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Could the Optiplex *Borrelia* assay replace the traditional, two-step method of diagnosing Lyme disease?

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Abstract

Introduction and Objective. Serological assays for Lyme disease (LD) routinely performed in laboratories often give inconclusive results, thereby making correct diagnosis difficult and delaying treatment. The aim of the study was to assess the usefulness of a commercial Optiplex *Borrelia* (OB) assay in the serological diagnostics of LD. Based on the results obtained in a previous study on the seroreactivity of the sera of patients with LD to *Borrelia* spp. antigens using enzyme immunoassays (ELISA) and immunoblotting (IB), the same sera were re-analyzed using the OB assay.

Results. The assays carried out with the use of OB method showed a statistically significant lower number of positive/borderline results for the presence of IgM antibodies, compared to the ELISA assay. Moreover, statistically lower positive/borderline results were obtained for antibodies in the IgG class with use of the OB method, compared to the IB assay and a two-stage diagnostic protocol (ELISA with IB). The specificity analysis showed that in both the IB and OB assays, anti-OspC IgM and anti-p41 antibodies were detected. Additionally, high positive/borderline values were found in the OB assay for native antigens derived from *B. afzelii* lysate. The IB assay most frequently detected antibodies against OspC, p39 (BmpA) and VlsE proteins in the IgG class. There were fewer positives/borderlines for anti-p41-I *B. afzelii* antibodies in the OB assay and a higher number for antigens: VlsE-C6, p18 B. afzelii (DbpA), and p39 *B. afzelii* (BmpA).

Conclusions. Answering the question whether the OB assay could replace the traditional, two-step method of LD diagnostics, it can be concluded that it could not. It can be used to diagnose LD only as a complementary assay and not as an optimal and dedicated method of *Borrelia* spp. infection detection.

Key words

Lyme disease, *Borrelia*, immunoreactivity, Optiplex

INTRODUCTION

Lyme disease (LD) is a bacterial disease transmitted by ticks belonging to the genus *Ixodes*. The multiformity of this disease in terms of the clinical picture and the antigenic heterogeneity of *Borrelia* genospecies very often make it difficult to diagnose [1]. In this situation, choosing an optimal antigen pattern for diagnostic tests seems to be problematic. Taking the above into account, the diagnostic methods used in detecting LD should be selected to carry the lowest risk of false-positive or negative results [2–4]. Currently, the diagnostics of choice are serological assays, which rely on the detection of anti-*Borrelia* antibodies in the IgM and IgG classes. According to the European Concerted Action on Lyme Borreliosis (EUCALB) recommendations, the diagnosis of LD requires a two-stage diagnostic protocol (with the exception of the occurrence of erythema migrans (EM) [5]. The first step involves running an enzyme-linked immunosorbent (ELISA) or indirect immunofluorescence (IIFT) assays. The obtained borderline or positive results in the screening assay require confirmation with the immunoblot (IB) or Western-blot (WB) assay in the second stage [2, 6–14].

Currently, manufacturers also offer new LD diagnostics assays which are not used in routine diagnostics [3]. A promising assay seems to be the commercial Optiplex *Borrelia* (OB) assay, which contains cell lysate and recombinant antigens in its antigen composition. According to the assay manufacturer, such a combination replaces the currently used two-stage LD diagnostics. Due to the use of a lysate, many immunogenic fractions can be detected; however, it is associated with the possibility of facing difficulties in distinguishing specific fractions from cross-reactive ones. Apart from native antigens (*B. afzelli* lysate for OB IgM assay and *B. garinii* lysate for OB IgG assay), the assay also includes recombinant antigens, the use of which ensures that the obtained reactions concern only specific proteins. The key antigens in the detection of IgM in the OB assay include the following: OspA *B. afzelii*, OspC *B. garinii*, OspC *B. afzelii*, p100 *B. afzelii*, p18 *B. afzelii* (DbpA), p39 *B. afzelii* (BmpA), p41-I *B. afzelii*, and VlsE-C6, while the key antigens in the

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detection of IgG include the following: OspC *B. afzelii*, p100 *B. afzelii*, p18 *B. afzelii*, p39 *B. afzelii*, p41-I *B. afzelii*, p58 *B. garinii* (OppA-2), and VlsE-C6 [15, 16].

The OspC proteins (*B. garinii* and *B. afzelii*) used in the assay are highly immunogenic and mainly responsible for the early humoral response, and are derived from *B. afzelii*: p41 int. (p41-I; the inner part of the flagellin molecule that does not cross-react with flagellin from other bacterial species), p100, p39, and p18 (crucial in IgG detection). *B. afzelii*-derived antigens are considered the most sensitive in Europe and are therefore recommended in LD serodiagnosis [6, 9, 17, 18]. The OB assay has also been supplemented with highly sensitive and specific diagnostic antigens to increase the assay's sensitivity: p58 *B. garinii* and a synthetic C6 peptide derived from the VlsE antigen [9,19,20]. The principle of the OB assay is based on the incubation of serum or cerebrospinal fluid with a mixture of antigens coated on the surface of polystyrene beads ('Bead Mix') (Fig. 1). Specific anti-*Borrelia* antibodies present in the patient's material, which have become bound to the surface of the beads, are detected by secondary anti-human IgM or IgG antibodies conjugated with the fluorescent dye phycoerythrin.

OBJECTIVE

In our previous work, we analyzed the immunoreactivity of sera from LD patients to species-specific *Borrelia* spp. antigens, using ELISA and IB assays [3]. However, to the best of our knowledge, to date no similar studies have been carried out using the OB assay. Therefore, in this study, we want to answer the question whether the commercial OB assay could serve as an innovative method of LD diagnostics, simultaneously replacing the basic two-stage serological diagnosis. Hence, this study aims to assess the diagnostic effectiveness of the OB assay, and compare it with the results obtained in the first part of the study [3].

MATERIALS AND METHODS

Patients. The material consisted of patient sera used in the previous study [3]. Briefly, sera were obtained from outpatients (experimental group, $n = 80$) suffering from LD, and healthy individuals (control group, $n = 22$). The first part of the work presented detailed data on the information collected and assessed in the interview.

The study was conducted following the principles set out in the Helsinki Declaration. The Ethical Committee of the Pomeranian Medical University in Szczecin approved the study (Approval No. KB-0012/147/18).

Optiplex *Borrelia* Assay. All the collected sera, which were analyzed with the ELISA (EUROIMMUN, Lübeck, Germany) screening assay and the IB (EUROIMMUN, Lübeck, Germany) confirmation assay in the previous study [3], were additionally assessed with the IgM and IgG OB assay by DiaMex (Heidelberg, Germany), following the manufacturer's recommendations [15, 16]. Fluorescence was measured using the LABScan 100 Flow analyzer (Luminex Corporation, Austin, TX, USA), whereas the Fusion 4.2 software (One Lambda, Inc., Canoga Park, CA, USA) was used to evaluate the results. OB results were analyzed in accordance with the interpretive criteria of the manufacturer [15, 16].

Statistical Analysis. McNemar's chi-square test was used to compare assays. The results of $p \leq 0.05$ were considered statistically significant. Statistical analysis of the results was carried out using the STATISTICA version 13.0 software

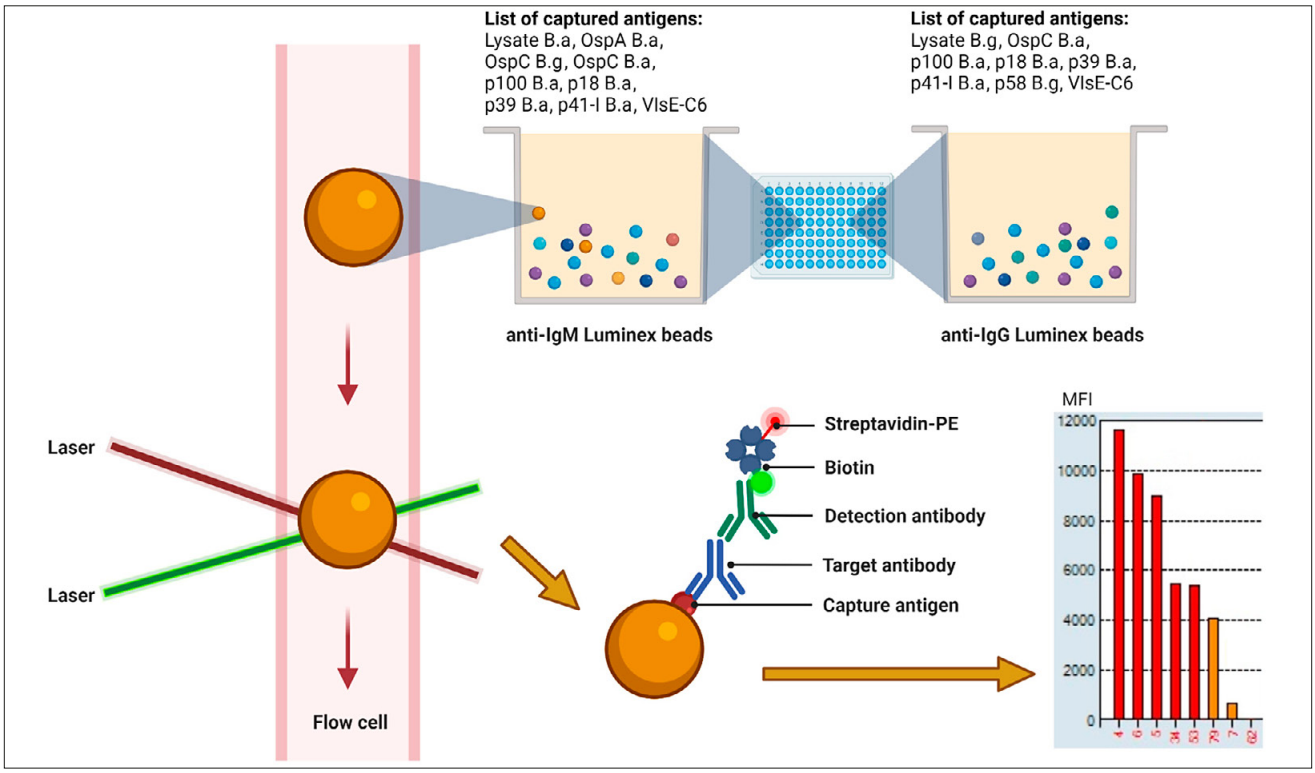

Figure 1. Principle of the Optiplex *Borrelia* assay operation. B.a – *Borrelia afzelii*, B.g – *Borrelia garinii*, MFI – mean fluorescence intensity. Created with BioRender.com

(StatSoft, Inc., Tulsa, USA). The description of calculations includes the number of cases (*n*) and the percentage (%). Data from an earlier study [3] was used to illustrate better the evaluation of the obtained results using the OB assay.

RESULTS

In the experimental group, in the case of IgM antibodies which were mainly detected in patients with EM, in the recommended ELISA screening assay a statistically higher number of positive/borderline results was obtained than in the OB assay. Although these results were not statistically significant, a comparable number of positives/borderlines was obtained for the same class of antibodies in both the IB and OB confirmation assays. Moreover, higher positive/borderline results were obtained for antibodies in the same class studied with use of two-stage diagnostic protocol (ELISA with IB), compared to the OB method. Nevertheless, these results were not statistically significant.

For IgG class antibodies which were detected in some of the patients with EM and late stage of LD (e.g. Lyme arthritis, borrelial lymphocytoma, peripheral neuropathy), the ELISA assay showed a higher number of positive/borderline results for sera from patients with confirmed LD. However, these results were not statistically significant. Furthermore, a statistically higher number of positives/borderlines was noted in the IB and a two-stage diagnostic protocol (ELISA with IB) assays than in the OB assay for the same class of antibodies.

For IgM and IgG antibodies in the control group, a comparable number of results was obtained in the individual assay combinations. The obtained results for the control group showed no statistically significant differences.

Detailed data on the correlation of serological detection of anti-*Borrelia* IgM and IgG antibodies present in sera of outpatients suffering from LD (experimental group) and healthy individuals (control group) using OB, ELISA, and IB assays, as well as a two-stage diagnostic protocol (ELISA with IB) (Tables 1 and 2; detailed results are included in Supplementary Table 1).

Table 1. Correlation of serological detection of anti-*Borrelia* IgM antibodies with Optiplex *Borrelia* (OB), enzyme-linked immunosorbent (ELISA), and immunoblot (IB) assays, as well as the two-stage diagnostic protocol (ELISA with IB) in Lyme disease patients (experimental group) and healthy individuals (control group)

		ELISA			IB			ELISA with IB		
		POS/BOR	NEG	Total	POS/BOR	NEG	Total	POS/BOR	NEG	Total
Experimental group										
OB	POS/BOR	30	10	40	20	20	40	17	7	24
	NEG	30	10	40	20	20	40	17	8	25
	Total	60	20	80	40	40	80	34	15	49
	McNemar's χ^2 (*p* value)	9.03 (0.0027)			0.03 (0.8744)			3.38 (0.0662)		
Control group										
OB	POS/BOR	0	2	2	0	2	2	0	2	2
	NEG	2	18	20	1	19	20	0	17	17
	Total	2	20	22	1	21	22	0	19	19
	McNemar's χ^2 (*p* value)	0.25 (0.6171)			0.0 (1.0)			0.5 (0.4795)		

CI – confidence interval; POS/BOR – positive/borderline results

Table 2. Correlation of serological detection of anti-*Borrelia* IgG antibodies with Optiplex *Borrelia* (OB), enzyme-linked immunosorbent (ELISA), and immunoblot (IB) assays, as well as the two-stage diagnostic protocol (ELISA with IB) in Lyme disease patients (experimental group) and healthy individuals (control group)

		ELISA			IB			ELISA with IB		
		POS/BOR	NEG	Total	POS/BOR	NEG	Total	POS/BOR	NEG	Total
Experimental group										
OB	POS/BOR	18	16	34	19	14	33	15	11	26
	NEG	28	18	46	34	13	47	24	9	33
	Total	46	34	80	53	27	80	39	20	59
	McNemar's χ^2 (*p* value)	2.75 (0.0973)			7.52 (0.0061)			4.11 (0.0425)		
Control group										
OB	POS/BOR	1	1	2	0	4	4	0	3	3
	NEG	1	19	20	2	16	18	1	16	17
	Total	2	20	22	2	20	22	1	19	20
	McNemar's χ^2 (*p* value)	0.5 (0.4795)			0.17 (0.6831)			0.25 (0.6171)		

CI – confidence interval; POS/BOR – positive/borderline results

To assess the specificity of the assays used in the study, which ultimately determined the final result, the seroreactivity of the sera against the antigens used in the IB and OB assays was additionally compared. In the IB and OB assays, anti-OspC antibodies were the most frequently detected antibodies in the IgM class (results comparable for the OspC antigens of all three genospecies used in both assays—*B. afzelii, B. garinii,* and *B. burgdorferi*) and anti-p41 in the IB. When analyzing IgG antibodies with the IB assay, it was found that the most frequently detected antibodies were those against the flagellin protein (p41), OspC, BmpA (p39), and VlsE (*B. afzelii, B. garinii,* and *B. burgdorferi*). On the other hand, the OB assay showed a lower number of positives/borderlines for anti-flagellin antibodies (p41-I *B. afzelii*), even though the highest number was found for VlsE-C6, p18 *B. afzelii* (DbpA), and p39 *B. afzelii* (BmpA). In terms of the control group, the positive/borderline results for anti-*Borrelia* antibodies in the IgM and IgG classes fluctuated at a similar, low level.

The number and frequency of patients with LD (experimental group) and healthy individuals (control group) with anti-*Borrelia* IgM and IgG antibodies after the analysis using IB and OB assays is presented in Tables 3 and 4. Moreover, a detailed summary of the results of anti-*Borrelia* antibodies determination in the IgM and IgG class obtained using the OB assay in the experimental and control groups are included in Supplementary Table 2. To better illustrate the assessment of anti-*Borrelia* antibody levels obtained by the OB assay, they should be compared with the data (ELISA and IB) presented in the previous part of the study [3].

Table 3. Number and frequency of patients with Lyme disease (experimental group) and healthy individuals (control group) with positive/borderline results from immunoblot (IB) and Optiplex *Borrelia* (OB) assays for anti-*Borrelia* IgM antibodies

Antigen	Experimental group		Control group	
	IB n (%)	OB n (%)	IB n (%)	OB n (%)
VlsE B.b	2 (2.5)	np	0 (0.0)	np
p41 (flagellin)	23 (28.8)	np	5 (22.7)	np
p39 (BmpA)	4 (5.0)	np	0 (0.0)	np
OspC B.a	36 (45.0)	33 (41.3)	1 (4.6)	3 (13.6)
OspC B.b	27 (33.8)	np	1 (4.6)	np
OspC B.g	31 (38.8)	36 (45.0)	0 (0.0)	4 (18.2)
OspC B.sp	0 (0.0)	np	0 (0.0)	np
Lysate B.a	np	29 (36.3)	np	2 (9.1)
OspA B.a.	np	10 (12.5)	np	0 (0.0)
p100 B.a	np	1 (1.3)	np	0 (0.0)
p18 B.a (DbpA)	np	8 (10.0)	np	2 (9.1)
p39 B.a (BmpA)	np	0 (0.0)	np	0 (0.0)
p41-I B.a	np	17 (21.3)	np	2 (9.1)
VlsE-C6	np	2 (2.5)	np	0 (0.0)

Np – antigen not present in the assay. B.a – *Borrelia afzelii*; B.b – *Borrelia burgdorferi*; B.g – *Borrelia garinii*; B.sp – *Borrelia spielmanii*

Table 4. The number and frequency of patients with Lyme disease (experimental group) and healthy individuals (control group) with positive/borderline results from immunoblot (IB) and Optiplex *Borrelia* (OB) assays for anti-*Borrelia* IgG antibodies

Antigen	Experimental group		Control group	
	IB *n* (%)	OB *n* (%)	IB *n* (%)	OB *n* (%)
VlsE B.a	20 (25.0)	np	0 (0.0)	np
VlsE B.b	24 (30.0)	np	0 (0.0)	np
VlsE B.g	30 (37.5)	np	1 (4.5)	np
Lipid B.a	1 (1.3)	np	0 (0.0)	np
Lipid B.b	3 (3.8)	np	0 (0.0)	np
p83	15 (18.8)	np	1 (4.5)	np
p41 (flagellin)	48 (60.0)	np	14 (63.6)	np
p39 (BmpA)	22 (27.5)	np	1 (4.5)	np
OspC	29 (36.3)	np	1 (4.5)	np
p58 (BB_A34)	6 (7.5)	np	0 (0.0)	np
p21 (BB_K53)	4 (5.0)	np	0 (0.0)	np
p20 (BB_Q03)	0 (0.0)	np	0 (0.0)	np
p19 (BB_N38)	3 (3.8)	np	0 (0.0)	np
p18 (BB_P38)	6 (7.5)	np	1 (4.5)	np
Lysate B.g	np	6 (7.5)	np	1 (4.5)
OspC B.a	np	1 (1.3)	np	2 (9.1)
p100 B.a	np	7 (8.8)	np	2 (9.1)
p18 B.a (DbpA)	np	17 (21.3)	np	3 (13.6)
p39 B.a (BmpA)	np	10 (12.5)	np	2 (9.1)
p41-I B.a	np	6 (7.5)	np	3 (13.6)
p58 B.g (OppA)	np	7 (8.8)	np	2 (9.1)
VlsE-C6	np	25 (31.3)	np	3 (13.6)

Np – antigen not present in the assay. B.a – *Borrelia afzelii;* B.b – *Borrelia burgdorferi;* B.g – *Borrelia garinii;* B.sp – *Borrelia spielmanii*

DISCUSSION

LD is a disease caused by the bacterial genospecies of *B. burgdorferi* sensu lato, which is highly heterogeneous. The compatibility of these genospecies in terms of the structure of antigens is different, for example, for the lipoprotein DbpA (p18), it is 51–63%, for OspC (p21), it is 71–75%, and for BmpA (p39), it is 88–90%. This variability causes difficulties in correctly diagnosing people suffering from LD [21]. Comparison of the results obtained with different assays results in many discrepancies. These discrepancies apply to both false-positive and false-negative results. The differences in compatibility regarding the structure of *Borrelia* spp. antigens also make it difficult to clearly define the genospecies responsible for LD. Correct diagnosis of LD mainly depends on a correctly selected diagnostic method because each of them has its advantages and limitations. In serological assays detecting specific anti-*Borrelia* antibodies, it is crucial to select appropriate diagnostic criteria and antigens [11, 12, 22–25]. Currently, two-stage serological diagnosis (except for EM patients) is recommended in the diagnostic procedure – ELISA screening assay and confirmation assay – IB or WB. According to the EUCALB recommendations, screening assays show a sensitivity of ≥90%. On the other hand, confirmation assays should have a specificity of at least 95% [6, 26]. As stated by many researchers, the IB assays containing mainly recombinant antigens are superior to the WB assay, which contains native antigens that can generate cross-reactions [2, 26, 27]. Currently, there are also new assays (not used in routine diagnostics) that can replace the two-step LD diagnosis. Examples include chemiluminescence-based assays or the OB assay that we have analyzed in this paper. Concerning the assays based on chemiluminescence, there are few reports of their use as part of replacing two-stage diagnostics with one-stage diagnostics; preliminary results seem promising but require further analysis [28]. However, there are currently no such reports in the case of the OB assay.

Compared to our first part of the study [3], based on the analysis of IgM and IgG anti-*Borrelia* antibodies carried out by ELISA and IB methods, the currently performed analysis found a statistically higher number of positives/borderlines in the ELISA (for IgM class), as well as in the IB assay and a two-stage diagnostic protocol (ELISA with IB) (for IgG class) than in the OB assay. Based on the results obtained in the IgG class, it can be concluded that the OB assay may generate false-negative results. It is worth emphasizing that in association with the above, the IB assay obtained in our study, especially in the case of the IgG class, has an advantage over the positive/borderline results obtained with both assays – OB and ELISA. A similar relationship has also been noticed by other authors [2, 29]. These studies confirm the necessity of an obligatory IB assay as a confirmation assay.

Currently in Europe, LD is caused mainly by two genospecies, *B. garinii* and *B. afzelii*, which are transmitted by *I. ricinus* and *I. persulcatus* ticks. Diseases caused by other genospecies, such as *B. burgdorferi* sensu stricto, *B. bavariensis*, *B. spielmanii,* and *B. lusitaniae*, are also recorded [30]. Specific genes may more often cause some symptoms of LD, that is, *B. afzelii* most often causes skin symptoms, *B. burgdorferi* is most associated with joint complications, and *B. garinii* is related to neurological symptoms [31].

Hauser et al. [32] conducted studies that established critical criteria for standardized IB assays in the diagnosis of LD.

These studies, carried out on a collection of sera obtained from various regions of Europe, have shown, among other things, that the *B. afzelii* strain is the most sensitive in the serological diagnosis of LD in Europe. In turn, Mavin et al. [33] conducted a study that consisted of two WB assays with different interpretation criteria. In the first reference assay, the local strain of *B. burgdorferi* was used, whereas in the second, two strains of *B. burgdorferi* and *B. afzelii* were used. This team obtained results that proved that the use of the WB assay with the *B. afzelii* strain increased the assay sensitivity compared to the reference assay.

In the previous part of the study, it was noticed that in the IB assay for the IgM and IgG class, antibodies were obtained that were directed against two or three genospecies, and they constituted the majority of the results [3]. The situation was different regarding the OB assay, which also obtained antibodies directed against more than one genera. However, most of the results concerned sera in which antibodies directed against one genus were detected in the IgG class. In the first part of the study, it was also noticed that in the analyzed IB assay in the IgM class, antibodies directed against three genospecies, *B. afzelii*, *B. garinii*, and *B. burgdorferi*, were most often obtained, less often against two, and the lowest number of positive/borderline results was obtained for antibodies against one genospecies. Concerning IgM antibodies directed against two genospecies, the highest number of positive/borderline results was obtained for *B. afzelii—B. garinii* and a comparable number was obtained for *B. burgdorferi—B. afzelii*. No antibodies against the *B. burgdorferi—B. garinii* system were found. In the case of antibodies against one genospecies in the same class, antibodies against *B. afzelii* and *B. garinii* were found. However, no antibodies against *B. burgdorferi* were detected. To evaluate IgM antibodies in the OB assay, two genospecies of *B. afzelii* and *B garinii* and the VlsE-C6 antigen-specific for all genospecies were used. In this study, the most frequent findings were IgM antibodies directed against two genospecies of *B. afzelii—B. garinii*. In only a few cases there were antibodies specific for one genospecies, namely *B. afzelii*. No antibodies to VlsE-C6 were found in this class of antibodies.

In the IgG class in the IB confirmation assay, the most common results were in the antibodies directed against the antigens of two and three genospecies [3]. The least frequent were antibodies directed against one genospecies. In a situation where antibodies directed against two genospecies were found, positive/borderline results were most often obtained for *B. afzelii—B. garinii* and *B. burgdorferi—B. garinii*. No positive/borderline result was obtained for the *B. afzelii—B. burgdorferi* system. Regarding detection of antibodies directed against the antigens of one genospecies, anti-*B. garinii* antibodies dominated, and antibodies against *B. burgdorferi* were detected in a few cases. There was no result that showed that the antibodies were only directed against *B. afzelii*. More antibodies directed against one genospecies were obtained in the same class of antibodies in the OB assay. In most cases of the sera assayed, *B. afzelii* was detected and *B. garinii* detected in a few cases. In contrast, antibodies to the two genospecies were less numerous. Antibodies directed against VlsE-C6 were also positive/borderline in this assay.

Analyzing the results of our research, a tendency was identified to detect antibodies against more than one *Borrelia* spp. This may be attributed to the infection of ticks by several genospecies or the occurrence of cross-reactions between the antigens used in the assays. Rauter and Hartung [34] carried out studies that assessed the presence of clinically significant genospecies in ticks using the real-time PCR technique. The results of these authors indicate that the most common genospecies were *B. afzelii* and the rarest were *B. garinii* and *B. burgdorferi*. Mixed infections were found in a few assayed ticks, and most often concerned the *B. afzelii—B. garinii* system, and less often *B. afzelii—B. burgdorferi*. However, no tick infection was detected in the *B. garinii—B. burgdorferi* system. Only one case was infected with three genospecies—*B. afzelii*, *B. garinii*, and *B. burgdorferi*. These authors additionally carried out a meta-analysis of the occurrence of *I. ricinus* tick infections by *B. burgdorferi* in Europe [34]. The data contained in this publication shows that mixed infections accounted for about one-tenth of all infections. Infections with two genospecies most often concerned the *B. garinii—B. valaisiana* system, and the combination of the three genospecies were the least common.

