VIROTECH Borrelia IgM ELISA
(Borrelia IgM ELISA)

Order no: EC022M00 Colour coding: gold/pale blue

Borrelia IgM Liquor/CSF Standards

Order no: EC022L80

Including performance data for CSF diagnosis

FOR IN VITRO DIAGNOSIS ONLY

Virotech Diagnostics GmbH
Waldstrasse 23 A2
63128 Dietzenbach, Germany

Tel.: +49(0)6074-23698-0
Fax.: +49(0)6074-23698-900
www.goldstandarddiagnostics.com

CE

Freigabedatum: 26.04.2022 08:33
REV 18 / VIROTECH Borrelia IgM ELISA EN

Contents

1. Intended Use 3

2. Diagnostic Relevance 3

3. Test Principle 4

4. Package Contents 4

4.1 Package Contents (IgM Testkit) 4
4.2 Package Contents (IgM CSF-Standards) 4

5. Storage and Shelf life of the Testkit and the ready to use reagents 4

6. Precautions and Warnings 5

7. Material required but not supplied 5

8. Test Procedure – SERUM DIAGNOSTIC 5

8.1 Examination Material 5
8.2 Preparation of Reagents 6
8.3 VIROTECH ELISA Test Procedure 6
8.4 Usage of ELISA processors 6

9. Test Evaluation – SERUM DIAGNOSTIC 7

9.1 Test function control 7
9.2 Calculation of the VIROTECH Units (VE) 7
9.3 Interpretation Scheme IgM 7
9.4 Limits of the Test 7

10. Performance Data – SERUM DIAGNOSTIC 8

10.1 Diagnostic sensitivity 8
10.2 Sensitivity and specificity 8
10.3 Cross reactivity 9
10.4 Seroprevalence (expected values) 9
10.5 Intra-assay coefficient of variation (repeatability) 9
10.6 Inter-assay coefficient of variation (reproducibility) 9

11. Performance Data CSF-DIAGNOSTIC 9

11.1 Sensitivity and Specificity 9
11.2 Cross Reactivity 10

12. References 10

13. Test Procedure Scheme 12

1. Intended Use

The Borrelia afzelii IgM ELISA serves as screening test for the semiquantitative and qualitative detection of IgG antibodies to Borrelia burgdorferi sensu lato in human serum. It can also be used in the parallel testing of paired serum and CSF samples, leading to the quantitative detection of endogenous synthesis of IgG and IgM antibodies.

2. Diagnostic Relevance

Lyme borreliosis (or Lyme disease) is a systemic disease which is caused by infection with the spirochaetes *Borrelia burgdorferi* (1,2). The spirochaetes are transmitted to humans from the bite of an infected tick. In Europe, the tick *Ixodes ricinus* has been identified as the main vector (5). At present, at least three human pathogenic *Borrelia burgdorferi* species are described for Europe (sensu lato), which are summarised under the term *Borrelia burgdorferi* sensu lato: *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii* (3,5,6).
Lyme borreliosis is a multisystem disease which develops in stages predominantly affecting the skin, the joints and the nervous system. Because of the wide range of clinical manifestations, the diagnosis of Lyme borreliosis is difficult (5). Among other things, a distinction between the different dermatological diseases (such as B-cell lymphoma of the skin and Lupus erythematosis), neurological diseases (such as multiple sclerosis) and internal diseases (such as arthritis and carditis) (15) is important for differential diagnostics.

The serological diagnostics of Lyme borreliosis is made more difficult by factors such as the following:

- a negative serology, particularly in the early stages, does not rule out Lyme borreliosis. The erythema migrans (primary stage) is seronegative in approximately 50% of cases (14)
- the formation of IgM antibodies can be completely absent
- IgM antibodies can persist over many months (10,11)
- IgG antibodies can still be detected years after clinical remission (10,11)
- cross reactions with other micro organisms have been observed (8,13). Diseases caused by bacteria, such as syphilis and herpes virus infections (particularly EBV), play an important role (12). False positive antibody responses can also occur in the presence of autoimmune antibodies (13).

Lyme borreliosis serology plays a supporting role in clarifying a clinically suspected case. Lyme borreliosis serology can supply important information about seronegativity or confirm a suspected case of fresh infection or advanced infection. However, it is essential that a positive antibody finding be assessed in association with the clinical picture (14).

We recommend carrying out the Lyme borreliosis serology in two stages (16). In the first stage, the samples to be tested should be examined with a sensitive screening test (MiQ12/2000 recommends using an ELISA as a screening test). After this, equivocal and positive sera should be examined in a confirmation test (Line immunoassay/Western Blot). The analysis in the Western Blot enables the antibody response to individual pathogen antigens to be analysed specifically.

