TestLine®

Instruction for use

EIA Borrelia recombinant IgM (192)

REF BrM192

Σ **192**

Kit for professional use

IVD CE

TestLine Clinical Diagnostics s.r.o.
Křižíkova 68, 612 00 Brno, Czech Republic
Tel.: +420 541 248 311
FAX: +420 541 243 390
E-mail: info@testlinecd.com
www.testlinecd.cz
www.testlinecd.com

CONTENT

1 Intended Use

Enzyme immunoassay for the detection of IgM antibodies to *Borrelia burgdorferi* sensu lato in human serum, plasma, cerebrospinal or synovial fluid and for the detection of intrathecal synthesis of specific antibodies.

2 Introduction

Lyme borreliosis (LB) is a multisystem infectious disease caused by spirochete *Borrelia burgdorferi.* The infection is transmitted by ticks of the genus *Ixodes*.

Lyme borreliosis is characterized by early and late clinical symptoms.

- Early localised infection – lasts days to weeks. The specific clinical sign of this phase is erythema migrans (EM), which appears in only 50% of patients. Early symptoms of the disease include flu-like symptoms, headaches and lymphadenitis.
- Early disseminated infection – lasts weeks to months. Borrelia bacteria are disseminated by blood and lymphatic system (CNS, joints, heart, eye and skin – secondary EM). In this phase, neuroborreliosis, neurofacialis paresis, borrelial lymphocytoma (swollen earlobes, knucklebones, etc.) and Bannwarth syndrome are the most frequently diagnosed symptoms.
- Late disseminated infection – lasts months to years. Immunopathological changes occur. The typical diagnostic findings are acrodermatitis chronica atrophicans (chronic skin lesions – ACA), chronic neuroborreliosis and borrelial arthritis.

The results of extensive studies demonstrate that all genospecies participate not only on the development of EM, but they cause many other clinical manifestations. However, *B. burgdorferi* sensu stricto is related mainly to joint disease, *B. garinii* is associated with neurological symptoms and *B. afzelii* with chronic skin disorders (especially ACA).

The diagnosis of the disease is based on anamnesis, clinical picture, and results of laboratory tests. At present, the diagnostic methods of choice are screening of specific IgG and IgM class antibodies by means of ELISA, and subsequent confirmation of the antibodies to specific antigens by means of immunoblot. Direct cultivation or electron microscopy is not applicable in a routine use.

Serological diagnosis of borreliosis is difficult regarding to the large genetic diversity of the species *Borrelia burgdorferi* s.l., possible cross reactivity with unrelated antigens of other microorganisms, and borrelia richness to heat shock proteins. Diagnosis is also complicated by different individual serological reactivity. The production of antibodies can be extremely slow in the early phase of the disease. On the other hand, the IgG and IgM antibodies can persists for more than ten years.

EIA Borrelia recombinant is an enzyme immunoanalytical 3rd generation kit with high diagnostic sensitivity and specificity. It is suitable for anti-borrelia antibody screening including detection of intrathecal synthesis in suspected neuroborreliosis patients.

3 Test Principle

The kit is intended for detection of specific IgM antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgM conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgM antibodies in the sample.

Antigen Used

Combination of recombinant antigens: OspC (B. afzelii, B. garinii, B. burgdorferi sensu stricto, B. spielmanii), VlsE, internal flagelin – p41i, p39, p17 and OspE of *Borrelia burgdorferi* sensu lato species.

4 Materials Provided

MICROPLATE	**Microtitre Plate** coated with antigen, 12 x 8 wells in bag with desiccant	2 pcs
CONTROL - CAL1	**Negative Control (Calibrator 1) 5 U/ml** Solution containing no specific human antibodies, ready to use	1 × 2 ml
CUTOFF CAL2	**CUT-OFF (Calibrator 2) 20 U/ml** Solution containing specific human antibodies in cut-off concentration, ready to use	1 × 3 ml
CONTROL + CAL3	**Positive Control (Calibrator 3) 200 U/ml** Solution containing specific human antibodies, ready to use	1 × 2 ml
CONJUGATE	**Conjugate** Solution containing peroxidase labelled animal immunoglobulin to human IgM, ready to use	1 × 28 ml
DILUENT 2	**Sample Diluent 2** Buffer with protein stabilisers, ready to use	1 × 105 ml
SUBSTRATE 2	**TMB-Complete 2** Chromogenic substrate solution containing TMB/H_2O_2, ready to use	1 × 28 ml
WASH 20x	**Wash Solution** 20× concentrated buffer	2 × 75 ml
STOP	**Stop Solution** Acid solution, ready to use	1 × 28 ml
	Instructions for use	1 pc

5 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

6 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum, citrate plasma, cerebrospinal and synovial fluid. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

7 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls (positive, negative and CUT-OFF) are ready to use, do not dilute further!

