

Multimetrix

Borrelia IgG Screening Test

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

Cat.No: MM2015
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 empleo: www.progen.de

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, 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).

If clinical findings are suspicious of neuroborreliosis it should be investigated whether IgG antibodies can be detected in CSF samples.

2. Principle of the Test

The MBA is designed to detect IgG class antibodies in properly diluted human sera to 2 *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):

| Antigen | Species | Antigen type |
|-----------|-------------------------|--------------|
| Lysat MMS | <i>Borrelia afzelii</i> | native |
| Lysat ZQ1 | <i>Borrelia garinii</i> | native |

In the second incubation step phycoerythrin-conjugated goat anti-human IgG reporter molecules („conjugate“) are added to detect IgG 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-IgG), which are indicative of anti-*Borrelia* antigen-specific antibodies. Conjugate binding to free IgG in the sample is not detected by the analyzer. Thus, there is no need for a washing step to remove unbound antibodies.

A third control bead set of the assay CR1 serves for the monitoring of the IgG level of the specimen and as a control of the reporter reaction of the conjugate.

3. Test Kit Components

- DIL** Assay buffer, 2 x 100 ml. Ready to use!
- NEG G** Negative control (human serum)*. Ready to use, 300 µl vial. The serum does not contain detectable *Borrelia*-specific IgG antibodies.
- REF G** Reference control (human serum)*. Ready to use, 300 µl vial. The serum contains *Borrelia*-specific IgG antibodies.
- POS G** Positive control (human serum)*. Ready to use, 300 µl vial. The serum contains *Borrelia*-specific IgG antibodies.
- BMG** Bead suspension („Bead Mix“). Ready to use, 2,6 ml vial. The suspension contains 3 antigen-specific bead sets.
- CON G** Phycoerythrin-conjugated goat anti-human IgG. Ready to use, 6,5 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 G**, **REF G** and **POS G** 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 sodium azide at a concentration of 0.09 % (w/v) 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.

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 (BMG)

Resuspend Bead Mix IgG (BMG) just prior to use by thorough vortexing. Avoid direct light exposure, beads are light sensitive!

Preparation of samples

1. Serum samples

Dilute serum samples 1:3015 with ready-to-use assay buffer in two steps. Ensure thorough mixing of dilutions!

Step I: 1+200** 5 µl serum + 1000 µl buffer

Step II: 1+14 50 µl 1:201 + 700 µl buffer

When testing for IgG and IgM simultaneously, serum dilution of the **Multimetrix *Borrelia* IgM Screening Test can be used.

2. Cerebrospinal fluid samples

Dilute CSF samples 1:80 with ready-to-use assay buffer in two steps. Ensure thorough mixing of dilutions!

Step I: 1+19** 5 µl CSF + 95 µl buffer

Step II: 1+3 25 µl 1:20 + 75 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 assay buffer (DIL)** to well A1 of microtitre plate.
2. Add **25 µl of negative control (NEG G)** to well B1.
3. Add **25 µl of reference control (REF G)** to well C1.
4. Add **25 µl of positive control (POS G)** to well D1.
5. Add **25 µl of diluted serum sample** to the microwells required.
6. Add **25 µl of bead mix (BMG)** to each well.
7. Seal microtitre plate with adhesive foil.
8. Incubate for **60 minutes at 37 °C in the dark**.
9. Add **50 µl of conjugate (CON G)** to each well.
10. Seal microtitre plate with adhesive foil.
11. Incute for **for 60 minutes at 37 °C in the dark**
12. 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 values of the positive and reference control 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:

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

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.

The single cut-off indices of the samples are summed up:

$$\sum \text{Index}_{\text{sample Ag}_n} = \text{Cut-off-index}_{\text{sample}}$$

Serum samples:

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.

Cerebrospinal fluid samples:

Negative: Cut-off index < 1,0

No antibodies to antigens of these bead sets detected in CSF samples.

Positive: Cut-off index ≥ 1,0

Antibodies to antigens of these bead sets detected in CSF samples.

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:

Serum samples

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 **Multimetrix *Borrelia* IgM / IgG Test**.

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

Cerebrospinal fluid samples

1. **Cut-off index ≤ 1,0**

→ **No detection of borreliosis specific antibodies in CSF samples.**

2. **Cut-off index > 1,0**

→ **Detection of borreliosis specific antibodies in CSF samples.**

In the case of 2 it should be investigated in the **Multimetrix *Borrelia* IgG Test** whether intrathecally produced IgG antibodies can be detected. 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.

