DRG

DRG® Borrelia burgdorferi IgM ELISA (EIA-4485)

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REVISED 15 MAY 15, 2008 **RUO IN THE USA**

1 INTRODUCTION

Lyme borreliosis is a systemic infectious disease with various clinical symptoms.

The disease can be divided into 3 stages involving different organ systems. Cardinal symptoms of the first stage (4 to 8 weeks p. i.) are the erythema chronicum migrans and local or general lymphadenopathies (lymphadenosis cutis benigna). The clinical manifestations of the second stage (1 to 12 months p. i.) are from meningitis, meningopolyneuritis, encephalitis up to hemiparesis, myoneuralgia and arthralgia, especially knee joint arthritis. Cardiac manifestations like life-threatening myocarditis/pancarditis are rarely detected. The third stage (months to years p. i.) is characterized by chronic infection of the nervous system (neuroborreliosis, progressive encephalomyelitis), the skin (acrodermatitis chronica atrophicans) and the joints (chronic errosive arthritis). The late manifestations of Lyme borreliosis, especially, can reduce quality of life and antibiotic therapy is difficult. An early diagnosis of borrelia infections is therefore particularly important.

The infectious agent of Lyme disease was isolated from ticks in 1982 by BURGDORFER et al. and belongs to spirochetes of the genus Borrelia.

The tick species Ixodes ricinus represents the main vector of Borrelia burgdorferi in Europe. The occurrence of ticks within densely wooded rural areas correlates with Lyme disease cases which are most frequently found in those areas with a peak in the summer and autumn months. While the tick is taking blood from the host the borrelia are transmitted from the contents of the tick intestine. Persons at risk for Borrelia infections are people who work in forestry or agriculture, hunters and campers, soldiers and all people that occasionally spend time in wooded areas.

The incidence of Lyme disease reaches from 2 to 40/100 000 in middle Germany to 300/100 000 in Austria. The estimated number of unrecognised cases is presumably higher, because a tick bite is not always recognised and the typical erythema chronicum migrans only develops in about 50 % of borrelia infections.

The direct detection of the infectious agent via culture from blood, liquor or skin biopsies undoubtedly represents the safest method for diagnosis of Lyme disease. Unfortunately this method is hampered by the long reproduction time of the borrelia, the complexity of the culture medium and the relatively low sensitivity in case of culture from patient material. Thus culture as well as borrelia detection from tissue sections via immunohistological methods are not suitable for routine diagnosis. Methods of choice are detection of IgG and/or IgM antibodies via immunofluorescence or enzyme imunoassay (ELISA). The ELISAs use borrelia sonicates, extracts or partially purified antigens for plate coating.

Because of the regionally differing occurrence of subtypes (genospecies) and the known variability of the cell surface proteins of Borrelia burgdorferi, antigen mixtures are often preferred. Confirmatory tests like westernblot or dot blot are essential and the serological test results have to be interpreted together with the clinical picture. Negative test results do not exclude Lyme disease (early stage of infection, seronegative cases).

2 INTENDED USE

The Borrelia burgdorferi-IgM ELISA is an in-vitro device for quantitative detection of human anti-Borrelia burgdorferi-IgM-Antibodies in serum, plasma or cerebrospinal fluid.

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3 PRINCIPLE OF THE TEST

The ***-Borrelia burgdorferi* IgM ELISA** is an immune enzymometric two step assay based on an insolubilized antigen mixture of a *Borrelia afzelii* strain **additionally supplemented with the highly specific VlsE antigen**.

Samples as well as positive and negative controls are pipetted into the microwells that are coated with the antigen. After 30 minutes of incubation at 37 °C, unbound components are removed and the wells are washed 3 times with wash buffer. After addition of anti-human-IgM-HRP conjugate, wells are incubated for 30 minutes at 37 °C, unbound components are then removed and washed 3 times with wash buffer. After that ready-to-use TMB/substrate solution (tetramethylbenzidine and hydrogen peroxide) is added to the wells. The incubation time is 15 minutes at room temperature and the reaction is stopped by addition of sulphuric acid to the wells. Absorbances are read with a microplate reader at 450 nm wavelength (reference filter 620 nm wavelength if possible). Results are interpreted in reference to the absorbances of the negative control.

4 PREPARATION AND STORAGE OF SAMPLES

Serum, plasma or cerebrospinal fluid can be investigated for anti *Borrelia burgdorferi* IgM antibodies with the *Borrelia burgdorferi* IgM ELISA.

Serum or plasma samples have to be diluted **1:101** with sample diluent,
e. g. 5 µl sample + 500 µl sample diluent.

Pay attention to a sterile sample collection. Samples can be stored at 2 - 8 °C for a maximum time of 48 hours. For longer storage times samples have to be stored at - 20 °C.

Frozen samples have to be warmed to room temperature slowly and mixed well before starting the test run. Repeated freezing and thawing of samples should be avoided.

