EUROIMMUN
a PerkinElmer company

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

Anti-Borrelia EUROLINE-RN-AT (IgG)

Anti-Borrelia EUROLINE-RN-AT-adv (IgM)

Anti-Borrelia EUROLINE-WB (IgG, IgM)

- **Certified according to EN ISO 13485 and EN ISO 9001**
- **In line with the recommendations of the German Society for Hygiene and Microbiology**
- **Anti-Borrelia EUROLINE-RN-AT (DN 2131 G or M)**
  with classic 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-RN-AT-adv (DN 2131-2 M)**
  well-established EUROLINE-RN-AT, NEW with recombinant dimeric OspC advanced from the species B. burgdorferi, B. garinii, B. afzelii and B. spielmanii: 30% more specific than conventional recombinant OspC
- **Anti-Borrelia EUROLINE-WB (DY 2131-1 G or M)**
  based on a classic Borrelia whole antigen extract with complete antigen spectrum plus recombinant VlsE

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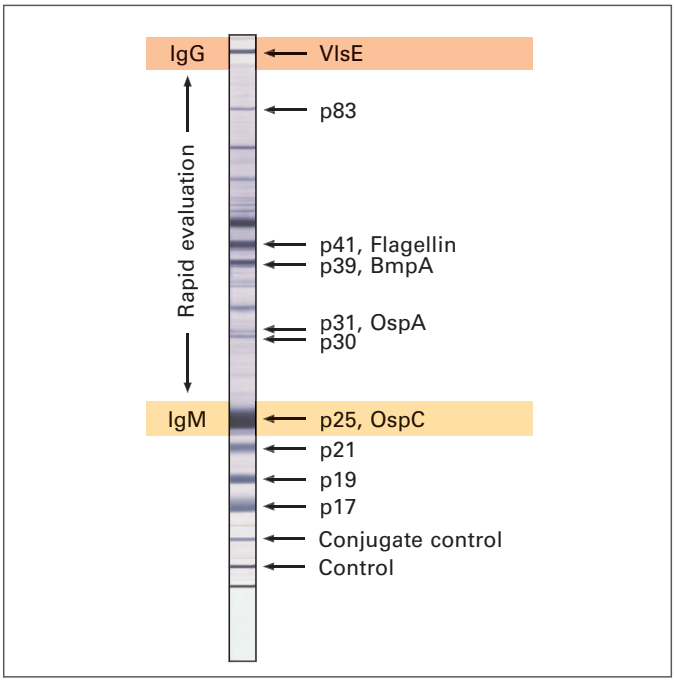
Anti-Borrelia EUROLINE-WB: Well-established borreliosis diagnostics using Borrelia whole antigen extract plus VlsE

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

The combination of a whole-antigen extract (B. afzelii) and recombinant VlsE as an early-stage disease marker (regardless of the species) enables the identification of atypical reactions and ensures a high sensitivity. Presence of a positive VlsE (IgG) or p25/OspC antigen band (IgM) allows-at-a-glance evaluation.

Clinical data: A panel of 115 clinically defined patient samples was investigated using the EUROIMMUN Anti-Borrelia EUROLINE-WB (IgG, IgM). The following prevalences were detected (only positive results were taken into account):

Panel	n	EUROIMMUN Anti-Borrelia EUROLINE-WB (IgG, IgM)		
		IgG [%]	IgM [%]	IgG/IgM[%]
Erythema migrans	47	51	57	83
Neuroborreliosis	27	78	33	81
Borreliosis arthritis	33	94	6	94
Acrodermatitis chronica atrophicans	8	100	13	100

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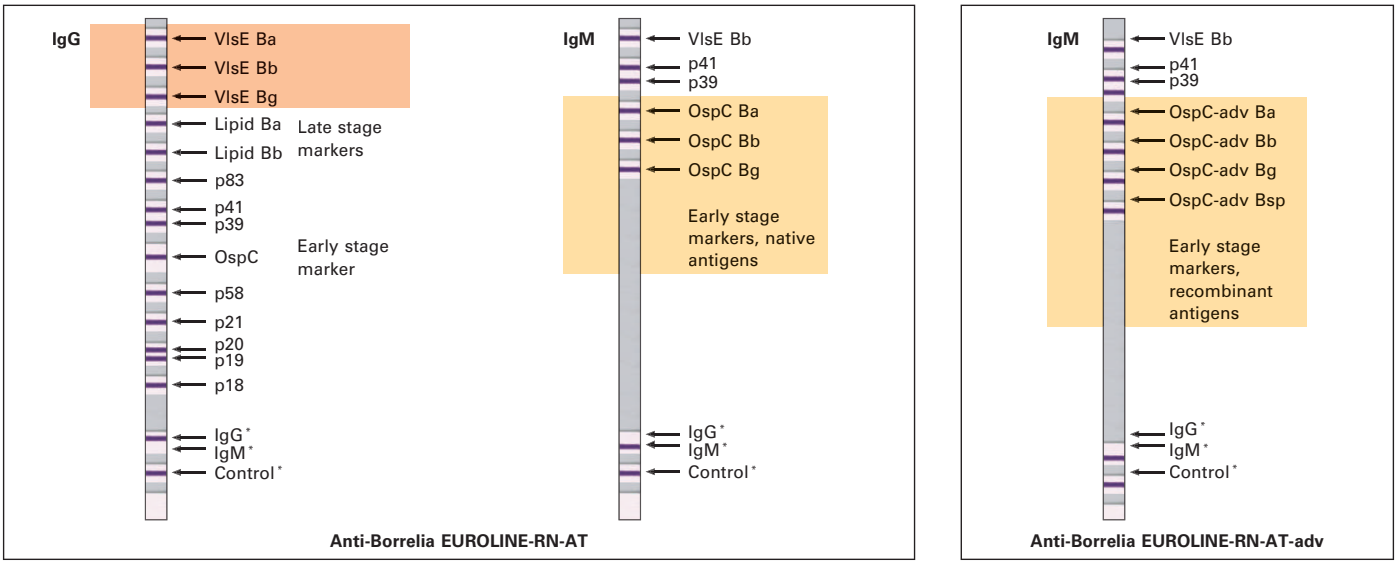
Anti-Borrelia EUROLINE-RN-AT and Anti-Borrelia EUROLINE-RN-AT-adv: New specific markers for comprehensive antibody diagnostics in serum and CSF