The Conjugate is ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

8 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

Dilution of cerebrospinal fluid (CSF) samples

Dilute well mixed CSF 1:2 with the Sample Diluent:

E.g.: 110 µl of CSF + 110 µl of the Sample Diluent

Mix well.

Dilution of synovial fluid samples

Dilute well mixed synovial fluids 1:21 and 1:41 with the Sample Diluent:

E.g.: 20 µl of fluid + 400 µl of the Sample Diluent and
10 µl of fluid + 400 µl of the Sample Diluent

Mix well.

9 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

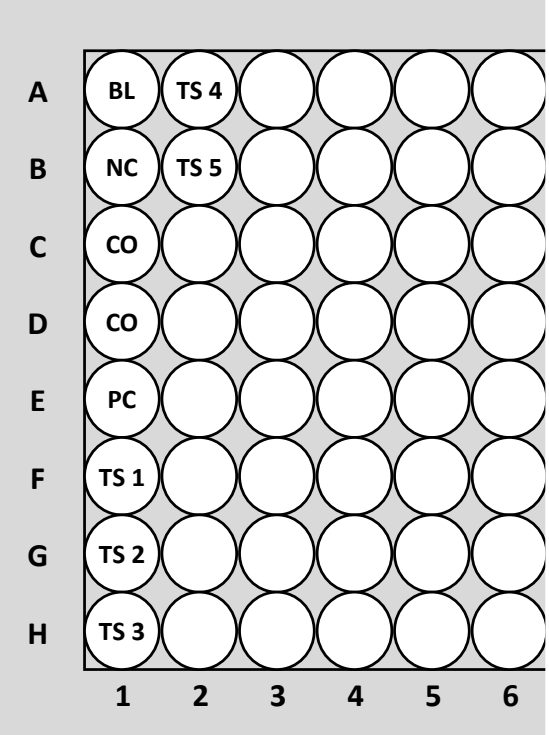
Quantitative evaluation in Units U/ml

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

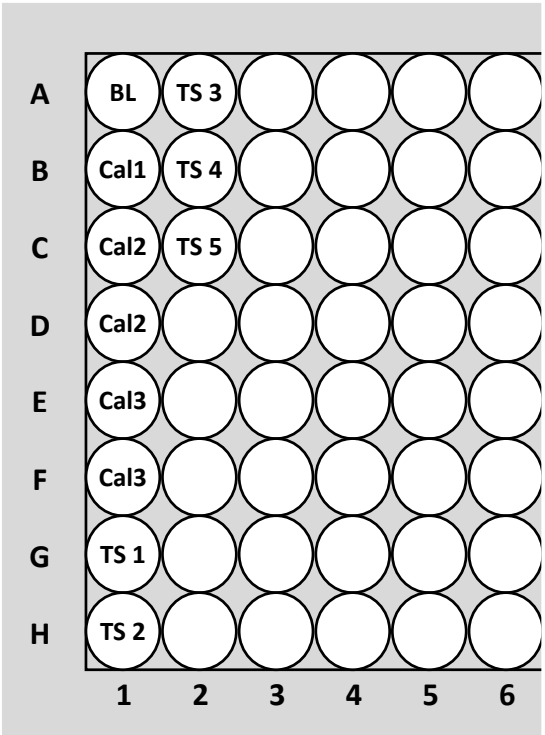
10 Working Schedule

Semiquantitative evaluation
Index of Positivity (IP)



BL	Blank (empty well)				
NC	100 µl	CONTROL	-	CAL1	
CO	100 µl	CUTOFF	CAL2		
PC	100 µl	CONTROL	+	CAL3	
TS 1-x	100 µl	diluted tested sample			

Quantitative evaluation
Units U/ml



BL	Blank (empty well)				
Cal1	100 µl	CONTROL	-	CAL1	
Cal2	100 µl	CUTOFF	CAL2		
Cal3	100 µl	CONTROL	+	CAL3	
TS 1-x	100 µl	diluted tested sample			

11 Quality Control

Test is valid if:

The absorbance of blank is lower than 0.150.

