



Blot systems for borreliosis diagnostics: Highly sensitive, highly specific, automatable



Anti-Borrelia EUROLINE-RN-AT (IgG)



Anti-Borrelia EUROLINE-RN-AT (IgM)



Anti-Borrelia EUROLINE-WB (IgG, IgM)



- Certified according to EN ISO 9001 / EN 46001 / EN ISO 13485
- In line with the recommendations of the German Society for Hygiene and Microbiology
- Two alternatives available:

1. Anti-Borrelia EUROLINE-WB

based on a classic Borrelia whole antigen extract with complete antigen spectrum plus recombinant VlsE

2. Anti-Borrelia EUROLINE-RN-AT

with classic, authentic, WB-purified antigens (p83, p39), recombinant VlsE or native OspC from *B. burgdorferi*, *B. garinii* and *B. afzelii*, recombinant designer antigens (p18, p19, p20, p21, p58) and new lipid antigens

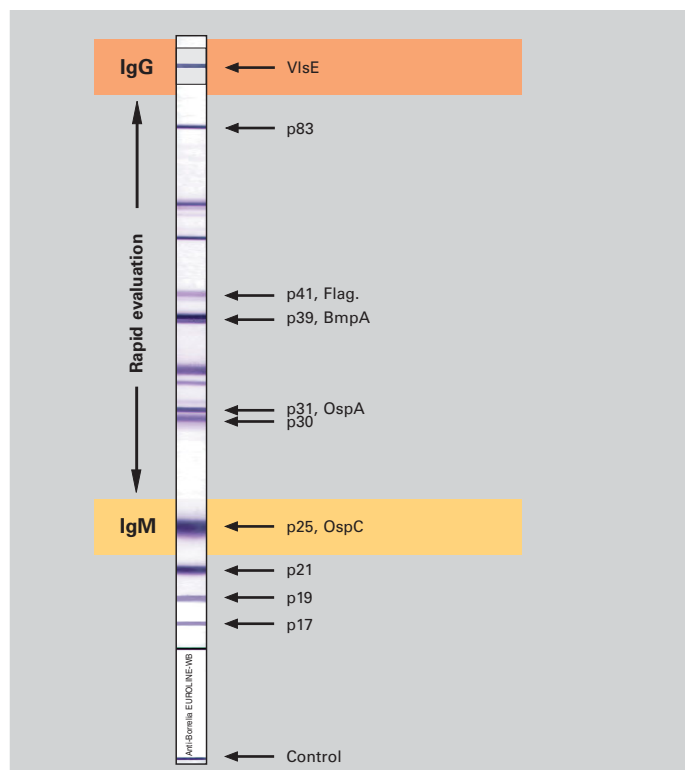


Anti-Borrelia EUROLINE-WB – well-established borreliosis diagnostics using Borrelia whole antigen extract plus VlsE

EUROLINE-WB – a combination of line blot and westernblot

In the **line blot** system **EUROLINE**, purified, biochemically characterised antigens are printed as parallel lines at defined positions on the membrane. The reactions can be evaluated effortlessly. However, the spectrum of available antigens is limited. Conventional native **westernblot** systems provide the complete antigen spectrum of cultured cells, tissue or infectious agents, whereby the individual proteins are separated electrophoretically according to size. But the variety of antigens on the membrane strip can make the evaluation difficult if the antigen bands lie very close to one another.

The **EUROLINE-WB** combines the advantages of both methods: Ready prepared **westernblot strips** are fitted with **EUROLINE membrane chips**, which are preprinted with either native, affinity chromatographically purified antigens or recombinant antigens. For each antigen the most suitable membrane is selected and the optimal coating procedure used.



Why is VlsE additionally employed as a recombinant antigen?

In a study performed by the Max Pettenkofer Institute (former Borrelia reference centre in Germany), it was shown that by additionally investigating for antibodies to VlsE, the serological hit rate can be increased by 20% compared with blots using whole extract. Of all recombinant antigens tested, VlsE possesses the **highest sensitivity for the detection of a Borrelia infection** (Schulte-Spechtel et al., J. Clin. Microbiol. 41:1299-1303, 2003). These results were confirmed in an internal study (Meyer et al., scientific presentation submitted to the 32nd Congress of the German Society for Rheumatology, Frankfurt, Germany, 2003). Over 85% of IgG positive sera could be identified at a glance by assessing the VlsE band. VlsE allows detection of antibodies against all Borrelia species, and the risk of a false negative reaction due to species differences is ten times lower.

