BlueBLOT-LINE Borrelia IgG

REF **BD-BGL024**

Σ **24**

IVD CE

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CONTENT

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

Extensive studies demonstrate that all *Borrelia* genospecies can be associated with the development of erythema migrans (EM) and the development of many other clinical manifestations. Still, infections with *B. burgdorferi* sensu stricto are mainly related to joint disease whereas an infection with *B. garinii* predominantly is linked to neurological symptoms. On average, infections with *B. afzelii* cause chronic skin disorders (especially ACA).

Due to the large genetic diversity of the species *Borrelia burgdorferi* s.l., the abundance of heat shock proteins and possible cross reactivity with unrelated antigens of other microorganisms, the serological diagnosis of borreliosis is complex. Furthermore, different individual serological response and persistent IgG and IgM antibodies (up to several years) hamper a correct diagnosis.

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

2 Test Principle

This kit is intended to be performed on the BlueDiver Instrument (hereafter BDI). The test is based on the principle of an enzyme immunoassay. 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 and evaluated. For test validity verification, the strips are equipped with a conjugate control band and with a control band indicating kit functionality and sensitivity.

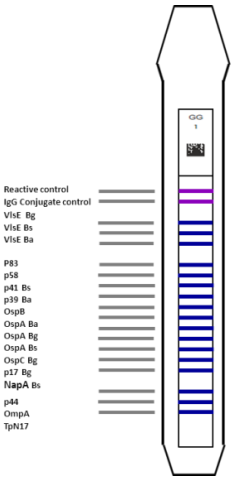
BlueDiver Protokol

Protocol No. 6

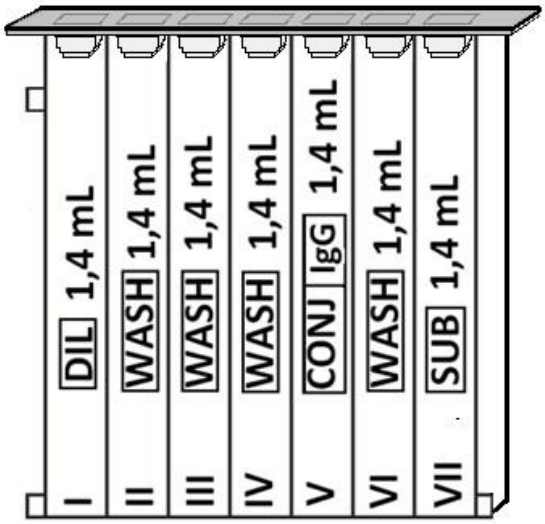
3 Materials Provided

BL strips	3 x 8 units on plastic supports breakable individually; sealed in aluminium pouch 19 bands on each: 1 Reactive control 1 Conjugate control 17 Antigens
Cartridge	24 units having each 7 compartments; sealed
Diluent buffer	Ist position, 1 x 1.4 ml (yellow) Buffer (TBS) for sample dilution containing antifoam emulsion
Wash buffer	IInd, IIIrd,IVth and VIth position, 4 x 1.4 ml (colourless) Buffer (TBS) for strip washing containing antifoam emulsion
Conjugate	Vth position, 1 x 1.4 ml (yellow) Solution containing AP labelled animal immunoglobulin, stabilizer and antifoam emulsion
Substrate	VIIth position, 1 x 1.4 ml (pale yellow solution) Buffer containing BCIP and NBT Buffer with BCIP and NBT
Other material	Absorbent paper; sealed with BL strips in aluminium pouch
Documents	Instructions for Use

STRIP



CARTRIDGE



4 Materials Required for Test Performance

BlueDiver Instrument

Micropipettes

Disposable tips

Laboratory gloves

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 13 months from the date of manufacture). The opened kit should be used within three months.