The latest development is *in vivo*-expressed antigens and these are also now available for diagnostic use. The special feature of these antigens is that they are only expressed *in vivo* by the borreliae in the infected mammal host (humans). Outstanding among these new *in vivo*-expressed antigens is the general genospecies protein VlsE (17, 18, 19). This acts as a second early marker next to OspC, particularly in IgG serology. Here, the tests have shown that, with early borrelioses, VlsE is frequently found in the IgG as well as the OspC in the IgM and that the sensitivity of the diagnosis of early Lyme borrelioses can be significantly increased.

Neuroborreliosis
In the content of a Borreliosis-infection, symptoms, that concern the nervous system are called Neuroborreliosis. 10 – 15% of patients with Borreliosis develop a Neuroborreliosis. It occurs 5 weeks after the bit of the tick in average. The clinical diagnostical suspicion of patients with Neuroborreliosis can be confirmed by inflammatory CSF-changes and the detection of a borrelia-specific intrathecal antibody-synthesis. The intrathecal specific antibody-production is detected by the determination of the antibody-index. The *B. afzelii* specific intrathecal antibody production is developing in untreated patients during the

second week of disease, after three weeks it is detectable in about 75% of the patients and after 8 weeks in more than 99% of the patients. If patients carry the symptoms over a period of more than 2-3 months, a negative Borrelia-antibody test does nearly exclude the possibility of a Neuroborreliosis. The positive detection of only borrelia-specific antibodies does not detect an active infection with *Borrelia afzelii*. On the other hand the Borrelia Serology might be negative in the early stage of a Borrelia infection – especially at an early treatment with antibiotics (9). During an acute Neuroborreliosis it is eventually possible that the IgG-synthesis might not happen, so that only IgM-antibodies can be found (20).

The antigen used is a mixture of the *B. afzelii* strain PKo (originally isolated from a human erythema migrans lesion in Germany), the *B. garinii* strain PBr (originally isolated from the cerebrospinal fluid of a Neuro Borreliosis patient in Germany), and the *B. burgdorferi* strain ZS7 (originally isolated from an infected tick in Germany) recommended for Europe.

Strain	Antigen	Description	Purification	Specificity of the antigens
Borrelia afzelii PKo	Lysate antigen	Bacterial cell lysate, contains all native antigens	Raw cell extract in phosphate buffer	sensitive
Borrelia burgdorferi ZS7	Lysate antigen	Bacterial cell lysate, contains all native antigens	Raw cell extract in phosphate buffer	sensitive
Borrelia garinii PBr	Lysate antigen	Bacterial cell lysate, contains all native antigens	Raw cell extract in phosphate buffer	sensitive

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents

4.1 Package Contents (IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgM negative Control, 2000µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgM cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgM positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 Package Contents (IgM CSF-Standards)

Borrelia ELISA IgM-Standards for the quantification of pathogen-specific antibody concentrations, 4 vials à 1000µl, human serum with protein-stabilizer and preservative, ready to use, 100wME; 25wME; 6,2wME;1,5wME (wME = arbitrary measurement units)

5. Storage and Shelf life of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure – SERUM DIAGNOSTIC

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient sera-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.

2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgM-positive, negative and cut-off control as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation – SERUM DIAGNOSTIC

The ready to use controls serve for a semiquantitative determination of specific IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$\text{VE (positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut - off control)}} \times 10$$

$$\text{VE (patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut - off control)}} \times 10$$

9.3 Interpretation Scheme IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. The course of the IgM-immune response is variable within the first 3 weeks after the infection (4). The treatment of the patients with antibiotics in the early stage of the disease may lead to a supression of the immune response, means no anti-*Borrelia burgdorferi* s.l. specific antibodies may be detected (8).
3. The cross-reaction between Borrelia and other spirochaeta may lead to a false positive result. Sera of patients with the following infections may cross-react: Syphilis (*Treponema pallidum*), Framboesia (*Treponema pertenue*), Recurrent Fever

(Borrelia spec.), Leptospirosis (Leptospira spec.). Furthermore there may occur cross-reactions with Herpes Diseases (CMV, HSV, Parvovirus) (12, 13).

4. Caused by a polyclonal stimulation of B-lymphocytes during the course of an EBV-infection (infectious mononucleosis), an unspecific development of anti-Borrelia-antibodies may occur, especially in the IgM-class (12, 13). In case of an isolated IgM-result and a missing Borrelia anamnesis, an infectious mononucleosis must therefore be excluded differential-diagnostically.

