Selected Aspects of Serology of Borrelia burgdorferi sensu lato

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Lyme-Borreliosis is gaining importance as an emerging disease. Estimations of incidences for central Europe reach to 237/100.000[1] and in some regions to 1.5%[2] per year. The variety of symptoms is complex and clinical diagnosis in the case of multiple "general symptoms" is difficult and in many cases not reliable. The antibody-diagnosis is often not able to solve problems of diagnostics either, due to "false-positive" and "false-negative" results. The serologic result gives a limited answer concerning the stage of Lyme-Borreliosis. However, the serologic result gives a clue whether an infection with Borrelia burgdorferi sensu lato has occurred in the past. It is of limited value for proving success of therapy. Generally a two-step serodiagnosis - similar to HIV-diagnostics - is used. If the result of the screening-assay (ELISA, i-IFT, HAT or KBR[3]) points towards the presence of antibody it is confirmed by Western-/ Immuno- Blot. The diagnosis is established by the presence of certain bands. It will be shown, that the diagnostic value of test-kits from different manufacturers may differ considerably. This may even result in missing the diagnosis in certain cases- if a non-appropriate system is used. This poster will demonstrate possible influences on serologic diagnostics. Examples are given by blot-stripes from the daily routine.

[1]Talaska, Brandenburgisches Ärzteblatt 11 / 2002; 338-340
[2]Poster: B. Reimer[†], A. Marschang[†], V. Fingerle[‡], B. Wilske[‡], F. v. Sonnenburg[†]
[†]Abteilung für Infektions- und Tropenmedizin, [‡]Max-von-Pettenkofer Institut für Mikrobiologie, Universität München, 1999
[3]Enzyme Linked Immuno Sorbent Assay, Indirect Immunfluorescence Test, Hemagglutination Test, Complement Binding Reaction

Technical effects on serological results

1. **Antigenes**:
   - Which antigenes are used? Borrelia burgdorferi sensu stricto, Borrelia garinii, Borrelia afzelii?
   - Production of Borrelia-strains: longtime "processed", e.g. cultured strains loose/change their antigenity.
   - Possibly a "wrong" Pko-/B.afzelii-strain has been distributed by "Stammsammlung" in Braunschweig (DSMZ).
   - Cleaning / preparing of antigenes / lysates.
   - Recombinant antigenes?
2. **Sera:**
   - Which dilution of sample (titre) is used ?
   - Which quantity of sample is used ?
   - Which adsorbents are used, e.g. RF-adsorbens[3] or TP-adsorbens[4] ?
   - Preanalytics: hemolysis, temperature of storage.
3. **ELISA:**
   - Thickness of preparation with antigenes.
   - Procedure: competitive or non-competitive, µ-capture.
   - The cut makes the decision about quality of test-result: positive, negative or borderline result.
4. **Indirect-IFT:** intensity of counter-stain.
5. **Western-/ Immuno- blot:**
   - Lined or gel-blotted stripes?
   - Interpretation-chart:
     - Manufactured by the user?
     - Or ready to use?
     - Which bands are shown by the bandlocator?

[4] Rheuma-factor-adsorbens
[5]Treponema-phagedenis-adsorbens

Individual realisation

- Are verifications (positive, negative, cut) carried out?
- Is the „blot-cut" developed enough?
- How much conjugate-drop is used in i-IFT?

Adjustment of tests

In absence of a "goldstandard" Bb-serologies are evaluated on different standards. Results are classified in "sensitivity" and "specifity". It´s usual to evaluate one serology by another. Another possibility is to evaluate clinical observation combined with serologic parameters of other tests. In rarely cases serology is compared with PCR or culture-results. Sometimes the ELISA-cut is established in comparison with pooled sera of blood-donors by estimation of their seroprevalence.