Sample collection, handling and storage

Blood samples can be collected in dry tubes or in tubes containing EDTA, heparin or citrate. After separation serum or plasma samples can generally be stored at 2-8°C for up to three days. Long term storage requires freezing at -20°C. Avoid repeated freezing/thawing cycles. After freezing always agitate samples before use to ensure homogeneity. Diluted samples should be used as soon as possible.

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

6 Assay Procedure

BASIC INFORMATION, HANDLING AND TIPS

TEST PROCESS (principle)

After manual loading of the strips and reagent cartridges, the incubation and washing steps of the procedure are automatically processed by the BDI which ensures an efficient circulation of fluids over the strips by continuously agitating them up and down in the wells of ready-to-use reagent cartridges. The whole test procedure is run at room temperature.

STRIPS (description)

The reactive (front) side of the strips is coated with antigens. This front side also displays a strip number and a 2-dimensional square barcode for traceability of the strips after removal from the BDI at the end of the test.

The non-reactive (back) side of the strips displays both alphanumeric and bar-coded information for identification of the strip type and lot number by the BDI.

The strips must be manually inserted into the dedicated clamp before starting the automated process. During this operation avoid touching the membrane zone of the strips with fingers. Always wear laboratory gloves and use the plastic parts (strip support) for handling or manipulation.

REAGENT CARTRIDGES (description)

The reagent cartridges are composed of 7 different wells filled with ready-to-use reagents. The cartridges are sealed, and the reagent wells are hermetically separated. The sealing has to be removed before starting the test. Once opened, manipulate the cartridges with care in order to avoid reagent spilling and contamination from well to well.

The back side of the cartridges is labelled with both alphanumeric and bar-coded information for identification of the cartridge type and lot number by the BDI.

The cartridges must be manually loaded onto the dedicated cartridge holder before starting the automated process. The front and back sides of the cartridges have, respectively, a bottom triangular and two (bottom + top) square plastic edges for secure position and orientation into the holder.

STRIPS/CARTRIDGES (associations)

The strips and cartridges of a same test kit share the same lot number and are dedicated to be associated in lot-specific pairs. Do not associate, in a same pair, a strip and a cartridge with different lot numbers as this will be detected as an invalid setting by the BDI and will stop the process.

As far as each strip/cartridge pair is valid, the BDI can process strips/cartridges associations of different kits; However, only kits having the same protocol number (same incubation time and sequence) can be processed together in one same run (please refer to the protocol number indicated under the kit reference at the top of first page).

6.1 Test preparation

Allow all kit components to equilibrate at room temperature (+18°C to +25°C) before use.

A working list should always be prepared for easy loading and correct association of strips, cartridges and patient samples.

Make sure that the cartridge holder is fixed in its emplacement in the BDI.

Make sure that the BDI is plugged in.

The following steps sequence summarizes the loading and preparation of the BDI, test strips, reagent cartridges and patient samples before starting the test:

