DRG® Borrelia 14kD and OspC IgM ELISA (EIA-4288)

RUO in the USA

Revised 12 May 2010 rm (Vers. 5.0)

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

Enzyme immunoassay for the *in-vitro* semi-quantitative or quantitative determination of IgM antibodies against the 14 kD and OspC antigens of Borrelia burgdorferi in human serum, plasma and CSF. Infections with all three B. burgdorferi subspecies (garinii, afzelii and senso strictu) are detected.

SUMMARY AND EXPLANATION

Borrelia burgdorferi, a bacterium of the Spirochaetaceae, is the ethiologic agent of Lyme disease (Borreliosis) being the most common disease in Europe and the USA transmitted by tics (Ixodes sp.). Lyme borreliosis is a multi-systemic disease with a broad spectrum of clinical symptoms. A typical symptom of the acute phase is the erythema chronicum migrans (ECM), often accompanied by flue-like symptoms. In later stages of the disease arthritis, carditis, as well as neurological and dermatological manifestations may occur.
Lyme borreliosis can be treated with antibiotics in all stages. Therefore, a safe and sensitive laboratory diagnosis of Lyme borreliosis, also detecting the early stage of diseases, is of major importance, since an early treatment is most appreciated. IgM antibodies usually appear approximately three weeks after the infection, IgG antibodies after four to six weeks. The early immune reaction is mainly directed against the 14 kD region of the flagellin and the OspC (Outer surface protein C) and is then spread on more and more bacterial proteins.
In the Borrelia burgdorferi 14 kD + OspC IgM ELISA the Borrelia burgdorferi-specific part of the flagellin is used as a recombinant protein for antibody binding. The results of extensive comparative studies with ELISA, IFA and agglutination tests as well as Western Blot demonstrate that the 14 kD IgM ELISA shows a higher specificity as well as an increased sensitivity for the early immune response in Lyme borreliosis. In addition to the recombinant 14 kD protein the native OspC protein is used as coating antigen in the DRG Borrelia 14 kD + OspC IgM ELISA.
Usually the acute phase is indicated by high titers of IgM antibodies. High IgG titers with low or without IgM antibodies occur when borreliosis is subsiding (due to therapy or spontaneously) or during the chronic stage. The Borrelia IgM test can be used for the diagnosis of Lyme borreliosis in the acute and chronic stage of the disease both requiring a therapy. Patients with a subsided borreliosis which does not require therapy any more will not show positive results.

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgM. After the substrate reaction the intensity of the color developed is proportional to the amount of IgM-specific antibodies detected. Results of samples can be determined directly using the standard curve or Cut-off standard.

WARNINGS AND PRECAUTIONS

1. For in-vitro use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact DRG or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to 3 mon in the broken, but tightly closed bag when stored at 2–8°C.

SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

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Storage Serum/Plasma/CSF:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability Serum/Plasma/CSF:	5 d	12 mon	

MATERIALS SUPPLIED

Quantity	Symbols	Component
1 x 12x8	MTP IgM	**Microtiter Plate** Break apart strips. Coated with specific antigen.
1 x 12 mL	ENZCONJ IgM	**Enzyme Conjugate** Ready to use. Red colored. Contains: anti-human IgM, conjugated to peroxidase.
4 x 1.5 mL	CAL A-D	**Standard A-D** 2; 10; 25; 100 U/mL Standard B = Cut-off Standard Ready to use. Contains: IgM antibodies against B. burgdorferi, stabilizers.
1 x 1.5 mL	CONTROL +	**Positive Control** Ready to use. Contains: IgM antibodies against B. burgdorferi, stabilizers.
1 x 1.5 mL	CONTROL -	**Negative Control** Ready to use. Contains: human serum, stabilizers.
1 x 100 mL	DILBUF M	**Diluent Buffer IgM** Ready to use. Blue colored. Contains: RF-Absorbent (goat anti-human IgG).
1 x 100 mL	WASHBUF CONC	**Wash Buffer, Concentrate (10x)** Contains: phosphate buffer.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 5; 10; 100; 1000 µL (adjustable)
2. Vortex mixer
3. Tubes (≥ 1 mL) for sample dilution
4. Incubator, 37°C
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
8. Bidistilled or deionised water
9. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

PRE-TEST SETUP INSTRUCTIONS

A. Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	Wash Buffer	ad 1000 mL	bidist. water	1:10	Resolve crystals at 18-25°C.	2-8°C	2 mon

B. Dilution of Samples

Serum, Plasma

Sample	to be diluted	with	Relation	Remarks
Serum, Plasma	generally	Diluent Buffer	1:101	e.g. 10 µL + 1 mL

Samples containing concentrations higher than the highest standard have to be diluted further.