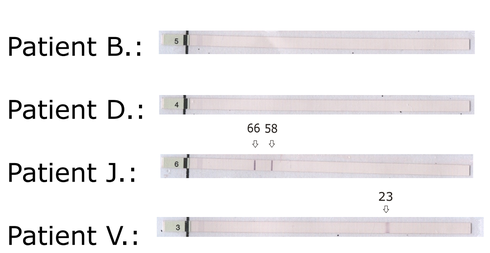
Methods:

We show the comparison of 8 sera measured on 2 to 4 different commercially available test-systems (blots). The tests have been performed as described by the providers. Test-kits based on different antigenes have been used:

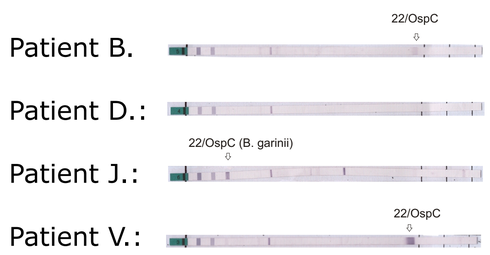
I. Full-cell-lysat-blot of Borrelia burgdorferi sensu stricto (Bb ss) strain 2531
II. Full-cell-lysat-blot of Borrelia afzelii plus OspC of B. garinii
III. Full-cell-lysat-blot of Borrelia afzelii
IV. Full-cell-lysat-blot of Borrelia-afzelii plus VlsE of Bb ss
V. Full-cell-lysat-blot of Bb ss plus Borrelia afzelii
VI. Full-cell-lysat-blot of Bb ss plus Borrelia afzelii plus Borrelia garinii
VII. Line-Blot containing OspC, VlsE, p39, p83, BBA36, BBO323, Crasp3, pG, EBV
VIII. Rekombinant-blot containing p100 (B. afzelii), p41 (B. afzelii), p39 (B. afzelii), OspA (B. afzelii), OspC (3 strains), p41 int. (B. afzelii, B. garinii), p18 (B. afzelii)
IX. Full-cell-lysat-blot of Bb ss plus B. afzelii plus VlsE

4 samples measured on 3 IgM-blots

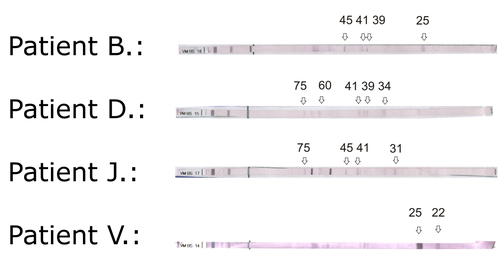
IgM, test I:



IgM, test II:



IgM, test V:



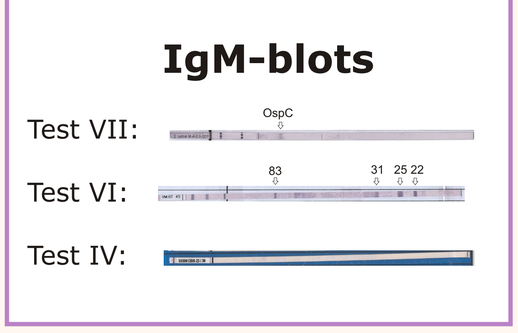
Results IgM-blots

	Test I	Test II	Test V
	Bands:	Bands:	Bands:
Patient B:		22/OspC	45, 41, 39, 25
Patient D:			75, 60, 41, 39, 34
Patient J:	66, 58	22/OspC (B. garinii)	75, 45, 41, 31
Patient V:	23	22 / OspC	25, 22

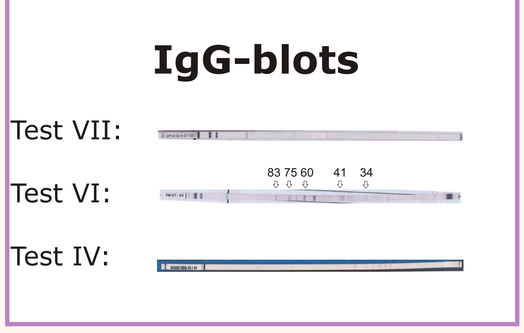
Patient J.: Reactivity with additional OspC (B. garinii) in test II