1. Switch ON the BDI and wait a few seconds until the date and time are displayed on the touch screen.

2. Confirm the correct Date and Time by pressing✓ on the touch screen (in case of first use or for reset, refer to the manual of use of the BDI) → "Initialize?" is displayed on the screen.
3. Confirm Initialization by pressing✓ on the touch screen → the horizontal arm of the instrument automatically moves forward to a central (stand-by) position → "Load strips (24)" is displayed on the screen.
4. Remove the clamp from its emplacement on the arm by gently pulling it upwards and load the strips to be tested: handle the clamp with numbered side facing up (open position) and insert the strips, also with numbered (reactive) side facing up, by slipping the upper plastic part (tongue) into the dedicated holes of the clamp. Apply a gentle pressure to ensure that the plastic tongue has reached the bottom end of the hole.
5. Replace the clamp in its emplacement on the arm by gently pushing it downwards.
6. Set the number of loaded strips using the up and down arrows on the touch screen.
7. Confirm the number of loaded strips by pressing ✓on the touch screen → the horizontal arm automatically moves backward to stand over the alignment holes of the cartridge holder → "Check alignment" is displayed on the screen.
8. Use the "JOG" function on the screen to check the correct alignment of the strips: maintain a gentle pressure on the down arrow on the touch screen until the bottom of the strips enters into the alignment holes of the cartridge holder. If correctly aligned, the strips will not touch the outlines of the holes.
9. Confirm the correct alignment of the strips by pressing✓on the touch screen → the BDI lowers the strips completely into the alignment holes and reads the barcodes of the strips → after complete barcode reading, "Load reagent" is displayed on the touch screen.
10. Unseal the reagent cartridges and insert them under their respective strips in the dedicated notches of the cartridge holder.
11. Confirm complete loading by pressing✓on the touch screen → the BDI reads the barcodes of the cartridges and checks the correct association with the strips → after complete barcode reading, the number of strips (validated strips/cartridges associations) is displayed on the screen.
12. Confirm the number of strips by pressing✓on the touch screen → the protocol number identified on the barcodes is displayed on the screen (Protocol ID xx).
13. Confirm the protocol number by pressing✓on the touch screen → "Please close cover." is displayed on the screen.
14. Close the cover of the BDI and confirm closing by pressing✓on the touch screen → the BDI proceeds to a first washing (pre-treatment) step by incubating

the strips into the 2nd well of the cartridges (processing time: 1 minute) → At the end of the wetting step, “Please open cover.” is displayed on the screen.

15. Open the cover of the BDI and confirm opening by pressing✓on the touch screen → the horizontal arm automatically moves forward to the front of the instrument and swings the strips to an oblique position → “Dry strips” is displayed on the screen.
16. Dry the strips by gently applying absorbent paper onto the basis of the bottom small cavity (sample loading hole).
17. Confirm drying by pressing✓on the touch screen → “Apply samples” is displayed on the screen.
18. Apply samples by pipetting 10 µl of patient serum/plasma into the bottom sample loading holes of the strips. If preferred the 10 µl of the serum can be directly pipetted into the Diluent Buffer (“Well I”) of the cartridge. This operation can be done at any time from opening of the cartridges. In case of cerebrospinal fluid analysis, remove 500 µl of the Diluent Buffer from the Well I and pipette 500 µl of the cerebrospinal fluid sample into this well.
19. Confirm samples’ loading by pressing✓on the touch screen → “Please close cover” is displayed on the screen.
20. Close the cover of the BDI and confirm closing by pressing✓on the touch screen → the BDI starts the test automatically by proceeding the steps sequence of the protocol.

For detailed information or in case of any problem met at one of the preceding steps, please refer to the Manual of Use of the BDI.

6.2 Test processing

Step	Description (Protocol 6)	Time (min)
01.	The strips are incubated into the 1st well of the cartridge (Diluent Buffer). Upon contact with the liquid in the wells and agitation, the pre-loaded patients' samples are released from the small cavity at the bottom of the strips and are diluted in the buffer.	30
02.	The clamp moves forwards and the strips are incubated into the 2nd well of the cartridge (Wash Buffer).	2
03.	The clamp moves forwards and the strips are incubated into the 3rd well of the cartridge (Wash Buffer).	2
04.	The clamp moves forwards and the strips are incubated into the 6th well of the cartridge (Wash Buffer).	2
05.	The clamp moves backwards and the strips are incubated into the 5th well of the cartridge (Conjugate).	20
06.	The clamp moves backwards and the strips are incubated into the 4th well of the cartridge (Wash Buffer).	2
07.	The clamp moves backwards and the strips are incubated into the 3rd well of the cartridge (Wash Buffer).	2
08.	The clamp moves backwards and the strips are incubated into the 2nd well of the cartridge (Wash Buffer).	2
09.	The clamp moves forwards and the strips are incubated into the 7th well of the cartridge (Substrate).	10
10.	The clamp moves backwards and the strips are incubated into the 6th well of the cartridge (Wash Buffer).	2

After completion of the process the clamp moves to a central (stand-by) position in the BDI to allow easy manipulation of the clamp. The instrument beeps and "Finished test" is displayed on the screen.

