Selected Aspects of Serology of Borrelia burgdorferi sensu lato II

Criteria of analytical efficiency using the example of commercially available assays for Borrelia burgdorferi antibodies (ELISA)

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Well-defined analytical test-criteria of serology of *Borrelia burgdorferi sensu lato* are indispensable for routine laboratory testing. They are also significant in the evaluation of seroprevalence of *Borrelia burgdorferi* antibodies and in upcoming efforts of standardization.

Analytical test-criteria can be subdivided into technical criteria such as precision and accuratness, linearity, detection limit, analytical sensitivity and methodical criteria such as selectivity, interferences, cut and hierarchy of reference-methods*.

*Keller, Klinisch-chemische Labordiagnostik für die Praxis, 2. Aufl. 1991
*Lothar Thomas, Labor und Diagnose, 6. Auflage

Assays for *B. burgdorferi* antibodies show significant differences with respect to their antigen spectra, preparation, and evaluation. Test kit manuals from the manufacturers contain usually only a sketchy documentation of their technical and methodical performance.

This poster evaluates ranges of evidence and deficiencies of some assays for *B. burgdorferi* antibodies.

The difference between analytical and diagnostic test-criteria will be shown:

- Reference methods are missing or not referred in 16 out of 19 tests.
- The specification of technical criteria and cut-off is carried out according to clinically classified sera, e.g. the estimated seroprevalence of pooled sera from blood-donors or from persons of non-endemic areas. This proceeding mixes analytical and diagnostic test-criteria.

An evidence-based diagnostic test interpretation is not possible without underlying analytical evaluation. According to the German standard DIN 58969-44 (specific requirements for the detection of antibodies against *B. burgdorferi*), the conclusion of the blot is restricted to the existence of antibodies. The test manufacturers should be required to substantiate all analytical test-criteria. And the range of evidence of searching assays should be limited to the analytic aspects.

Based on analytical evaluation there can be made conclusions for clinical / diagnostic means: sensitivity, specifity and predictive values.

Technical Criteria

Precision and accuratness are characteristics for the repeatability of results.

Linearity of the calibration curve allows a quantification.

The **analytical sensitivity** determines the resolution capability of a system.

The **detection limit** defines the lowest definitely detectable amount of an analyte. Two methods are used: 3-fold standard-deviation of the blank value or the 5%-overlap of blank value and the standard value. The blank value contains matrix or buffer.

For this purpose a reliable negative sample or blank value (substrate) is necessary.

Methodical Criteria

Selectivity (analytical specifity) allows to detect only the designated analyte.

Interferences are interactions, e. g. cross-reactivity or matrix effects.

The **cut** is the determining analytical criterion and the indicator for reactivity or non-reactivity of the sample. There is no rule for its preparation. In most cases it is estimated by the "clinical picture" or the approximated seroprevalence of pooled sera.

If pooled samples of blood donation are used for the cut, the result will provide information on antibody-levels in the sample in comparison with antibody-levels in averaged pooled sera.

Hierarchy of quality of techniques:
I. **Definite method (Gold-standard)**
II. **Reference-method:**
  a. verified by the definite method
  b. not like a., but standard-sera are available
  c. like b. without standard-sera.
III. **Routine-method:**
  a. recommended method with defined interference
  b. recommended method with undefined interference.

For Borrelia-serology there are no gold-standard, no reference-method, no standard-sample. Making conclusions of *Borrelia burgdorferi* antibodies is possible up to a limited degree. "Comparison-methods" are rarely declared by the manufacturers. Antigens of all human-pathogen strains are necessary to get evidence-based conclusions about the "real" status of antibodies. Reasons of "false-negative" results need to be considered, e. g. immune-complexes and limits, which are caused by the analytical selectivity.

