

Lyme disease serology
studies and experiences with
EUROLINE Borrelia RN-AT

Further Blot Tests
HSV, Rubella, EBV, T. pallidum

Wolfgang Meyer

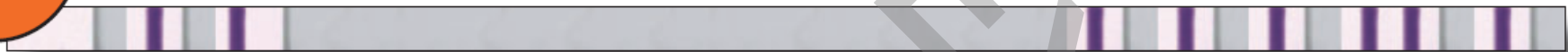
Westernblot plus VisE – EUROLINE RN-AT



Anti-Borrelia EUROLINE-RN-AT (IgG)

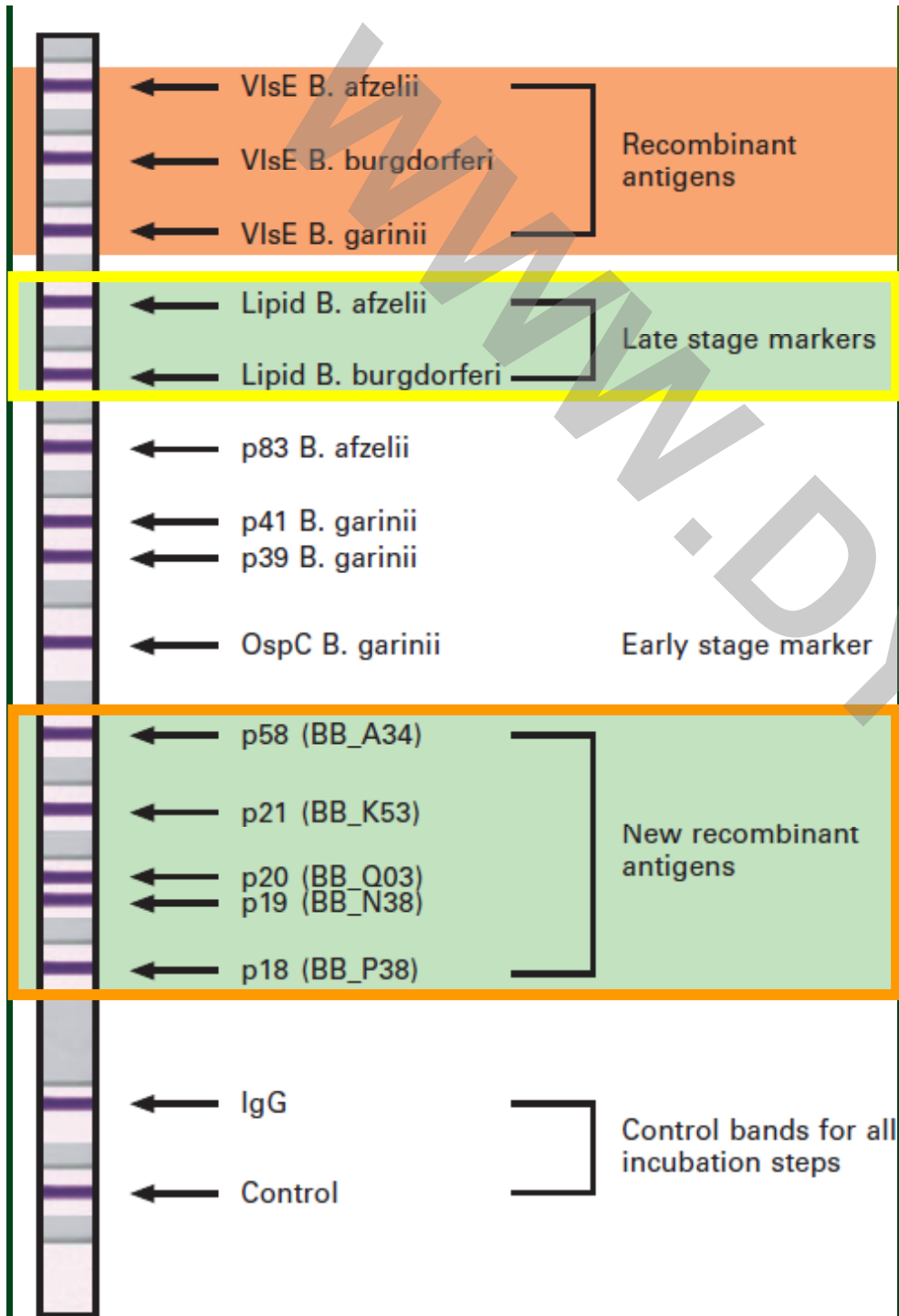


Anti-Borrelia EUROLINE-RN-AT (IgM)