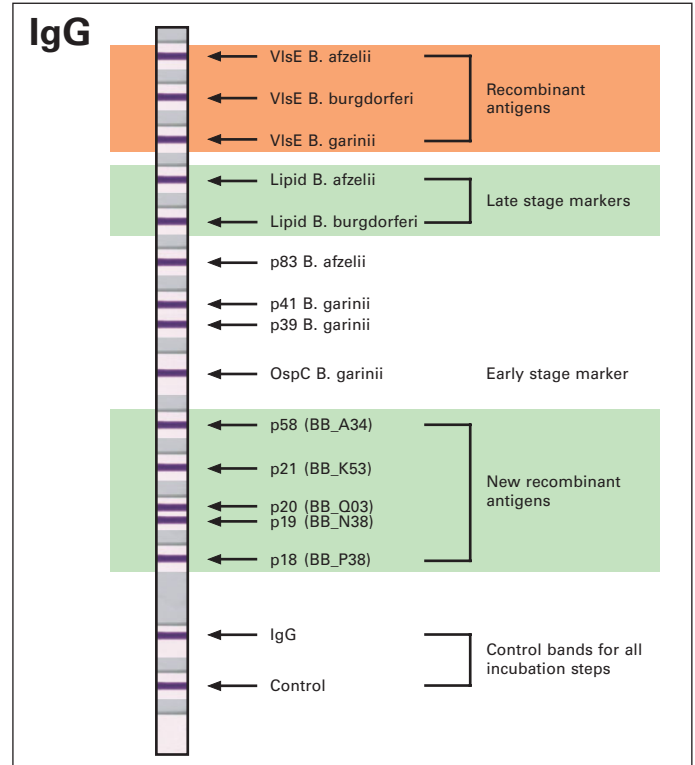
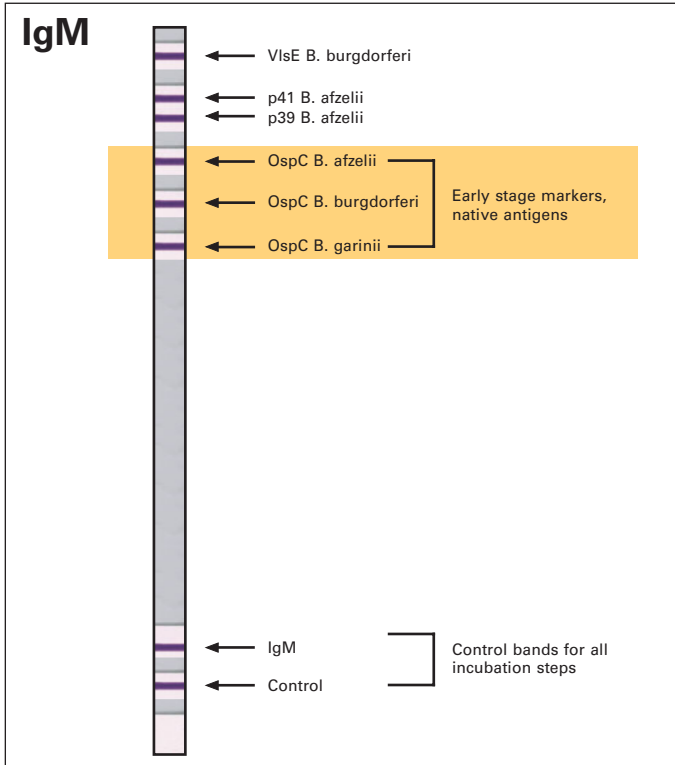
Quality features of the EUROIMMUN Anti-Borrelia EUROLINE-WB (whole antigen plus VlsE)

Various anti-Borrelia westernblots were compared and the usefulness of the VlsE component of the Anti-Borrelia EUROLINE-WB was investigated. By using VlsE it was possible to increase the serological hit rate for IgG antibodies from 40% to 62% in patients with erythema migrans (n=47), and from 78% to 93% in patients with neuroborreliosis (n=27). In the case of IgM antibodies, no significant increase in sensitivity was found through the use of VlsE, as here antibodies against OspC (p25) play the decisive role. When both IgG **and** IgM antibodies are investigated using the Anti-Borrelia EUROLINE-WB, a hit rate of 89 to 100% is achieved throughout the various stages of infection (sera from Prof. H.-J. Hagedorn, Herford; Dr. F. Blaes, Giessen and others).