BLANK < 0.150

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL	-	CAL1	< 0.5 ×	CUTOFF	CAL2

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.150 – 0.900.

0.150 <	CUTOFF	CAL2	< 0.900

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL	+	CAL3	> 1.5 ×	CUTOFF	CAL2

12 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of serum, plasma, CSF, synovial fluid}}{\text{Mean absorbance od CUT-OFF}}$$

Interpretation of the test results is described in the tables (Table 1 and Table 2).

Table 1 Interpretation of serum, plasma and CSF test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Table 2 Interpretation of synovial fluid test results

Sample	Dilution 1:21	Dilution 1:41	Interpretation
	IP	IP	
sample X	lower than 1.0	lower than 1.0	negative
sample Y	higher than 1.0	lower than 1.0	borderline
sample Z	higher than 1.0	higher than 1.0	positive

The interpretation is based on comparison of IP values obtained with two different dilutions of the sample.

Quantitative evaluation in Units (U/ml)

Construct a calibration curve by plotting the concentration (X) of the calibrators in U/ml against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (U/ml) in samples from the calibration curve. Interpretation of the quantitative test results is described in the table (Table 3).

Table 3 Quantitative interpretation of serum, plasma and CSF test results in Units (U/ml)

Antibody level (U/ml)	Evaluation
lower than 18	negative
18 to 22	borderline
higher than 22	positive

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

Detection of intrathecal antibody synthesis using Antibody Index

Determination of Antibody Index is described in the Chapter Detection of intrathecal synthesis of specific antibodies.

13 Test Performance

13.1 Specificity and Sensitivity

Diagnostic specificity was determined in the panel of negative sera. Diagnostic sensitivity was determined in the panel of positive sera. The number of sera tested and the results obtained are described in the table (Table 4).

13.2 Reproducibility

Reference control samples were tested in a statistically significant number of replicates, either in one or several analyzes. Acquired data was used for Intra assay and Precision within the laboratory. The obtained results are described in the table (Table 4).

13.3 Analytical Sensitivity – maximum threshold sensitivity

The analytical sensitivity is the maximum binary dilution of CUT-OFF-like or low positive sample, possibly international recognized standards, giving absorbance significantly different from the background. The value is expressed in units of U/ml. This value is a minimum limit of detection and quantification. The obtained results are described in the table (Table 4).

13.4 Intra-homogeneity

The intra-homogeneity is expressed as the amount of agreement among 100 replicates of CUT OFF-like or low positive serum in one analyze. The value is expressed as a coefficient of variation. The obtained results are described in the table (Table 4).

13.5 Measuring range of the kits

The measuring range of each kit lies between the values of the lowest and the highest calibrators.

Table 4 Test Performance

Parameter	Value
Specificity (n 124)	97.72%
Sensitivity (n 114)	99.07%
Intra-assay	33.43%
Within-laboratory precision	6.42%
Analytical sensitivity limit – index of positivity (IP)	0.07
Intra-homogeneity	3.64%

13.6 Interference

Two samples (one negative plasma pool and one positive plasma pool) were spiked with potentially interfering endogenous substances. Results of interference testing are shown in the table (Table 5).

Table 5 Interference Results

Interfering substance	The result was not affected up to concentration:
Bilirubin	0.4 mg/ml
Triacylglycerols	20 mg/ml
Hemoglobin	5 mg/ml

13.7 Cross-reactivity

The assay was evaluated for potential cross-reactivity using samples positive for selected pathogens and factors. Results of testing are shown in the table (Table 6).

Table 6 Results of Cross-Reacting Pathogens or Factors

Category	n	Positive Result
Treponema pallidum	15	0
Chlamydia pneumoniae	8	0
Mycoplasma pneumoniae	13	0
TBEV	8	0
RF	13	0
ANA	19	0
EBV	9	0
Total	**85**	**0**

14 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

15 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

Protected by the European Patents No.0894143 and No.1012181.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

16 Detection of intrathecal synthesis of specific antibodies

16.1 Introduction

Detection of intrathecal antibody production is necessary for diagnosis of early and late neuroborreliosis according to the international recommendation (EFNS guidelines). Diagnosis of neuroborreliosis is based on the detection of specific antibodies to *Borrelia* sp. produced in cerebrospinal fluid (CSF). Calculation of the Antibody Index (AI) was established to evaluate intrathecal antibody synthesis.

