Borrelia-IgM-ELISA medac

English

CE

201-VPE/010610

MANUFACTURER

medac
Gesellschaft für klinische
Spezialpräparate mbH
Fehlandtstraße 3
D-20354 Hamburg

MARKETING

medac
Gesellschaft für klinische
Spezialpräparate mbH
Geschäftseinheit Diagnostika
Theaterstraße 6
D-22880 Wedel

Phone: ++49/ 4103 / 80 06-351
Fax: ++49/ 4103 / 80 06-359

ORDERING ADDRESS

Phone: ++49/ 4103 / 80 06-111
Fax: ++49/ 4103 / 80 06-113

Borrelia-IgM-ELISA medac

Enzyme immunoassay for quantitative detection of
Borrelia IgM antibodies in serum and cerebrospinal fluid (CSF)

Cat.no.: 201

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

Lyme borreliosis is the most common tick associated illness of the northern hemisphere. It is a multisystemic disease with different stage depending symptoms and affected organs (skin, nervous system, joint).

The causative bacteria *Borrelia burgdorferi sensu lato* belong to the spirochetes. In Europe mainly three species (*B. burgdorferi sensu stricto, B. afzelii, B. garinii*) are described. The transfer of the pathogen results from a bite of an infected tick.

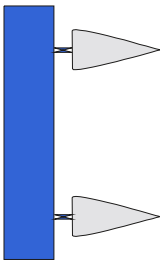
Lyme borreliosis is primarily a clinical diagnosis. Criteria like assured contact to ticks and symptoms are essential factors for interpretation of laboratory results. A serological stepwise diagnosis is recommended to ascertain diagnosis. The detection of IgM and IgG antibodies using enzyme immunoassays with high sensitivity and specificity is the first step (screening method). In the event of positive or equivocal IgM and/or IgG results a confirmatory diagnosis has to be carried out with immunoblots using recombinant proteins as antigens.

Specific serum antibodies are determined in 20 to 50 % of cases with illness in stage I (erythema migrans), in 70 to 90 % of cases in stage II (neuroborreliosis) and in almost 100 % of patients in stage III (acrodermatitis, Lyme arthritis).
Hence, a negative serological result in an early stage of infection does not rule out a disease. In the case a borreliosis is suspected, serological follow-up controls should be carried out. Borrelia IgG as well as IgM antibodies may persist for a longer period of time even after successful therapy. A positive IgM without detectable IgG normally excludes a late manifestation of disease.

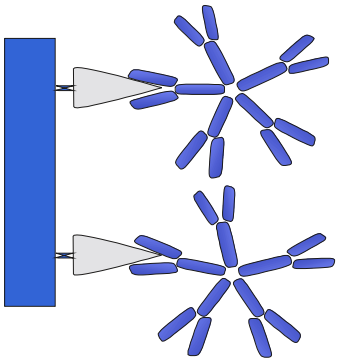
An inflammatory changed CSF is an essential criterion for acute neuroborreliosis. The detection of intrathecally produced antibodies is an additional means to confirm the diagnosis of neuroborreliosis. For this purpose Borrelia-specific antibody indices (AI) in serum-CSF pairs are determined. Depending on the duration of neurological symptoms, intrathecally produced antibodies can be determined in 80 to 100 % of the cases. Pathological AI can be measured even years after successful therapy. Furthermore an intrathecal synthesis of Borrelia-specific antibodies is described as part of a polyspecific reaction in patients with multiple sclerosis.

The Borrelia-IgM-ELISA medac is an assay for the quantitative detection of IgM antibodies based on a Borrelia-specific VlsE/OspC-peptide antigen. The test is suitable for the detection of a pathogen-specific antibody index in serum-CSF pairs.

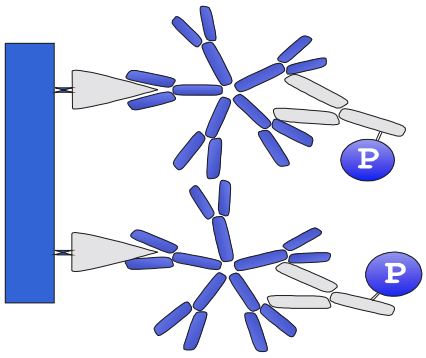
TEST PRINCIPLE



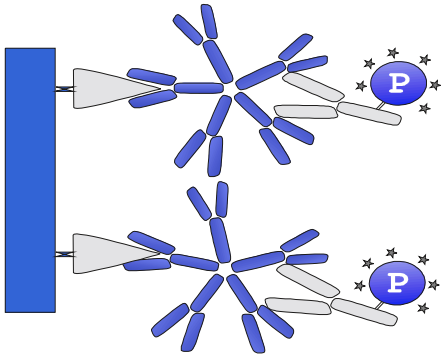
The plate is coated with Borrelia-specific VlsE/OspC-peptide antigen.



The Borrelia-specific antibodies from the specimen bind to the antigen.



Peroxidase-conjugated anti-human IgM antibodies bind to the Borrelia-IgM antibodies (P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- ☞ The breakable microwell strips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.
- ☞ Single-point quantification, no standard curve needed.
- ☞ No additional calibration curve for investigation of CSF needed.

KIT CONTENTS
Cat. no.: 201

1. **MTP**
   Microplate: 12 x 8 wells, grey coded, (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with VlsE/OspC-peptide antigen and BSA, ready to use.

2. **CONTROL –**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, stained orange, contains NBCS, phenol, ProClin™ 300 and gentamycin sulfate.

3. **CONTROL +**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, stained orange, contains FCS, BSA, phenol, ProClin™ 300 and gentamycin sulfate.

4. **CAL**
   Calibrator: 1 vial with 1.5 ml, human serum, ready to use, stained orange, contains FCS, BSA, phenol, ProClin™ 300 and gentamycine sulfate.

5. **WB**
   Wash buffer: 1 bottle with 100 ml PBS/Tween (10x), pH 7.2 –7.4, contains ProClin™ 300.

6. **BO-DIL**
   Sample diluent: 1 bottle with 110 ml PBS/Tween/NBCS, pH 7.0 – 7.2, ready to use, stained orange, contains ProClin™ 300.

7. **CON**
   Conjugate: 3 vials with 4.5 ml each, goat anti-human IgM, HRP-conjugated, ready to use, stained red, contains BSA, phenol, ProClin™ 300 and gentamycin sulfate.

8. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

9. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

10. **RF-ABS**
    IgG/RF-absorbent: 1 vial with 4 ml, goat anti-human IgG antibody, ready to use, contains < 0.1 % sodium azide.

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls/Calibrator	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Conjugate	opened	2...8 °C	6 weeks
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date
IgG/Rf-absorbent	opened	2...8 °C	6 weeks

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37 °C.

2.6. Microplate reader with filters for 450 nm and 620 - 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate
The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated under point 1.

3.2. Wash buffer

Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

Do not mix test specific reagents (microplate, controls, calibrator and conjugate) from different kit lots. In contrast to that, wash buffer, IgG/RF-absorbent, TMB-substrate and stop solution are generally exchangeable in all serologic ELISA of medac. Different lots of sample diluent are exchangeable within the Borrelia kits of medac.

Reagents from other manufacturers must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum and CSF samples (for investigation of CSF see 8.).

4.2. In order to avoid interferences with high IgG titres and rheumatoid factors (RF) an absorption of IgG/RF shall be performed for all sera. The sera are used in a final dilution of 1:150. They are diluted 1:100 with sample diluent first following a 1:1.5 dilution with IgG/RF absorbent. Samples above the measuring range can be diluted further with sample diluent after RF absorption.

4.3. An additional pretreatment of sera, e.g. inactivation, is not necessary. However, sera should neither be contaminated with microorganisms nor contain any red blood cells.

4.4. The diagnostic investigation of serum-CSF pairs is described in detail under 8.

5. TEST PROCEDURE

5.A. IgG/RF-ABSORPTION

Attention:

* **The controls are ready to use (no absorption necessary).**
* **The following volumes indicated apply for single determinations only.**

5.A.1. Serum: 10 µl serum are diluted with 990 µl sample diluent (dilution 1:100).

5.A.2. Absorption: 20 µl IgG/RF-Absorbent and 40 µl diluted serum are mixed (dilution 1:150) and incubated for 15 min at room temperature.

5.A.3. Now the test dilution is 1:150.

5.B. TEST RUN

5.B.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be prewashed.

5.B.2. Pipette 50 µl of the sample diluent into the well A1 as blank (see 6.A.). Add 50 µl of the negative control, positive control as well as the diluted samples in single determination and 50 µl of the calibrator in duplicate to the wells.