Gently apply absorbent paper onto the basis of the strips to remove liquid from the bottom small cavity (sample loading hole) and allow the strips to dry for 30 minutes before interpretation of the results. Leave the processed strips attached to the clamp. The interpretation has to be done in the 24 hours following the test processing.

TEST DATA REGISTRATION

The test protocol can be downloaded by pressing the USB stick symbol and following the indications on the screen (Insert USB → Writing USB → Remove USB). This step is not obligatory but is highly recommended for traceability and regulatory matters.

7 Quality Control

The test is valid if:

1. The control band is present on the strips.
2. The conjugate control band is present on the strips.

8 Results Interpretation

8.1 Use of Immunoblot Software and Scanning system

1. Remove the clamp from the BDI. Leave the processed strips attached to the clamp.
2. Insert the clamp, the reactive side of the strips facing down, into the dedicated emplacement in the cover of the BlueScan scanner.
3. Start scanning the strips using the Immunoblot Software.

8.2 Overview of specific antigens

Highly specific antigens are described in Table 1.

Table 1 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)
p58 Bg	OppA-2 (Oligopeptide permease 2) – membrane transporter, is considered a marker of disseminated stage of Lyme disease
p41 Bs	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
NapA Bs	Neutrophil activating protein A – strong immunogen, main marker of Lyme arthritis pathogenesis
p44	*Anaplasma phagocytophila* – main marker of HGA antibody response
OmpA	Outer membrane protein A of *Anaplasma phagocytophila,* peptidoglycan-associated lipoprotein, significant virulence marker
TpN17	Highly specific membrane protein of *Treponema pallidum*

8.3 Test evaluation

Test evaluation is based on the combination of the presence of the specific antigen lines and their intensity (AU) as described in Table 2.

Table 2 Test evaluation

Band intensity (AU)	Evaluation	Interpretation
< 5.5	No band	Negative
5.5 – 13	Weak band	Borderline
> 13	Positive band	Positive

Final evaluation of the **Borrelia test** should be performed according to Table 3. Combine the presence of VlsE band with presence of other specific bands.

Table 3 Borrelia test final evaluation

	p83, p58, p41, p39, OspB, OspA, OspC, p17, NapA			
At least 1 VlsE band	more than 2 positive bands	2 positive bands	1 positive band or 2 weak bands	1 weak band or no band
intensive	**positive**	**positive**	**positive**	**borderline**
weak	**positive**	**positive**	**borderline**	**negative**
no band	**positive**	**borderline**	**negative**	**negative**

Analysis should be repeated in case of borderline results. A new sample has to be collected after 2 to 6 weeks according to the disease state.

Evaluation of **Anaplasma test** should be performed according to Table 4.

Table 4 Anaplasma test evaluation

Specific antigens p44, OmpA	**Evaluation**
At least 1 positive band and 1 weak band	positive
At least 2 weak bands	borderline
1 positive band	
no positive band	negative
at most 1 weak band	

Presence of anti-p44 and anti-Borrelia antibodies may indicate 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 an alternative test to exclude HGA.

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

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

Evaluation of **Treponema test** is performed according to Table 5.

Table 5 Treponema test evaluation

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

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

9 Test Performance

9.1 Reproducibility

Reference control samples were tested in statistically relevant repetitions in a same run or over several runs for the calculation of intra- and inter-assay variation, respectively. In every case the intensity of the bands was within the specified range and standard deviations were less than 10%.

9.2 Sensitivity and Specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) were analysed according to the test instructions. Sensitivity and specificity were calculated from the results generated by the Immunoblot Software (see Table 6 and **Table 7**).