Anti-Borrelia EUROLINE-WB (IgG, IgM)





EUROLINE Borrelia RN-AT IgG

New specific native Borrelia antigens:

Lipids of *Borrelia afzelii*

Lipids of *Borrelia burgdorferi*

New specific recombinant Borrelia antigens

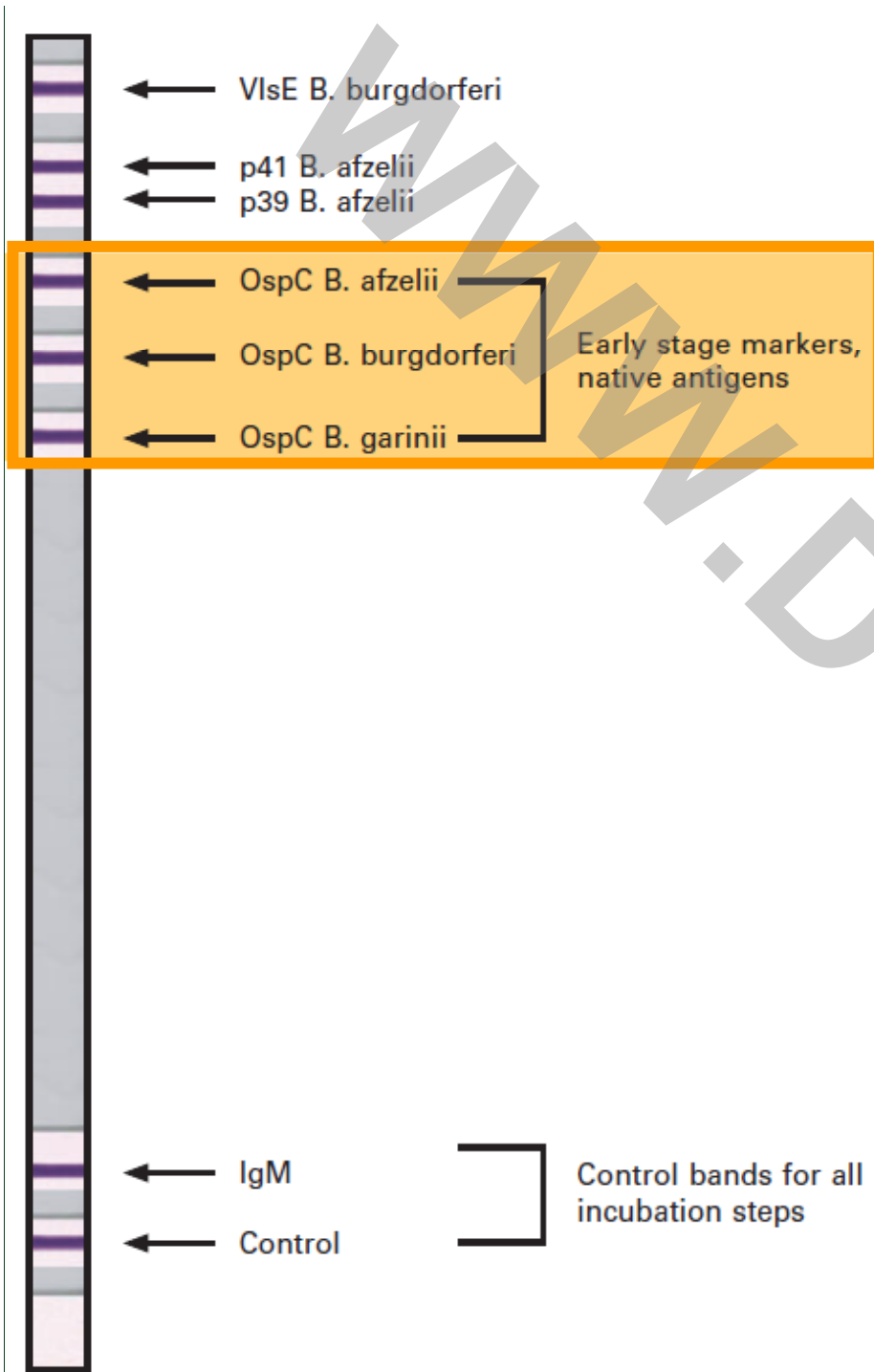
p18 – BB_P38

p19 – BB_N38

p20 – BB_Q03

p21 – BB_K53

p58 – BB_A34



EUROLINE Borrelia RN-AT IgM

native OspC-antigens:

