



# Anti-Borrelia IgM

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgM antibodies to *Borrelia burgdorferi* in human serum, joint fluid and CSF

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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## INTENDED USE

**Anti-Borrelia IgM is used for the qualitative and quantitative determination of IgM antibodies to *Borrelia burgdorferi* antigens in human serum, joint fluid and cerebrospinal fluid (CSF).**

The spirochete bacterium *Borrelia burgdorferi* is the causative agent of the systemic infectious disease called Borreliosis or Lyme disease. *Borrelia burgdorferi* is solely transferred to humans by ticks (*Ixodes ricinus*). Consequently, Borreliosis is endemic in areas where ticks are found (e.g. Austria, Italy, several parts of Germany).

Clinical illness brought about by an infection with *Borrelia burgdorferi* is diverse and demonstrates three distinct stages:

- I Early stage (4 - 8 weeks) Erythema migrans
- II Generalization (1 - 12 months) meningitis, meningopolyneuritis, myalgia, Lymphadenitis cutis benigna, carditis
- III Late Stage (months to years) Acrodermatitis chronica atrophicans, arthritis, neuro-Borreliosis

The possible severe outcome of an infection with *Borrelia burgdorferi* and the complicated treatment of late stages of this infection demand diagnosis as early as possible.

The determination of IgM and IgG antibodies to *Borrelia burgdorferi* by enzyme immunoassay provides the first important step towards a serological diagnosis of Borreliosis. Anti-Borrelia burgdorferi IgM antibodies are detected mainly during the first stage of infection. Following the course of infection anti-Borrelia burgdorferi IgG antibodies occur, whereas the specific IgM antibodies disappear steadily.

Positive results should be confirmed by western blot analysis. Results obtained by *in vitro* diagnostics are to be interpreted in context with the clinical signs of the infection.

For the diagnosis of neuro-Borreliosis we suggest the determination of the antibody index using both serum and CSF samples. On request an instruction manual will be provided.

Wilske B et al.: Intrathecal production of antibodies against *B. burgdorferi* in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome). *J Infect Dis*, 1986, 153:304-314

Tumani H et al.: Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. *Neurology*, 1995, 45(9):1663-1670

Kaiser R Lücking CH: Intrathecal synthesis of specific antibodies in neuroborreliosis. Comparison of different ELISA techniques and calculation methods. *J Neurol Sciences*, 1993, 118:64-72

## PRINCIPLE of the TEST

Anti-Borrelia IgM is an enzyme immunoassay for the qualitative or semi-quantitative determination of IgM antibodies to *Borrelia burgdorferi*. Alternatively a calibrator set (3301) can be ordered to run the assay quantitatively.

The antibodies of the controls and diluted patient samples react with purified *Borrelia afzelii* antigens enriched with OspC and synthetic VlsE antigen immobilized on the solid phase of microtiter plates. Purified antigens of an European isolate of *Borrelia afzelii* coated on the microtiter plate guarantees the specific binding of *Borrelia burgdorferi* IgM antibodies of the specimen under investigation. Following an incubation period of 30 min at 37 °C, unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgM-antibodies conjugated to horse radish peroxidase (HRP). Following an incubation period of 30 min at 37 °C, excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

The horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H<sub>2</sub>SO<sub>4</sub>) into the wells after 15 min at 37°C turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

**Note:** *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1,0 ml sample diluent (C), prior to assay. (controls of the kit are ready for use, prediluted accordingly)*

## TEST COMPONENTS for 96 determinations

<b>A</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with <i>Borrelia afzelii</i> antigens enriched with OspC and synthetic VlsE	1	vacuum sealed with desiccant
<b>Ag</b> <b>96</b>			
<b>B</b>	<b>Concentrated wash buffer</b> sufficient for 1000 ml solution each	100 ml	concentrate capped white
<b>BUF</b> <b>WASH</b>	<b>10x</b>		
<b>C</b>	<b>Sample diluent</b> containing rheumatoid factor absorbent	100 ml	ready for use capped black
<b>DIL</b>			
<b>D</b>	<b>Conjugate</b> containing polyclonal anti-human-IgM (sheep) coupled with horse radish peroxidase	15 ml	ready for use capped green
<b>CONJ</b>			
<b>E</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml	ready for use capped blue
<b>SOLN</b> <b>TMB</b>			
<b>F</b>	<b>Stop solution</b> 0.25 M sulfuric acid	15 ml	ready for use capped yellow
<b>H2SO4</b>	<b>0.25M</b>		
<b>P</b>	<b>Positive control</b> (human serum diluted)	1 ml	ready for use capped red
<b>CONTROL</b>	<b>+</b>		
<b>N</b>	<b>Negative control</b> (human serum diluted)	1 ml	ready for use capped green
<b>CONTROL</b>	<b>-</b>		

For the quantitative determination of anti-*Borrelia* IgM antibodies in serum and liquor specimens to calculate antibody indices in the diagnosis of neuroborreliosis 4 anti-*Borrelia* IgM calibrators are separately available at Generic Assays GmbH (Order No. 3301).

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or micro-plate washer

- incubator (37 °C)
- microplate reader with optical filters for 450 nm and 620 or 690 nm
- graduated cylinders 100 ml
- distilled or de-ionized water

### Size and storage

Anti-*Borrelia* IgM has been designed for 96 determinations.

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

Upon receipt, all components of the Anti-*Borrelia* IgM 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.