Comparing the results from our previous part of the study [3], it was observed that in the IB assay in the IgM class, antibodies directed against antigens were detected in the following order: OspC *B. afzelii* < OspC *B. garinii* < OspC *B. burgdorferi* < flagellin (p41) < p39 (BmpA) < VlsE *B. burgdorferi*. However, no positive/borderline result for OspC *B. spielmanii* was revealed. In turn, in the current study (analyzed using the OB assay), the presence of antibodies was found in the following order: OspC *B. garinii* < OspC *B. afzelii* < native *B. afzelii* antigens < p41-I *B. afzelii* < OspA *B. afzelii*. Other authors also obtained similar results [2, 35]. On the other hand, in the IgG class, the antibodies against flagellin (p41) < VlsE *B. garinii* < OspC < VlsE *B. burgdorferi* < p39 BmpA < VlsE *B. afzelii* proteins were most often detected in the IB confirmation assay. In the OB assay, the highest number of positive/borderline bands was obtained for VlsE-C6 < p18 *B. afzelii* (DbpA) < p39 *B. afzelii* (BmpA). A significant number of positives/borderlines for the *B. afzelii* p18 (DbpA) antigen was also obtained in the OB assay. The research studies conducted by Heikkilä et al. [23] indicate high interspecific heterogeneity in the structure of this antigen. DbpA shows high antigen specificity, provided that the diagnostic assay includes protein variants from the three genospecies of *B. afzelii*, *B. burgdorferi,* and *B. garinii*. This antigen variation can reduce the number of false-negative results. On the other hand, the antibodies against *B. afzelii* OspC antigens > p58 *B. garinii* (OppA-2) > 100 *B. afzelii* were the least frequently detected in the IgG OB assay. The p83 and p58 proteins are highly specific and characteristic of the long duration of *Borrelia* spp. infection. The low reactivity of these proteins may be related to the stage of infection of the patients in the study group (mainly the initial phase of the disease). Furthermore, a low value was obtained for the native *B. garinii* lysate and the *B. afzelii* p41-I antigens. On the other hand, in the IB assay, the antigens, that is, lipids of *B. afzelii* and *B. burgdorferi,* as well as BB_P38, BB_A34, BB_K53, BB_N38, which belong to *B. burgdorferi*, reacted least frequently. However, no positive/borderline result was revealed for BB_Q03 *B. burgdorferi*. These antigens were obtained through molecular biology methods. The assay manufacturer provides information that they show a very low sensitivity (7.1—22.4%) with high specificity (99.3 – 100%), which can be observed in the results of own research.

CONCLUSIONS

Answering the question whether the OB assay could replace the traditional, two-step method of LD diagnostics recommended by the EUCALB, it can be stated that it is not replacing the traditional method. This fact is supported by a significantly higher number of positives/borderlines in the ELISA (for IgM class), as well as in the IB and a two-stage diagnostic protocol (ELISA with IB) (for IgG class) than in the OB assay. When comparing the results obtained with the IB confirmation assay and the OB assay, a decrease in the number of cross-reactions for the inner part of the flagellin molecule (the p41-I *B. afzelii* antigen) occurs when the unmodified p41 antigen is used in the IB assay. In conclusion, OB assay can be used to diagnose the LD only as a complementary assay and not as an optimal form of detection of *Borrelia* spp. infection.

REFERENCES

1. Steere AC, Coburn J, Glickstein L. The emergence of Lyme disease. J Clin Invest. 2004; 113(8): 1093–1101. https://doi.org/10.1172/JCI21681
2. Wojciechowska-Koszko I, Mączyńska I, Szych Z, et al. Serodiagnosis of borreliosis: indirect immunofluorescence assay, enzyme-linked immunosorbent assay and immunoblotting. Arch Immunol Ther Exp (Warsz). 2011; 59(1): 69–77. https://doi.org/10.1007/s00005-010-0111-0
3. Wojciechowska-Koszko I, Mnichowska-Polanowska M, Kwiatkowski P, et al. Immunoreactivity of polish Lyme disease patient sera to specific *Borrelia* antigens – Part 1. Diagnostics. 2021; 11(11): 2157. https://doi.org/10.3390/diagnostics11112157
4. Wojciechowska-Koszko I, Kwiatkowski P, Sienkiewicz M, et al. Cross-reactive results in serological tests for borreliosis in patients with active viral infections. Pathogens. 2022; 11(2): 203. https://doi.org/10.3390/pathogens11020203
5. O Connell S. European Concerted Action on Lyme Borreliosis (EUCALB). Euro Surveill. 1996; 1(3): 23–24.
6. Robertson J, Guy E, Andrews N, et al. A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. J Clin Microbiol. 2000; 38(6): 2097–2102. https://doi.org/10.1128/JCM.38.6.2097-2102.2000
7. Wilske B. Diagnosis of Lyme borreliosis in Europe. Vector Borne Zoonotic Dis. 2003; 3(4): 215–227. https://doi.org/10.1089/153036603322662200
8. Aguero-Rosenfeld ME, Wang G, Schwartz I, et al. Diagnosis of Lyme borreliosis. Clin Microbiol Rev. 2005; 18(3): 484–509. https://doi.org/10.1128/CMR.18.3.484-509.2005
9. Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol. 2007; 49(1): 13–21. https://doi.org/10.1111/j.1574-695X.2006.00139.x
10. Hunfeld KP, Kraiczy P. When is the best time to order a Western blot and how should it be interpreted? Curr Probl Dermatol. 2009; 37: 167–177. https://doi.org/10.1159/000213074
11. Seriburi V, Ndukwe N, Chang Z, et al. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. Clin Microbiol Infect. 2012; 18(12): 1236–1240. https://doi.org/10.1111/j.1469-0691.2011.03749.x
12. Leeflang MMG, Ang CW, Berkhout J, et al. The diagnostic accuracy of serological tests for Lyme borreliosis in Europe: a systematic review and meta-analysis. BMC Infect Dis. 2016; 16: 140. https://doi.org/10.1186/s12879-016-1468-4
13. Lohr B, Fingerle V, Norris DE, et al. Laboratory diagnosis of Lyme borreliosis: current state of the art and future perspectives. Crit Rev Clin Lab Sci. 2018; 55(4): 219–245. https://doi.org/10.1080/10408363.2018.1450353
14. Theel ES. The past, present, and (possible) future of serologic testing for Lyme disease. J Clin Microbiol. 2016; 54(5): 1191–1196. https://doi.org/10.1128/JCM.03394-15
15. DiaMex. Optiplex *Borrelia* IgM Test. https://www.diamex.com/fileadmin/media/diamex.com/image/pdf-Dateien/Borr-IgM-insert_eng.pdf (access: 2021.11.20).
16. DiaMex. Optiplex *Borrelia* IgG Test. https://www.diamex.com/fileadmin/media/diamex.com/image/pdf-Dateien/Borr-IgG-insert_eng_10–15.pdf (access: 2021.11.20).
17. Rauer S, Spohn N, Rasiah C, et al. Enzyme-linked immunosorbent assay using recombinant OspC and the internal 14-kDa flagellin fragment for serodiagnosis of early Lyme disease. J Clin Microbiol. 1998; 36(4): 857–861. https://doi.org/10.1128/JCM.36.4.857-861.1998
18. Arnaboldi PM, Seedarnee R, Sambir M, et al. Outer surface protein C peptide derived from *Borrelia burgdorferi* sensu stricto as a target for serodiagnosis of early Lyme disease. Clin Vaccine Immunol. 2013; 20(4): 474–481. https://doi.org/10.1128/CVI.00608-12
19. Krupka I, Knauer J, Lorentzen L, et al. *Borrelia burgdorferi* sensu lato species in Europe induce diverse immune responses against C6 peptides in infected mice. Clin Vaccine Immunol. 2009; 16(11): 1546–1562. https://doi.org/10.1128/CVI.00201-09
20. Porwancher RB, Hagerty CG, Fan J, et al. Multiplex immunoassay for Lyme disease using VlsE1-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. Clin Vaccine Immunol. 2011; 18(5): 851–859. https://doi.org/10.1128/CVI.00409-10
21. Probst C, Ott A, Scheper T, et al. N-terminal disulfide-bridging of *Borrelia* outer surface protein C increases its diagnostic and vaccine potentials. Ticks Tick Borne Dis. 2012; 3(1): 1–7. https://doi.org/10.1016/j.ttbdis.2011.10.001
22. Magnarelli LA, Ijdo JW, Padula SJ, et al. Serologic diagnosis of Lyme borreliosis by using enzyme-linked immunosorbent assays with recombinant antigens. J Clin Microbiol. 2000; 38(5): 1735–1739. https://doi.org/10.1128/JCM.38.5.1735-1739.2000
23. Heikkilä T, Seppälä I, Saxen H, et al. Species-specific serodiagnosis of Lyme arthritis and neuroborreliosis due to *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii* by using decorin binding protein A. J Clin Microbiol. 2002; 40(2): 453–460. https://doi.org/10.1128/JCM.40.02.453-460.2002
24. Reed KD. Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol. 2002; 40(2): 319–324. https://doi.org/10.1128/JCM.40.2.319-324.2002
25. Branda JA, Linskey K, Kim YA, et al. Two-tiered antibody testing for Lyme disease with use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay. Clin Infect Dis. 2011; 53(6): 541–547. https://doi.org/10.1093/cid/cir464
26. Fawcett PT, Rosé CD, Gibney KM, et al. Comparison of immunodot and Western blot assays for diagnosing Lyme borreliosis. Clin Diagn Lab Immunol. 1998; 5(4): 503–506. https://doi.org/10.1128/CDLI.5.4.503-506.1998
27. Mogilyansky E, Loa CC, Adelson ME, et al. Comparison of Western immunoblotting and the C6 Lyme antibody test for laboratory detection of Lyme disease. Clin Diagn Lab Immunol. 2004; 11(5): 924–929. https://doi.org/10.1128/CDLI.11.5.924-929.2004
28. Ledue TB, Collins MF, Young J, et al. Evaluation of the recombinant VlsE-based liaison chemiluminescence immunoassay for detection of *Borrelia burgdorferi* and diagnosis of Lyme disease. Clin Diagn Lab Immunol. 2008; 15(12): 1796–1804. https://doi.org/10.1128/CVI.00195-08
29. Marangoni A, Sparacino M, Mondardini V, et al. Comparative evaluation of two enzyme linked immunosorbent assay methods and three Western blot methods for the diagnosis of culture-confirmed early Lyme borreliosis in Italy. New Microbiol. 2005; 28(1): 37–43.
30. Marques AR, Strle F, Wormser GP. Comparison of Lyme disease in the United States and Europe. Emerg Infect Dis. 2021; 27(8): 2017–2024. https://doi.org/10.3201/eid2708.204763
31. Stanek G, Wormser GP, Gray J, et al. Lyme borreliosis. Lancet. 2012; 379(9814): 461–473. https://doi.org/10.1016/S0140-6736(11)60103-7
32. Hauser U, Lehnert G, Wilske B. Validity of interpretation criteria for standardized Western blots (immunoblots) for serodiagnosis of Lyme borreliosis based on sera collected throughout Europe. J Clin Microbiol. 1999; 37(7): 2241–2247. https://doi.org/10.1128/JCM.37.7.2241-2247.1999
33. Mavin S, Evans R, Milner RM, et al. Local *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* strains in a single mixed antigen improves western blot sensitivity. J Clin Pathol. 2009; 62(6): 552–554. https://doi.org/10.1136/jcp.2008.063461
34. Rauter C, Hartung T. Prevalence of *Borrelia burgdorferi* sensu lato genospecies in Ixodes ricinus ticks in Europe: a metaanalysis. Appl Environ Microbiol. 2005; 71(11): 7203–7216. https://doi.org/10.1128/AEM.71.11.7203-7216.2005
35. Ivanova L, Christova I, Neves V, et al. Comprehensive seroprofiling of sixteen *B. burgdorferi* OspC: implications for Lyme disease diagnostics design. Clin Immunol. 2009; 132(3): 393–400. https://doi.org/10.1016/j.clim.2009.05.017

Supplementary Table 1. Detailed results of enzyme-linked immunosorbent (ELISA), immunoblot (IB) and Optiplex *Borrelia* (OB) assays results for anti-*Borrelia* IgM and IgG antibodies in sera samples of Lyme disease patients (experimental group) and healthy individuals (control group)