Borrelia afzelii

Borrelia burgdorferi

Borrelia garinii

Experiences with the new EUROLINE Borrelia RN-AT (1)

EUROLINE Borrelia RN-AT: Marriage of authentic recombinant and native borrelia antigens in a line immunoassay format

**W. Meyer, A. Janssen, B. Kuehn, T. Scheper, W. Suer, L. Komorowski,
C. Probst, W. Schlumberger, and W. Stoecker**

Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany

International Journal of Medical Microbiology (in press)

EUROLINE Borrelia RN-AT IgG: Sensitivity and specificity

EUROLINE Borrelia- IgG	Antibodies class IgG	
	sensitivity [%]	specificity [%]
VlsE Ba	65,5	98,6
VlsE Bb	88,5	98,6
VlsE Bg	67,6	95,3
Lipid Ba	25,1	100,0
Lipid Bb	25,1	99,6
p83	53,7	95,3

Roc analysis, Evaluation of 617 sera from different patient groups

(suspected borreliosis, clinically characterized borreliosis, control groups (healthy blood donors, pregnant women, other infections))

EUROLINE Borrelia RN-AT IgG: Sensitivity and specificity

EUROLINE Borrelia- IgG	Antibodies class IgG	
	sensitivity [%]	specificity [%]
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

Roc analysis, Evaluation of 617 sera from different patient groups

(suspected borreliosis, clinically characterized borreliosis, control groups (healthy blood donors, pregnant women, other infections))

EUROLINE Borrelia RN-AT IgM: Sensitivity and specificity

EUROLINE Borrelia- IgM	Antibodies class IgM	
	sensitivity [%]	specificity[%]
VlsE Bb	4,9	99,4
p39	15,9	99,0
OspC nativ Ba	88,2	99,0
OspC nativ Bb	77,1	99,2
OspC nativ Bg	84,1	96,8

Roc analysis, 644 sera from different patient groups

Experiences with the new EUROLINE Borrelia RN-AT (2)

Lyme borreliosis: Prevalence of antibodies against non-proteinic (lipid) antigens

W. Meyer, A. Janssen, T. Scheper, W. Schlumberger, W. Stoecker

	n	Positive IgG reaction with lipids	
		Borrelia afzelii	Borrelia burgdorferi
Erythema migrans	62	4,8%	8,1%
Borrelia induced arthritis	17	29,4%	70,6%

International Journal of Medical Microbiology (in press)

Antibody response in early and late Lyme borreliosis

External study patients from Austria/Slovenia

Samples (n=430):