### Preparation before use

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

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

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable at 2-8 °C up to 30 days. Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the substrate to light.

## ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense **100 µl** controls (P, N) or calibrators 1-4 **100 µl** diluted patient samples into the respective wells.
3. Seal plate, incubate **30 min** at 37 °C.
4. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at 37 °C.
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min in the dark at 37°C**.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the optical density at **450 nm** versus 620 or 690 nm within **15 min** after adding the stop solution.

## DATA PROCESSING

### 1. QUALITATIVE/SEMI-QUANTITATIVE EVALUATION

#### Cut-off determination

The cut-off for qualitative evaluation of the test is calculated according to the following criterion:

$$\text{OD of the negative control} + 0.45$$

Alternatively the results can be interpreted by calculating the binding index (BI – ratio between OD of the sample and OD of cut-off) using the following formula:

$$\text{BI} = \text{OD}_{\text{sample}} / (\text{OD}_{\text{negative control}} + 0.45)$$

#### Example of typical assay results

Wells	OD (a)	OD (b)	OD (mean)	BI
Negative control	0.109	0.101	0.105	0.19
Positive control	1.716	1.734	1.725	3.11
Positive	$> 0.105 + 0.450 = 0.555$			1.0
Negative	$< 0.605 \times 0.9 = 0.499$			0.9
Patient 1	1.116	1.124	1.120 - positive	2.00
Patient 2	0.501	0.518	0.509 - borderline	0.92
Patient 3	0.129	0.111	0.120 - negative	0.21

## REFERENCE VALUES

Anti-Borrelia IgM	OD	BI
negative	$< \text{cut-off} \times 0,9$	$< 0,9$
positive	$> \text{cut-off}$	$> 1,0$

Specimens with concentrations detected in the grey zone ( $0.9 \times \text{cut-off} < \text{OD sample} < \text{cut-off}$ ,  $0.9 < \text{BI sample} < 1.0$ ) should be tested again. Alternatively, the patient is to be re-examined within 1 to 2 weeks.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-Borrelia burgdorferi levels, as usually done for other diagnostic parameters, too.

#### Test validity

The test run is valid if:

- the mean OD of the positive control is  $\geq 0.80$
- the mean OD of the negative control is  $\leq 0.15$

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.

### 2. QUANTITATIVE EVALUATION

For the calculation of antibody indices regarding the diagnosis of neuroborreliosis quantitative determination of IgM antibodies to Borrelia burgdorferi is recommended. An instruction manual for the calculation of antibody indices can be ordered from Generic Assays.

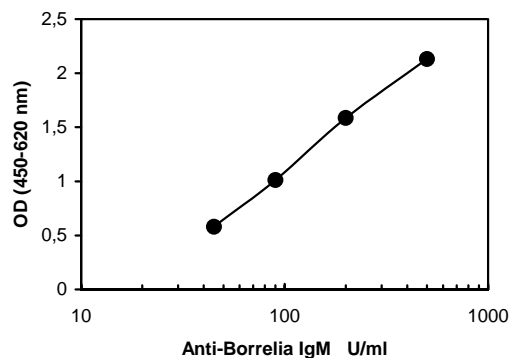
The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (separately available – Order No. 3301) on the ordinate, y-axis, (lin. scale) versus their respective anti-Borrelia concentrations on the abscissa, x-axis, (log. scale).

The anti-Borrelia IgM concentrations of the unknown samples are directly read off in U/ml against the respective OD values. The reference values for the decision positive-negative in quantitative evaluation are stated on the leaflet enclosed to the separately available calibrators.

## Example of typical assay results

Well	OD 450/620 nm	U/ml
Calibrator 1	0.579	45
Calibrator 2	1.011	90
Calibrator 3	1.586	200
Calibrator 4	2.133	500
Patient 1	1.102	102

### TYPICAL STANDARD CURVE



#### Limits of the Method

The early immune response of patients infected with Borrelia burgdorferi is directed towards a protein of the flagella of these spirochete bacteria, called flagellin. However, the flagellin of B. burgdorferi shows sequence homologies at the C- and N-terminus with flagellin proteins of other spirochete species (e.g. Treponema pallidum). Consequently, an infection with other spirochete bacteria may trigger cross-reacting antibodies. These antibodies will lead to false-positive results if produced abundantly.

The in vitro results should be interpreted always in context with the clinical status of the patient. Repeated testing over several weeks is recommended in order to discriminate an active infection from long term persistent antibody titers without clinical implication.

Positive anti-Borrelia IgM antibody sera should be confirmed by Western blot (3500) analysis.

## CHARACTERISTIC ASSAY DATA

#### Calibration

Due to the lack of an international reference material the Anti-Borrelia IgM is calibrated in arbitrary units (U/ml) or interpreted qualitatively.

#### Linearity

Dilutions of specimens in anti-Borrelia burgdorferi-IgM antibody free human serum are determined according to the expected theoretical values with Anti-Borrelia IgM.

#### Diagnostic sensitivity and specificity

Specificity data of the Anti-Borrelia IgM have been determined by examining more than 1000 patients with no clinical signs of an infection with Borrelia burgdorferi and 70 patients with the clinical diagnosis of Borreliosis.

Specificity: 97 %

Sensitivity: 90 %

#### Precision

Intraassay		Interassay	
OD (mean)	CV %	OD (mean)	CV %
0.85	3.6	0.78	4.7
1.22	5.1	1.13	5.8
1.79	4.6	1.85	5.7