Table 6 Sensitivity and specificity of BlueBLOT-LINE Borrelia IgG

Neuroborreliosis (n = 39)		reactive	negative	
confirmation	reactive	24	0	**Sensitivity 100.00%**
	negative	0	15	**Specificity 100.00%**
Erythema migrans (n = 26)		reactive	negative	
confirmation	reactive	12	0	**Sensitivity 100.00%**
	negative	1	13	**Specificity 96.15%**

Lyme arthritis (n = 28)		reactive	negative	
confirmation	reactive	23	1	**Sensitivity 96.42%**
	negative	0	4	**Specificity 100.00%**

Controls (n = 69)		reactive	negative	
confirmation	reactive	0	0	**Sensitivity 100.00%**
	negative	0	69	**Specificity 100.00%**

Control group composition:

n = 49 blood donor samples

n = 20 diseases known to show cross reactivity with Lyme disease (tick-borne meningoencephalitis, aseptic meningitis syndrome)

Table 7 Parameters of BlueBLOT-LINE Borrelia IgG

	Diagnostic Sensitivity (%)	**Diagnostic Specificity (%)**
Borrelia IgG (n = 162)	**98.20**	**99.00**
Anaplasma IgG (n = 50)	**91.96**	**93.94**
TpN17 IgG (n = 99)	**97.96**	**99.00**

Table 8 Kit parameters for Borrelia detection in CSF

Neuroborreliosis (n = 30)		reactive	negative	
confirmation	reactive	29	2	**Sensitivity 96,67 %**

Controls (n = 33)		reactive	negative	
confirmation	negative	1	32	**Specificity 96.97 %**

Test group composition:

n = 28 patients diagnosed with lyme neuroboreliosis

n = 20 other neurological diseases (tick-born meningoencephalitis, viral meningitis, cerebral palsy, etc.)

10 Test Limitations

A clinical diagnosis should not be made on the basis of a single in vitro diagnostic method.

A complete clinical investigation, as well as other laboratory test results, should be considered to determine a diagnosis, since no technique used alone can rule out the possibility of false-positive or false-negative results.

In any case, GLP should be applied with all general and individual regulations to the use of this kit.

11 Safety Precautions

The kit is intended for in vitro diagnostic use only. The test kit should be processed by trained technical staff only.

The kit contains potentially hazardous components, thus contact with skin, eyes or mucosae has to be avoided.

Patient samples must be handled with care as being a potential infection hazard. Local safety rules and regulations must be observed.

First aid

In case of contact with eyes, flush with extensive 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.

12 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.

Always start loading into position 1 of the clamp (left side) and do not leave empty spaces between the strips!

After complete loading, check visually the vertical, horizontal and lateral alignment of the strips. Any obvious misalignment should be corrected by unloading the strip(s) from the clamp and loading them again.

Be careful: any plastic bits remaining after breaking apart the individual strip holders may hinder the processing on the instrument and/or the reading with the BlueScan scanner; please remove them with scissors.

In case of the following problems refer to the Manual of the BDI:

- failure to read one or more cartridge barcode(s), or in case of detection of a wrong strip/cartridge association (flashing LED at the corresponding position),
- misalignment (contact of the strips with the cartridge holder),
- failure to read one or more strip barcode(s) (flashing LED at the unread position)

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

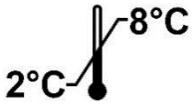
- reagent exposure to excessive temperature (summer sun) or massive bacterial contamination
- using reagents from different kit lots
- assay performed before reagents were allowed to come to room temperature
- contact of reagents with oxidants, heavy metals and their salts

TestLine Clinical Diagnostics s.r.o. and its authorised distributors shall not be liable for any damages resulting from a change or modification in the procedure indicated.

Notes

Notes

13 IFU Symbols

	Temperature limitation
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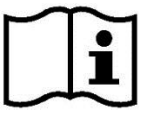
	Do not expose to sunlight
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device