patients

50 Erythema migrans

48 Arthritis induced by borrelia

controls

12 CMV

30 RF

26 rubella

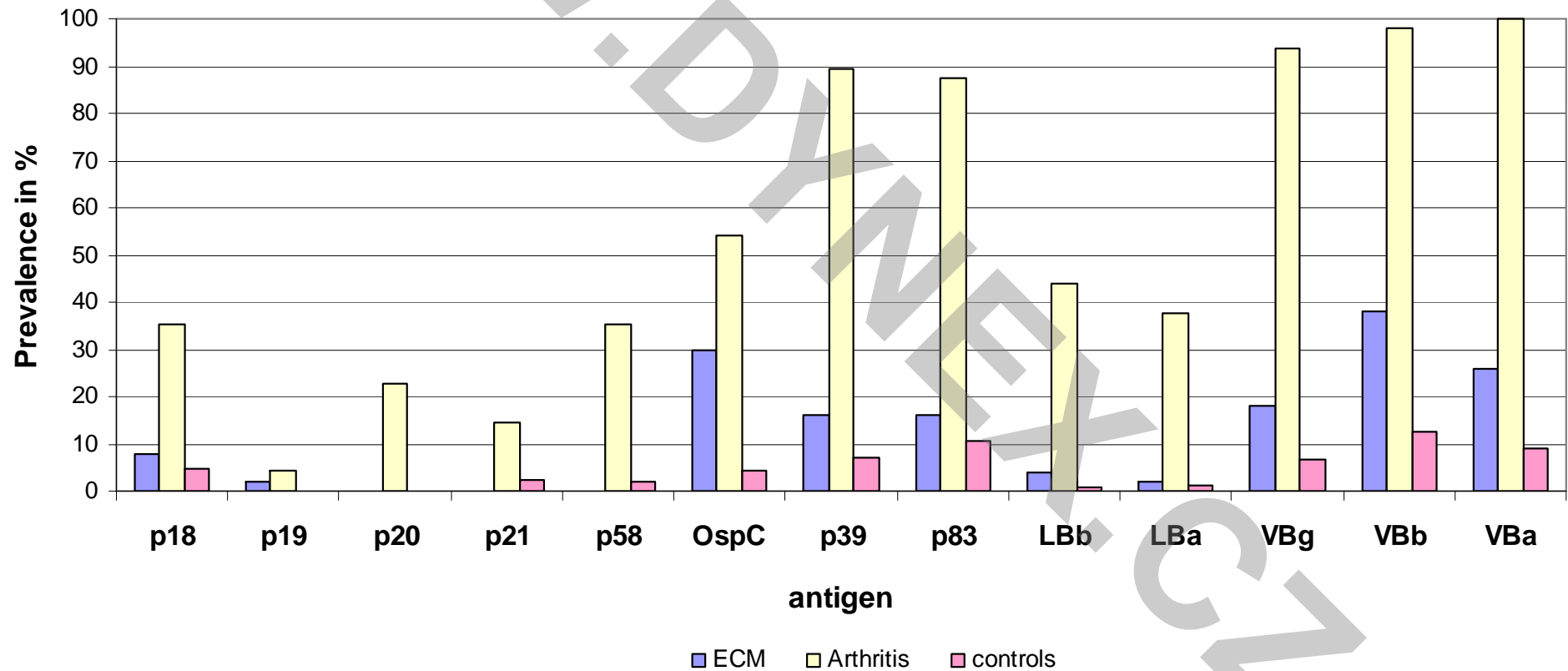
80 Toxoplasma

84 Treponema pallidum

100 blood donors

Antibody response in early and late Lyme borreliosis

Prevalence of antibodies against different antigens in early and late borreliosis



Antibody prevalence in a group of high risk forest workers

Forest workers from poland

Prevalence (%) in the blot positive cases	EUROLINE RN-AT IgG	
	Forest workers 184 of 574	Blood donors 4 of 100
VlsE Ba	80.4	25.0 (1 of 4)
VlsE Bb	88.0	100.0 (4 of 4)
VlsE Bg	52.7	0.0
Lipid Ba	8.7	0.0
Lipid Bb	17.4	0.0
p83 Ba	43.5	50.0 (2 of 4)
p41 Bg*	79.3	100.0 (4 of 4)
p39 Bg	51.1	25.0 (1 of 4)
OspC	29.3	0.0
p58	13.0	0.0
p21	5.4	0.0
p20	2.2	0.0
p19	4.9	0.0
p18	37.0	0.0

Blood donors from Lübeck

Features EUROLINE Borrelia RN-AT (1)

- The EUROLINE Borrelia has the **same sensitivity and specificity** as the whole extract Borrelia EUROLINE-WB.
- Same incubation procedure!
- **Further advantages of the EUROLINE Borrelia.**
- Evaluation is **easier** .
- **No** evaluation matrix required.
- Optimized **epitope presentation** (membrane type, stabilisation, blocking, ...).

Features EUROLINE Borrelia RN-AT (2)

- Specific surface antigens are present, **lipids and proteins**.
- The main antigens like **VlsE** for **IgG** detection and **OspC** for **IgM** are present from **all three pathogenic genospecies**.
- Highest specificity due to the use of **native OspC** instead of recombinant OspC.
- Separate strips with control bands for detection of anti-human-**IgG** and anti-human-**IgM**.
- **Automation** with **DYNABLOT** and evaluation with **EUROLineScan** and EUROBlotScanner or -Camera.

Herpes simplex virus

Anti-HSV-1/HSV-2-gG2 EUROLINE-WB (IgG/IgM)



↑
Control

↑
gG2
HSV-2 type-specific antigen
(additional membrane chip)

↑
gG1
HSV-1 type-specific antigens
(whole extract)

↑
gC1

HSV-1 specific antigens: gC-1 and gG-1

Both antigens **gG1** or the **gC1** are discussed to be specific for HSV 1.

