum and CSF were matched for the same immune globuline concentration prior to measurement.

The examination of serum / CSF pairs with the recomBead Borrelia IgG/IgM 2.0, in case of suspected neuroborreliosis, is possible without previous examination by a screening ELISA.

4.1 Sampling and preparation of reagents

4.1.1 Samples (serum and CSF)

Serum (EDTA, citrate, heparin, CPD) and cerebrospinal fluid from the patient must be collected on the same day. Serum (EDTA, citrate, heparin, CPD) and cerebrospinal fluid from the patient must be prepared in the microtitre plate immediately after the sampling and preparation of reagents.

To prevent deviations from test to test, serum and CSF must always be prepared in the microtitre plate immediately after the same immune globuline concentration prior to measurement.

Haemolytic CSF, in particular, can produce incorrect results.

Caution!

If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at +2°C to +8°C. Extended storage of the samples is possible at -20°C or below. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results.

4.1.2 Preparation of ready-to-use wash and dilution buffer

This buffer is required for the washing stages and for the dilution of samples.

The buffer concentrate is diluted 1 + 5 with deionised water. A total of 24 ml of ready-to-use buffer is prepared (4 ml concentrate + 20 ml H2O deion.) for each microlitre plate strip with 8 wells.

4.2 Testing of CSF/serum pairs with recomBead Borrelia

To prevent deviations from test to test, serum and CSF must always be measured at the same time in the same test run. CSF samples must be prepared in the microtitre plate immediately after the associated serum sample.

4.2.1 Sample dilution

As written above, an intrathecal synthesis of Borrelia-specific antibodies can be confirmed with the recomBead Borrelia IgG/IgM 2.0 in two different ways:

a) Calculation of antigen specific antibody indices (according to Reiber) via a standardized dilution.

b) Calculation of antigen specific antibody indices (according to Reiber) after serum and CSF were matched for the same immune globuline concentration prior to measurement.

Depending on the desired approach, different dilutions of the serum samples are required.

a) Antigen specific antibody index:

The dilution of the sample is then prepared as follows:

Serum 1:816 (IgG)

According to instructions for use patient sera are prediluted 1:51 (500 µl dilution buffer + 10 µl serum) with dilution buffer. In a second step IgG is finally diluted 1:16 (300 µl dilution buffer + 20 µl of the predilution) and IgM is finally diluted 1:4 (300 µl dilution buffer + 100 µl of the predilution).

CSF 1:5

80 µl dilution buffer + 20 µl CSF

b) Antigen specific antibody index with matched immune globuline concentration in CSF and serum:

Individual serum dilution

The individual serum dilution (matched immune globuline concentration in CSF and serum) is calculated in the recomWell evaluation software (see chapter 5).

CSF 1:5

80 µl dilution buffer + 20 µl CSF

4.2.2 Test procedure

The test procedure is carried out in line with the instructions for use for the recomBead Borrelia IgG/IgM 2.0.

No. | Execution | Note
--- | --- | ---
1 | Preparation of samples and controls | see 4.2.1
2 | Preparation of particle mixture | Thoroughly mix the particle mixture (for 30-60 seconds, max. rpm).
3 | Preparation of the microplate | Before each use, the particle mixture (bead mix) must be vortexed in order to ensure a consistent suspension.
4 | Incubation of samples | Establish at least one value for each control and sample. The CSF sample has to be placed directly behind the corresponding serum well.
5 | Washing (5x) | Attention, the microtiter plate must not be knocked out! The microtiter plate must be properly adjusted on the magnetic plate!
6 | Incubation with conjugate | Carry out the washing steps 8.5b-8.5c five times in total.
7 | Washing (5x) | The microtiter plate must be properly adjusted on the magnetic plate!
8 | Resuspension in system fluid | Attention, the microtiter plate must not be knocked out! The microtiter plate must be properly adjusted on the magnetic plate!
9 | Measuring fluorescence | Carry out the washing steps 8.7b-8.7c three times in total.

4.3 Results

4.3.1 Qualitative evaluation

4.3.1.1 Validation – Quality Control

The test can be evaluated under the following conditions:

- As per recomQuant, each determination of cerebrospinal fluid and serum must be valid. EXCEPTION: The error message “no serum” may appear when determining the CSF. This is due to the lower concentration of antibodies in cerebrospinal fluid. The measurement is still valid and can be used for analysis.
- Some antigens cannot be evaluated if reactivity is outside the linear range (>15000 MFI) and the value in CSF or serum is shown as “over”.