Serum/CSF

For diagnostics of cerebrospinal fluid (CSF) according to Reiber, it is necessary to use approximately similar concentrations or Cut-off indices (COI) in the OD range of 2.0 to 0.3 for serum and CSF. This is generally ensured with the following dilutions:

Sample	to be diluted	with	Relation	Remarks
Serum	generally	Diluent Buffer	1:401	e.g. 5 µL + 2 mL
CSF	generally	Diluent Buffer	1:4	50 µL + 150 µL

The Cut-off indices are corrected by the dilution factors of each dilution in relation to the 1:101 dilution:
The Cut-off index for the 1:401 serum dilution must be multiplied by 4 and the 1:4 CSF dilution must be divided by 25.
A set of dilutions should be performed, if the test sample results are not within the range of 2.0 to 0.3 OD.
The following dilutions are recommended:

Serum	1:100	1:200	1:400	1:800	1:1600
CSF	1:2	1:4	1:8	1:16	1:32

⚠ IgM samples must not be treated with RF-Absorbent, because the RF-Absorbent is already part of the Diluent Buffer. The time until the samples are dispensed should be < 15-20 min.

TEST PROCEDURE

1. Pipette **100 µL** of **each Standard**, **Control and diluted sample** into the respective wells of the Microtiter Plate. In the semiquantitative test only Standard B (Cut-off Standard) is used.
2. **Incubate 1 h** at **37°C**. Use cover or moisture chamber.
3. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Pipette 100 µL of Enzyme Conjugate into each well.
5. **Incubate 30 min** at **37°C**. Use cover or moisture chamber.
6. Remove adhesive foil. Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
7. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8. Pipette **100 µL** of **TMB Substrate Solution** into each well.
9. **Incubate 30 min at RT in the dark.**
10. Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11. Measure optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 min** after pipetting of the Stop Solution.

QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.
It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

CALCULATION OF RESULTS

The evaluation of the test can be performed either semi-quantitatively or quantitatively.

C. Semi-Quantitative Evaluation

The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical density of the sample and Cut-off value. If the optical density of the sample is within a range of 10 % around the Cut-off value (grey zone), the sample has to be considered as borderline. Samples with higher ODs are positive; samples with lower ODs are negative.

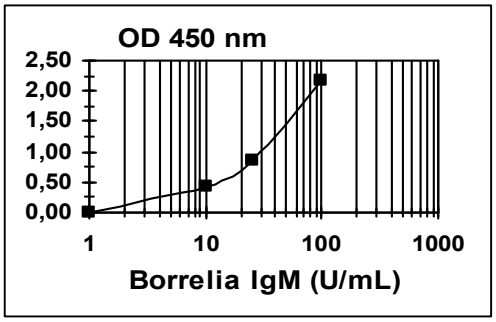
Typical Example:
Cut-off = OD (Standard B, Cut-off standard) = 0.45
Sample OD = 0.60
Cut-off index (COI): 0.60 / 0.45 = 1.33. The sample has to be considered positive.

D. Quantitative Evaluation

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.
For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).
The concentration of the samples can be read from the standard curve.
The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.
Samples showing concentrations above the highest standard can be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve
(Example. Do not use for calculation!)

Standard	U/mL	Mean OD
A	2	0.011
B	10	0.414
C	25	0.856
D	100	2.167



INTERPRETATION OF RESULTS / EXPECTED VALUES

Method	Range	Interpretation
Quantitative (Standard curve):	>11 U/mL	positive
	9 – 11 U/mL	borderline
	<9 U/mL	negative

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical

Semi-Quantitative (Cut-off Index, COI):	>1.1	positive
	0.9 – 1.1	borderline
	<0.9	negative

observations and diagnostic tests.

In case of **IgM negative results** with negative IgG an acute borreliosis is unlikely. However, a fresh infection can not be fully excluded if the sample has been collected within less than three weeks after infection as no specific antibodies are formed within this period. If the sample is borderline or positive for IgG, the result indicates a late or chronic infection as well as polyclonal antibody stimulation caused by other infections. A polyclonal stimulation can be excluded by a Western Blot analysis. Results should be confirmed by a follow-up control after 14 days.
IgM borderline results accompanied by negative IgG results may occur in acute infection and should be confirmed by a follow-up control after 14 days (titers constant or increasing) or by Western Blot analysis. If IgG results are positive or borderline, this result is indicative for a persisting acute infection requiring therapy. However, polyclonal stimulation should be excluded as above.
IgM positive values with coincident negative IgG results are indicative for an acute infection in the early phase. IgM positive results with positive or borderline IgG are indicative for a persisting acute infection.
The Borrelia 14 kD + OspC IgM ELISA shows high sensitivity and specificity for the detection of the early immune response to Borrelia burgdorferi infection. Due to the use of the recombinant 14 kD fragment of Borrelia flagellin and the purified native OspC, to which the early immune response is mainly directed, this test recognizes a Borrelia infection much earlier than other ELISA, hemagglutination or Western Blot techniques which employ an antigen prepared from ultrasonicated Borreliae. During further course of infection antibodies are formed against various other antigens. This results in a decrease of the absolute concentration of antibodies against the 14 kD fragment and the OspC. However, these antibodies will not disappear totally, so that also persisting or chronic infections are detected with high reliability.
The Borrelia 14 kD + OspC IgM ELISA is also suitable for the **follow-up control of a successful therapy**. In this case it has to be taken into account that antibody titers do not decrease significantly until 2 - 4 months after the infection is cured.
The results for the IgM assay may be increased unspecifically in **pregnants**. In such a case a reconfirmation of the results with a blot and observation of the course after 14 days is advised.

LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.
For cross-reactivities, see PERFORMANCE.
Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.
The following blood components do not have a significant effect (+/- 20 %of expected) on the test results up to the below stated concentrations:

Hemoglobin	2.0 mg/mL
Bilirubin	0.3 mg/mL
Triglyceride	2.5 mg/mL

PERFORMANCE

Analytical Specificity (Cross Reactivity)	Patient Group	Negative Results / tested samples		
	Lues (Treponema pallidum)	8/8		
	Rubella IgM positive	7/9		
	Parvovirus IgM positive	18/19		
	Measles IgM positive	8/8		
	CMV IgM/IgG positive	8/8		
	HSV IgM/IgG positive	8/8		
	VZV IgM positive	15/18		
	EBV IgM positive	6/8		
Precision	Range COI / U/mL	CV (%)		
Intra-Assay n=20	<1 / <10	4.4		
	>1 / >10	1.3		
Inter-Assay n=20	0.3 / 3.3	9.9		
	0.5 / 5	8.3		
	2.8 / 35	4.3		
	5.2 / 89	6.6		
Linearity	Range (OD)	Serial dilution range	Range (%)	
	2.0 – 0.3	1:1 – 1:16	80 – 120	
Method Comparison versus ELISA & Western Blot	Rel. Sensitivity	100 %		
	Rel. Specificity	> 95 %		
Automation	This test has been validated with, e.g, BEPIII (Dade Behring), TRITURUS (Grifols)			

CSF Determination	The ELISA for Lyme specific IgM has been performed with serum and CSF in 5 appropriate dilutions each: Serum 1:100-1:1600 and CSF 1:2-1:32. Serum/CSF pairs have been taken from the same day and the determination has been based on the evaluation program for CSF diagnosis by Prof. Reiber. For the evaluation serum/CSF pairs with intrathecally produced Lyme specific IgM with and without pathologic diffusion rate from blood to brain were taken. All DRG ELISA test results were in accordance to the clinical symptoms and reference test results.

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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità