Evaluation of the BioPlex 2200 Lyme IgG/IgM immunoassay system

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Background

Lyme borreliosis (LB) is the most prevalent tick-born infection in Europe. Serological evaluation for antibodies to *Borrelia burgdorferi* sensu lato remains the recommended method for diagnosis in early disseminated disease and in late disease.

Aim

In this study, the performance of the BioPlex 2200 assays were compared to the LIAISON® Lyme IgG and IgM assays (DiaSorin).

Materials and methods

Assay

The BioPlex 2200 Lyme IgG and IgM assays (Bio-Rad Laboratories) are fully automated, multiplex immunoassays using recombinant proteins including p58, DbpA, peptide FVlsE for IgG and VlsE (*B. bavariensis*), OspC (*B. burgdorferi* and *B. afzelli*) for IgM.

Specimens

Four groups of samples have been taken into account, in total 760 were tested:
Group 1: 402 unselected samples
Group 2: 200 known positive samples (100 IgG positive, 100 IgM positive)

Group 3: 110 test-ordered serum /CSF sample pairs
Group 4: 48 known positive Lyme IgG serum/CSF samples pairs
The results were compared to the Liaison Lyme IgG and IgM assays performed on the Liaison® XL. The Immunoblot RecomLine Borrelia IgG and IgM (Mikrogen Diagnostik) were used to arbitrate discordant results.
For the serum/CSF pairs, total IgG and albumin were performed (Cobas Roche) and used to calculate the antibody index (AI) to estimate the presence of intrathecally synthesized antibodies.

Results

Group 1 402 unselected samples

Table I: Agreement analysis for IgG

Total Nb Samples N=402		BioPlex 2200 Lyme IgG				
		Non-reactive	Equivocal	Reactive	Exclud	Total
Liaison Borrelia IgG	Non-reactive	349	1	11	0	361
	Equivocal	8	0	5	0	13
	Reactive	11	0	16	1*	28
	Total	368	1	32	1	402

*one specimen excluded because not tested on with BioPlex

The Lyme IgG negative and positive agreements were 96.7% (349/361) and 59.3% (16/27) respectively.

Table II: Agreement analysis for IgM

Total Nb Samples N=402		BioPlex 2200 Lyme IgM			
		Non-reactive	Equivocal	Reactive	Total
Liaison Borrelia IgM	Non-reactive	324	4	4	332
	Equivocal	11	2	6	19
	Reactive	21	8	22	52
	Total	356	14	32	402

The Lyme IgM negative and positive agreements were 97% (324/332) and 43.1% (22/51) respectively.

The overall agreements, removing equivocal, were 94.3% for IgG (365/387) and 93.3% for IgM (346/371).

Table III: discrepancies IgG samples by Immunoblot

Discrepant Serum Samples N=33		BioPlex Lyme IgG					
		Non-reactive		Equivocal		Reactive	
		blot -	blot +	blot -	blot +	blot -	blot +
Liaison Borrelia IgG	Non-reactive			1		10**	1
	Equivocal	6 *				4	
	Reactive	11 *					

* 17 samples: true negative BioPlex
**10 samples: true negative Liaison

Table IV: discrepancies IgM samples by Immunoblot

Discrepant Serum Samples N=47		BioPlex Lyme IgM					
		Non-reactive		Equivocal		Reactive	
		blot -	blot +	blot -	blot +	blot -	blot +
Liaison Borrelia IgM	Non-reactive			4***		3***	
	Equivocal	8*		**1		5**	
	Reactive	18*		8**			

* 26 samples true negative BioPlex
** 14 samples false positive/equivocal BioPlex and Liaison
*** 7 samples false positive BioPlex

Group 2 200 known positive samples (confirmed by Immunoblot) table V and VI

Table V: analysis for IgG

Total samples N=100	BioPlex Lyme IgG				Positive agreement (sensitivity)	
	Non-reactive	Equivocal	Reactive	Total	%	CI95
Lyme IgG positive	1	0	99	100	99	94,6-100

Table VI: analysis for IgM

Total samples N=100	BioPlex Lyme IgG				Positive agreement (sensitivity)	
	Non-reactive	Equivocal	Reactive	Total	%	CI95
Lyme IgM positive	12	0	82	100	82	73,1-89

Group 3 110 test-ordered serum / CSF sample pairs

Table VII: agreement analysis with serum

Total Nb Samples N=110		BioPlex Lyme IgG serum			
		Non-reactive	Equivocal	Reactive	Total
Liaison Borrelia IgG	Non-reactive	79	0	6	85
	Equivocal	1	0	3	4
	Reactive	0	0	21	21
	Total	80	0	30	110

The overall agreement (equivocal samples excluded) is 94.3% (100/106) with 100% (21/21) and 92.9 % (79/85) positive negative agreement respectively.

Table VIII: agreement analysis with CSF

Total Nb Samples N=110		BioPlex Lyme IgG CSF			
		Non-reactive	Equivocal	Reactive	Total
Liaison Borrelia IgG	Non-reactive	77	0	21	100
	Equivocal	0	0	1	0
	Reactive	0	0	10	10
	Total	77	0	32	110

The overall agreement (one specimen excluded because not tested) is 79.8% 87/109) with 100% (10/10) and 78.6% (77/98) positive negative agreement respectively.

Group 4 48 known positive Lyme IgG serum / CSF pairs specimen (prospective and retrospective)

Table IX: agreement analysis with serum and CSF

	Negative agreement		Positive agreement		Overall agreement	
	%	CI 95	%	CI 95	%	CI 95
Serums N=48	100	NA	100	92,4-100	92,3	81,5-97,9
CSF N=48	13,6	NA	100	88,4-100	63,5	49-76,4

Table X: comparison of Reiber index

Calculation of the IA intrathecal synthesis index IA (manufacturer's formula derived from the Reiber approach) is done by comparing serum / LCS specific antibody levels and adjusting the 2 compartments according to the total IgG levels and serum albumin / LCS (Cobas® 6000C-C501 Roche).

Serum/CF Samples positive N=48			BioPlex Lyme IgG	
			IA	
			Negative < 1,5	Positive > 1,5
Liaison Borrelia IgG	IA	Negative < 1,5	12	1
		Positive > 1,5	3	14

Positive agreement: 82.4% (14/17)   Negative agreement: 92.3% (12/13)

Discussion-Conclusion

The BioPlex 2200 Lyme IgG and IgM is the first fully automated multiplex platform based capable of reporting individual protein results and showed good performance in this study with less false positive results for serum specimen.

The cutoff needs to be refined to improve the agreement between the two techniques and clinical specifications of the patients are needed for ROC analysis.