EUROLINE-WB HSV 1/HSV 2 contains both bands!

n=101		gG-1 (IgG)	
		positive	negative
gC-1 (IgG)	positive	76	0
	negative	0	25

Interpretation HSV-1 positive : gC-1 + and gG-1 +

Rubella

Anti Rubella-Westernblot IgG

In pregnancy fresh rubella infections often cause **severe affections to the fetus**.

According to the **German maternity protection guidelines**: In addition to a **careful medical history** further serological tests are requested. Especially the **detection of IgM antibodies**.

However, it should be borne in mind that anti-rubella **IgM** antibodies may **persist for several months**. Further **interference** for the **IgM antibody detection** are **rheumatic factor** and **polyclonal B cell stimulation**.

To limit the infection period, the **avidity of specific IgG** antibodies can be tested. A high avidity makes an **infection within the last 4-6 weeks unlikely**.

Another distinction between a primary and a long past infection gives the determination of specific IgG antibodies against the surface **proteins E1 and E2**. According to literature **IgG antibodies against E1** were generated from **4-6 days after infection**. Whereas **IgG antibodies against E2** are detectable **3-4 months after infection**.

Anti Rubella-Westernblot IgG

Antigen description:

Band	Antigen	Description
96-98 kDa	E1-E2 dimer	dimer of envelope protein 1 und envelope protein 2
60 kDa	c-protein	capsid protein
50 kDa	E1	envelope protein <u>E1</u>
46-48 kDa	E2	envelope protein <u>E2</u>