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5 TEST COMPONENTS FOR 96 WELLS

Microtitrer plate	12 single breakable 8-well strips (96 wells) coated with purified *Borrelia afzelii* antigen containing additionally a VlsE supplement	1 vacuum-sealed with desiccant
Wash buffer, 10X	10-fold; for 1000 ml solution	1 x 100 ml concentrate white cap
Sample Diluent		50 ml ready to use black cap
Positive Control		1 ml, ready to use, red cap
Negative Control		1 ml, ready to use, green cap
HRP Conjugate	HRP-labelled, polyclonal anti-bodies (sheep)	15 ml ready to use green cap
Substrate Solution	Substrate: 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml ready to use blue cap
Stop Solution	0.25 M sulphuric acid	15 ml ready to use yellow cap

6 MATERIALS REQUIRED BUT NOT PROVIDED

- micropipettes
- multi-channel pipette or multi-pipette
- glassware
- tubes (1 ml) for sample preparation
- pipette tips
- Reagent container for multi-channel pipette
- Incubator (37 °C)
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

7 PREPARATION AND STORAGE OF REAGENTS

7.1 Kit size and expiry

One kit is designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.

Upon receipt, all test components have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

7.2 Reagent preparation

Allow all components to reach room temperature prior to use in the assay.

The **microtitration plate** is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Washing solution

Prepare a sufficient amount of wash solution by diluting the 10fold concentrated wash buffer **1 + 9** with distilled or de-ionized water.

For Example:

10 ml wash buffer concentrate + 90 ml distilled water.

This ready to use wash buffer solution is stable for at least 30 days when stored at 2 - 8 °C.

Make sure that the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!

Avoid light exposure of the TMB substrate solution!

8 ASSAY PROCEDURE

- **Dilute samples with sample diluent 1 + 100,**
  e.g. 5 µl serum + 0.5 ml sample diluent
- **Avoid any time shift during dispensing of reagents and samples.**

Note: Using an automatic system it is recommended to wash 5 times (instead of 3 times) during each washing step.

8.1 Working steps

1. Warm all reagents to room temperature before use. Mix gently without foaming.

2. Dispense
   100 µl Negative and Positive Control and **100 µl of the diluted sample**
3. Cover plate and incubate for 30 min at 37°C.
4. Aspirate, then wash each well **3x** with **300 µl** diluted wash solution and tap dry onto absorbent paper.
5. Dispense **100 µl HRP-Conjugate**.
6. Cover plate and incubate for 30 min at 37°C.
7. Aspirate, then wash each well **3x** with **300 µl** diluted wash solution and tap dry onto absorbent paper.
8. Dispense **100 µl Substrate Solution**.
9. Incubate for 15 min at room temperature protected from light.
10. Dispense **100 µl Stop Solution** mix gently.
11. Read OD at 450 nm (reference filter 620 or 690 nm) with a microplate reader within 30 min after reaction stop.

9 RESULT INTERPRETATION

9.1 Determination of the cut off and the grey area

The cut off is calculated from:

mean absorbance of the negative control + 0.40 absorbance units

Grey area spans the region from:

0.9 x cut off absorbance up to the cut off absorbance

9.2 Test validity

The test run is valid if:

the mean absorbance of the negative control is ≤ 0.15

the mean absorbance of the positive control is ≥ 1.30

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria, contact your supplier.

10 REFERENCE VALUES

Borrelia burgdorferi-IgM ELISA

Negative	< 0,9 x cut off
Positive	> cut off

Serum samples with absorbances within the grey area should be retested. If necessary an additional sample collected after 1 to 2 weeks should be investigated.

11 LIMITATIONS OF THE PROCEDURE

In the course of borreliosis the immune reaction follows certain regularities. Antibody production in the early stage of infection is at first directed against the flagellin protein. Although of low variation within the species the flagellin of Borrelia burgdorferi unfortunately shows C- and N-terminal sequence homologies to the flagellin protein of other spirochetes. Thus an infection with other spirochetes than B. burgdorferi can induce cross reactive antibodies that in certain cases and with high titers can cause falsely reactive ELISA results.

Correct interpretation of the results should always take into account the clinical findings. In certain cases repeated investigations of patient sera collected with a distance of several weeks may be helpful.

Serum samples reactive for Borrelia burgdorferi IgM and/or IgG antibodies in the ELISA should be verified with a confirmatory test like western blot (e. g. Serablot Human Anti-Borrelia burgdorferi IgG/IgM).

Microbial contaminations of reagents or samples as well as cross contaminations of test kit components and samples can cause false results.

Incorrect washing for separation of unbound sample or reagent components as well as incorrect incubation times can cause false results.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

Intra-Assay:

Probe	Mean OD	CV (%)
1	0.85	3.6
2	1.22	5.1
3	1.79	4.6

Inter-Assay:

Probe	Mean OD	CV (%)
I	0.78	4.7
II	1.13	4.8
III	1.85	5.7

12.2 DRG ELISA vs comparative ELISA

A total of 56 serum samples was investigated in parallel in the DRG Borrelia burgdorferi-IgM ELISA and in another commercially available ELISA.

		comparative ELISA		
		negative	grey area	positive
DRG Anti-Borrelia IgM	negative	20	0	3
	grey area	2	1	4
	positive	5	1	22

12.3 Specificity and Sensitivity

For estimation of specificity and sensitivity more than 1000 sera from healthy persons and 70 Sera from patients with clinical confirmed diagnosis of Borreliosis were investigated.

Specificity: 96 %

Sensitivity: 91 %

13 COMMON ADVICE AND PRECAUTIONS

This kit is for in-vitro use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
The expiry dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.
Do not use reagents from other manufacturers.
Avoid time shift during dispensing of reagents.
All reagents should be kept at 2 °C to 8 °C before use.
Some of the reagents contain small amounts of Thimerosal (< 0.01 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.
Handle all components and all patient samples as if potentially hazardous.
Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

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SYMBOLS USED WITH DRG ASSAY´S

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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				

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Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..