



REF 3500

6<sup>th</sup> December, 2007

# Anti-Borrelia Blot IgM

- 27 determinations -



Immunoblot for the determination of IgM antibodies to *Borrelia burgdorferi* sensu lato in human serum or plasma

<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 Blot IgM is used for the quantitative determination of IgM antibodies to *Borrelia burgdorferi* sensu lato in human serum.** 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*).

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. 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.

The determination of IgG and IgM antibodies to *Borrelia burgdorferi* by enzyme immunoassay provides the first important step towards a serological diagnosis of Borreliosis. However positive results should always be confirmed by **immunoblot analysis** as well. Results obtained by in vitro diagnostics are to be interpreted in context with the clinical signs of the infection.

In the last years the meaning of the VlsE (variable major protein-like sequence, expressed) antigen for the diagnosis of Lyme disease was recognized. In vivo the bacterium is hidden from the immunosystem by variation of surface proteins expressed by VlsE genes. So in early stages of *Borrelia* infection no antibodies to VlsE are produced. In later stages the antibodies to VlsE serve as a diagnostic marker of infection. Cultured bacteria do not express VlsE antigens, so a synthetic antigen has to be used in the diagnostics of Lyme disease.

Burgdorfer W, Barbour A G, Hayes S F, Benach J L, Grunwaldt E, Davis J P: Lyme disease, a tick-borne spirochetosis? *Science* 216, 1982, 1317-1319

Bingnan M, Christen B, Leung D, Vigo-Pelfrey C: Serodiagnosis of Lyme Borreliosis by Western Immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. *J Clin Microbiol* 30, 1992, 370-376.

Lawrenz MB, Hardham JM, Owens RT, Nowakowski J, Steere AC, Wormser GP, Norris SJ: Human antibody responses to VlsE antigenic variation protein of *Borrelia burgdorferi*. *J Clin Microbiol* 1999, 37, 3997-4004

## PRINCIPLE of the TEST

Anti-*Borrelia* Blot IgM is a sensitive immunoblot for the determination of IgM antibodies to *Borrelia burgdorferi* sensu lato.

Proteins derived from *Borrelia afzelii* were separated electrophoretically according to their molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. VlsE antigen was added as a separate line. After blocking of free binding sites, the membrane was cut into ready-for-use strips, provided in the kit.

Patient sera and strips are incubated in the test tray. During the first incubation antibodies of the patient samples bind to the target antigens immobilized on the solid phase of the strips. Following an period of 45 min unbound serum components are removed by a washing step.

The bound antibodies react specifically with anti-human-IgM or anti-human-IgM conjugated to horse radish peroxidase (HRP). Following an incubation period of 45 min excessive conjugate is separated from the solid-phase immune complexes by an additional washing step.

Horse radish peroxidase converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a dark blue precipitating product. Defined by the antibodies in the individual serum samples different patterns of bands reflecting the target antigens become visible. Using the provided reference pattern in the kit positives and negatives are discriminated.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used. Plasma can be used too. The use of different

The samples may be kept at 2...8°C for up to three days. Long-term storage requires -20°C.

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:** *Neat patient samples have to be used for the assay. Final dilution of 1 + 100 (v/v) is done in the incubation tray.*

## TEST COMPONENTS for 27 determinations

<b>A</b> <b>Ag</b>	<b>Blot strips</b> 27 numbered strips coated with <i>Borrelia afzelii</i> antigens (electrophoretically separated) and VlsE	27 Blot strips
<b>B</b> <b>BUF</b> <b>WASH</b>	<b>Buffer</b> sufficient for 350 ml solution <b>5x</b>	2 x 35 ml concentrate capped black
<b>D</b> <b>CONJ</b> <b>M</b>	<b>Conjugate</b> anti-human-IgM (goat) coupled with horse radish peroxidase	50 ml ready for use capped green
<b>E</b> <b>SOLN</b> <b>TMB</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide (black bottle)	50 ml ready for use capped blue
<b>F</b>	<b>Incubation tray for 9 strips</b>	2 x
<b>G</b>	<b>Lot specific blot pattern</b>	1 x

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- pipette tips
- multi-channel pipette
- trough for multi-channel pipette
- graduated cylinders
- distilled or de-ionized water
- 2 ml tubes
- horizontal plate shaker or rocking shaker
- plastic pincers

## Size and storage

Anti-Borrelia Blot IgM has been designed for 27 determinations.

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

Upon receipt, all components of the Anti-Borrelia Blot 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 4 weeks, provided proper storage.

### Preparation before use

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

Prepare a sufficient amount of buffer solution by diluting the concentrated buffer (B) 5 times (1 + 4) with de-ionized or distilled water. For example, dilute 15 ml of the concentrate with 60 ml of distilled water per strip. The buffer solution prepared is stable at 2...8°C up to 4 weeks.

Avoid exposure of the TMB substrate solution to light.

## ASSAY PROCEDURE

- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (20...25°C) before use. Mix gently without causing foam.
2. Place the strips with the reactive side up (labels on top) Note number of serum sample and strips as well as lot number and antibody isotype (IgG/ IgM). Dispense **1.5 ml** of buffer solution (made of B) into the respective wells.
3. Cover tray, incubate 5 min on a horizontal shaker.
4. Add **15 µl** neat patient serum to the respective wells.
5. Cover tray and incubate 45 min while shaking at room temperature (20...25°C).
6. Decant or aspirate, wash each well three times 5 min with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
7. Add **1.5 ml ready to use conjugate** anti-human IgM POD (D) to each well.
8. Cover tray, incubate 45 min at room temperature (20...25°C) while shaking.
9. Decant or aspirate, and wash each well three times 5 min with **1.5 ml** buffer solution (made of B) while shaking. (Discard the solution contained in the wells by slowly inverting the plate. Dry the edges of the tray with absorbent paper.)
10. Add with **1.5 ml** of substrate (E) to each well.
11. Cover tray, incubate 10 min on a horizontal shaker at room temperature (20...25°C) till a blue color develops.
12. Decant or aspirate, wash each well three times with 2 ml distilled water to stop the reaction. \*
13. Collect the strips from the wells and dry the membrane by pressing briefly the reactive side of the strip on the absorbent paper. After approximately 30 min the strips are interpreted with the lot specific pattern.

\* **Some sera show unspecific background staining of the strips hypergammaglobulinaemia, circulating immune complexes, antibodies to milk proteins). In such cases the reaction has to be stopped immediately by rinsing the strips three times with 2 ml aqua dest..**

## DATA PROCESSING

### Validation criteria:

Results can be interpreted only if the specific control line and the cut-off line are clearly visible.

### Evaluation:

Only dried blot strips shall be interpreted. The interpretation of the blot strips is based upon the comparison of the exposed protein bands with the lot specific pattern.

Interpreting the intensity of the antigen bands:

weak band	(+)	comparable with cut-off intensity
clearly visible band	+	stronger than cut-off intensity
strong band	++	comparable with control band intensity

The relevance of the diagnostic important proteins is mentioned in the table below:

IgM	Blotted antigens
<b>negative</b>	<b>no band of the proteins listed below</b>
<b>borderline</b>	<b>strong p41 band</b>
<b>positive</b>	<ul style="list-style-type: none"> <li>• p39 band</li> <li style="text-align: center;">or</li> <li>• OspC band (p23-25)</li> <li style="text-align: center;">or</li> <li>• p17 band</li> </ul>

Because of the heterogeneity of the outer surface protein the antibody response to OspC may be expressed as a band as well at 23kD, at 25kD or at both bands.

Due to the variability of VlsE this surface structure is not recognized by the immunosystem in early stages of a Borrelia infection. The occurrence of anti-VlsE IgM therefore is improbable. The meaning of IgM to VlsE is still unclear.

The individual immune response following an infection with B. burgdorferi species may expose more Borrelia specific or unspecific protein bands which have no influence on the above mentioned diagnose criteria but may be helpful to distinguish between the different infection stages. The relevance of the immunoblot bands is shown on the schedule.

### 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, a past infection with these spirochetes may cause cross-reactive false-positive results.

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 titer without clinical implication.

The prevalence of Borrelia antibody positive apparently healthy individuals may amount to 15 % depending on the population.

Antibody production may be insufficient or suppressed in patients with immune-deficiency syndromes, under antibiotic therapy or in the early phase of an infection with B. burgdorferi.

In all immunologic methods bacteriological or fungal contaminations of the kit components and samples as well as cross-reactivity may cause wrong results.

**Insufficient washing or bad time management may lead to false negative results.**

## CHARACTERISTIC ASSAY DATA

### Relevance of the IMMUNOBLOT bands

band	antigen characterization	Antibody relevance
<b>VlsE</b>	variable surface structure	highly specific, frequently occurrence of IgG antibodies in later stages; significance of IgM antibodies unclear
<b>p83 (p100, p94)</b>	protein of membrane, vesicula at the surface	very high; characteristic for stage III
<b>p75</b>	heat shock protein	non specific
<b>p66</b>	heat shock protein	non specific; often in stage I-II
<b>p60</b>	heat shock protein, overlap of species	non specific, ab also detectable in E. coli, Trep. phagedenis, Legionella, Mycobacterium tuberculosis infections
<b>p58</b>	not sufficiently characterized; duplex bands	specific
<b>p43</b>	not sufficiently characterized	specific
<b>p41 (flagellin)</b>	structure protein of endoflagellum	less specific; already strong expressed at stage I
<b>p39 (BmpA)</b>	flagellin complex, (Borrelia membrane protein A)	highly specific; detectable at all stages
<b>p35 (OspB)</b>	Outer surface protein B	highly specific, antibodies rarely found
<b>p31 (OspA)</b>	Outer surface protein A	highly specific; often low expressed
<b>p30</b>	not sufficiently characterized	highly specific
<b>p23-25</b>	Outer surface protein C; several bands	highly specific; ab in stage I already detectable; important IgM marker
<b>p21</b>	not sufficiently characterized	highly specific; antibody in stage I already detectable
<b>p19</b>	not sufficiently characterized	low specific
<b>p17</b>	not sufficiently characterized	specific

**The nomenclature of the bands concerning molecular weight, specificity and validity in different disease stages is differently interpreted in literature.**

### Diagnostic sensitivity and specificity

For the determination of the diagnostic sensitivity and specificity 176 sera of apparently healthy blood donors as well as 29 patients with Erythema migrans, 21 patients with meningopolyneuritis and 23 patients with Lyme arthritis or Acrodermatitis chronica atrophicans were investigated. The following values were determined for the Anti-Borrelia Blot IgM:

Diagnostic specificity: 95%

Diagnostic sensitivity:

1. Stadium (Erythema migrans): 86%
2. Stadium (Meningopolyneuritis) 62 %
3. Stadium (Lyme Arthritis oder Acrodermatitis) 78%

Diagnostic sensitivity of IgG (3400) and/or IgM:

1. Stadium (Erythema migrans): 89%
2. Stadium (Meningopolyneuritis) 86 %
3. Stadium (Lyme Arthritis oder Acrodermatitis) 100%

## INCUBATION SCHEME

# Anti-Borrelia Blot IgM (3500)

1.	Bring all reagents and the requested number of strips to room temperature (20...25 °C)
2.	Place the strips with the reactive side upside in the tray and dispense 1.5 ml of <b>buffer solution</b> (made of B) into the respective wells
3.	Cover tray and incubate while shaking <span style="float: right;">5 min, room temperature (20...25°C)</span>
4.	Pipette <b>15 µl neat patient serum</b>
5.	Incubate while shaking <span style="float: right;">45 min, room temperature (20...25°C)</span>
6.	Decant, wash strips while shaking <span style="float: right;">3 x 5 min with <b>1.5 ml</b> (made of B)</span>
7.	Pipette <b>1.5 ml ready to use anti-human IgM conjugate (D)</b>
8.	Incubate while shaking <span style="float: right;">45 min, room temperature (20...25°C)</span>
9.	Decant, wash strips while shaking <span style="float: right;">3 x 5 min with 1.5 ml (made of B)</span>
10.	Pipette <b>1.5 ml substrate (E)</b>
11.	Incubate while shaking <span style="float: right;">10 min, room temperature (20...25°C)</span>
12.	Decant, wash strips with aqua dest. <span style="float: right;">3 x with 2ml</span>
13.	Interpret blotstrips with the lot specific pattern

## 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 re-constituted 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 Kathon (1.0 % v/v) as preservatives. 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.