



INSTRUCTION MANUAL

REF 3200

March 28, 2008

Anti-Borrelia IgG

- 96 determinations -



IVD *In vitro* diagnostic device

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

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



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INTENDED USE

Anti-Borrelia IgG is used for the quantitative determination of IgG 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, neuroborreliosis

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 IgG and IgM 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 neuroborreliosis 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 IgG is an enzyme immunoassay for the quantitative determination of IgG antibodies to *Borrelia burgdorferi*.

The antibodies of the calibrators, controls and diluted patient samples react with purified *Borrelia afzelii* antigens enriched with OspC 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* IgG 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-IgG-(Fab)₂ 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 colourless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H₂SO₄) 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. The standard curve is plotted by using the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

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. (calibrators of the kit are ready for use, prediluted accordingly)*

For the diagnosis of neuroborreliosis liquor samples have to be diluted to 20 mg/l total IgG according to the total IgG determination in liquor.

TEST COMPONENTS for 96 determinations

A	Microtiter plate , 12 breakable strips per 8 wells (total 96 individual wells) coated with <i>Borrelia afzelii</i> antigens enriched with purified OspC and synthetic VisE	1	vacuum sealed with desiccant 2 adhesive foils
Ag 96			
B	Concentrated wash buffer sufficient for 1000 ml solution each	100 ml	concentrate capped white
BUF WASH	10x		
C	Sample diluent	100 ml	ready for use capped black
DIL			
D	Conjugate containing polyclonal anti-human-IgG-(Fab) ₂ (sheep) coupled with HRP	15 ml	ready for use capped red
CONJ			
E	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml	ready for use capped blue
SOLN TMB			
F	Stop solution 0.25 M sulfuric acid	15 ml	ready for use capped yellow
H2SO4	0.25M		
1 - 4	Calibrators (human serum diluted) conc.: see leaflet enclosed	1 ml each	ready for use capped white
CAL			
P	Positive Control (human serum diluted) conc.: see leaflet enclosed	1 ml	ready for use capped red
CAL	+		
N	Negative Control (human serum diluted)	1 ml	ready for use capped green
CAL	-		

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 microplate washer
- incubator (37 °C)

- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

Size and storage

Anti-Borrelia IgG 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 IgG 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. 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.

All other assay components are ready for use and can be stored up to the expiry date stated on the label.

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 calibrators (1 - 4)
100 µl controls (P, N)
100 µl diluted patient samples
 into the respective wells.
3. Seal plate; incubate **30 min** at 37 °C.
4. Decant, then wash each well **three** 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 **three** 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 30 min after adding the stop solution.

DATA PROCESSING

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective anti-Borrelia-concentrations on the abscissa, x-axis, (log. scale).

The anti-Borrelia IgG concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

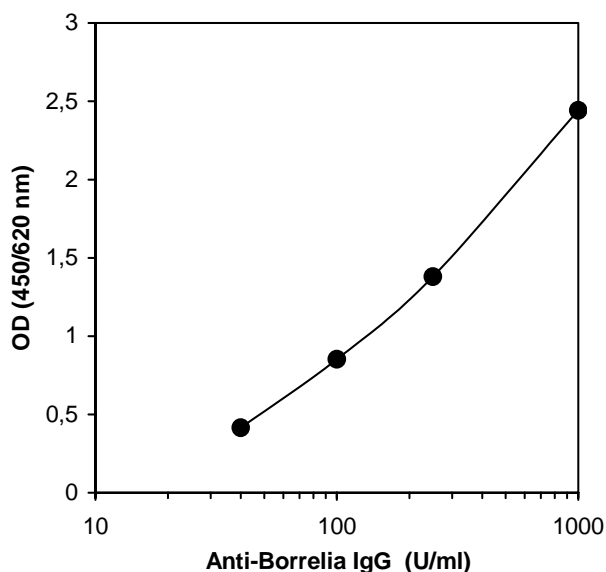
Anti-Borrelia IgG may be used also with Computer Assisted Analysis using software able to plot log/lin curves with spline smoothing or sigmoid fit.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly. According to the different dilution a correction factor is necessary for liquors.

Example of typical assay results

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 1	0.406	0.424	0.415	40
Calibrator 2	0.856	0.850	0.853	100
Calibrator 3	1.370	1.391	1.381	250
Calibrator 4	2.425	2.461	2.443	1000
Patient 1	1.118	1.086	1.102	158

TYPICAL STANDARD CURVE



Specimens with an OD > Calibrator 4 should be diluted with anti-Borrelia antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the standard 1 is ≤ 0.5
- the mean OD of the standard 4 is ≥ 1.2

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.

REFERENCE VALUES

Anti-Borrelia IgG	
negative	< 50 U/ml
positive	> 61 U/ml
grey zone	50 – 61 U/ml

Specimens with concentrations detected in the grey zone should be tested again. Alternatively, the patient is to be reexamined within 1 to 2 weeks.

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

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 always be interpreted 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 IgG antibody sera should be confirmed by Western blot analysis.

CHARACTERISTIC ASSAY DATA

Calibration

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

Linearity

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

Sensitivity

The analytical sensitivity of the Anti-Borrelia IgG is 20 U/ml.

Diagnostic sensitivity and specificity

Specificity and sensitivity data of the Anti-Borrelia IgG have been determined by examining more than 3000 patients with no clinical signs of an infection with Borrelia burgdorferi and 70 patients with the clinical diagnosis of Borreliosis.

Sensitivity: 95 %

Specificity: 98 %.

Precision

Intra-Assay (n=8)		Inter-Assay (n=4 x 8)	
Mean U/ml	CV %	Mean OD	CV %
35	3.2	39	3.1
73	5.0	70	5.9
201	5.5	212	5.8
621	5.6	611	6.9

INCUBATION SCHEME

Anti-Borrelia IgG (3200)

Dilute patient sera	10 µl serum + 1.0 ml sample diluent (C)
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1	Bring all reagents to room temperature (18-25°C) before use.				
			Calibrators (1 - 4)	Controls (P, N)	Samples
2	Pipette	Calibrators (1 - 4) Controls (P, N) 1 + 100 prediluted samples	100 µl	100 µl	100 µl
3	Seal plate, incubate 30 minutes at 37°C				
4	Wash Decant, Dispense 3 x 300 µl (made of B)				
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Seal plate, incubate 30 minutes at 37°C				
7	Wash Decant, Dispense 3 x 300 µl (made of B)				
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light 15 minutes at 37°C				
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration 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.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material.
 - Always use protective gloves.
 - Never pipette material by mouth.
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-Borrelia levels. as usually done for other diagnostic parameters, too. Therefore, the above mentioned data only provide a guide to values which might be expected.