13. Test Characteristics

Intra-assay precision was determined from 12 assays of sera and CSF reactive to all antigens used in

Multimetrix *Borrelia* IgG Screening Test.

Intra-assay coefficient of variation: CV < 8,0 %

Inter-assay precision was determined from 5 independent tests of sera and CSF reactive to all antigens used in **Multimetrix *Borrelia* IgG Screening Test.**

Inter-assay coefficient of variation: CV < 12 %

14. Limitations

The results obtained with the **Multimetrix *Borrelia* IgG 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 **Multimetrix *Borrelia* IgM Test** and the **Multimetrix *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 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 of an inhomogeneous dilution or poor mixing.

16. References

1. Hauser U., Lehnert G., Wilske B. (1998): Diagnostic Value of Proteins of Three *Borrelia* Species (*Borrelia burgdorferi* Sensu Lato) and Implications for Development and Use of Recombinant Antigens for Serodiagnosis of Lyme Borreliosis in Europe. *Clin. Diagn. Lab. Immunol.* 5: 456-462
2. Vogt A (1990): Die Labordiagnostik der *Borrelia burgdorferi*-Infektion, *Fortschr Med* 108:194-197
3. Wilske B, Fingerle V, Herzer P, Hofmann A, Lehnert G, Peters H, Pfister H-W, Preac-Mursic V, Soutschek E, Weber K (1993): Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. *Med Microbiol Immunol* 182:255-270
4. Steere A C (1989): Medical progress - Lyme disease. *N Engl J Med* 321:586-596
5. Putzker M, Sauer H (1995): Labordiagnostik von Infektionen mit *Borrelia burgdorferi* sensu lato, *Klin. Lab.*; 41:431-439
6. Herzer P, Wilske B, Preac-Mursic V, Schierz G, Schattenkirchner M, Zöllner N (1986): Lyme arthritis: clinical features, serological and radiographic findings of cases in Germany. *Klin Wochenschr* 64:206-215
7. Bruckbauer H, Preac-Mursic V, Fuchs R, Wilske B (1992): Cross-reactive proteins of *Borrelia burgdorferi*. *Eur J Clin Microbiol Infect Dis* 11:1-9

8. Horst H (1997): Einheimische Zeckenborreliose (Lyme-Krankheit) bei Mensch und Tier, 3. überarb.Aufl., Spitta Verlag 126-130
9. Teward F, Braun R (1998): Durchführung und Interpretation serologischer Tests bei Verdacht auf *Borrelia*-Infektionen, *Clin.Lab* 44: 697-902
10. Goosens HAT, van den Boogard AE, Nohlmans MKE (1999): Epstein-Barr-Virus and Cytomegalovirus infections cause false positive results in IgM two-test protocol for early lyme borreliosis, *Infection* 27: 231
11. Wie-Gang Q, Schutzer SE, Bruno JF, Attie O, Xu Y, Dunn JJ, Fraser CM, Casjens SR, Luft BJ (2004): Genetic exchange and plasmid transfers in *Borrelia burgdorferi* sensu stricto revealed by three-way genome comparisons and multilocus sequence typing, *PNAS* 101: 14150-14155



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Protocol Summary for Multimatrix *Borrelia* IgG Screening Test

| | |
|---|--|
| Prepare serum dilution, 1:3015: Step I: 1+200: 5 µl serum+ 1000 µl buffer Step II: 1+14: 50 µl 1:201 + 700 µl buffer | Prepare CSF dilution, 1:80: Step I: 1+19 5 µl CSF + 95 µl buffer Step II: 1+3 25 µl 1:20 + 75 µl buffer |
|---|--|

| | |
|--|--------------|
| Samples: | 25 µl |
| DIL, NEG G, REF G, POS G , (undiluted), Serum /CSF samples (diluted), see pipetting scheme | |
| Add BMG 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 G 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 | DIL | | | | | | | | | | | |
| B | NEG G | | | | | | | | | | | |
| C | REF G | | | | | | | | | | | |
| D | POS G | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

Settings for the Creation of Templates for Multimatrix *Borrelia* IgG Screening Test

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

Entries - page 1:

Template Name: **MM_BorScreen_IgG**
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: **3**

| Name | Units | Description | Bead ID | MinBeads |
|---------------|--------------|--------------------|----------------|-----------------|
| CR1 | MFI | | 61 | 70 |
| LysMMS | MFI | | 9 | 70 |
| LysZQ1 | MFI | | 85 | 70 |

When using the evaluation software provided by PROGEN Biotechnik 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**