16.2 Test Principle

The Antibody Index expresses the ratio of specific antibodies in the CSF to specific antibodies in blood serum in relation to the condition of the blood-CSF barrier and concentration of the total immunoglobulins in the CSF and serum. EIA test values are sufficient for the calculation. Of these, the Antibody Index Software by Testline Clinical Diagnostics s.r.o. constructs a calibration curve. On the basis of the calibration curve, arbitrary units are read and subsequently the Antibody Index of an analysed sample is calculated by Testline Clinical Diagnostics s.r.o. Antibody Index Software.

16.3 Material for test performance

To determine the Antibody Index, you need both CSF and serum (plasma) samples taken at the same time.

16.4 Data and equipment for Antibody Index evaluation

Test evaluation requires:

1. Values of albumin and total immunoglobulins IgM concentration in CSF and serum. Determination is performed e.g. nephelometrically. Ratio of albumin concentrations in CSF and serum expresses haematoencephalic barrier condition.
2. Antibody Index Software

16.5 1.1 Dilution of cerebrospinal fluids (CSF) and sera samples

Use the same dilution scheme as for detection of specific antibody level (see Chapter Preparation of Samples).

Additional dilution of CSF and sera samples

The additional dilution is performed only if absorbance of either CSF, or both CSF and serum, exceeds the absorbance of the highest point of calibration curve and the AI result in such pair of samples is negative.

Dilute further the diluted CSF and sera samples (see Chapter Preparation of Samples) 1:10 with the Sample Diluent:

E.g.: 15 µl of diluted serum sample + 135 µl of Sample Diluent and
15 µl of diluted CSF sample + 135 µl of Sample Diluent

Mix well.

16.6 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

The performance of the Antibody Index assay is identical to that of conventional sample testing.

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the sample at appropriate dilution into the other wells.
- Pipette 100 µl of the diluted samples (CSF, serum) into the other wells.

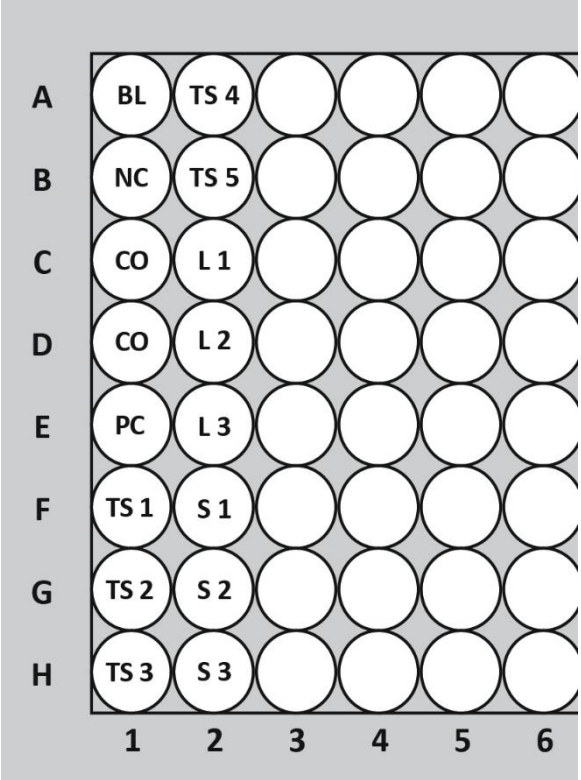
Quantitative evaluation in Units U/ml

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the sample at appropriate dilution into the remaining wells.
- Pipette 100 µl of the diluted samples (CSF, serum) into the remaining wells.

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

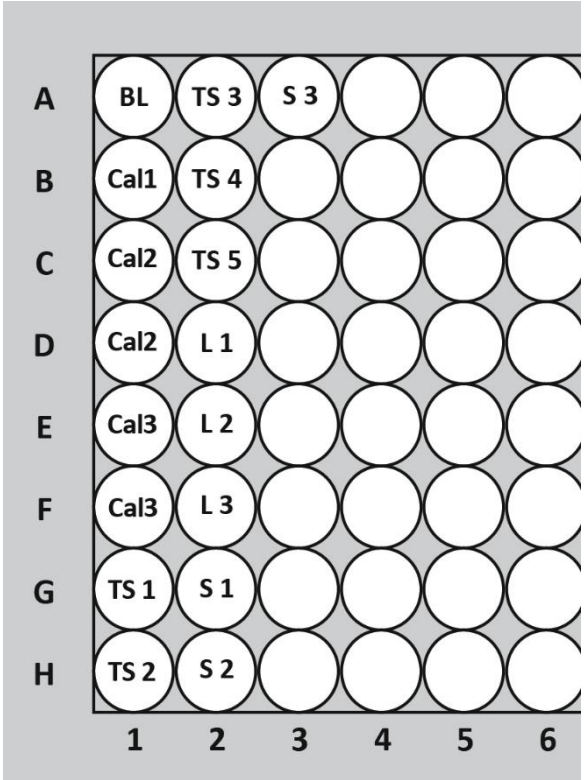
16.7 Working Schedule

Semiquantitative evaluation
Index of Positivity (IP)



BL	Blank (empty well)				
NC	100 µl	CONTROL	-	CAL1	
CO	100 µl	CUTOFF	CAL2		
PC	100 µl	CONTROL	+	CAL3	
TS 1-x	100 µl	diluted tested sample			
L 1-x	100 µl	diluted CSF sample			
S 1-x	100 µl	diluted serum sample			

Quantitative evaluation
Units U/ml



BL	Blank (empty well)				
Cal1	100 µl	CONTROL	-	CAL1	
Cal2	100 µl	CUTOFF	CAL2		
Cal3	100 µl	CONTROL	+	CAL3	
TS 1-x	100 µl	diluted tested sample			
L 1-x	100 µl	diluted CSF sample			
S 1-x	100 µl	diluted serum sample			

16.8 Quality Control

The test is valid:

The absorbance of blank is lower than 0.150.

BLANK < 0.150

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.350 – 0.750.

0.350 < | CUTOFF | CAL2 | < 0.750

16.9 Results Interpretation

Determination of Antibody Index (AI)

Use the Antibody Index Software (TestLine Clinical Diagnostics s.r.o.) for intrathecal synthesis detection and follow the software instructions.

For interpretation of the test results, see table below (Table 7).

Table 7 Interpretation of the investigation results

AI Value	Results Interpretation
< 1.4	negative AI, intrathecal synthesis unconfirmed
1.4 – 1.5	borderline AI, the result should be evaluated in correlation with clinical symptoms and antibody response in serum
> 1.5	positive AI, intrathecal synthesis confirmed

In rare cases, absorbance of either CSF, or both CSF and serum, exceed absorbance of the highest point of calibration curve, and AI of these paired samples is negative. You will be notified of such occurrence by the software. Then dilute further the diluted CSF and serum samples with the Sample Diluent (see Additional dilution of CSF and sera samples, Chap. Preparation of Samples) and repeat the test.

Notes to the interpretation of results:

Regarding individual capacity to develop an immune response to *B. burgdorferi* s. l., it is necessary to consider following eventualities:

- slow production of antibodies during the early phase of the disease
- possible effect of previous administration of antibiotics on antibody production
- atypical dynamics of immune response
- persistence of antibodies after the completion of therapy does not necessarily mean that the treatment has failed
- seronegativity in small part of patients
- a negative result in CSF does not exclude Lyme neuborreliosis.

Antibody levels in cerebrospinal fluid depend on following parameters:

- antibody level in blood serum
- permeability of haematoencephalic barrier
- intrathecal antibody production.

Intrathecal production of specific antibodies may continue for several years after antibiotic therapy. In some cases, specific antibodies can be detected in cerebrospinal fluid sooner than in serum.

17 References

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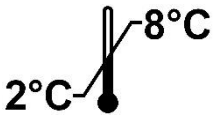
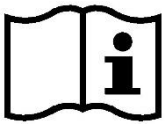
18 IFU Symbols

	Temperature limitation
	Keep dry
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

Notes

Notes

Summary of EIA Borrelia recombinant IgM (192) Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml) cerebrospinal fluids 1:2 (110 µl + 110 µl) synovial fluids 1:21 (20 µl + 400 µl), 1:41 (10 µl + 400 µl)
2		Pipette Controls and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 min
4		Aspirate and wash the wells 5×
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5×
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 15 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm