BLOT-LINE Borrelia/HGA IgG

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IVD CE

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ISO 9001 ISO 13485

CONTENT

BLOT-LINE kit for the detection of specific IgG antibodies to recombinant antigens of *Borrelia* species and *Anaplasma phagocytophila* (HGA) in human serum, plasma, cerebrospinal fluid and synovial fluid

1 Introduction

Lyme borreliosis (LB) is a multisystem infectious disease caused by spirochete *Borrelia burgdorferi.* The infection is transmitted by ticks of the genus *Ixodes*.

Lyme borreliosis is characterized by early and late clinical symptoms.

- Early localised infection – lasts days to weeks. The specific clinical sign of this phase is erythema migrans (EM), which appears in only 50% of patients. Early symptoms of the disease include flu-like symptoms, headaches and lymphadenitis.
- Early disseminated infection – lasts weeks to months. Borrelia bacteria are disseminated by blood and lymphatic system (CNS, joints, heart, eye and skin – secondary EM). In this phase, neuroborreliosis, neurofacialis paresis, borrelial lymphocytoma (swollen earlobes, knucklebones, etc.) and Bannwarth syndrome are the most frequently diagnosed symptoms.
- Late disseminated infection – lasts months to years. Immunopathological changes occur. The typical diagnostic findings are acrodermatitis chronica atrophicans (chronic skin lesions – ACA), chronic neuroborreliosis and borrelial arthritis.

The results of extensive studies demonstrate that all genospecies participate not only on the development of EM, but they cause many other clinical manifestations. However, *B. burgdorferi* sensu stricto is related mainly to joint disease, *B. garinii* is associated with neurological symptoms and *B. afzelii* with chronic skin disorders (especially ACA).

The diagnosis of the disease is based on anamnesis, clinical picture, and results of laboratory tests. At present, the diagnostic methods of choice are screening of specific IgG and IgM class antibodies by means of ELISA, and subsequent confirmation of the antibodies to specific antigens by means of immunoblot. Direct cultivation or electron microscopy is not applicable in a routine use.

Serological diagnosis of borreliosis is difficult regarding to the large genetic diversity of the species *Borrelia burgdorferi* s.l., possible cross reactivity with unrelated antigens of other microorganisms, and borrelia richness to heat shock proteins. [1] Diagnosis is also complicated as well by different individual serological reactivity. The production of antibodies can be extremely slow in the early phase of the

disease. On the other hand, the IgG and IgM antibodies can persists for more than ten years [2].

Human granulocytic anaplasmosis (HGA) is a disease caused by bacterium *Anaplasma phagocytophila*. The infection is transmitted by ticks – *Ixodes ricinus*.

The clinical course of the disease varies from asymptomatic infection to severe manifestation with respiratory, gastrointestinal, renal, neurologic complications and other complications.

BLOT-LINE Borrelia/HGA is a 3rd generation kit with high diagnostic sensitivity and specificity. Examination enables detailed evaluation of specific antibodies and serves for confirmation of borderline and positive ELISA or other serological test results.

1. L.A.Magnarelli, J.F.Anderson, R.C.Johnson; Cross-Reactivity in Serological Tests for Lyme Disease and Other Spirochetal Infections; The Journal of Infectious Diseases; 1987; 156(1):183-8
2. R.A.Kalish, G.McHugh, J.Granquist, B.Shea, R.Ruthazer, A.C.Steere; Persistence of Immunoglobulin M or Immunoglobulin G Antibody Resposes to Borrelia burgdorferi 10-20 Years after Active Lyme Disease; Clinical Infectious Diseases; 2001; 33:780-5

2 Test Principle

Recombinant antigens are transferred to a nitrocellulose membrane fixed on a plastic pad. In the first reaction step, as the individual strips are incubated with the tested sample, the specific antibodies (if present in the sample) bind to the corresponding antigenic bands on the strip. After washing, the strips are further incubated with a conjugate. Visualisation is performed by incubation with a substrate solution. After colour development, the test strips are dried out and evaluated by means of the enclosed protocol with a scanned validation strip where individual positions of the specific bands are indicated. For test validity verification, the strips are equipped with a conjugate control band and with a control band indicating kit functionality and sensitivity.

The bands intensity can be evaluated visually or by means of software (the band intensity is expressed in percentage).

3 Materials Provided

BL STRIP	**BL Strips** Test strips with recombinant antigens	1 × 20 pcs
CONTROL -	**Negative Control** Solution containing no specific antibodies, ready to use	1 × 5 ml
CONTROL +	**Positive Control** Solution containing specific antibodies, ready to use	1 × 5 ml
CONJUGATE	**Conjugate** Solution containing AP labelled animal immunoglobulin, ready to use	2 × 20 ml
SUBSTRATE	**Substrate Solution** Buffer with BCIP and NBT, ready to use	2 × 20 ml
UNIVERSAL	**Universal Solution** Buffer for sample dilution and strip washing, ready to use	1 × 300 ml
	Instructions for use	1 pc
	Protocol with displayed template strip	1 pc
	Mobile template for visual evaluation	1 pc
	Transparent self-adhesive foil	2 pcs
	Sealing clip	1 pc

4 Other Materials Required for Test Performance

Single and multichannel pipettes

Disposable tips

Aspirator (e.g. NUNC)

Timer

Test tubes for serum dilution

Filter paper

Distilled water

Shaker for blot incubation (alternatively, an ELISA shaker adjusted to the lowest frequency can be used)

Scanner, PC and Immunoblot Software for software evaluation

5 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid (the shelf life of the kit is 18 months from the date of manufacture). The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum, citrate plasma, cerebrospinal and synovial fluid. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

6 Preparation of Samples

Use the Positive and Negative Controls (ready to use) in case of test validation.

Dilution of serum and plasma sample

Dilute well mixed samples 1:51 with the Universal Solution.

30 µl of sample + 1.5 ml of the Universal Solution

Mix well.

Dilution of cerebrospinal fluid (CSF) samples

Dilute well mixed samples 1:2 with the Universal Solution.

0.75 ml of CSF + 0.75 ml of the Universal Solution

Mix well.

Dilution of synovial fluid samples

Dilute well mixed samples 1:17.5 with the Universal Solution.

90 µl of Synovial Fluid + 1.5 ml of the Universal Solution

Mix well.

7 Assay Procedure

1. Before use take all the components out of the kit box and allow them to equilibrate at room temperature for approximately 60 minutes. Mix thoroughly.
2. Pipette 2.5 ml of the Universal Solution into each well of the incubation tray. One well is required per sample to be tested.
3. Use forceps to grasp the lower part of the strip which is not covered by membrane, remove the strip from a stock pad and place one strip into each well. The strip must be completely immersed in the Universal Solution. Incubate the BL strips at room temperature on a rocking shaker for 10 minutes.

   Return unused strips immediately into the bag with desiccant and seal the bag tightly with the enclosed sealing clip.
4. Aspirate off the Universal Solution from the wells.
5. Pipette 1.5 ml of the diluted samples into the wells and incubate at room temperature on a rocking shaker for 30 minutes. **When using the Negative and Positive Control do not dilute them.** They are ready to use.

   (Alternatively, samples can be diluted directly in wells. Pipette 1.5 ml of the Universal Solution into wells with wet strips. Then add 30 µl of samples into wells; in case of CSF, pipette 0.75 ml of the Universal Solution and 0.75 µl of CSF. In case of Synovial Fluid pipette 1.5 ml of Universal Solution and 90 µl of Synovial Fluid. This way of dilution demands thorough mixing content of the well.)
6. Aspirate off the diluted samples.
7. Wash the strips with 1.5 ml of the Universal Solution 3 × 5 minutes each time on a rocking shaker.
8. Aspirate off the Universal Solution, pipette 1.5 ml of the Conjugate into each well and incubate at room temperature on a rocking shaker for 30 minutes.
9. Aspirate off the Conjugate.
10. Wash the strips 3 x 5 minutes each time with 1.5 ml of the Universal Solution on a rocking shaker.
11. Aspirate off the Universal Solution, pipette 1.5 ml of the Substrate Solution into each well and incubate at room temperature on a rocking shaker for 15 minutes.
12. Aspirate off the Substrate Solution and wash each strip with 2 ml of distilled water 2 x 5 minutes on a rocking shaker.

13. Take the strips out of the incubation tray and transfer them into the self-adhesive frames on the evaluation protocol. Lay a sheet of filter paper over the strips and press them gently to the protocol. Let the strips air dry.
14. Evaluate the dried strips using the enclosed template or by software (Immunoblot Software). For long-term storage, protect the strips from light exposure by covering them with the enclosed transparent self-adhesive foil.

WARNING! When using evaluation software, cover the strips with the foil after scanning!

8 Quality Control

The test is valid if:

1. The control band is present on the strips.
2. The conjugate control band of respective antibody class is present on the strips.
3. The Positive Control contains the following specific antigen bands (Table 1).

Table 1 Positive Control evaluation

Antigen band	**Intensity**
at least one VlsE band	intensively positive band
at least 4 other bands of VlsE, p83 Ba, p41 Ba, p39 Ba, OspB Bs, OspA Ba, OspA Bg, OspA Bs, OspC Bs, p17 Bg and p44	positive band

The Positive Control included in the kit may not contain all the specific antigen bands.

9 Results Interpretation

9.1 Visual and software evaluation of specific bands

The template for visual evaluation, which is a part of the kit, facilitates evaluation of the band intensity not using the software. The position of specific antigen bands is identified at the left side of the template. The example strip with bands of borderline intensity is situated at the right side of the template. The band intensity evaluation scale: positive, borderline and negative is situated by the upper edge of the example strip. Visual evaluation is described in Table 2.

Table 2 Visual evaluation of specific bands

Band intensity	Band evaluation
lower than cut-off	negative
equal as cut-off	borderline
higher than cut-off	positive

Positive bands can be further discriminated on the basis of their intensity as weakly intensive, intensive and highly intensive.

Generally, negative samples can cause weak signal on the strips.

Use Immunoblot Software for software evaluation of the test.

9.2 Overview of specific antigens

Highly specific antigens are described in Table 3.

Table 3 Specific antigens

Antigen	Description
VlsE Ba	Expressed part of variable major protein-like sequence, significant for IgG antibody response, species-specific antigen (Ba – *B.afzelii,* Bg – *B. garinii,* Bs – *B. burgdorferi* sensu stricto)
VlsE Bg	
VlsE Bs	
p83 Ba	Main extracellular protein (product of p100 degradation)
p41 Ba	Internal flagellin, highly specific antigen of early antibody response
p39 Ba	BmpA (glycosaminopeptide receptor) – marker of late IgG antibody response
OspB Bs	Outer surface protein B
OspA Ba	Outer surface protein A, highly specific marker of Borrelia infection in IgG class
OspA Bg	
OspA Bs	
OspC Bg	Outer surface protein C – main antigen of early antibody response, immunodominant marker of IgM antibody response
p17 Bg	DbpA (decorin-binding protein A) – outer membrane protein
p44	*Anaplasma phagocytophila* – main marker of HGA antibody response
TpN17	Highly specific membrane protein of *Treponema pallidum*

9.3 Test evaluation

Final evaluation of the Borrelia test should be performed according to the Table 4. Combine the presence of VlsE band with presence of other specific bands.

Table 4 Borrelia test final evaluation

	p83, p41, p39, OspB, OspA, OspC, p17			
At least 1 VlsE band	more than 2 positive bands	2 positive bands	1 positive band or 2 weak bands	no band or 1 weak band
intensive	**positive**	**positive**	**positive**	**borderline**
weak	**positive**	**positive**	**borderline**	**negative**
no band	**positive**	**borderline**	**negative**	**negative**

Examination should be repeated in case of borderline results. Collect and test a new sample after 2 to 6 weeks according to the disease specifics.

Result interpretation of Anaplasma test should be performed according to the Table 5.

Table 5 Anaplasma test evaluation

Specific antigen band	**Evaluation**
intensive p44 band	positive
weakly intensive p44 band	borderline
no p44 band	negative

Parallel presence of anti-p44 and anti-Borrelia antibodies may signify HGA and borreliosis coinfection.

Sensitivity of HGA screening examination corresponds with strong positivity of the samples analysed by immunofluorescence.

In case of Anaplasma positive test result, we recommend to perform another examination to confirm or exclude HGA.

The test is based on highly specific recombinant Borrelia antigens. Nevertheless, cross-reaction with other spirochaetes, especially *T. pallidum*, may occur in rare cases.

Test uses highly specific and sensitive marker of syphilis infection antigen – TpN17 to eliminate Borrelia false positive results.

Result interpretation of Treponema test is performed according to the Table 6 .

Table 6 Treponema test evaluation

Specific antigen band	**Evaluation**
intensive TpN17 band	positive
weakly intensive TpN17 band	borderline
no TpN17 band	negative

In case of Treponema borderline/positive test result, it is necessary to perform another examination to confirm or exclude syphilis.

Serological finding should be interpreted in the context of the results of other laboratory tests and patient's clinical picture.

10 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amounts of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

11 Procedural Notes

In order to obtain reliable results, it is necessary to have sufficient technical skills and strictly follow the Instructions for Use. Always use clean preferably disposable tips and glassware.

The strips should be handled only in the part which is not covered by membrane or in the area labelled with the strip's lot and serial number.

The BL strip should always face upwards in the well during the detection – i.e. facing up the front side with the starting line.

The incubation trays for blotting should not be used repeatedly in order to avoid possible contamination of used reagents.

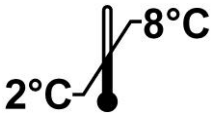
Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps

- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature (summer sun) or massive bacterial contamination
- insufficient washing of the wells with the strips
- using reagents from different kit lots
- assay performed before reagents were allowed to come to room temperature
- contact of reagents with oxidants, heavy metals and their salts

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

12 IFU Symbols

	Temperature limitation
	Keep dry
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

Summary of BLOT-LINE Borrelia/HGA IgG Protocol

Step No.	Symbol	Test steps
1		Pipette Universal Solution – 2.5 ml
2		Strips soaking at room temperature for 10 min Shaker
3		Aspirate off
4		Dilute samples[1] serum/plasma 1:51 (30 µl + 1.5 ml) cerebrospinal fluid 1:2 (0.75 ml + 0.75 ml) synovial fluid 1:17.5 (90 µl + 1.5 ml)
5		Pipette controls and diluted samples – 1.5 ml
6		Incubate at room temperature for 30 min Shaker
7		Aspirate and wash 3 × 5 min with 1.5 ml of Universal Solution Shaker
8		Pipette Conjugate – 1.5 ml
9		Incubate at room temperature for 30 min Shaker
10		Aspirate and wash 3 × 5 min with 1.5 ml of Universal Solution Shaker
11		Pipette Substrate Solution (BCIP/NBT) – 1.5 ml
12		Incubate at room temperature for 15 min Shaker
13		Aspirate and wash 2 × 5 min with 2 ml of distilled water Shaker
14		Dry and evaluate strips

[1] Alternatively, samples can be diluted directly in wells