DRG® Borrelia Blot IgG (DOT-4539)

RUO in the USA

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The Borrelia Blot-IgG is a device (immunoblot) for the detection of specific IgG-antibodies against the proteins of *Borrelia burgdorferi* in human serum or samples from CSF.

2 PRINCIPLE OF THE TEST

Proteins of *Borrelia afzelii* sonicate are separated by SDS polyacrylamide gel electrophoresis according to their molecular mass and then transferred to a nitrocellulose membrane. Additional protein lines (control and VlsE antigen) are applied to the membrane by a microdispensing system. After blocking remaining free protein binding sites the membrane is cut into strips.

The procedure for detection of IgG or IgM antibodies may be divided into three steps.

1. Step
The nitrocellulose strips are incubated in the diluted serum samples for 45 minutes. Specific antibodies to borrelia proteins that may be present in the samples form immune complexes with the membrane bound antigens during that time. After incubation unbound proteins are removed by 3 washing cycles.

2. Step
The second incubation starts with the addition of horse radish peroxidase labelled anti-human IgG- or IgM-antibodies to the nitrocelluose strips respectively. After 45 minutes incubation unbound conjugate is removed by 3 washing cycles.

3. Step
The addition of the colourless precipitating TMB/substrate solution stains the membrane bound immune complexes by formation of blue precipitates. The substrate reaction should not exceed 10 minutes due to growing background by prolongation of reaction time. Reaction is stopped by aspirating the substrate solution followed by rinsing the strips with sufficient quantity of distilled water. After drying the strips, interpretation of the resulting pattern is done by comparison with the lot specific template.

For computer-based evaluation with the BloTrix-system strips have to be transferred into a black incubation tray (available from DRG)

3 PREPARATION AND STORAGE OF SAMPLES

Serum, plasma or other biological fluids can be investigated for anti *Borrelia burgdorferi* IgG antibodies with the Borrelia Blot-IgG.

Sample collection should be done in a sterile manner.

Samples can be stored at 2 - 8 °C for a maximum of 48 hours.

For longer storage times samples have to be stored at - 20 °C. Frozen samples have to be warmed to room temperature and mixed well before starting the test run. Repeated freezing and thawing of samples should be avoided.
Serum or plasma samples have to be diluted 1:101 with *Wash and Incubation Buffer*, WIB (2) in the incubation tray.

4 KIT COMPONENTS

1 **[TESTSTR]**	Nitrocellulose strips	27 strips with separated and fixed *Borrelia afzelii* antigens
2 **[WIB CONC 5X]**	Wash- and Incubation buffer	2 x 35 ml, 5-fold concentrated, white bottle, black cap
3 **[CONJ HRP IgG]**	Anti human IgG (red)-HRP-conjugate (goat) colour coded	50 ml, ready for use transparent bottle, red cap
4 **[SUBSTR TMB]**	TMB Substrate Solution Hydrogen peroxide and 3,3´,5,5´-Tetramethyl-benzidine	50 ml ready to use, black bottle, blue cap
5 **[INCUTRAY]**	Incubation tray	2
6 **[LOTEVAL]**	lot specific template	1

5 MATERIALS REQUIRED BUT NOT PROVIDED

- glassware
- pipettes and tips
- plastic forceps
- rocking platform (vertical)
- distilled or de-ionized water
- filter paper
- black incubation trays when using the BloTrix-system for evaluation

6 PREPARATION AND STORAGE OF REAGENTS

Kit size and expiry

One kit is designed for 27 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.
Upon receipt, all test components 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.

Reagent preparation

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

Prepare a sufficient amount of Wash and Incubation buffer solution by diluting the WIB concentrate 5X (2) 1 + 4 with distilled or de-ionized water. Vortex before use.

For Example:

20 ml WIB concentrate 5X (2) + 80 ml distilled water.