10. Performance Data – SERUM DIAGNOSTIC

10.1 Diagnostic sensitivity

In order to evaluate the diagnostic sensitivity, 43 clinically characterised sera (early manifestation) were tested.

Borrelia afzelii IgM ELISA		
positive	**borderline**	**negative**
35	5	3

With reference to the diagnostic findings, the diagnostic sensitivity worked out to be 92,1%. Borderline sera were not included in the calculation of diagnostic sensitivity.

10.2 Sensitivity and specificity

For the determination of the sensitivity and specificity, clinically characterised Lyme borreliosis sera, blood donor sera and sera from pregnant women were tested in the Borrelia afzelii IgM ELISA and in a Borrelia burgdorferi ELISA for comparison.

Sensitivity

Sera collective (n=106); Lyme borreliosis early manifestations (n=43), neuroborreliosis (n=19), Lyme arthritis (n=19), ACA (n=25).

VIROTECH ELISA Borrelia afzelii IgM	Comparison test		
	positive	negative	borderline
positive	63	3	5
negative	0	21	2
borderline	2	7	3

For the Borrelia afzelii IgM ELISA, the sensitivity > 99.0%.
Borderline sera were not included in the calculation.

Stage	Sensitivity [IgM]
Stage I (EM, n=43)	92.1, %
Stage II (NB, n=18)	72.2 %
Stage III (ACA, Lyme, n=44)	60.5 %

Specificity

Sera collectively (n=88), blood bank sera (n=78), sera from pregnant women (n=10).

VIROTECH ELISA Borrelia afzelii IgM	Comparison test

	positive	negative	borderline
positive	2	1	1
negative	0	81	1
borderline	0	2	0

For the Borrelia afzelii IgM ELISA, the specificity > 98.8%.

Borderline sera were not included in the calculation.

10.3 Cross reactivity

Cross reactions are known with Treponema-positive sera.

Borrelia reactive sera can be produced during infection associated with Herpes virus infections (predominantly with EBV).

Cross-reactions with mycoplasma, Helicobacter pylori, CMV, Parvo and Yersinia sera as well as autoimmune sera are less common.

Treponema pallidum positive sera, EBV positive sera as well as autoimmune sera were investigated on the B. afzelii IgM ELISA with regard to their cross-reactivity:

Collective	**pos**	**neg**	**limit**	**Sum**
Treponema pallidum pos. sera	6	14	2	22
EBV pos. sera	6	3	1	10
Autoimmune sera	1	13	1	15

10.4 Seroprevalence (expected values)

The following table shows tests for 78 blood donor sera:

	IgM
negative	74
borderline	3
positive	1

This corresponds to an infection rate of 1.3%:

10.5 Intra-assay coefficient of variation (repeatability)

In one assay, the strips of different plates from one lot were tested with a serum. The coefficient of variation determined was < 9%.

10.6 Inter-assay coefficient of variation (reproducibility)

In 11 independent test runs, a positive, an borderline and a negative serum were tested in different laboratories by different test persons.

Serum	**Average OD**	**Coefficient of variation of VEs**
negative	0.14	13.66
borderline	0.31	10.41
positive	1.49	10.52

11. Performance Data CSF-DIAGNOSTIC

Proof of a specific intrathecal antibody production based on the antibody index determination according to Reiber (21)

11.1 Sensitivity and Specificity

For the detection of the **diagnostic** sensitivity, defined Neuroborreliosis-positive CSF/Serum-pairs have been tested using the VIROTECH ELISA.

Diagnostic Sensitivity IgM

	n	%
total	25	100
pathological	24	96
normal	1	4

The sensitivity is 96%. It therefore fits to the range for sensitivity of the antibody detection procedures in the Lyme-Borreliosis-Diagnostic stage II / III (70-100%) mentioned in the MIQ.

For the detection of the **diagnostic** specificity, defined CNS-negative CSF-serum pairs have been tested using the VIROTECH ELISA.

Diagnostic Specificity IgM

	n	%
total	20	100
pathological	0	0
normal	20	100

The specificity is > 99,9%.

For the detection of the sensitivity and specificity, CSF-serum-pairs of ensured Neuroborreliosis, CSF-serum-pairs with suspicion of Neuroborreliosis samples have been tested using the VIROTECH Borrelia afzelii IgM ELISA and a reference-test.

Sensitivity and Specificity IgM

CSF/serum-pairs (n=58)

VIROTECH ELISA	Reference-test	
	pathological	normal
pathological	22	1
normal	1	34

In IgM the sensitivity for the VIROTECH Borrelia afzelii IgM ELISA is 95,7% and the specificity is 97,1%.