No. of patient	ELISA	IB	OB	ELISA	IB	OB
		IgM			IgG	
Experimental group						
1.	POS	NEG	NEG	POS	NEG	NEG
2.	POS	NEG	NEG	NEG	NEG	NEG
3.	POS	POS	NEG	NEG	NEG	NEG
4.	POS	NEG	POS	POS	POS	NEG
5.	NEG	NEG	NEG	POS	NEG	NEG
6.	POS	BOR	POS	NEG	NEG	NEG
7.	POS	NEG	BOR	NEG	NEG	POS
8.	POS	NEG	NEG	POS	POS	BOR
9.	BOR	NEG	NEG	NEG	POS	NEG
10.	POS	POS	NEG	NEG	BOR	NEG
11.	NEG	NEG	POS	NEG	NEG	POS
12.	NEG	NEG	NEG	POS	POS	NEG
13.	POS	POS	POS	NEG	NEG	NEG
14.	NEG	NEG	NEG	NEG	NEG	NEG
15.	POS	BOR	NEG	POS	BOR	NEG
16.	NEG	NEG	POS	POS	POS	NEG
17.	POS	POS	NEG	POS	POS	NEG
18.	NEG	NEG	NEG	NEG	NEG	BOR
19.	BOR	NEG	POS	NEG	NEG	BOR
20.	POS	NEG	POS	NEG	NEG	POS
21.	BOR	NEG	NEG	POS	POS	NEG
22.	BOR	NEG	NEG	NEG	NEG	BOR
23.	BOR	POS	BOR	POS	POS	NEG
24.	NEG	BOR	POS	POS	POS	POS
25.	NEG	NEG	NEG	NEG	POS	NEG
26.	NEG	NEG	POS	POS	POS	NEG
27.	BOR	NEG	BOR	POS	BOR	NEG
28.	BOR	NEG	NEG	NEG	NEG	BOR
29.	POS	NEG	NEG	POS	POS	BOR
30.	POS	NEG	NEG	NEG	POS	BOR
31.	BOR	BOR	BOR	NEG	NEG	NEG
32.	NEG	NEG	NEG	POS	BOR	NEG
33.	POS	POS	NEG	POS	POS	NEG
34.	POS	POS	NEG	BOR	POS	NEG
35.	POS	POS	POS	POS	POS	NEG
36.	NEG	NEG	NEG	NEG	BOR	NEG
37.	POS	NEG	POS	NEG	POS	NEG
38.	BOR	NEG	POS	POS	POS	POS
39.	POS	NEG	NEG	BOR	BOR	BOR
40.	NEG	BOR	NEG	BOR	POS	NEG
41.	POS	POS	NEG	POS	POS	POS
42.	POS	POS	NEG	POS	POS	POS
43.	POS	NEG	POS	NEG	BOR	NEG
44.	NEG	POS	POS	POS	POS	POS
45.	POS	POS	POS	NEG	NEG	POS
46.	NEG	NEG	NEG	POS	POS	NEG
47.	NEG	NEG	BOR	POS	NEG	NEG
48.	POS	POS	NEG	NEG	NEG	NEG
49.	POS	POS	NEG	BOR	BOR	POS
50.	POS	POS	NEG	POS	NEG	BOR
51.	NEG	BOR	NEG	NEG	POS	NEG
52.	POS	POS	POS	POS	POS	NEG
53.	POS	POS	NEG	NEG	POS	NEG
54.	POS	POS	POS	POS	POS	BOR
55.	POS	POS	POS	NEG	NEG	BOR
56.	POS	POS	POS	NEG	BOR	NEG
57.	POS	BOR	NEG	POS	BOR	NEG
58.	NEG	NEG	POS	NEG	NEG	BOR
59.	POS	POS	POS	POS	POS	POS
60.	POS	POS	NEG	NEG	BOR	NEG
61.	POS	POS	POS	NEG	NEG	NEG
62.	NEG	NEG	BOR	NEG	BOR	BOR
63.	POS	POS	POS	NEG	NEG	NEG
64.	POS	POS	NEG	POS	POS	NEG
65.	POS	NEG	POS	NEG	POS	NEG
66.	POS	NEG	NEG	POS	POS	NEG
67.	POS	NEG	NEG	POS	POS	NEG
68.	NEG	BOR	POS	POS	NEG	POS
69.	POS	BOR	POS	POS	POS	NEG
70.	POS	NEG	NEG	POS	POS	BOR
71.	POS	NEG	POS	POS	BOR	BOR
72.	POS	NEG	POS	POS	POS	POS
73.	POS	POS	POS	POS	POS	NEG
74.	POS	POS	BOR	POS	POS	POS
75.	POS	BOR	NEG	NEG	POS	BOR
76.	POS	NEG	POS	POS	NEG	POS
77.	POS	POS	POS	NEG	NEG	POS
78.	POS	POS	NEG	POS	POS	NEG
79.	NEG	NEG	POS	NEG	POS	POS
80.	POS	NEG	POS	POS	NEG	NEG
Control group						
1.	NEG	NEG	NEG	NEG	NEG	NEG
2.	NEG	NEG	POS	NEG	NEG	POS
3.	NEG	NEG	POS	NEG	NEG	POS
4.	NEG	NEG	NEG	NEG	NEG	POS
5.	NEG	NEG	NEG	POS	BOR	NEG
6.	NEG	NEG	NEG	NEG	NEG	NEG
7.	NEG	NEG	NEG	NEG	NEG	NEG
8.	NEG	NEG	NEG	NEG	NEG	NEG
9.	NEG	NEG	NEG	NEG	NEG	NEG
10.	NEG	NEG	NEG	POS	NEG	NEG
11.	NEG	NEG	NEG	NEG	BOR	NEG
12.	NEG	NEG	NEG	NEG	NEG	NEG
13.	NEG	NEG	NEG	NEG	NEG	NEG
14.	NEG	BOR	NEG	NEG	NEG	NEG
15.	NEG	NEG	NEG	NEG	NEG	NEG
16.	NEG	NEG	NEG	NEG	NEG	NEG
17.	NEG	NEG	NEG	NEG	NEG	NEG
18.	NEG	NEG	NEG	NEG	NEG	NEG
19.	NEG	NEG	NEG	NEG	NEG	NEG
20.	NEG	NEG	NEG	NEG	NEG	NEG
21.	NEG	NEG	NEG	NEG	NEG	NEG
22.	NEG	NEG	NEG	NEG	NEG	NEG

NEG - negative result; POS - positive result; BOR - borderline result

Supplementary Table 2. Detailed summary of Optiplex *Borrelia* results for anti-*Borrelia* IgM and IgG antibodies in sera samples of Lyme disease patients (experimental group) and healthy individuals (control group).

	IgM										IgG								
No. of patient	Lysate B.a	OspA B.a	OspC B.g	OspC B.a	p100 B.a	p18 B.a (DbpA)	p39 B.a (BmpA)	p41-I B.a	VlsE-C6	Result	Lysate B.g	OspC B.a	p100 B.a	p18 B.a (DbpA)	p39 B.a (BmpA)	p41-I B.a	p58 B.g (OppA)	VlsE-C6	Result
Experimental group																			
1.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
2.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
3.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
4.	+	-	(+)	-	+	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
5.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
6.	+	-	+	+	-	-	-	(+)	-	POS	-	-	-	-	-	-	-	-	NEG
7.	(+)	-	(+)	(+)	-	+	-	-	-	BOR	-	-	+	+	(+)	-	-	+	POS
8.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	+	BOR
9.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
10.	-	-	(+)	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
11.	+	-	+	+	-	-	-	-	-	POS	-	-	-	+	-	-	-	+	POS
12.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
13.	+	+	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
14.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
15.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
16.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
17.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
18.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	+	BOR
19.	-	-	-	-	-	+	-	(+)	-	POS	-	-	-	-	-	-	-	+	BOR
20.	+	(+)	+	+	-	-	-	(+)	-	POS	+	-	+	+	+	(+)	+	+	POS
21.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
22.	-	-	-	-	-	-	-	(+)	-	NEG	-	-	-	-	-	-	-	+	BOR
23.	-	-	(+)	-	-	-	-	+	-	BOR	-	-	-	-	-	-	-	-	NEG
24.	(+)	-	(+)	-	-	+	-	+	-	POS	-	-	-	+	-	+	-	+	POS
25.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
26.	-	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
27.	+	-	+	(+)	-	-	-	(+)	-	BOR	-	-	-	-	-	-	-	-	NEG
28.	-	-	-	-	-	-	-	-	-	NEG	+	-	-	-	-	-	+	-	BOR
29.	-	-	-	-	-	-	-	-	-	NEG	-	-	(+)	-	-	-	-	-	BOR
30.	-	-	-	-	-	-	-	-	-	NEG	(+)	-	-	-	-	-	+	-	BOR
31.	(+)	-	-	-	-	(+)	-	(+)	(+)	BOR	-	-	-	-	-	-	-	-	NEG
32.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
33.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
34.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
35.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
36.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
37.	-	+	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
38.	+	+	+	+	-	+	-	+	(+)	POS	+	-	-	+	+	-	+	+	POS
39.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	+	BOR
40.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
41.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	+	-	-	-	+	POS
42.	-	-	-	-	-	-	-	(+)	-	NEG	-	-	-	+	-	-	-	+	POS
43.	+	(+)	-	-	-	-	-	+	-	POS	-	-	-	-	-	-	-	-	NEG
44.	+	-	+	+	-	-	-	-	-	POS	-	-	-	+	-	-	-	+	POS
45.	-	+	+	+	-	-	-	-	-	POS	-	-	+	+	(+)	-	-	+	POS
46.	-	-	(+)	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
47.	-	-	+	(+)	-	-	(+)	-	-	BOR	-	-	-	-	-	-	-	-	NEG
48.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
49.	-	-	-	-	-	-	-	+	-	NEG	-	-	-	-	(+)	-	-	+	POS
50.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	(+)	-	(+)	(+)	BOR
51.	-	-	(+)	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG

No. of patient	IgM										IgG								
	Lysate B.a	OspA B.a	OspC B.g	OspC B.a	p100 B.a	p18 B.a (DbpA)	p39 B.a (BmpA)	p41-I B.a	VlsE-C6	Result	Lysate B.g	OspC B.a	p100 B.a	p18 B.a (DbpA)	p39 B.a (BmpA)	p41-I B.a	p58 B.g (OppA)	VlsE-C6	Result
52.	(+)	(+)	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
53.	-	-	-	-	-	-	-	-	+	NEG	-	-	-	-	-	-	-	-	NEG
54.	-	-	-	-	-	-	-	+	-	POS	-	-	-	-	-	-	-	+	BOR
55.	+	-	+	+	-	-	-	-	-	POS	-	-	-	+	-	-	-	-	BOR
56.	-	+	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
57.	-	-	(+)	+	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
58.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	+	BOR
59.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	+	POS
60.	-	-	-	-	-	-	-	-	(+)	NEG	-	-	-	-	-	-	-	-	NEG
61.	+	(+)	+	+	-	-	-	+	-	POS	-	-	-	-	-	-	-	-	NEG
62.	(+)	-	(+)	(+)	-	-	-	-	-	BOR	-	-	-	-	-	+	-	-	BOR
63.	+	-	+	+	-	-	-	+	-	POS	-	-	-	-	-	-	-	-	NEG
64.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
65.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
66.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
67.	-	-	-	-	-	-	-	(+)	-	NEG	-	-	-	-	-	-	-	(+)	NEG
68.	-	-	+	(+)	-	-	-	-	-	POS	-	-	-	+	-	-	-	+	POS
69.	+	-	+	+	-	-	-	-	-	POS	-	-	-	-	-	-	-	-	NEG
70.	-	-	(+)	-	-	-	-	-	-	NEG	-	-	-	+	-	-	-	-	BOR
71.	-	+	+	+	-	-	-	-	-	POS	-	-	-	+	-	-	-	-	BOR
72.	(+)	-	+	+	-	+	-	-	-	POS	-	-	-	+	-	-	-	+	POS
73.	+	-	+	+	-	-	-	+	-	POS	-	-	-	-	-	-	-	-	NEG
74.	-	-	+	(+)	-	-	-	-	-	BOR	-	-	(+)	+	-	(+)	-	+	POS
75.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	+	-	-	-	BOR
76.	+	-	+	+	-	+	-	+	-	POS	+	+	+	+	+	+	+	+	POS
77.	+	-	+	+	-	+	-	+	-	POS	+	-	+	+	+	+	+	+	POS
78.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	(+)	-	-	NEG
79.	+	-	+	+	-	-	-	+	-	POS	-	-	-	-	(+)	-	-	+	POS
80.	+	-	+	+	-	-	-	+	-	POS	-	-	-	(+)	-	-	-	(+)	NEG
Control group																			
1.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
2.	(+)	-	+	+	-	+	-	+	-	POS	+	+	+	+	+	+	+	+	POS
3.	+	-	+	+	-	+	-	+	-	POS	-	(+)	+	+	+	+	+	+	POS
4.	-	-	+	(+)	-	-	-	-	-	NEG	-	-	-	+	-	-	-	+	POS
5.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	(+)	-	-	NEG
6.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
7.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
8.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
9.	-	-	(+)	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
10.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
11.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
12.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
13.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
14.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
15.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
16.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
17.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
18.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
19.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
20.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
21.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG
22.	-	-	-	-	-	-	-	-	-	NEG	-	-	-	-	-	-	-	-	NEG

"-" NEG – negative result (cut-off-index <1); "+" POS – positive result (cut-off-index ≥1.5); "(+)" BOR – borderline result (1≤ cut-off-index <1.5); B.a – *Borrelia afzelii*, B.g – *Borrelia garinii*.