7 ASSAY PROCEDURE

- **The test has to be performed at room temperature (20 - 25°C).**
- **The nitrocellulose strips have to be transferred into the incubation tray by using plastic forceps in a way that the printed strip number on the top is visible**
- **The following procedure has to adhere strictly to the time table.**
- **Any time shift during dispensing of samples and reagents should be avoided.**

7.1 Working steps

1. Incubate the nitrocellulose strips (1) in the Incubation tray (5) with **1.5 ml** Wash and Incubation buffer solution (from (2)) on a rocking platform for **5 min**.
2. Add **15µl of** the samples to each strip.
3. Cover the Incubation tray (5) and incubate **45 min** on a rocking platform.
4. Aspirate, then wash **3 x 5 min** with **1.5 ml** Wash and Incubation buffer solution (from (2)).
5. Incubate the nitrocellulose strips with **1.5 ml** conjugate ready for use (3)
6. Cover the Incubation tray (5) and incubate **45 min** on a rocking platform.
7. Aspirate, then wash **3 x 5 min** with **1.5 ml** Wash and Incubation buffer solution (from (2)).
8. Incubate nitrocellulose strips with **1.5 ml** TMB/substrate solution (4) normally 10 min (3).
9. Aspirate the substrate solution (4) and rinse the strips **3 times** with distilled water to stop the reaction.
10. The developed strips have to be dried between filter paper and should be evaluated within **6 hours**.

Please note:

With so called „problematic" serum samples (e.g. from specimens from donors with hypergammaglobulinaemia, circulating immune complexes, antibodies to milk proteins) the background develops rapidly and the nitrocellulose strips discolour blue. In such cases the substrate reaction has to be terminated earlier.

8 TEST EVALUATION

8.1 Test validity

The test run is valid if the control band just below the printed strip number is clearly visible.

Comment:

If no VlsE-band is visible in the IgG-immunoblot an infection with Borrelia is not necessarily excluded.

The VlsE-band is unimportant for interpretation of the IgM immunoblot.

Comments to individual bands:

- Some samples may show a strong reactivity with the p30 antigen. In these cases the p30 and the OspA-band may appear as one band making a clear differentiation impossible.
- The reactivity to OspC is reflected either as 23 kDa band or as 25 kDa band or as double band due to the protein heterogeneity.

The occurrence of further bands which are either specific or non-specific for an infection with *Borrelia burgdorferi* is possible and reflects the individual immune response.

9 LIMITATIONS OF THE PROCEDURE

After an infection with *Borrelia burgdorferi* antibodies of IgM isotype directed to flagellin (p41) and to the outer surface protein C (Ospc) become detectable first. Flagellin is highly immunogenic with only rare variations among the different strains but it shares sequence homologies with flagellar proteins of other spirochaetes at its C- and N – terminus. Thus infections with *Treponema phagendensis* or *Treponema pallidum* but also with Yersinia, Salmonella and E.coli can provoke false positive results. In cases of isolated antibodies to flagellin and continuing suspicion for Lyme-Borreliosis, antibody status should be repeatedly checked.

In a very early phase of infection with borrelia antibody response can be still too low to result in visible bands. Therefore reinvestigation after two to three weeks is recommendable.

Persons treated with antibiotics or persons with immunosuppression show a delayed immune response especially regarding the switch from IgG to IgM which can lead to false negative results.

Therefore a negative result does not necessarily exclude an infection with *Borrelia burgdorferi*.

As in other immunoassays cross contamination of reagents and/or samples by fungi and bacteria can produce false positive as well as false negative results.

Incorrect dilutions of samples and conjugates, incorrect washing of the strips or insufficient rinsing after substrate reaction but also incorrect timing can produce erroneous results.

10 COMMON ADVICES AND PRECAUTIONS

Follow the working instruction carefully. The test should be performed by trained technical laboratory staff only.

The expiry dates stated on the respective labels are to be observed.

Do not use or mix reagents from different lots except for wash and incubation buffer and for TMB/substrate solution.

Do not use reagents from other manufacturers to complete the kit.

All reagents should be stored at 2 - 8 °C but warmed to room temperature before use.

Handle the blot strips only with plastic forceps.

Prior to testing the identification numbers of the serum samples, the numbers of the blot strips and the Lot number of the kit should be fixed on the data sheet.

Some of the reagents contain small amounts of Kathon (1,0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes. In case of contact immediately remove under rinsing water.

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 by mouth,
- Note safety precautions of the single test components.

LITERATURE

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