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* lyseate. 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-p41-1 *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. afzelii* 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. affelii* (DbpA), p39 *B. afzelii* (BmpA), p41-1 *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-\textit{Borrelia} IgM and IgG antibodies present in sera of outpatients suffering from LD (experimental group) and healthy individuals (control group) using OB, ELISA, and supplementary Table 1.

#### Table 1. Correlation of serological detection of anti-\textit{Borrelia} IgM antibodies with Optiplex \textit{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      | 30    | 10 | 40            |
| Total    | 60    | 20 | 80            |
| McNemar’s \( \chi^2 \) (p value) | 9.03 (0.0027) | 0.03 (0.8744) | 3.38 (0.0662) |
| OB       |       |    |               |
| POS/BOR  | 0     | 2  | 2             |
| NEG      | 2     | 18 | 20            |
| Total    | 2     | 20 | 22            |

#### Table 2. Correlation of serological detection of anti-\textit{Borrelia} IgG antibodies with Optiplex \textit{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      | 28    | 18 | 46            |
| Total    | 46    | 34 | 80            |
| McNemar’s \( \chi^2 \) (p value) | 2.75 (0.0973) | 7.52 (0.0061) | 4.11 (0.0425) |
| OB       |       |    |               |
| POS/BOR  | 1     | 1  | 2             |
| NEG      | 1     | 19 | 20            |
| Total    | 2     | 20 | 22            |

\( CI \) – confidence interval; \( POS/BOR \) – positive/borderline results.

For IgG class antibodies, as well as for a two-stage diagnostic protocol (ELISA with IB) (Tables 1 and 2; detailed results are included in Supplementary Table 1).

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 OB and IB 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—\textit{B. afzelii}, \textit{B. garinii}, and \textit{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. \textit{afzelii}, B. \textit{garinii}, and B. \textit{burgdorferi}). On the other hand, the OB assay showed a lower number of positives/borderlines for anti-flagellin antibodies (p41-1 B. \textit{afzelii}), even though the highest number was found for VlsE-C6, p18 B. \textit{afzelii} (DbpA), and p39 B. \textit{afzelii} (BmpA). In terms of the control group, the positive/borderline results for anti-\textit{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-\textit{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-\textit{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-\textit{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].
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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), 0 (0.0) | np, np |
| p14 (flagellin) | 23 (28.8), 5 (22.7) | np, np |
| p39 (BmpA) | 4 (5.0), 0 (0.0) | np, np |
| OspC B.a | 36 (45.0), 33 (41.3) | 1 (4.6), 3 (13.6) |
| OspC B.b | 27 (33.8), 1 (4.6) | np, np |
| OspC B.g | 31 (38.8), 36 (45.0) | 0 (0.0), 4 (18.2) |
| Lysate B.a | np, 29 (36.3) | np, 2 (9.1) |
| OspA B.a | np, 10 (12.5) | 0 (0.0) |
| p100 B.a | np, 1 (1.3) | 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-L B.a | np, 17 (21.3) | np, 2 (9.1) |
| VlsE-C6 | np, 2 (2.5) | 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 spielmani

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. spielmani*, and *B. lonestari*, 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 < p41-1 *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 Heikillé 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* lysoase and the *B. afzelii* p41-1 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_M38, 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) occurs when the unmodified p41 antigen is used in the IB assay. In conclusion, OB assay can be used to diagnose the LB only as a complementary assay and not as an optimal form of detection of Borrelia spp. infection.

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### 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 | Experimental group | Control group |
|----------------|-------------------|---------------|
|                | ELISA IgM | IB IgM | OB IgM | ELISA IgM | IB IgM | OB IgM |
| 1.             | POS        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 2.             | POS        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 3.             | POS        | POS    | NEG    | NEG       | NEG    | NEG    |
| 4.             | NEG        | NEG    | POS    | POS       | NEG    | NEG    |
| 5.             | NEG        | BOR    | NEG    | NEG       | NEG    | NEG    |
| 6.             | POS        | NEG    | BOR    | NEG       | NEG    | NEG    |
| 7.             | POS        | NEG    | NEG    | POS       | NEG    | NEG    |
| 8.             | POS        | NEG    | BOR    | NEG       | NEG    | NEG    |
| 9.             | BOR        | NEG    | NEG    | NEG       | POS    | NEG    |
| 10.            | POS        | POS    | NEG    | NEG       | NEG    | NEG    |
| 11.            | NEG        | NEG    | POS    | NEG       | NEG    | NEG    |
| 12.            | NEG        | NEG    | POS    | POS       | NEG    | NEG    |
| 13.            | POS        | NEG    | POS    | NEG       | NEG    | NEG    |
| 14.            | NEG        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 15.            | NEG        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 16.            | POS        | POS    | NEG    | NEG       | NEG    | NEG    |
| 17.            | POS        | POS    | POS    | NEG       | NEG    | NEG    |
| 18.            | NEG        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 19.            | BOR        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 20.            | POS        | NEG    | POS    | NEG       | NEG    | NEG    |
| 21.            | BOR        | NEG    | NEG    | POS       | NEG    | NEG    |
| 22.            | BOR        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 23.            | BOR        | POS    | BOR    | POS       | NEG    | NEG    |
| 24.            | NEG        | BOR    | POS    | POS       | POS    | NEG    |
| 25.            | NEG        | NEG    | POS    | POS       | POS    | NEG    |
| 26.            | NEG        | NEG    | NEG    | POS       | POS    | NEG    |
| 27.            | NEG        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 28.            | BOR        | NEG    | NEG    | NEG       | NEG    | NEG    |
| 29.            | POS        | NEG    | NEG    | POS       | NEG    | NEG    |
| 30.            | POS        | NEG    | NEG    | POS       | NEG    | NEG    |
| 31.            | BOR        | BOR    | NEG    | NEG       | NEG    | NEG    |
| 32.            | NEG        | NEG    | NEP    | POS       | BOR    | NEG    |
| 33.            | POS        | POS    | NEP    | POS       | NEG    | NEG    |
| 34.            | POS        | POS    | NEG    | BOR       | NEG    | NEG    |
| 35.            | POS        | POS    | POS    | POS       | NEG    | NEG    |
| 36.            | NEG        | NEG    | NEG    | NEG       | BOR    | NEG    |
| 37.            | POS        | NEG    | POS    | POS       | POS    | NEG    |
| 38.            | POS        | NEG    | BOR    | NEG       | NEG    | NEG    |
| 39.            | POS        | NEG    | NEG    | BOR       | NEG    | NEG    |
| 40.            | POS        | POS    | POS    | POS       | POS    | NEG    |
| 41.            | POS        | POS    | POS    | POS       | POS    | NEG    |
| 42.            | POS        | POS    | POS    | POS       | POS    | NEG    |
| 43.            | NEG        | POS    | BOR    | NEG       | NEG    | NEG    |
| 44.            | NEG        | POS    | NEG    | BOR       | NEG    | NEG    |
| 45.            | POS        | POS    | NEP    | POS       | POS    | NEG    |
| 46.            | POS        | POS    | NEG    | POS       | NEP    | NEG    |
| 47.            | NEG        | NEG    | POS    | BOR       | POS    | NEG    |
| 48.            | NEG        | POS    | NEG    | NEG       | NEG    | NEG    |
| 49.            | NEG        | POS    | NEG    | NEG       | NEG    | NEG    |
| 50.            | NEG        | POS    | NEG    | BOR       | NEG    | NEG    |
| 51.            | NEG        | BOR    | POS    | NEG       | NEG    | NEG    |
| 52.            | POS        | POS    | POS    | POS       | POS    | NEG    |
**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).

| No. of patient | IgM       |          | IgG       |          |
|---------------|-----------|----------|-----------|----------|
|               | Lyz A B     | OspA B C D | Lys A B     | OspA B C D |
| Experimental group | |
| 1.            | - - - -     | - - - -   | - - - -     | - - - -   |
| 2.            | - - - -     | - - - -   | - - - -     | - - - -   |
| 3.            | - - + +     | - - - -   | - - - -     | - - - -   |
| 4.            | + - (+) +   | - - - -   | POS         | - - - -   |
| 5.            | - - + (+) + | - - - -   | - - - -     | - - - -   |
| 6.            | + - + +     | - - - -   | POS         | - - - -   |
| 7.            | (+) - (+) + | - - - -   | BOR         | - - + +   |
| 8.            | - - - +     | - - - -   | NEG         | - - - -   |
| 9.            | - - + (+) + | - - - -   | NEG         | - - - -   |
| 10.           | - - - (+) + | - - - -   | NEG         | - - - -   |
| 11.           | + - + +     | - - - -   | POS         | - - - -   |
| 12.           | - - + (+) + | - - - -   | NEG         | - - - -   |
| 13.           | + + + +     | - - - -   | POS         | - - - -   |
| 14.           | - - - -     | - - - -   | NEG         | - - - -   |
| 15.           | - - - -     | - - - -   | NEG         | - - - -   |
| 16.           | + - + +     | - - - -   | POS         | - - - -   |
| 17.           | - - - -     | - - - -   | NEG         | - - - -   |
| 18.           | - - - -     | - - - -   | NEG         | - - - -   |
| 19.           | - - - -     | - - - -   | NEG         | - - - -   |
| 20.           | + (+) + + + | - - - -   | POS         | - - - -   |
| 21.           | - - - -     | - - - -   | NEG         | - - - -   |
| 22.           | - - - -     | - - - -   | NEG         | - - - -   |
| 23.           | - - (+) +   | - - - -   | BOR         | - - - -   |
| 24.           | (+) - (+) + | - - - -   | POS         | - - - -   |
| 25.           | - - - -     | - - - -   | NEG         | - - - -   |
| 26.           | - - - +     | - - - -   | NEG         | - - - -   |
| 27.           | + - + (+) + | - - - -   | BOR         | - - - -   |
| 28.           | - - - -     | - - - -   | NEG         | - - - -   |
| 29.           | - - - -     | - - - -   | NEG         | - - - -   |
| 30.           | - - - -     | - - - -   | NEG         | - - - -   |
| 31.           | (-) - - -   | - (+) + (+) | BOR     | - - - -   |
| 32.           | - - - -     | - - - -   | NEG         | - - - -   |
| 33.           | - - - -     | - - - -   | NEG         | - - - -   |
| 34.           | - - - -     | - - - -   | NEG         | - - - -   |
| 35.           | + - - +     | - - - -   | POS         | - - - -   |
| 36.           | - - - +     | - - - -   | NEG         | - - - -   |
| 37.           | - - - -     | - - - -   | POS         | - - - -   |
| 38.           | + - - -     | - - - -   | NEG         | - - - -   |
| 39.           | - - - -     | - - - -   | POS         | - - - -   |
| 40.           | - - - -     | - - - -   | NEG         | - - - -   |
| 41.           | - - - -     | - - - -   | NEG         | - - - -   |
| 42.           | - - - -     | - - - -   | NEG         | - - - -   |
| 43.           | + (+) - -   | - - - -   | POS         | - - - -   |
| 44.           | + + + -     | - - - -   | NEG         | - - - -   |
| 45.           | - - - -     | - - - -   | POS         | - - - -   |
| 46.           | - - (+) +   | - - - -   | NEG         | - - - -   |
| 47.           | - - - + (+) | - - - -   | BOR         | - - - -   |
| 48.           | - - - -     | - - - -   | NEG         | - - - -   |
| 49.           | - - - -     | - - - -   | NEG         | - - - -   |
| 50.           | - - - -     | - - - -   | NEG         | - - - -   |
| 51.           | - - (+) +   | - - - -   | NEG         | - - - -   |
| No. of patient | IgM | IgG |
|---------------|-----|-----|
|               | Lysate B.a | OspC B.g | OspC B.g | p100 B.a | p18 B.a (DbpA) | p39 B.a (BmpA) | VlsE-C6 | Result | Lysate B.g | OspC B.g | p100 B.a | p18 B.a (DbpA) | p39 B.a (BmpA) | p41-I B.a | OspC B.g | 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 | - - - - - - - - | + | BOR |
| 62.           | (+) - (+) + - - - | BOR | - - - - - - - - | - | NEG |
| 63.           | + + + + - - - - | POS | - - - - - - - - | - | NEG |
| 64.           | - - - - - - - - | NEG | - - - - - - - - | - | NEG |
| 65.           | + + + + - - - - | POS | - - - - - - - - | - | NEG |
| 66.           | - - + (+) - - - - | NEG | - - - - - - - - | - | NEG |
| 67.           | - - + (+) - - - - | POS | - - - - - - - - | + | POS |
| 68.           | + + + + - - - - | POS | - - - - - - - - | - | NEG |
| 69.           | - - + (+) - - - - | NEG | - - - - - - - - | - | 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

| No. of patient | IgM | IgG |
|---------------|-----|-----|
| 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.