4.3.1.2 Threshold values

In the case of the qualitative evaluation, the threshold for the evaluation of sera and CSF is calculated as follows:

Threshold for serum IgG / IgM:

Signal height (MFI) ≥ 1.0x cut-off

Threshold for CSF IgG / IgM:

Signal height (MFI) ≥ 0.5x cut-off

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4.3.1.3 Interpretation of test results

The antibody index for recomBead Borrelia is calculated individually for each antigen if the following conditions are met:

<table>
<thead>
<tr>
<th>Result recomBead Borrelia IgG/IgM 2.0</th>
<th>Next steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI&lt;sub&gt;serum&lt;/sub&gt; &lt; threshold for serum and COI&lt;sub&gt;CFS&lt;/sub&gt; &lt; threshold for CSF</td>
<td>no evidence of bb-specific antibodies in serum</td>
</tr>
<tr>
<td>COI&lt;sub&gt;serum&lt;/sub&gt; ≥ threshold for serum and COI&lt;sub&gt;CFS&lt;/sub&gt; &lt; threshold for CSF</td>
<td>Evidence of Bb-specific antibodies in serum</td>
</tr>
<tr>
<td>COI&lt;sub&gt;serum&lt;/sub&gt; &lt; threshold for serum and COI&lt;sub&gt;CFS&lt;/sub&gt; ≥ threshold for CSF</td>
<td>no evidence of Bb-specific antibodies in serum</td>
</tr>
<tr>
<td>COI&lt;sub&gt;serum&lt;/sub&gt; ≥ threshold for serum and COI&lt;sub&gt;CFS&lt;/sub&gt; ≥ threshold for CSF</td>
<td>Evidence of Bb-specific antibodies in serum</td>
</tr>
</tbody>
</table>

4.3.2 Calculation of antigen specific antibody indices

The antigen specific antibody index (AI) can be determined if the values of chemical analysis (albumin, total IgG, total IgM) are entered in the recomQuant software (see 6 CSF diagnostics recomQuant). The antigen specific antibody index is calculated for each antigen separately, when following rules apply:

- Threshold value for CSF (IgG/IgM) ≥ 0.5x cut-off
- Reactivity (signal height) of serum and CSF is within the linear measuring range (<15000 MFI)

4.3.3 Calculation of antigen specific antibody indices with matched immune globuline concentration in CSF and serum

In the case of the calculation of antigen specific antibody indices with matched immune globuline concentration in CSF and serum, the reactivities (signal heights) of the individual antigens (in serum and CSF) are set in direct relation to each other (see 6 CSF diagnostics recomQuant). The dilution data or chemical analysis data are not required.

4.3.4 Interpretation of antibody index values

The following evaluation criteria apply to the interpretation of antigen specific antibody indices:

<table>
<thead>
<tr>
<th>Antibody index IgG and IgM</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6 ≤ AI ≤ 1.3</td>
<td>normal</td>
</tr>
<tr>
<td>1.3 &lt; AI &lt; 1.5</td>
<td>borderline</td>
</tr>
<tr>
<td>≥ 1.5</td>
<td>pathological</td>
</tr>
</tbody>
</table>

- Antibodies against individual Borrelia burgdorferi s.l. antigens are determined separately in parallel with recomBead Borrelia IgG/IgM 2.0. Thus the antibody index values for the individual Borrelia burgdorferi s.l. antigens are calculated antigen specific. The individual antigen specific antibody index values can differ from antibody index values that were determined by ELISA.
- A single pathological index value is sufficient to suggest an intrathecal synthesis of Borrelia antibodies and therefore a pathological interpretation:
  - Antibody index values between 0.6 ≤ AI ≤ 1.3 are within the normal range. An intrathecal synthesis of Borrelia antibodies is unlikely. The detected Borrelia-specific antibodies have entered the subarachnoid space by passive transfer from the serum via the blood–CSF barrier.
  - Index values <0.6 have no pathological significance as a rule. But they can indicate an error in the analysis (e.g. chemical analysis). It is recommended to verify whether the serum and CSF were sampled on the same day and/or whether the samples got mixed up. Where necessary, the determination of clinical chemistry and/or Borrelia-specific antibodies may be repeated.
  - Antibody index values between 1.3 and 1.5 are borderline. We recommend assaying the CSF/serum pair again and, in the case of clinical issues, assaying a new CSF/serum pair over the course of the illness.
  - Antibody index values ≥1.5 are considered to be pathological. They indicate the presence of an intrathecal synthesis of Borrelia-specific antibodies, like those found in patients with neuroborreliosis.