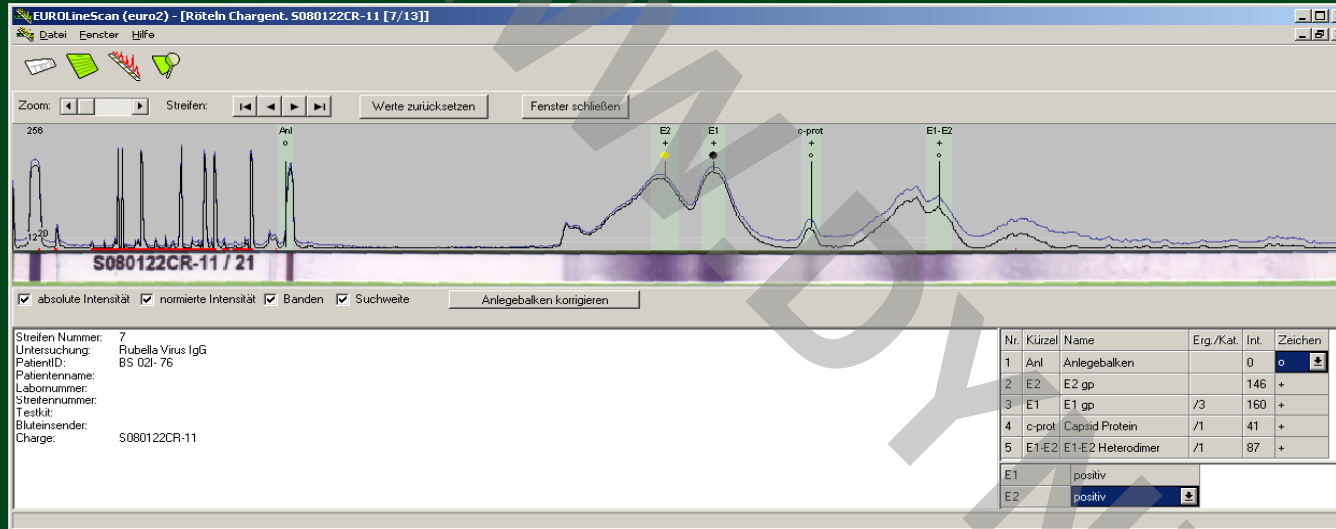
Evaluation: EUROLINEScan Software

Anti Rubella-Westernblot IgG

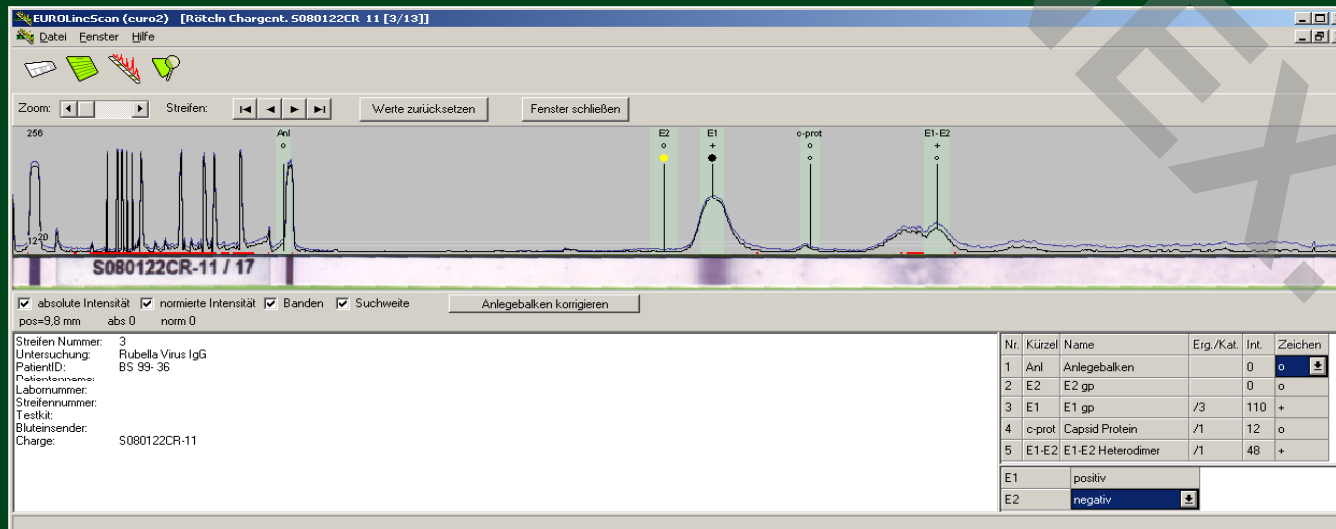
Band pattern	E1	E2	serological assessment
1	negative	negative	<u>No indication of a rubella infection or vaccination.</u> The possibility of a primary infection or vaccination in the last few days remains.
2	positive	negative	<u>Indication of a rubella infection in the early stages or a fresh rubella vaccination.</u> A delayed or lack of formation of antibodies against E2 can't be excluded.
3	positive	positive	Band pattern indicates <u>not an early stage of a rubella infection or vaccination.</u>
4	E1 and/or E2 borderline		Verification with other methodes

External validation march / april 2009: Labor Prof. Dr. med. Gisela Enders and Partner; Stuttgart