If necessary the microplate wells can be kept for max. 30 min at RT before proceeding.

5.B.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.B.4. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.B.5. Add conjugate (coloured red) to each well.

50 µl of conjugate have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of conjugate have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.B.6. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.B.7. After incubation wash microplate wells again (see 5.B.4.).

5.B.8. Add 50 µl of TMB-substrate to each well and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.B.9. Stop the reaction by adding 100 µl of stop solution to each well. Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading must be done within 15 min after adding the stop solution.

5.C. TABLE FOR THE IgG/RF-ABSORPTION OF SERUM

Indication per determination

10 µl serum
+
990 µl sample diluent

1000 µl; dil. 1:100

↓

20 µl IgG/RF-absorbent
+
40 µl diluted serum

60 µl; dil. 1:150

↓

Vortex

↓

Incubate for 15 min at
room temperature

↓

Test dilution 1:150

5.D. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Calibrator	Sample
Sample diluent	50 µl	–	–	–	–
Negative control	–	50 µl	–	–	–
Positive control	–	–	50 µl	–	–
Calibrator	–	–	–	50 µl	–
Absorbed sample	–	–	–	–	50 µl
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer					
Conjugate	50/60 µl*)	50/60 µl*)	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37 °C, wash 3x with 200 µl wash buffer					
TMB-substrate	50 µl	50 µl	50 µl	50 µl	50 µl
Incubate for 30 min at 37 °C in the dark					
Stop solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 – 650 nm)					

*) manual/automatic procedure (see 5.B.5.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* The evaluation is performed using arbitrary units (AU).

* Read OD values at 450 nm (reference wavelength 620 – 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* Lot-specific data

  The lot-specific data sheet provided with the kit contains the following information:

  - Lot-specific calibration curve
  - Curve parameters a and b
  - Nominal OD value of the calibrator
  - Lower OD limit of the calibrator
  - Nominal concentration range (AU/ml) of the positive control

* Validity criteria

- The OD value of the **blank** has to be **< 0.100.**
- The OD value of the **negative control** has to be **< 0.150.**
- The unit value of the **positive control** has to be within the nominal range indicated in the lot-specific data sheet.
- The mean OD value of the calibrator has to be above the lower OD limit indicated in the lot-specific data sheet.
- Additional validity criteria for the detection of serum-CSF pairs see 8.

Repeat the run if the results do not meet the specification.

* Correction of the results

The measured OD values of the positive control and the samples have to be corrected as follows:

$$OD_{corrected} = \frac{\text{Nominal OD value of the calibrator}}{\text{Measured OD value of the calibrator}} \times OD_{measured}$$

* Quantification of the results

The corresponding concentrations of the corrected OD values in AU/ml can be read from the lot-specific calibration curve (see lot-specific data sheet).

Alternatively, the concentrations can be calculated using the following formula:

$$\text{Concentration [AU/ml]} = b/\left(\frac{a}{OD_{corrected}} - 1\right)$$

Most of the new ELISA readers allow to program the formula, thus enabling an automated data processing.

The measuring range for serum samples spans from 12.6 to 200 AU/ml. Samples below this range have to be interpreted as < 12.6 AU/ml, those above as > 200 AU/ml. These values must not be extrapolated.

The cut-off is 14 AU/ml.

Grey zone = 12.6 - 15.4 AU/ml

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with Unit values below the lower limit of the grey zone are reported as **NEGATIVE.**

* Samples with Unit values within the grey zone are reported as **EQUIVOCAL.**

  These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with Unit values exceeding the upper limit of the grey zone are reported as **POSITIVE.**

* The results should always be interpreted in connection with clinical data, the Borrelia-IgG results and additional diagnostic parameters.

* According to the quality standards for the microbiological diagnosis of Lyme borreliosis (MiQ 12) equivocal and positive ELISA results have to be confirmed by Western blot.

* High concentrations of hemoglobin and lipids in serum do not influence the test results.

* Cross-reactivities with antibodies against **T. pallidum** cannot be excluded in individual cases (see 7.B.).

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SEROPREVALENCE

100 sera of blood donors were investigated in comparison to a competitor's ELISA. A prevalence of 1 % (competitor 2 %) was determined.

