DiaMex GmbH
Highend diagnostic products

Optiplex Borrelia IgM Test

Multiplex Bead Assay (MBA) for Simultaneous Detection of IgM Antibodies based on Semi-Quantitative Measurements to Separate Borrelia Antigens in Human Serum and Cerebrospinal Fluid (CSF)

Cat.No: IN0503
Content: 96 Tests
Store at: 2–8 °C

CE IVD

Instruction sheet / Gebrauchsanweisung/ Carnet d'instruction / instrucciones de uso / gebruiksaanwijzing / istruzioni per l'uso / σελίδα οδηγιών / bruksanvisning / modo de emprego
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1. Introduction

Lyme borreliosis is the most common tick-borne disease in the northern hemisphere. The prevalence of antibodies to Borrelia spec. 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, increasing IgG antibody titres with spreading specificity to Borrelia antigens are 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).
If clinical findings are suspicious of neuroborreliosis it should be investigated whether intrathecally produced IgM/IgG antibodies can be detected.
In association with clinical symptoms the presence of intrathecally produced Borrelia-specific IgM/IgG antibodies is considered as a positive proof of neuroborreliosis. For this purpose, the Borrelia-specific antibodies are determined in serum/CSF pairs and related to the total concentration of immunoglobulins in the serum and CSF. With this procedure one can calculate the Borrelia-specific antibody index AI which allows to differentiate between antibody fractions which have passively diffused into the cerebrospinal space and intrathecally synthesized antibodies.

2. Principle of the Test

The MBA is designed to detect IgM class antibodies in properly diluted human sera and CSF samples to 8 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 and cerebrospinal fluids 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 bead sets of the MBA.
The various sets of beads are coated with the following antigens isolated from various Borrelia species (1):

Antigen	Species	Antigen type
Lysate	B. afzelii	native
OspC	B. garinii PBi	recombinant
OspC	B. afzelii PKo	recombinant
p100	B. afzelii PKo	recombinant
p18	B. afzelii PKo	recombinant
p39	B. afzelii PKo	recombinant
p41-I	B. afzelii PKo	recombinant
VlsE-C6	All species	peptide (IR6)

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 ninth control bead set of the assay, CR1, serves for the monitoring of 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 Human negative control serum*. Ready to use, 300 µl vial. The serum does not contain detectable Borrelia-specific IgM antibodies
POS M Human positive control serum*. Ready to use, 300 µl vial. The serum contains Borrelia-specific IgM antibodies.
REF M Human reference control 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 9 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 Luminex™, 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, POS M and REF 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 and CSF 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.

If serum/CSF pairs are tested it must under all circumstances be ensured that the serum and CSF samples are taken the same day and that the serum/CSF pairs are analyzed during the same test run.

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

1. 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

2. Cerebrospinal fluid samples

Dilute cerebrospinal fluid samples 1:20 with ready-to-use assay buffer. Ensure thorough mixing of dilutions!

1+19: 5 µl cerebrospinal fluid + 95 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/CSF sample** to the microwells 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. Incubate for **60 minutes at 37°C in the dark**.
11. Read plate on the Luminex analyzer **immediately**.

10. Quality Control

The values of the reference, positive and negative control 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 the positive control (quotient) must be within the range indicated in the batch certificate. For antigens with a fixed cut-off the values of the reference control (MFI) must be within the indicated range. 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 values of the different antigenes are differently generated, depending on their relevance for the diagnosis of early Borreliosis in the IgM-state. The cut-off values for the early Borreliosis marker antigens Lysat, OspCPBi and OspCPKo are calculated from the result of the reference control in well C1 for each bead set divided by the control indices given in the batch certificate as follows:

$$\frac{\text{Measured value}_{\text{REF M, Ag}_n}}{\text{Index}_{\text{REF M, Ag}_n}} = \text{Cut - off}_{\text{Ag}_n}$$

The antigens p100, p18, p39, p41-I and VlsE are recognized by the immune system at later stage Borreliosis and are specific IgG-phase markers. These antigens are therefore only rarely recognized by IgM antibodies. Their respective cut-off values are given as fixed values in the batch certificate.
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:

$$\frac{\text{Measured value}_{\text{xy sample, Ag}_n}}{\text{Cut - off}_{\text{Ag}_n}} = \text{Cut - off - Index}_{\text{sample, Ag}_n}$$

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.

Evaluation of Serum Samples:

Negative: Cut-off-Index < 1
No antibodies to antigens of this bead set detected.
Weakly positive: 1 ≤ Cut-off-Index < 1.5
Antibodies to the antigen of this bead set suspected.
Positive: Cut-off-Index ≥ 1.5
Antibodies to the antigen of this bead set detected.

Evaluation of Serum-CSF-paired Samples: Antibody-Index calculation
To differentiate between an intrathecal response and a blood-brain barrier dysfunction it will be necessary to calculate the **antibody index according to REIBER** (12).
The calculation of the AI should take into account cut-off indices ≥ 0.5 for the individual antigens and the total lysate.
It is not necessary to provide a virtual standard curve.
The reaction index is multiplied by the original dilution and the resulting product is then substituted into the known REIBER formula (12). The detailed AI calculation is described in the annex.

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. All bead sets „negative“ or no more than two sets borderline/“weakly positive“ or one set “positive” and no more than one set borderline/”weakly positive”
   → **No suspicion of Lyme borreliosis immunoserologically**
2. One bead set „positive“ and at least two sets borderline/”weakly positive” or at least three sets „weakly positive“
   → **Lyme borreliosis not reliably excluded immunoserologically**

In this case the sample should be tested using **Optiplex Borrelia IgG Test**. One „positive“ antigen group in each of the two tests strengthens the suspicion of Lyme borreliosis. With unclear findings persisting, a second testing should be performed after approximately 10 days.
3. More than one bead set „positive“
   → **Strong suspicion of Lyme borreliosis immunoserologically**

Presentation in tabular form:

Number of weakly positive bead sets	Number of positive bead sets	Overall result*	Overall evaluation: Lyme borreliosis
0	0	neg[a]	No suspicion
≤ 2	0	neg	No suspicion
0	1	neg	No suspicion
1	1	neg	No suspicion
> 2	0	spos[b]	Not reliably excluded
> 1	1	spos	Not reliably excluded
0	≥ 2	pos[c]	Strong suspicion

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

Inter-assay precision was determined from 5 independent tests on 2 different Luminex Analyzers. In the calculation there were included tests showing at least one weakly positive result (cut-off index > 1).
Inter-assay coefficient of variation: CV < 10 %
Intra-assay precision was determined from twelve assays of sera reactive to all antigens used in **Optiplex Borrelia IgM Test.**
Intra-assay coefficient of variation: CV < 11 %

In a study a total of 106 clinical well characterized sera of patients with Lyme borreliosis were tested. Both weakly positive and positive results were included. The results are summarized in the following tab.

	Number	IgG	Recovery
Acrodermatitis	11	10	91%
(Neuro-) Borreliosis	13	13	100%
Chronic Borreliosis	38	38	100%
Lyme-Arthritis	11	11	100%
Borreliosis Reinfection	4	4	100%
Erythema migrans	29	17	59%

	Number	IgM	Recovery
Acrodermatitis	11	1	9%
(Neuro-) Borreliosis	13	2	15%
Chronic Borreliosis	38	10	26%
Lyme-Arthritis	11	3	27%
Borreliosis Reinfection	4	2	50%
Erythema migrans	29	15	52%

[a]neg, „negative“
[b]spos, „weakly positive“
[c]pos, „positive

	Number	IgG/gM	Recovery
Acrodermatitis	11	10	91%
(Neuro-) Borreliosis	13	13	100%
Chronic Borreliosis	38	38	100%
Lyme-Arthritis	11	11	100%
Borreliosis Reinfection	4	4	100%
Erythema migrans	29	21	72%

Furthermore blood donor sera were examined. With the IgG test a seroprevalence of up to 12 % and with the IgM test up to 8 % was found, which results in a total (IgM/IgG) seroprevalence of up to 19 %. Both weakly positive and positive results were included.

232 Blood Donor Sera

	IgM	IgG	IgM / IgG
pos / spos	19	27	43
Seroprevalence	8%	12%	19%

14. Limitations

The results obtained with the **Optiplex Borrelia IgM 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.
The results obtained for the serum/CSF pairs should only be interpreted by a specialist. If CSF results are not consistent with the clinical symptoms the sample should be reanalyzed with a higher CSF dilution. For calculation, the used dilution has to be considered.
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 typic for an IgG-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 an inhomogeneous dilution or poor mixing.

16. Literature

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Protocol Summary for Optiplex Borrelia IgM Test

Prepare **serum dilution, 1:201:**	Prepare **CSF dilution, 1:20:**
1+200: 5 µl serum + 1000 µl buffer	**1+19:** 5 µl CSF + 95 µl buffer

Samples: **NEG M, POS M, REF M,** (undiluted), Serum/CSF samples (diluted), see pipetting scheme	**25 µl**
Add **BMM** to each well (mix just prior to use!)	**25 µl**

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

Add **CON M** to each well	**50 µl**

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG M											
B	POS M											
C	REF M											
D												
E												
F												
G												
H												

Settings for the Creation of Templates for Multimetrix Borrelia IgM Test

with the Luminex IS Software (version 2.2 SP1 and higher)

Entries page 1:

Template Name:	**DM_Borrelia_IgM**	
Version No.:	**3.0**	
Template Type:	**Data collection only**	
Sample Vol. (µl):	**70**	
Sample Timeout (sec):	**100**	
Doublet Discriminator Gate	Low Limit:	**6800**
	High Limit:	**16000**

Entries page 2:

Tests: **9**

Name	Units	Description	Bead ID	MinBeads
CR1	**MFI**		**62**	**70**
Lysat	**MFI**		**17**	**70**
OspCPBi	**MFI**		**54**	**70**
OspCPKo	**MFI**		**52**	**70**
p100	**MFI**		**9**	**70**
p18	**MFI**		**58**	**70**
p39	**MFI**		**34**	**70**
p41-I	**MFI**		**30**	**70**
VlsE-C6	**MFI**		**56**	**70**

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

Consult instructions for use	Tests per kit	LOT Lot Number	REF Catalog Number	Store at
IVD For *in vitro* diagnostic use	Use by	Manufacturer	CE European Conformity	

AI – Calculation according to Reiber to Evaluate serum/CSF Pairs

1 Correction of the calculated cut-off indices of the CSF samples per antigen

$$\text{cut-off-index}_{\text{corr, CSF, agn}} = \frac{\text{actual CSF dilution}}{\text{serum dilution}} * \text{cut-off-index}_{\text{CSF, agn}}$$

Serum dilution (acc. to instruction sheet): with IgM Test = 201
with IgG Test = 3015

For CSF the quotient of the actual CSF dilution divided by the serum dilution acc. to the instruction sheet yields the following correction factor:

With the IgM test: 20/201 = 0.0995
With the IgG test: 80/3015 = 0.02653

2 Calculation of the Borrelia-specific AI with the IgM and IgG test
Note: The results are multiplied with the factor of 1000 to display readily legible values

$$Q_{spec} = \frac{\text{cut-off-index}_{\text{corr, SCSF, agn}}}{\text{cut-off-index}_{\text{serum, agn}}} * 1000 = Q_{spec} * 10^{-3}$$

3 Calculation of the total albumin quotient (IgM and IgG):

$$Q_{Alb} = \frac{\text{Albumin conc.}_{CSF}}{\text{Albumin conc.}_{serum}} * 1000 = Q_{Alb} * 10^{-3}$$

4 Calculation of the total immunoglobulin quotient (IgM and IgG):

$$Q_{total} = \frac{\text{Immunglobulin conc.}_{CSF}}{\text{Immunglobulin conc.}_{serum}} * 1000 = Q_{total} * 10^{-3}$$

5 Calculation of Q_{Lim}

$$Q_{Lim\ IgG} = 0.93 * \sqrt{(Q_{Alb})^2 + 6 * 10^{-6}} - 1.7 * 10^{-3}$$

$$Q_{Lim\ IgM} = 0.67 * \sqrt{(Q_{Alb})^2 + 120 * 10^{-6}} - 7.1 * 10^{-3}$$

6 Calculation of the AI taking into account Q_{Lim}

$Q_{Lim} > Q_{total}$ obeys:

$$AI = \frac{Q_{spec}}{Q_{total}}$$

$Q_{Lim} < Q_{total}$ obeys:

$$AI = \frac{Q_{spec}}{Q_{Lim}}$$

7 Interpretation aids for the specialist

AI	< 1.3	passive diffusion
AI	1.3 – 1.5	suspicious / borderline
AI	> 1.5	strong suspicion of intrathecal synthesis of Borrelia antibodies

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