Manufacturer	**Technical Criteria**						**Methodical Criteria**			**Diagnostic Criteria**		
	Precision / accuratness	Detection limit	cut-off	Linearity	Resolution capability	Analytical sensitivity & specifity	Selectivity	Interference	Reference - / comparison- method	Diagnostic sensitivity	Diagnostic specifity
A	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Recomwell, Liaison	35 clinically characterised sera and 204 unselected sera	
B	+	IgM: negative-control plus cut-off-factor, IgG: Standard		Ø	Ø	Ø	Ø	+	External laboratory	84 samples	194 "healthy" donors
C	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Reference-EIAs	154 sera of LB-patients, clinical study German NRC for Borreliae	100 serum specimens from subjects living in an endemic area and without history of tick contact or Lyme disease
D	+	Ø	Standard B	+	Ø	Analytical specifity (cross-reactivity): HSV, VZV, EBV ...	Ø	+	Comparison-test 1, comparison-test 2, Blot BEPIII (Dade Behring), TRITURUS (Grifols)	Relative sensitivity: ELISA + blot-comparison 94%-100%	Relative specifity: ELISA + blot-comparison, >95%-98%
E	+	Ø	Ø	+	Ø	Ø	Ø	+	Commercially available ELISA	Positively declared sera, 88%	"Negative" tested samples, 97%
F	+	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	208 clinically characterized sera	200 blood donors, estimated seroprevalence 9,5% / 4,5%
G	+	Substrate blank value	Standard	Ø	Ø	Ø	Ø	?	Test A: commercially available & 2 commercially available ELISAs and 2 blots	IgG: 14 control-sera, 26 sera of pregnant women,159 sera of patients with possible Borrelia-infection, IgM: 172 selected sera, 26 sera from the daily routine, 46 sera from blood donors, 100 sera (NRC) 97,3%	100%
H	+	Ø	Ø	Ø	Ø	Clinically characterized sera and sera from Blood-donors: estimated seroprevalence 7% (IgG), 1,5 % (IgM)	Ø	+	Borrelia burgdorferi ELISA	105 sera	Ø
I	+	Ø	Ø	Ø	Ø		Ø	+	Ø	539, 103 clinical characterized sera / estimated seroprevalence 5-20 %	100, 105, 150, 300 healthy blood donors; 98-100 %
K	+	+	4 calibrators	?	Ø	20 U /mL (only IgG)	Ø	+	„Due to the lack of an international reference-preparation the calibration is carried out in arbitrary Units (U/mL)"	70 sera	1000 / 3000 unconspicuous sera
L	+	Ø	Standard	+	Ø	Sensitivity: 0,99 U / mL	Ø	+	Ø	88% (IgG); 100 % (IgM)	97% (IgG), 100% (IgM)
M	+	Ø	Pooled normal-sera from endemic & non-endemic areas (N= 96 + 98)	Ø	Ø	Ø	Ø	+	Clinical laboratory, BioWhittaker Lyme Stat	CDC-Panel, 25 sera	s. cut, 9,1 % positive und conspicuous samples
N	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	70 sera, 91%	1000 clinically unconspicuous sera, 96 %
O	+	Ø	Standard	Ø	Ø	Ø	Ø	+	Ø	Ø	Ø
P	+	Ø	Standard	Ø	Ø	Ø	Ø	+	Ø	Ø	Ø
Q	+	Ø	2,5% estimated seroprevalence in blood donors (IgG)	+	Ø	Ø	Ø	Ø	Common anti-Borrelia ELISA	89-96 %	281 healthy blood-donors – 97%
R	Like blot	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Higher than comparative tests, 54 positive sera.	Avoiding false-positive results, 199 healthy donors
S	+	Ø	Ø, precursor test with detection limit	Ø	Ø	Ø	Ø	+	Ø	257 preliminary inspected sera	Ø
T	+	Ø	Standard	Ø	Ø	Ø	Ø	+	Comparison-methods	62 IgG,65 IgM, 96% -100 %	90-94 %

Summary: Specifications of the manufacturers to analytical criteria

Precision / accuratness: Data supplied in 14 of 19 tests.

Detection limit: by means of the substrate blank-value: manufacturer G.

Linearity: information supplied by manufacturer D, E, L and Q.

Resolution capability: Ø

Selectivity: Ø

Interference: data supplied in 12 tests.

Reference methods: data supplied by 3 of 19 manufacturers. 16 of 19 tests without data or without named comparison-tests.

Apart from interferences, precision and accuracy there are only few data about analytical efficiency. This can be due to the fact, that there is no consensus about Borrelia strains to be proved. There is also no consensus about the range of evidence (analytical or diagnostic) to be declared by a manufacturer.

Diagnostic sensitivity and diagnostic specifity

Diagnostic sensitivity:
Probability to get positive reaction of sick persons´ sera.

Diagnostic Specifity:
Probability to get a negative reaction of healthy persons´ sera.

Positive predictive value:
Probability, that a reactive test-result detects a sick person.

Negative predictive value:
Probability, that a negative test-result is obtained from a healthy person.

Specifications of the manufacturers:

Diagnostic sensitivity:
6 manufacturers prove their tests on clinical defined sera, 8 manufacturers take „comparison-samples", 2 x serological comparisons, 2 x percentaged data, 2 x no data.

Diagnostic Specifity:
5 x „healthy" donors, 1 x non-preselected sera, 2 x inconspicuous sera, 3 x percentaged data, 1 x sera of pregnant women, 3 x estimated seroprevalence, 3 x blood donors, 1x serological comparisons, 4 x no data.

Positive predictive value: Ø

Negative predictive value: Ø

The actual situation of test adjustment:

The diagnostic specifity and the cut are determined (1) by measuring sera of „healthy" or „ill" persons, (2) by estimated seroprevalence of pooled sera of blood-donors or (3) by measuring sera from endemic and not-endemic areas and (4) other methods.

The diagnostic sensitivity is specified by comparison samples, clinically defined sera, percentage data and serological data. This approach substitutes and blurs an analytical test-evaluation by a diagnostic procedure. Searching assays are solely designed to detect antibodies.

Conclusions

A well defined detection limit is necessary for detection of *Borrelia burgdorferi* antibodies as well as the disclosure of reference- / comparative-methods with their restrictions. Subsequently, conclusions can be made about clinical aspects such as sensitivity, specifity and predictive values.

Data acquisition and comparison with PCR- and culture- positive samples are indispensable for this purpose. Making the cut by estimation of the seroprevalence of blood-donors or other comparative methods is not evidence-based. Analytical test-criteria should rank before diagnostic conclusions.

"It does not surprise, that there are so few data about sensitivity and specifity ... of tests. Costs for gaining them are high in comparison to the benefit. Despite of this there should be obtained as much data as possible in the era of evidence-based medicine."
(www.biorama.ch)