		Competitor's ELISA			
		negative	**equivocal**	**positive**	**total**
Borrelia-IgM-ELISA medac	**negative**	**95**	**2**	**1**	**98**
	equivocal	**0**	**1**	**0**	**1**
	positive	**0**	**0**	**1**	**1**
	total	**95**	**3**	**2**	**100**

7.B. CROSS REACTIVITY

75 sera of patients with antibodies against **T. pallidum** were measured in comparison to a competitor's ELISA. The results obtained are shown in the table below:

		Competitor's ELISA			
		negative	**equivocal**	**positive**	**total**
Borrelia-IgM-ELISA medac	**negative**	**60**	**5**	**4**	**69**
	equivocal	**0**	**1**	**1**	**2**
	positive	**1**	**1**	**2**	**4**
	total	**61**	**7**	**7**	**75**

7.C. SENSITIVITY AND SPECIFICITY

We investigated 322 samples from patients with suspected Borrelia infection during the diagnostic evaluation. Our data were compared with the nominal results. The nominal results were defined according to the MiQ standards for the diagnosis of Lyme borreliosis (MiQ 12) by means of a competitor's ELISA in combination with a Western blot assay. Sera with borderline ELISA and blot results were eliminated and not considered for the evaluation.

		Nominal results			
		negative	**equivocal**	**positive**	**total**
Borrelia-IgM-ELISA medac	**negative**	**154**	**0**	**36**	**190**
	equivocal	**2**	**0**	**5**	**7**
	positive	**11**	**0**	**114**	**125**
	total	**167**	**0**	**155**	**322**

Specificity = 92 %
Sensitivity = 74 %

Concordance: 83 %

The combination of IgG and IgM results in comparison to the nominal results defined above, is shown in the table below:

		Nominal results			
		negative	**equivocal**	**positive**	**total**
Borrelia-IgG/IgM-ELISA medac	**negative**	**48**	**0**	**5**	**53**
	equivocal	**1**	**0**	**0**	**1**
	positive	**11**	**0**	**232**	**243**
	total	**60**	**0**	**237**	**297**

Specificity = 80 %
Sensitivity = 98 %

Concordance: 94 %

7.D. PRECISION

Sample	Intra-assay variation				Sample	Inter-assay variation			
	mean AU	SD	CV (%)	n		mean AU	SD	CV (%)	n
PC	27.6	0.9	3.3	22	**PC**	29.7	2.6	8.8	11
N° 1	80.5	1.8	2.2	22	**N° 4**	62.2	7.3	11.7	11
N° 2	29.8	0.8	2.7	22	**N° 5**	150.1	16.7	11.1	11
N° 3	185.9	3.9	2.1	22	**N° 6**	66.3	6.2	9.4	11
					N° 7	28.7	1.3	4.5	11

PC = positive control

8. DIAGNOSTIC INVESTIGATION OF CEREBROSPINAL FLUID (CSF)

The detection of Borrelia-specific antibody synthesis in the central nervous system (CNS) as part of the diagnostic investigation of CSF is an essential component of the differential diagnosis of neuro-borreliosis.

The identification of Borrelia-specific intrathecal antibody synthesis is performed by estimation of the antibody index (AI) according to Reiber (Reiber 1987, 1999). In order to calculate the AI the following conditions must be fulfilled:

- estimation of the albumin quotient (Q_{alb}) so as to assess the function of the blood-brain-barrier and to calculate the Limes value in patients with elevated total IgM quotients ($Q_{tot} > Q_{lim}$)
- estimation of the total IgM quotient (Q_{tot})

8.1. SPECIMEN

8.1.1. The test is suitable for serum-CSF paired samples.

8.1.2. In order to avoid interferences with high IgG titres and rheumatoid factors (RF) an absorption of IgG/RF shall be performed for all sera and CSF.

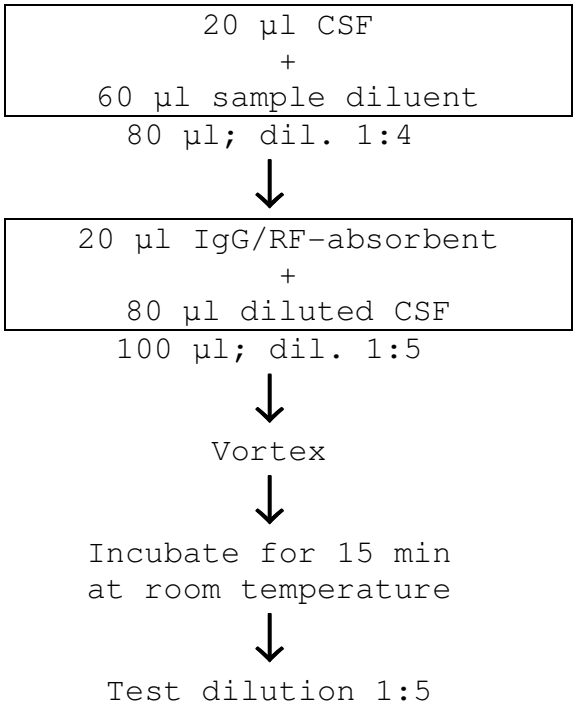
8.1.3. Further pre-treatment of sera and CSF samples, e.g. inactivation, is not necessary. However sera should neither be contaminated with microorganisms nor contain any red blood cells.

8.1.4. Serum
Sera have to be diluted 1:150 (see 5.A.). If the AU value is above 200 AU/ml, the sample has to be further diluted in 10 fold steps with sample diluent after RF absorption (see 8.4.).

8.1.5. Cerebrospinal fluid
The CSF samples are diluted to a standard dilution 1:5. They are diluted 1:4 with sample diluent first, following a 1:1.25 dilution with IgG/RF absorbent. If the measured antibody concentration is above 200 AU/ml, the sample has to be further diluted in 10 fold steps after RF absorption (see 8.4.).

Serum and CSF have always to be assayed in parallel in the same test run (this also applies to repeat measurements).

8.1.6 Table for the IgG/RF absorption of CSF
Indication per determination



8.2. TEST PROCEDURE

The test setup for Borrelia-IgM determination in serum-CSF pairs is performed as described under 5.B.

8.3. DETERMINATION OF TOTAL IgM AND ALBUMIN CONCENTRATIONS

In addition to the Borrelia-specific IgM determinations, in each sample pair the total IgM concentration and the albumin concentration in serum and CSF have to be determined.

8.4. CALCULATION OF RESULTS/VALIDITY

* Validity

The validity criteria specified under 6.A. are applicable.

Repeat the run if the results do not meet the specification.

The following points also apply to CSF investigations:

- The assay range for sera extends from 12.6 – 200 AU/ml.
- Sera with an antibody content < 12.6 AU/ml in a dilution of 1:150 are considered as sero-negative. In such cases the antibody index cannot be determined.
- In single cases, sero-negative patients may have demonstrable intrathecal Borrelia IgM antibodies indicating a suspected neuroborreliosis, which has to be confirmed with further differential diagnosis measures.
- The assay range for CSF extends from 6.25 – 200 AU/ml.
- CSF samples from patients with positive serostatus which are below the assay range at the dilution of 1:5 cannot be calculated. In this case an intrathecal Borrelia IgM antibody synthesis is most unlikely.

* Evaluation

- Calculation of AU values see 6.A.
- Calculation of the pathogen-specific IgM quotient (Q_{spec}).

$$Q_{spec} = \frac{\text{AU CSF x dilution CSF}}{\text{AU serum x dilution serum}}$$

- Calculation of the antibody index

The pathogen-specific index is calculated from the formula:

1. $AI = Q_{spec}/Q_{tot}$ (for $Q_{tot} < Q_{lim}$)
2. $AI = Q_{spec}/Q_{lim}$ (for $Q_{tot} > Q_{lim}$)
3. $Q_{lim} = 0{,}67 \times \sqrt{Q_{alb}^{2} + 120 \times 10^{-6}} - 7{,}1 \times 10^{-3}$

8.5. INTERPRETATION OF RESULTS

* AI values from 0.6 – 1.3 are regarded as the normal range.

* AI values > 1.3 and ≤ 1.5 are regarded as borderline.

* **The pathological range is defined as AI > 1.5.**

* AI values < 0.6 point to analytical errors and cannot be interpreted.

* The CSF diagnostic criteria for an acute, active disease of the CNS are inflammatory changed CSF and a raised albumin quotient. These reflect obstruction to CSF flow due to some inflammatory condition.

* Elevated antibody indices are not reliable evidence of the acute phase of an infective CNS disease, because antibodies, even intrathecally, may persist for long periods, and because polyspecific CNS-intrinsic antibody synthesis may occur. In some circumstances it may be advisable to look for a significant change in the AI value by testing second paired serum-CSF samples. For this purpose further sample collection will be necessary and should be performed after a time interval chosen in the light of the clinical circumstances.

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents), should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated by national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

Date of issue: 01.06.2010

REFERENCES

Aguero-Rosenfeld, M.E., Wang, G., Schwartz, I., Wormser, G. P.: Diagnosis of Lyme Borreliosis. Clin Microbiol Rev 18 (3), 484-509 (2005)

Aguero-Rosenfeld, M.E.: Lyme Disease: Laboratory Issues. Infect Dis Clin N Am 22, 301-313 (2008)

Goettner, G., Schulte-Spechtel, U., Hillermann, R., et al.: Improvement of Lyme Borreliosis Serodiagnosis by Newly Developed Recombinant Immunoglobulin G (IgG) and IgM Line Immunoblot Assay and Addition of VlsE and DbpA Homologues. J Clin Microbiol 43 (8), 3602-3609 (2005)

Hofmann, H.: Lyme Borreliose. Kutane Manifestationen. Hautarzt 56 (8), 783-796 (2005)

Jobe, D.A., Lovrich, S.D., Asp, K.E. et al.: Significantly Improved Accuracy of Diagnosis of Early Lyme Disease by Peptide Enzyme-Linked Immunosorbent Assay Based on the Borreliacidal Antibody Epitope of *Borrelia burgdorferi* OspC. Clin Vaccine Immunol 15 (6), 981-985 (2008)

Kaiser, R., Fingerle, V.: Neuroborreliose. Nervenarzt 80 (10), 1239-1251 (2009)

Krause, A., Fingerle, V.: Lyme Borreliose. Z Rheumatol 68 (3), 239-254 (2009)

Leitlinien der Deutschen Gesellschaft für Neurologie zur Neuro-borreliose, 2008

Mathiesen, M.J., Christiansen, M., Hansen, K. et al.: Peptide-Based OspC Enzyme-linked Immunosorbent Assay for Serodiagnosis of Lyme Borreliosis. J Clin Microbiol 36 (12), 3474-3479 (1998)

Mathiesen, M.J., Holm, A., Christiansen, M. et al.: The Dominant Epitope of *Borrelia garinii* Outer Surface Protein C Recognized by Sera from Patients with Neuroborreliosis Has a Surface-Exposed Conserved Structural Motif. Infect Immun 66 (9), 4073-4079 (1998)

Nau, R., Christen, H.-J., Eiffert, H.: Lyme Disease-Current State of Knowledge. Dtsch Arztebl Int 106 (5), 72-82 (2009)

Reiber, H., Felgenhauer, K.: Protein transfer at the blood cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. Clin Chim Acta. 319-328 (1987)

Reiber, H.: Liquordiagnostik, in: Klinische Neurologie, Berlit, P.(Hrsg.): Springer Verlag, Heidelberg, 148–177 (1999)

Stanek, G., Strle, F.: Lyme borreliosis: a European perspective on diagnosis and clinical management. Curr Opin Infect Dis 22, 450–454 (2009)

Strle, F., Stanek, G.: Clinical Manifestations and Diagnosis of Lyme Borreliosis, in: Lyme Borreliosis. Curr Probl Dermatol 37, Lipsker, D., Jaulhac, B. (eds.): Karger Basel, 51–110 (2009)

Wildemann, B., Oschmann, P., Reiber, H. (Hrsg.): Neurologische Labor-diagnostik: Liquordiagnostik. 30–73, Georg Thieme Verlag Stuttgart New York (2006)

Wilske, B., Zöller, L., Brade, H., Eiffert, U.B., Stanek, G., Pfister, H.-W.: Quality Standards for the Microbiological Diagnosis of Infectious Diseases.: Lyme Borreliosis. (MIQ 12) Urban und Fischer München (2000)

Wilske, B.: Epidemiology and diagnosis of Lyme borreliosis. Annals of Medicine 37, 568–579 (2005)

Wormser, G.P., Nowakowski, J., Nadelmann, R.B. et al.: Impact of Clinical Variables on *Borrelia burgdorferi*-Specific Antibody Seropositivity in Acute Phase Sera from Patients in North America with Culture-Confirmed Early Lyme Disease. Clin Vaccine Immunol 15 (10), 1519–1522 (2008)