4.4 Limitations of the method - restrictions

- Serological test results must always be seen in the context of the patient's clinical picture. Any treatment resulting from the serological findings must always be based on the clinical data.
- A normal result does not exclude CSF infection with Borrelia burgdorferi. Particularly in the early stages of infection, antibodies may not be present or not present in a detectable quantity. In the early stages, antibiotic treatment may prevent the development of detectable antibodies. With clinically suspected neuroborreliosis and negative and/or inconclusive serum results, further sampling and testing should be carried out.
- Pathological IgG and/or IgM results are not always an indication for neuroborreliosis. Since intrathecal antibodies have a long-term persistence, the presence of antibodies is evidence of a previous infection.
- Intrathecal synthesis of IgM antibodies is no indication of a recent infection.
- Cross-reactions with antibodies, produced by infection with syphilis pathogens (Treponema pallidum), recurrent fever (Borrelia recurrentis, Borrelia duttonii, Borrelia hermsii) or leptospirosis (Leptospira sp.) can be largely excluded due to the selective use of recombinant Borrelia burgdorferi antigens. With inconclusive Borrelia serology, a Lues infection should be excluded.
- An infectious mononucleosis (Pfeiffer’s gland fever, EBV infection) may result in polyclonal stimulation of B lymphocytes. This can lead to unspecific reactions during the detection of antibodies of the IgM class. If the medical history is unclear and in the presence of a weak IgM response, it is recommended to exclude an EBV infection by differential diagnosis.

4.5 Test performance

General Features

see 3.4 Test performance recomWell Borrelia IgG/IgM
5 Evaluation software recomWell

The Excel program developed by Mikrogen is intended for the calculation of the intrathecal antibody index in serum and CSF, using an ELISA. For the subsequent confirmation assay, it also calculates the dilutions for CSF/serum pairs for a matched immunoglobulin concentration.

5.1 Required
- Computer with Excel program

5.2 Warnings and precautions
- For In vitro diagnostic use only.
- Please check whether you have the current version. The current version is available on request from Mikrogen (+ 49 89 54801-0).
- Alternatively, you can download it from www.mikrogen.de → Downloads.
- Please carefully read the entire Instructions for Use before you carry out the assay and then follow the individual steps. Deviations from the Instructions for Use may lead to erroneous results.

5.3 Execution
When opening the evaluation program, this window appears:

![Evaluation software recomWell](image)

After selecting the assay and general data, enter the patient data in the field “Sample”, and albumin and immunoglobulin levels in the field “Clinical chemistry”. Select the appropriate clinical chemistry units (g/l; mg/l; g/dl; mg/dl).

In the field “Specific antibodies” enter the measured ELISA extinctions. With a serum value of <0.100 with positive cerebrospinal fluid, an extinction of 0.100 is assumed for the unit calculation of the serum.

The results of recomWell must be entered with the appropriate dilution factor. The default setting for serum dilution is 1:101, and IgG 1:4 or IgM 1:2 for CSF dilution. The programme also uses the absorbance of the cut-off control to calculate the CSF, since serum and CSF must be entered in the same run.

Using the clinical chemistry results and the recomWell data, the program automatically calculates the ratio and antibody index values (AI). Where test strip verification is required after AI calculation, the required dilution can be determined by clicking on the field “Calculation of strip dilution”. This takes you to a second spreadsheet:

![Second spreadsheet](image)
6 CSF diagnostics recomQuant

With recomQuant by MIKROGEN, the analysis of the antigen specific antibody index and Q_{spez} calculation can be carried out automatically.

6.1 Warnings and precautions
- For In vitro diagnostic use only.
- Please check whether you have the current version. The current version is available on request from Mikrogen (+ 49 89 54801-0).
- Please carefully read the entire Instructions for Use before you carry out the assay and then follow the individual steps. Deviations from the Instructions for Use may lead to erroneous results.

6.2 Execution
Select the test and batch used and importing the measured data (see current user manual recomQuant). Depending on the chosen method for antigen specific antibody index calculation ((a) standard dilution or (b) Ig-matched dilution), please click on the respective button to start CSF diagnostics:

In the case a) standard dilution for the antigen specific antibody index calculation in the CSF diagnostics, clinical data must be entered in the following window:
- a) Select units for serum and CSF
- b) Select serum/CSF pairs
- c) Enter/verify serum and CSF dilutions
- d) Enter albumin and antibody concentrations

For the determination of antigen specific antibody indices with matched immunoglobulin concentration the selection of the serum/CSF pairs in the following window is sufficient:

Once all of the relevant data has been entered, the antibody index calculation can be started here:

As an alternative to the antibody index calculation, you can also request the Q_{spez} values for each antigen (only for (a) standard dilution method). Follow this link:
7 Literature
See Instructions for Use recomWell Borrelia IgG, IgM or recomBead Borrelia IgG, IgM 2.0

8 Manufacturer and version information

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Instructions for Use valid from AVrXBBLS004EN 2014-07

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