11.2 Cross Reactivity

The same antibodies are present in the cerebrospinal fluid and the serum (22). As a consequence, cross-reactions with antibodies against the same pathogens can occur in serological diagnostics as well as in cerebrospinal fluid diagnostics. For this reason, the data from serology can be applied to cerebrospinal fluid diagnostics.

12. References

1. Burgdorfer, W., Barbour, A.G., Hayes S.F. et al. (1982), Lyme disease - a tick -borne spirochetosis?, Science 216:1317-19.
2. Steere, A.C. (1989), Lyme Disease, N. Engl. J. Med. 321:586-96.
3. Dressler, F., Ackermann, R. and Steere, A.C. (1994), Antibody responses to the three genomic groups of Borrelia burgdorferi in European Lyme Borreliosis, J. Infect. Dis. 169: 313-318.
4. Hofmann, H. (1996) Lyme Borreliosis – Problems of Serological Diagnosis, Infection 24, No. 6 :470-472.
5. Pfister,H-W., Wilske, B. (1994) Lyme borreliosis: basic science and clinical aspects, The Lancet Vol. 343: 1013-1015.
6. Dressler, F. (1994) Lyme borreliosis in European children and adolescents, Clinical and Experimental Rheumatology 12 (Suppl. 10) :49-54.
7. Hansen, K. (1993), Laboratory Diagnistic Methods in Lyme Borreliosis, Elsevier Science Publishing Co., Inc.:12.

8. Tewald,F. Braun, R. (1998), Durchführung und Interpretation serologischer Tests bei Verdacht auf Borrelieninfektion [*Procedure and Interpretation of Serological Tests for Suspected Borreliosis Infection*], Clin.Lab. 44: 897-902.
9. Goosens, H.A.T., Bogaard, van den A.E., Nohlmans, M.K.E., (1999), Evaluation of Fifteen comercially available serological tests for diagnosis of Lyme borreliosis,Eur.J. Microbiol.Infect.Dis 18: 551-560.
10. Craft, J.E., Fischer, D.K., Shimamoto, G.T. and Steere, A.C. (1986), Antigens of Borrelia burgdorferi recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G late in the illness., J. Clin. Invest. 78:934-39.
11. Craft, J.E., Grodzicki, R.L. and Steere, A.C. (1984), Antibody response in Lyme disease: evaluation of diagnostic tests, J. Inf. Dis. 149:789-95.
12. Goosens, H.A.T., Bogaard, van den A.E., Nohlmans, M.K.E., (1999), Epstein-Barr Virus and Cytomegalovirus Infections cause false.positive results in IgM two-test protocol for early Lyme-Borreliosis, Infection 27 No.3: 231.
13. Horst, H. (1997), Einheimische Zeckenborreliose (Lyme-Krankeit) bei Mensch und Tier [*Endemic Tick Borreliosis (Lyme disease) in Humans and Animals*], 3., überarbeitete Auflage, Spitta Verlag: 128-130.
14. RKI (1999), Ratgeber Infektionskrankheiten, Lyme-Borreliose [*Encyclopaedia of Infectious Diseases, Lyme Borreliosis*], Epidemiologisches Bulletin, überarbeitete Auflage
15. Oschmann und Kraiczy (1998) Lyme-Borreliose und Frühsommer-Meningoenzephalitis [*Lyme Borreliosis and Spring-Summer Meningo Encephalitis*], UNI-MED-Verlag 48-67.
16. Wilske et al. MiQ12/2000; Urban&Fischer
17. Zhang, J-R. et al.; Antigenic variation in Lyme disease Borrelia by promiscuous recombination of VMP-like sequence cassettes (1997); Cell; 89:275-285
18. Lawrenz, M.B. et al.; Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi*; American Society of Clinical Microbiology; Dec. 1999: 3997-4004.
19. Wang, D., Botkin, D.J. and Norris, S.J.; Characterization of the vls antigenic variation loci of the Lyme disease spirochaetes *Borrelia garinii* Ip90 and *Borrelia afzelii* ACAI (2003); Molecular Microbiology; 47(5): 1407-1417.
20. Felgenhauer K, Beuche W: Labordiagnostik neurologischer Erkrankungen; Thieme Verlag, Stuttgart, New York, 1999
21. Reiber H., und Lange P. (1991) Quantification of virus-specific antibodies in cerebrospinal fluid and serum: sensitive and specific detection of antibody synthesis in brain, Clin Chem 37: 1153-60
22. Reiber, H. und Peter J. B. (2001) Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs, Journal of the Neurological Sciences 184: 101-122.

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgM-Samples – Dilution**
1:101
Rheumafactor-absorption with RF-SorboTech
e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgM
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**