Panel	n	Anti-Borrelia antibodies (IgG)		Anti-Borrelia anti-bodies (IgM)		Anti-Borrelia antibodies (IgG/IgM)	
		Conventional Anti-Borrelia Westernblot	Anti-Borrelia-EUROLINE-WB with VlsE	Conventional Anti-Borrelia Westernblot	Anti-Borrelia-EUROLINE-WB with VlsE	Conventional Anti-Borrelia Westernblot	Anti-Borrelia-EUROLINE-WB with VlsE
Erythema migrans	47	40%	62%	68%	70%	80%	89%
Neuroborreliosis	27	78%	93%	48%	48%	85%	96%
Borreliosis arthritis	33	94%	94%	15%	15%	94%	94%
Acrodermatitis chronica atrophicans	8	100%	100%	13%	13%	100%	100%



Anti-Borrelia EUROLINE-RN-AT – new specific markers for comprehensive antibody diagnostics



Unique composition of Borrelia-specific antigens

When selecting the antigens for the new Anti-Borrelia EUROLINE-RN-AT, the biochemical characteristics of each individual Borrelia antigen were taken into account in order to ensure optimal presentation of the proteins on the test strip. Many patients with acute Borrelia infection show reactions to native OspC. So far, however, it has been impossible to develop recombinant OspC of sufficient diagnostic quality. Unlike OspC, VlsE cannot be obtained from a Borrelia culture because it is produced by bacteria only when they are “stressed” by the immune response of the host organism during an infection. But VlsE can be produced using recombinant techniques. The examples show that recombinant and native antigens should be combined to create an ideal test system. When using tests which are only based on recombinant antigens, it must be accepted that 25% of control sera show a false positive IgM result.

The new Borrelia EUROLINE combines the advantages of a westernblot and a line blot in one test strip. Antigens such as p83, p39 and OspC, which are most specific in their native form, were accurately cut from a classic westernblot after digital evaluation and placed onto a EUROLINE strip. Additionally, immunoreactive lipids were extracted from Borrelia membranes and applied in lines to membranes. For optimisation of the sensitivity, 68 potentially immunoreactive proteins were identified in a bioinformatic analysis of the Borrelia genome and produced recombinantly by means of molecular biological methods. Subsequently, the obtained antigens were enhanced by molecular designing to yield maximum specificity. The performance characteristics of the Borrelia EUROLINE were determined by comparing the results with the EUROLINE-WB (n=617). The sensitivity and specificity of the individual antigens calculated by ROC (receiver operating characteristics) analysis can be found in the following tables.

Borrelia antigens	EUROLINE-RN-AT, IgM (n=617)	
	Sensitivity (%)	Specificity (%)
VlsE B. burgdorferi	4.9	99.4
p39	15.9	99.0
OspC native B. afzelii	88.2	99.0
OspC native B. burgdorferi	77.1	99.2
OspC native B. garinii	84.1	96.8

Borrelia antigens	EUROLINE-RN-AT, IgG (n=617)	
	Sensitivity (%)	Specificity (%)
VlsE B. afzelii	65.5	98.6
VlsE B. burgdorferi	88.5	98.6
VlsE B. garinii	67.6	95.3
Lipid B. afzelii	25.1	100.0
Lipid B. burgdorferi	25.1	99.6
p83	53.7	95.3
p39	61.3	98.6
OspC	48.7	95.7
p58 (BB_A34)	20.7	97.5
p21 (BB_K53)	8.9	99.3
p20 (BB_Q03)	7.1	100.0
p19 (BB_N38)	9.1	99.3
p18 (BB_P38)	22.4	99.3

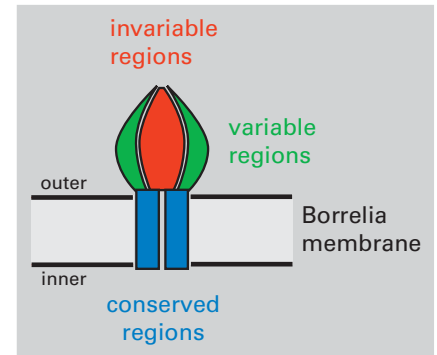


VlsE – the main antigen for Borrelia serology

What is VlsE and what is its function?