Bb Borrelia burgdorferi, Ba Borrelia afzelii, Bg Borrelia garinii, Bsp Borrelia spielmanii, *Control bands for all incubation steps

EUROLINE-RN-AT: Unique combination of Borrelia-specific antigens

The Anti-Borrelia EUROLINE-RN-AT provides a comprehensive range of diagnostically relevant Borrelia antigens in a user-friendly line blot format. In addition to the most important serological early phase markers OspC and VlsE from different genospecies, it includes highly specific p39 (Bmp) and the late phase marker p83. The test also contains native immunogenic lipids which were extracted in native form from Borrelia and printed as lines onto membranes.Furthermore, immunoreactive antigens with a high specificity for the detection of anti-Borrelia antibodies were produced by means of bioinformatic analysis of the Borrelia genome and molecular designing.

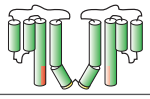
Anti-Borrelia antigens	EUROLINE-RN-AT, IgG (n=617)	
	Sensitivity [%]	Specificity [%]
VlsE B. afzelii	65.5	98.6
VlsE B. burgdorferi	88.5	98.6
VlsE B. garinii	67.6	95.3
Lipid B. afzelii	25.1	100.0
Lipid B. burgdorferi	25.1	99.6
p83	53.7	95.3
p39	61.3	98.6
OspC	48.7	95.7
p58	20.7	97.5
p21	8.9	99.3
p20	7.1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

Anti-Borrelia antigens	EUROLINE-RN-AT, IgM (n=644)	
	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

EUROLINE-RN-AT-adv: 30% more specific due to OspC advanced

Antibodies against OspC are the most important serological marker for the detection of Borrelia infections (sensitivity: up to 90%). Numerous research results have shown that native OspC purified from Borrelia (dimeric form) is the ideal antigen substrate. However, the standardised production of native OspC dimers is complicated, which means that the use of recombinant, monomeric OspC is often preferred. Since Borrelia infections cannot be detected using only moderate amounts of monomeric OspC, high concentrations must be employed. In this way, an acceptable detection rate is achieved, but this comes at the price of a higher number of unspecific reactions. Scientists from EUROIMMUN AG have successfully produced recombinant covalently bonded dimeric OspC (European patent EP 2 199 303 B1) by genetic engineering. This **OspC advanced is 30% more specific than conventional recombinant OspC** (Probst et al., ICLB, 2010), with the same sensitivity as native OspC (Ott et al., ECCMID/ICC, 2011). The OspC advanced is used in the **EUROIMMUN Anti-Borrelia EUROLINE-RN-AT-adv (IgM)**. This line blot allows reliable detection of antibodies against all relevant human pathogenic Borrelia genospecies, since OspC advanced from B. spielmanii is also included.

Dimeric OspC advanced



Panel	n	Prevalence of anti-OspC (IgM) [%]					
		B. afzelii		B. burgdorferi		B. garinii	
		adv	native	adv	native	adv	native
Active borreliosis	150	65	61	69	54	66	64
Past Borrelia infection (persistent IgM)	16	13	13	13	13	13	19
Acute EBV	10	0	0	0	0	0	0
Pregnant women	50	2	2	2	2	2	2
Blood donors	50	4	4	6	4	4	2

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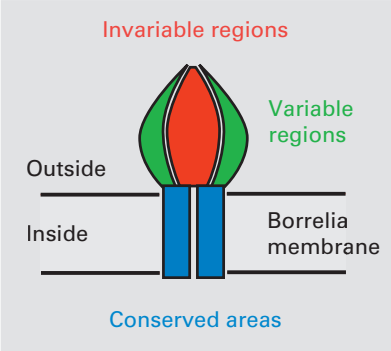
VlsE: The main antigen for Borrelia serology

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.



VlsE on the Borrelia surface

The VlsE protein is divided into several sections: conserved regions, which serve as transmembrane 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.

Automatic processing of immunoblot strips

EUROBlotOne is a fully automatic device for the standardised processing of EUROIMMUN line assays (EUROLINE, EUROLINE- WB, Westernblot) – from sample recognition to the final test result. Samples are pipetted by the device and all incubation and washing steps are carried out automatically. Finally the data of the pictures taken by the integrated camera are automatically evaluated and archived by the **EUROLineScan software**. Alternatively, the immunoblot strips can be incubated by the **EUROBlotMaster** and scanned using the **EUROBlotScanner** or photographed directly in the incubation tray using the **EUROBlotCamera**. Also in this case, the automatic evaluation is carried out by the EUROLineScan software. The bidirectional communication with a laboratory information management system for import of work lists and export of results is enabled by **EUROLineScan** or, optionally, the laboratory management software **EUROLabOffice**.