Anti Rubella-Westernblot IgG



Serological interpretation:
past rubella infection or rubella vaccination

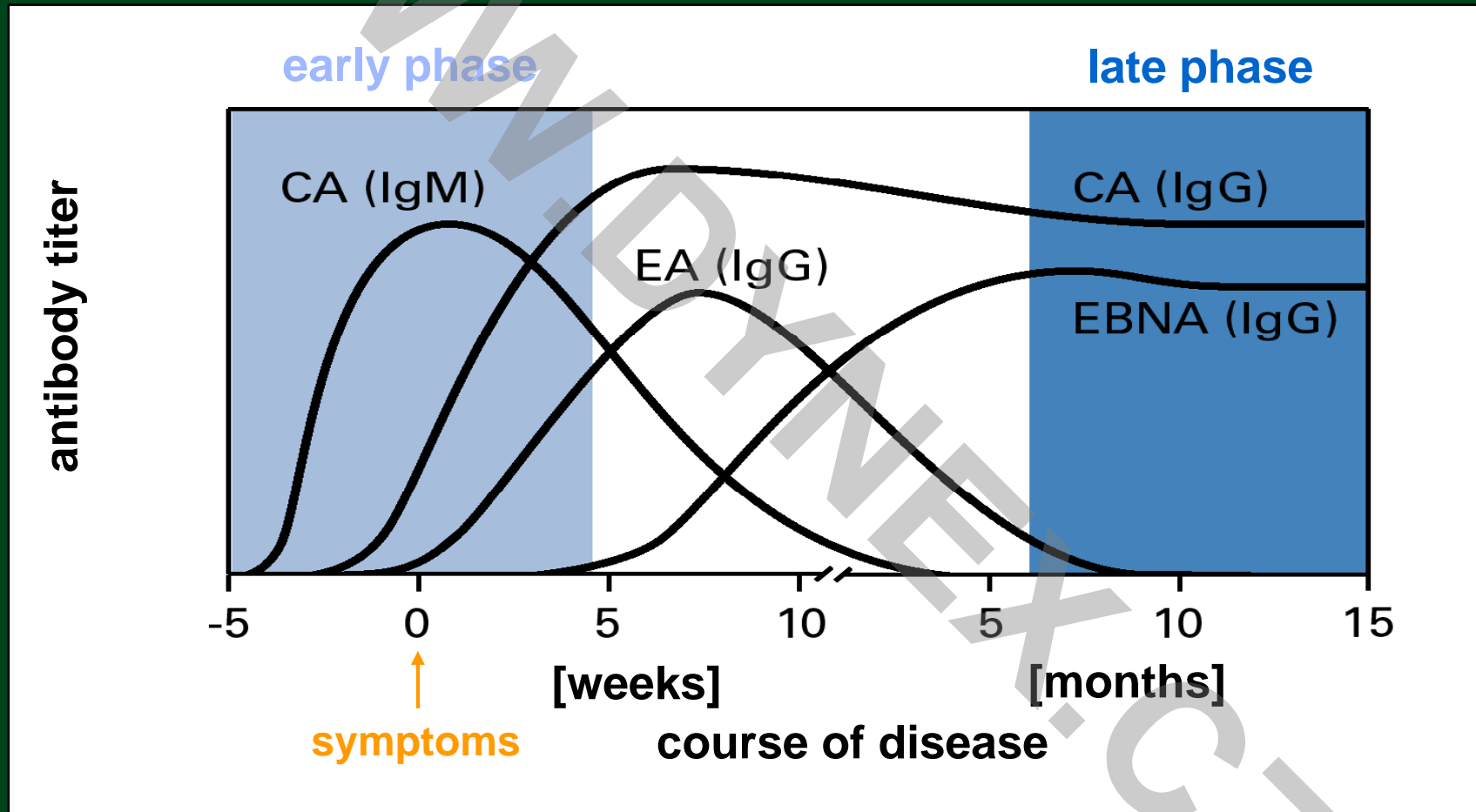


Serological interpretation:
indication of a rubella infection in the early stages, or a fresh rubella vaccination.

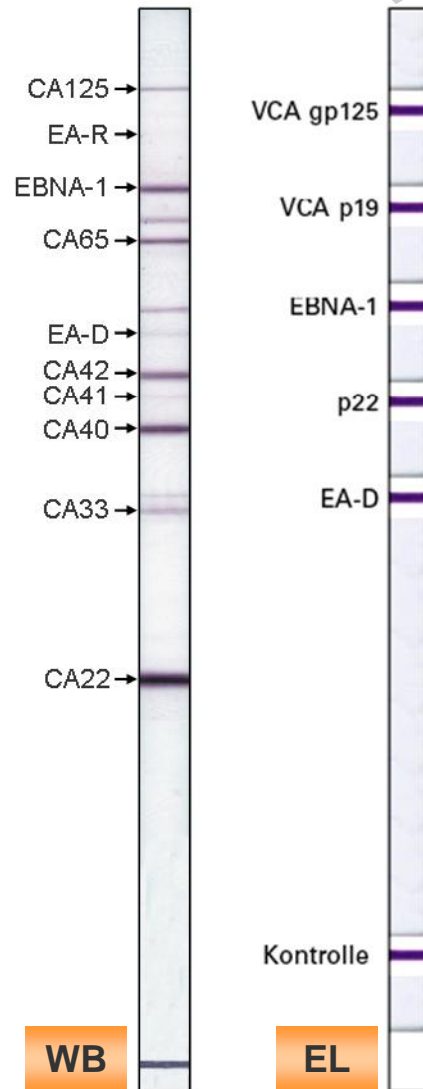
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EBV