VlsE (variable major protein-like sequence, expressed) is a **surface protein of Borrelia burgdorferi**. Its significance for the serological diagnosis of borreliosis was overlooked until several years ago. VlsE plays a key role in the survival strategy of Borrelia. After penetration into the host organism, Borrelia bacteria constantly change their surface-expressed VlsE and, in this way, try to escape recognition and elimination by the immune system.

The VlsE protein is divided into several sections: conserved regions, which serve as trans-membrane domains and anchor VlsE in the bacterial membrane, as well as variable and invariable regions. The variable regions of VlsE facing outwards are constantly changed by recombination, whereby the attacking immune system consistently encounters new, altered antigen epitopes. The invariable regions are masked by the variable regions and, in living Borrelia bacteria, are protected from direct attack by the immune system. When deceased Borrelia bacteria are processed by antigen-presenting cells, the complete VlsE protein is presented to the immune system and the host also forms **antibodies against the invariable and conserved regions of VlsE**. These antibodies are highly suitable for diagnosis of borreliosis because of the high level of conservation of their target antigens: Using enzyme immunoassays (ELISA, EUROLINE-WB, EUROLINE) Lyme borreliosis can be diagnosed in 85% of cases, regardless of the species, through the detection of antibodies against VlsE alone.



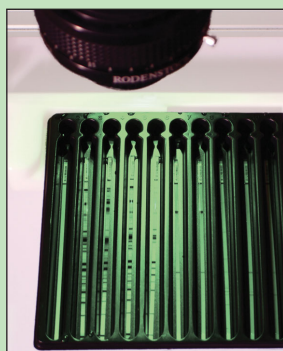
VlsE on the surface of Borrelia

Automatic processing of immunoblot strips

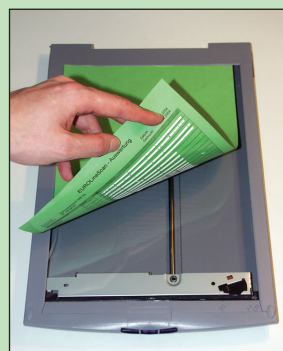
Not only Borrelia immunoblots, but all EUROIMMUN immunoblots (EUROLINE, EUROLINE-WB, Westernblot) can be processed in a standardised manner using the **EUROBlotMaster**, allowing higher precision and better reproducibility. The incubated strips are either scanned using a flatbed scanner (**EUROBlotScanner**) or photographed in the incubation tray by means of a camera system (**EUROBlotCamera**). Subsequently, the immunoblots are automatically evaluated and archived using the computer programmes **EUROLineScan** and **EUROLabOffice** offered by EUROIMMUN.



EUROBlotMaster



EUROBlotCamera



EUROBlotScanner

Patients ID's: Serum 3 Test: EUROLINE Borrelia IgG
Date of receipt: 14.03.2008 Number: 3
Results from: 08.05.2008

EUROIMMUN Medizinische Labordiagnostika AG Automatic evaluation of test strips using the EUROLineScan software

Antigen	char	o	(+)	+
VlsE Borrelia afzelii (VBa)				+
VlsE Borrelia burgdorferi (VBb)				+
VlsE Borrelia garinii (VBg)				+
Lipid Borrelia afzelii (LBa)				+
Lipid Borrelia burgdorferi (LBb)				+
p83 Borrelia afzelii (p83)				+
Flagellin Borrelia garinii (p41)				+
BmpA Borrelia garinii (p39)				+
OspC Borrelia garinii (OspC)				+
BB_A34 (p58)				+
BB_K53 (p21)				+
BB_Q13 (p20)				+
BB_N38 (p19)				+
BB_P38 (p18)				+
Anti-human-IgG (IgG)				+
Anti-human-IgM (IgM)				o
Control (Co)				+
Label (Et)				o

Class	Explanation
o	no staining
(+)	weak staining
+	strong staining

Test	Result
EUROLINE Borrelia IgG	positive

Signature: *Manhagen*

Lab result sheet created automatically by the EUROLineScan software