4 samples measured on 3 IgG-blots

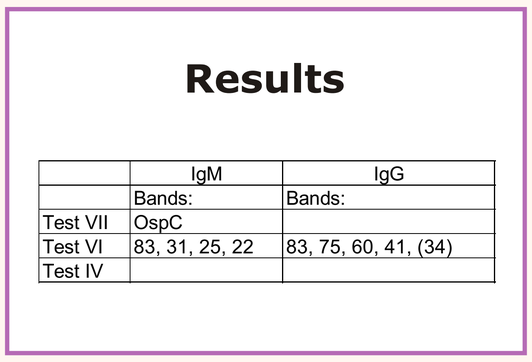
IgG, test I :



IgG, test II :



IgG, test V :

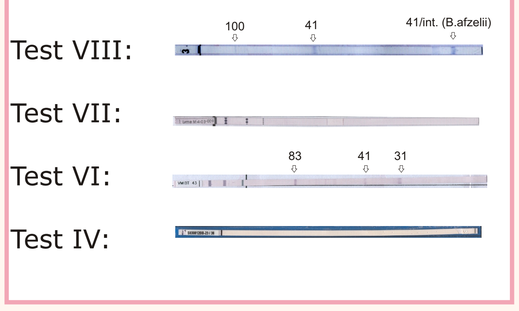


Results IgG-blots

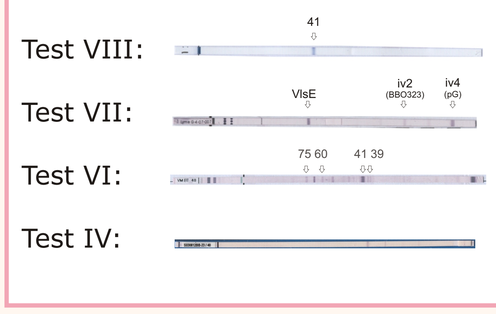
	Test I	Test II	Test V
	Bands:	Bands:	Bands:
Patient B:	41, 34	60, 58, 43, 41	83, 60, 41, 34, 30/31
Patient D:		41	60, 41, 25, 22
Patient J:	41	41	41, 34, 31
Patient V:		60, 43, 41, 22/OspC	41, 25

Same sample measured on 4 IgM-blots and the corresponding IgG-blots

IgM-blots



IgG-blots



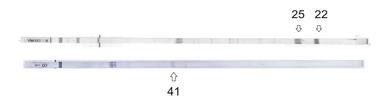
Results

	IgM	IgG
	Bands:	Bands:
Test VIII	100, 41, 41 int.(B. afzelii)	41
Test VII		VlsE, iv2, iv4
Test VI	83, 41, 31	75, 60, 41, 39
Test IV		

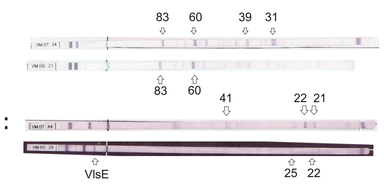
Difference in VlsE-expression between test VII and test IV (IgG)

Three more..

Patient M., IgM



Patient E.:



Patient M.: Difference in OspC-expression
Patient E.: Additional p39 and p31 in a blot with additional B. garinii strain (IgG-blot)
Patient E.: VlsE in IgM-blot

Another sample measured on 3 IgM-blots and the corresponding IgG-blots

IgM-blots

IgG-blots

Results

	IgM	IgG
	Bands:	Bands:
Test VII	OspC	
Test VI	83, 31, 25, 22	83, 75, 60, 41, (34)
Test IV		

Conclusion:

Identical sera react differently in the used test-systems. This may explain contradictory results, if the same sample was tested in various laboratories. Differences of the results could be better explained, if the used test-system was stated in addition on the laboratory-report. The selection of the test-system may be crucial for establishing the diagnosis.