Anti-EBV antibodies: Early and Late Phase

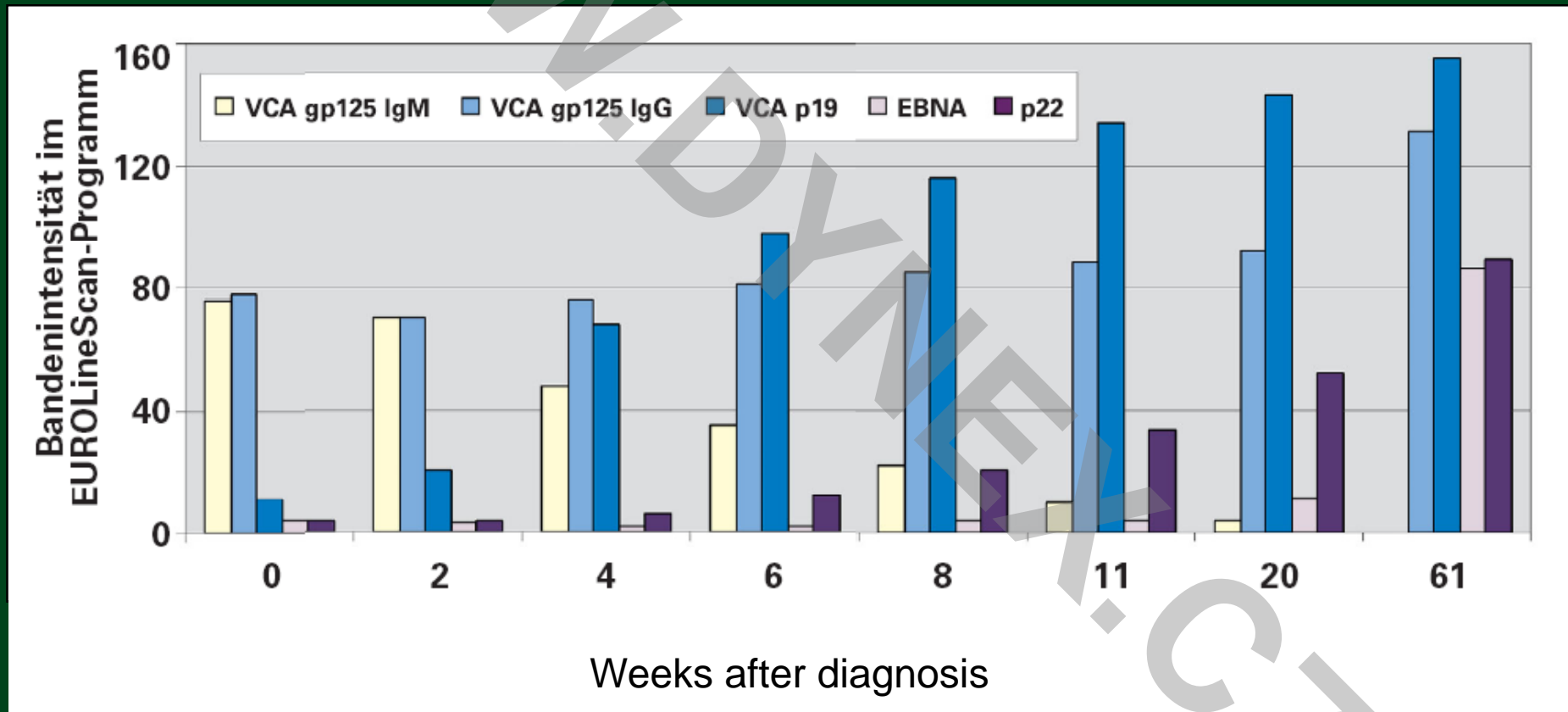


Anti-EBV-Westernblot & EUROLINE-EBV-Profil 2



- all relevant antigens on one strip:
Early phase (gp125) and Late phase marker (EBNA-1 und p22)
- Westernblot: specific evaluation matrix for each gel
- EUROLINE: defined position for each band
- incubation with Dynablot
- evaluation with EUROLINEScan

Antibodies response in a fresh EBV infection



Treponema pallidum

Treponema pallidum

Confirmation test: EUROLINE-WB

unspecific:
activity marker / therapy control

specific:
antibodies against *Treponema pallidum*



- optimal presentation of the specific antigens
TpN 15, TpN 17, tmpA und TpN 47
- membrane chip with cardiolipin in three different concentrations
- Westernblot: specific evaluation matrix for each gel
- incubation with Dynablot
- evaluation with EUROLINEScan

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Thank you very much for your attention