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Identification of immunoreactive linear epitopes of *Borrelia miyamotoi*

Rafal Tokarz[a]#, Teresa Tagliafierro[a], Adrian Caciula[a], Nischay Mishra[a], Riddhi Thakkar[a], Lokendra V Chauhan[a], Stephen Sameroff[a], Shannon Delaney[b], Gary P. Wormser[c], Adriana Marques[d], W. Ian Lipkin[a]

[a]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY

[b]Department of Psychiatry, Columbia University, New York, NY

[c] Division of Infectious Diseases, New York Medical College, Valhalla, NY

[d] Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

#Corresponding author

Rafal Tokarz

Center for Infection and Immunity

Mailman School of Public Health, Columbia University

722 West 168th Street, Room 1701, New York, NY 10032

phone: (212) 631 335 5021

Email: rt2249@columbia.edu

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Abstract

Borrelia miyamotoi is an emerging tick-borne spirochete transmitted by *Ixodid* ticks. Current serologic assays for *B. miyamotoi* are impacted by genetic similarities to other *Borrelia* and limited understanding of optimal antigenic targets. In this study, we employed the TBD-Serochip, a peptide array platform, to identify new linear targets for serologic detection of *B. miyamotoi*. We examined a wide range of suspected *B. miyamotoi* antigens and identified 352 IgM and 91 IgG reactive peptides, with the majority mapping to variable membrane proteins. These included peptides within conserved fragments of variable membrane proteins that may have greater potential for differential diagnosis. We also identified reactive regions on FlaB, and demonstrate crossreactivity of *B. burgdorferi* C6 with a *B. miyamotoi* C6-like peptide. The panel of linear peptides identified in this study can be used to enhance serodiagnosis of *B. miyamotoi*.

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Introduction

Borrelia miyamotoi is a tick-borne relapsing fever spirochete found throughout temperate regions worldwide that is transmitted by hard ticks of the genus *Ixodes* (1-5). *B. miyamotoi* was discovered in 1995; however, the link to disease was first established in 2011 when it was implicated in an outbreak of tick-borne illness in Russia (1, 6). *B. miyamotoi* is primarily transmitted by *I. scapularis* and *I. pacificus* in North America, and *I. ricinus* and *I. persulcatus* in Europe and Asia (6-9). These tick species also transmit *Borreliae* that cause Lyme borreliosis (10). Lyme borreliosis is the most common vector-borne disease in the United States, where most infections are caused by *Borrelia burgdorferi* and transmitted by *Ixodes scapularis.* Despite sharing the same vectors, *B. miyamotoi* differs from *B. burgdorferi* in a number of ecological aspects, including the ability for transovarial transmission, quicker transmission during tick feeding, and lower infection rates in vector ticks. The prevalence of *B. miyamotoi* in nymphs is typically 1% to 5% versus 15% to 25% for *B. burgdorferi* (11-15). Nonetheless, *B. miyamotoi* and *B. burgdorferi* can occasionally infect the same tick, and concurrent human infections have been reported (5, 15, 16).

Symptomatic infections with *B. miyamotoi* (*Borrelia miyamotoi* disease; BMD) usually present with fever and other non-specific symptoms including fatigue, headache, chills and nausea (17, 18). Bouts of relapsing fever may occur in untreated patients. In the United States, reports of BMD are rare with less than 200 cases identified between 2011 and 2017 (3, 19, 20). This is considerably less than would be expected based on the high incidence of other tick-borne diseases and suggests a substantial underreporting of *B. miyamotoi* infections (21). A portion of infections may be asymptomatic while symptomatic infections may not be identified because of a similar presentation to certain other tick-borne illnesses and the lack of optimal diagnostic tests (22-24). Patients with

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BMD may test positive on the C6 ELISA, a serologic assay used in the diagnosis of Lyme disease (20, 25, 26). Current methods of BMD diagnosis include PCR (in the acute stage), and a two-tiered antibody assay (ELISA and western blot) based on immunoreactivity to glycerophosphodiester phosphodiesterase (GlpQ), an enzyme present in *B. miyamotoi* but absent in Lyme *Borrelia* (17, 27). Because GlpQ homologs are present in other relapsing fever spirochetes and in other bacteria, its specificity for *B. miyamotoi* is limited (28-31). In addition, serologic assays based on reactivity to GlpQ are only reactive in 56% to 78% of sera from convalescent BMD patients and only 16% of sera from individuals with acute disease (3, 32). Thus, other targets are needed in order to develop more sensitive and specific serologic tests.

An alternative approach for BMD serologic diagnosis is the development of assays that target outer surface antigens. Although this approach has been applied for diagnosis of Lyme borreliosis, relatively little is known about the utility of outer surface antigens in diagnosis of BMD. The primary challenge in assay development is the rapid antigenic variation that is a characteristic of relapsing fever spirochetes (33-36). Like all relapsing fever *Borreliae*, the genome of *B. miyamotoi* contains linear plasmids that encode multiple alleles of variable major proteins (Vmps) (36-38). Vmps consist of two types of lipoproteins that are dissimilar in sequence and length: the variable small proteins (Vsps) and variable large proteins (Vlps)(39). At any given time, only one Vmp is expressed by the spirochete when a single allele is copied into the Vmp expression site. Relapsing fever *Borreliae* alternate expression of these Vmps in order to evade the host immune response (31, 32, 38). The alleles are genetically heterogenous; thus, it is unlikely that host IgM raised against one Vmp will neutralize spirochetes expressing another Vmp. Despite high genetic diversity, Vmps share conserved regions that may also be a target of neutralizing antibodies. Identifying these regions could be useful for diagnosis,

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104 particularly early in disease (37, 39). In this study, we employed the Tick-borne disease
105 Serochip (TBD-Serochip), a novel serologic peptide array platform, to examine the
106 antibody response in human patients with BMD to a wide array of *B. miyamotoi* antigens.
107 Our work identified linear epitopes that could be applied in the development of improved
108 diagnostic tests (40).

109

110 **Materials and Methods**

111 ***TBD-Serochip***. The TBD-Serochip is a peptide array that consists of approximately
112 170,000 12-mer linear peptides designed from the protein sequence of the primary
113 antigens of *Anaplasma phagocytophilum*, *B. burgdorferi*, *B. miyamotoi*, *Babesia microti*,
114 *Rickettsia rickettsii*, *Ehrlichia chaffeensis*, Powassan virus and Heartland virus (40). The
115 12-mer peptides tile the sequence of each antigen with an 11 amino acid (aa) overlap to
116 the preceding 12-mer peptide in a sliding window pattern. Antigens selected for inclusion
117 on the TBD-Serochip were all previously reported to elicit an antibody response in
118 humans. For *B. miyamotoi*, the TBD-Serochip includes 12-mer peptides designed from
119 a wide array of Vmp and non-Vmp antigens (**Table 1**). For each selected antigen, we
120 downloaded every available homologous protein sequence from the NCBI protein
121 database. Sequences were aligned and used to design 12-mer peptides, with redundant
122 peptides excluded prior to synthesis. This approach resulted in the TBD-Serochip
123 incorporating peptides to all genetic variants of every included antigen.
124 ***Sera***. We tested seven convalescent-phase sera from patients diagnosed with BMD.
125 Five sera originated from New York Medical College, and 2 from Tufts University.
126 Diagnosis of BMD was made during the acute disease phase, six by PCR, and one by a
127 GlpQ-based serologic assay (**Table 2**). We contrasted these data with TBD-Serochip
128 data obtained from sera from patients with Lyme disease (N=100), and healthy

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individuals with no history of Lyme disease (N=16). All sera were tested at a 1:50 dilution. We used de-identified human sera from our previous work with the TBD-Serochip, and additional samples acquired form the National Institutes of Health (40). These samples were obtained under clinical protocols (ClinicalTrials.gov Identifier NCT00028080 and NCT00001539) approved by the institutional review board of the National Institute of Allergy and Infectious Diseases, and all participants signed informed consent. Sample types ranged from early to late Lyme disease (confirmed by the two-tiered testing algorithm), All methods used for sample collection were performed in accordance with the proper guidelines and regulations.

Epitope identification. Following antibody incubations, the arrays were scanned on NimbleGen MS 200 Microarray Scanner (Roche) to generate raw fluorescent intensity data sets that were then subjected to quantile normalization. IgM and IgG reactivity were examined separately. Based on the data obtained from healthy controls, we computed a cut-off threshold for each epitope that was defined by calculating the mean plus 3 standard deviations of the signal intensity for the same epitope. We then filtered out any peptides with signal intensities below threshold in all experiments. To identify peptides that could be used to differentiate between BMD and healthy control sera, we used the non-parametric Wilcoxon rank sum test and identified peptides that yielded a statistically significant signal (q value ≤0.05 for both IgM and IgG, corrected for multiple testing using Benjamini-Hochberg Procedure). This identified the optimal sets of IgM and IgG 12-mer peptides that were reactive in sera with BMD, but not in healthy individuals. A portion of these 12-mer peptides were assembled into longer contigs that were then ranked by the average signal intensity.

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Luciferase ImmunoPrecipitation System assay (LIPS). We generated a *Renilla* luciferase-GlpQ construct for detection of anti-GlpQ IgG antibody. The complete GlpQ coding sequence was amplified by PCR from a *B. miyamotoi*-positive *I. scapularis* using primers 5'-GGATCCATGAAATTAAAATTACTAATGC-3' and 5'-GAGCTCTTATTTTTTTATGAAGTTCATT-3'. The PCR product was cloned into pREN2 vector and the fusion proteins were generated in Cos-1 cells. Light units were measured in a Berthold LB 960 Centro microplate luminometer (Berthold Technologies) using a Renilla Luciferase Assay System kit (Promega) and MikroWin 2010 software.

Results

Of the 23,946 *B. miyamotoi* 12-mer peptides present on the TBD-Serochip, we identified 1491 peptides that were significantly reactive with IgM antibodies present in the seven BMD sera tested. Through assembly of overlapping reactive 12-mer peptides, we identified 352 reactive epitopes made up of 2 or more consecutive reactive peptides. For IgG, we identified 429 peptides that were clustered into 91 putative epitopes. All IgM and IgG epitopes were then mapped to specific regions within *B. miyamotoi* proteins. The majority of the epitopes mapped to Vmps (**Table 3**).

In addition to *B. miyamotoi* peptides, we examined the reactivity to peptides from other tick-borne agents. In 2 samples, we detected IgG reactivity to specific epitopes of *B. burgdorferi* that were identified in our previous work (40). Subsequent examination of clinical history of these 2 individuals revealed that both had previously been diagnosed with Lyme disease.

Reactivity to Vsp peptides. The TBD-Serochip contains 12-mer peptides designed from the amino acid (aa) sequence of 13 full length and 4 partial Vsp paralogs (**Table 1**). *B. miyamotoi* Vsps are approximately 220 aa in length and consist of a conserved N-

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terminal fragment of approximately 85 aa, followed by highly variable regions (**Figure 1A**). We identified IgM-reactive epitopes within both the conserved and variable fragments within every Vsp homolog. Although previous studies that were focused on Vsp1 suggested that it may be a major immunodominant antigen of *B. miyamotoi*, our data revealed Vsp4 to be more immunoreactive (**Figure 1B** and **1C**). Among the immunodominant regions of Vsps included a region within the N-terminal fragment that was reactive with IgM in 5 out of 7 sera (sera 2 and 4 - 7). This 16 aa peptide, designated VS-C-1, spans aa 52 to 66 of Vsp1 (mapped to accession number AGS80212) and is conserved in the majority of sequenced *B. miyamotoi* Vsp paralogs (**Figure 2**).

One Vsp paralog (accession number BAA11304) contains substantial aa variation within the central portion of the VS-C-1 sequence. The aa at positions 8-12 in this paralog deviates from AVEFA to SELME (**Figure 2**). Presumably, antibodies to VS-C-1 region would not react with peptides with this divergent amino acid sequence (designated VS-C-2). Nonetheless, peptides corresponding to VS-C-2 (mapped to accession number BAA11304) were reactive with 4 of the 7 VS-C-1-reactive samples (sera 4-7), but did not react with sera from patient 2 (**Figure 3**). The presence of antibodies to both peptide variants indicates that these 4 patients were exposed to Vsp paralogs with both VS-C-1 and VS-C-2 sequences.

We identified other reactive regions on Vsps, including VS-C-3 located at the end of the conserved N-terminal portion that was highly reactive in sera from patient 7 (**Figure 1B**). We also identified epitopes that were mapped to the variable fragments of Vsps. However, these epitopes were less frequently reactive than VS-C-1 or VS-C-2 (**Table 3**). OspC, a highly immunogenic *B. burgdorferi* lipoprotein, is the closest homolog to Vsp.

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Both proteins have a high degree of homology within the N terminal region. We and others previously identified a reactive epitope within this region of OspC (39, 41, 42). The alignment of Vsp1 (AGS80212) with OspC (CAA59253) revealed that the VS-C-1 and OspC epitopes do not overlap and map to non-homologous portions of these lipoproteins (**Figure 4**).

Reactivity to Vlp peptides. Vlps of relapsing fever *Borrelia* are typically 330 aa to 350 aa in length and are classified into α, β, δ, and γ sub-families (38). The N-terminal 70 aa to 120 aa fragments are conserved within each subfamily, whereas the remaining portions display a substantially greater heterogeneity in aa sequence. *B. miyamotoi* encodes multiple α, δ, and γ alleles and only a single putative β-like allele. To account for this genomic diversity, the TBD-Serochip includes peptides for 33 full length and 26 partial α, δ, and γ homologs from multiple *B. miyamotoi* strains (**Table 1**).

All 7 tested sera reacted with a wide range of peptides from Vlps. Although IgM reactivity was predominant, several Vlp regions were also reactive with IgG. Overall, the largest number of Vlp-reactive peptides mapped to δ Vlps. **Figure 5** displays the location of all reactive peptides mapped to Vlp15/16 (accession number ALM31565), a Vlp homolog with the highest number of immunoreactive epitopes. A δ Vlp-specific region, located between aa 8-24 of Vlp15/16, reacted with all seven BMD sera (**Figure 5**). We designated this region Vlpδ-C-1 (**Table 4**). Six sera were IgM-positive to Vlpδ-C-1, including two (samples 1 and 3) that were also IgG-positive. One serum (sample 2) was reactive with only IgG. Six sera were reactive with another reactive region designated Vlpδ-C-2. Two sera were reactive with IgM and IgG, two with IgM, and two with IgG. Vlp-C-2 was mapped to aa 51-66 of Vlp15/16, a fragment that is also partially conserved in

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231 α, δ, and γ Vlps. γ Vlps peptides corresponding to this region (designated Vlpγ-C-2) were
232 reactive with four sera (all IgG) (**Figure 6, Table 3**). α peptides (Vlpα-C2) were reactive
233 with IgG from three sera (**Table 3**).
234
235 Only one region within the variable fragment of δ Vlps was reactive with all seven sera.
236 This region, mapped to aa 222-240 of Vlp 15/16, was designated VlpδV-1. In three sera,
237 the reactive fragment extended approximately 25 aa downstream, and overlapped with a
238 conserved region that is homologous to the C6 peptide of *B. burgdorferi* (**Figure 7A**).
239 VlpδV-1 corresponds to a poorly conserved fragment in Vlps, even within paralogs of the
240 same subfamily (**Figure 7A**). Nonetheless, all seven sera were reactive to a wide range
241 of non-homologous peptides from different δ Vlp homologs that mapped to this region
242 (**Figure 7B**). Although the corresponding peptides from Vlp α and Vlp γ have limited
243 homology to VlpδV-1 peptides from Vlp δ, 4 sera also had reactivity to Vlp γ peptides and
244 3 sera reacted with Vlp α peptides from within this region. Our results indicate that the
245 region corresponding to Vlp-V-1 in Vlps represents a major immunogenic region within
246 *B. miyamotoi* Vlps, irrespective of sequence similarity.
247
248 ***Crossreactivity of the C6 peptides.*** VlsE, the closest homolog to Vlp, is a major
249 immunodominant *B. burgdorferi* lipoprotein and includes a 26 aa C6 epitope that is
250 employed in a peptide ELISA for Lyme disease diagnosis. Recent studies have reported
251 cross-reactivity in the Lyme disease C6 ELISA with sera from BMD patients (20, 26).
252 The similarity between a C6-like region in *B. miyamotoi* and the C6 was cited as a
253 potential cause. Comparison of *B. burgdorferi* C6 and corresponding homologous Vlp
254 sequences indicate that some δ Vlp15/16 homologs share 20 out of 26 aa residues with
255 the C6 epitope (**Figure 7A**). The homology is most pronounced at the C terminal portion,

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with 14 out of 15 identical residues in both peptides. To more clearly delineate antibody responses to these two fragments, we compared the TBD-Serochip data to results obtained with a commercial C6 ELISA. Of the seven BMD sera tested, four samples (2, 5, 6, and 7) were positive on the TBD-Serochip for reactivity to the *B. miyamotoi* C6-like peptides (**Figure 8**). The same 4 samples, along with sample 3, had reactivity to the *B. burgdorferi* C6 peptides and all were also positive on the C6 ELISA. Sera 1 and 4 were negative with both assays. Serum 3 was positive for *B. burgdorferi* with the C6 ELISA but did not react with *B. miyamotoi* peptides on the TBD-Serochip. This discrepancy was likely due to past exposure to *B. burgdorferi*. Samples 3 and 5 both contained IgG antibodies to the C6 peptides from a prior infection with *B. burgdorferi.* These antibodies were specific for *B. burgdorferi* peptides and did not crossreact with the corresponding peptides of *B. miyamotoi* **(Figure 8)**. Serum 3 did not contain antibodies to the *B. miyamotoi* C6-like region; thus the positive result on the *B. burgdorferi* ELISA and TBD-Serochip result was likely exclusively triggered by anti-*B. burgdorferi* antibodies. Serum 5 had IgG antibodies to *B. burgdorferi*, and IgM antibodies to *B. miyamotoi* C6-like peptides. Sera 2, 6 and 7 had IgM or IgG antibodies to the *B. miyamotoi* C6-like peptides; they also reacted with the highly conserved C-terminal portion of the *B. burgdorferi* C6 and were the likely cause of the positive result on the C6 ELISA.

In summary, only 4 of the 7 patients with a history of *B. miyamotoi* infection had antibodies to the *B. miyamotoi* C6-like peptide. This is in contrast to the C6 which is one of the most frequently reactive linear peptides in *B. burgdorferi.* However, when they are present, the antibodies to the *B. miyamotoi* C6-like peptide will likely crossreact on the C6 ELISA.

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FlaB. FlaB was the most reactive non-Vmp antigen. IgM antibodies to FlaB were detected in six out of seven BMD sera tested. FlaB comprises the major component of the spirochete flagellum and is among the most immunogenic of all *Borrelia* antigens (43, 44). However, its utility in diagnosis is compromised by its high cross-reactivity (45). FlaB bands can be recorded on both IgM and IgG Lyme disease western blots even in specimens from healthy individuals (46). FlaB is also highly conserved amongst *Borrelia,* with *B. miyamotoi* and *B. burgdorferi* FlaB sharing 90% aa identity. This further limits its utility for differential diagnosis. In tests of BMD or Lyme disease sera, we detected reactivity to a wide range of corresponding FlaB 12-mer peptides from both *B. miyamotoi* and *B. burgdorferi* (**Figure 9 A and B**). The same was true when we tested sera from patients with Lyme disease. We mapped the primary reactive portion of FlaB to a 45 aa fragment located between aa 192 and 236 of *B. miyamotoi* (accession number AHH05270) and its corresponding region in *B. burgdorferi* located between aa 190 and 236 (accession number AAC66541) (**Figure 9 C**). We found this region to be among the most frequently reactive peptide fragments when testing sera from Lyme disease or BMD. Despite this high reactivity, the majority of 12-mer peptides within these fragments were occasionally cross-reactive with IgM antibodies present in sera from healthy individuals. Nonetheless, we identified a 14 aa fragment within this region that was reactive only with BMD and Lyme disease sera but not with control sera. This peptide, AQEGAQQEGVQAVP, was located within aa 210 and 223 of *B. miyamotoi* FlaB. The corresponding 13 aa peptide in *B. burgdorferi* VQEGVQQEGAQQP was located within aa 211 and 223. Although these peptides cannot be used to discriminate between *B. miyamotoi* and *B. burgdorferi*, they may have utility for diagnosis of *Borrelia* infections.

GlpQ. We did not identify a specific epitope for GlpQ. To determine whether this was due to the absence of anti-GlpQ antibodies, we established a LIPS assay using a full

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length GlpQ as a target antigen. We tested 5 samples and IgG antibodies were present in 3 sera. Our combined TBD-Serochip and LIPS data suggests that these 3 samples contained anti-GlpQ antibodies to conformational but not linear epitopes. We propose that detection of antibodies to conformational epitopes likely constitutes the primary means of GlpQ serologic detection.

We did not identify unique epitopes on the remaining non-Vmp antigens. Although reactive peptides were detected, they were not consistently reactive among the samples tested.

Additional specimens. We analyzed three additional sera from patients who had received a BMD diagnosis based on a positive index on a GlpQ ELISA from a commercial laboratory. Upon testing these specimens on the TBD-Serochip, we did not observe reactivity to any *B. miyamotoi* antigens. When we examined these by our LIPS assay, one sample had a very low positive reading and the remaining two were negative.

Discussion

Lack of standardized assays, coupled with limited understanding of optimal target antigens contribute to the challenge of serologic diagnosis of TBD (47-49). The identification of superior targets, particularly of immunodominant specific epitopes has the potential to improve serodiagnosis. Among the primary challenges of differential serologic diagnosis of BMD is the insufficient sensitivity of GlpQ and the antigenic similarities between *B. miyamotoi* and Lyme borreliosis *Borrelia*. Through accurate mapping of specific linear immunoreactive peptides, the TBD-Serochip provides an unparalleled opportunity for identification of agent-specific linear epitopes that could facilitate differential diagnosis. In this study we used the TBD-Serochip to identify

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peptides that can potentially serve as diagnostic targets for *B. miyamotoi* and differentiate between patients with Lyme disease and BMD.

The most promising candidate diagnostic peptide targets were found on flagella and Vmps, both well-known antigens within relapsing fever *Borreliae*. Flagellar proteins are among the most immunogenic components in spirochetes, highlighted by their inclusion as diagnostic targets in western blot assays for Lyme disease. In previous work, we identified an immunoreactive 13 aa peptide within *B. burgdorferi* FlaB with high diagnostic utility. We subsequently found that this peptide, along with the C6 fragment, were the most frequently reactive *B. burgdorferi* linear peptides in patients with Lyme disease. In addition, we observed that both peptides are often reactive in patients where reactivity to other linear peptides was not detectable. The diagnostic utility of this FlaB fragment extends to *B. miyamotoi,* as the corresponding 14 aa peptide in *B. miyamotoi* was reactive of all samples tested. Although its utility for differential diagnosis is partially diminished by the inability to distinguish between Lyme disease and BMD sera, we found this peptide to be potentially highly useful for diagnosis of *Borrelia* infections, both in early disease (with IgM) or later disease and convalescence (with IgG).

The majority of immunoreactive epitopes identified in our study were located on Vmps (32, 50, 51). Vmps have been shown to be key antigens for neutralization in *Borrelia hermsii* (50). Recent studies in mice have shown the potential of Vsps as a possible target for diagnostic serologic assays for BMD (32). In our study, the majority of reactive peptides were mapped to the variable portions of Vsps and Vlps, but we also identified reactive regions within the conserved protein fragments. Because of higher degree of aa conservation, these reactive peptides could have greater utility for BMD diagnosis.

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Our work supports findings from previous studies that reported the C6 ELISA cannot effectively discriminate between antibodies to *B. burgdorferi* and *B. miyamotoi* (20, 26). We also demonstrate that the corresponding C6-like peptide in *B. miyamotoi* is the likely cause of crossreactive signals. Our findings also raise concerns about the specificity of GlpQ as a diagnostic antigen. Three sera that were positive on the GlpQ ELISA did not have reactivity with *B. miyamotoi* peptides in the TBD Serochip assay. We cannot explain the positive commercial ELISA results in the two samples that were negative on the TBD-Serochip and by LIPS. One ELISA-positive sample had a low positive result by LIPS but not the TBD-Serochip. We consider it very unlikely that this patient would have antibodies to *B. miyamotoi* GlpQ but not to any other immunodominant antigens. We conclude that the positive result may have been due to cross-reacting antibodies. Although GlpQ is not present in *B. burgdorferi*, this enzyme is present in a wide range of bacteria, and in some cases, shares substantial aa sequence similarity. For example, the *Escherichia coli* GlpQ shares 49% aa identity with *B. miyamotoi* GlpQ, and in one 39 aa stretch, 34 aa are identical in the two proteins. Thus, it is plausible that in some instances, antibodies to GlpQ from other bacteria may react with *B. miyamotoi* GlpQ ELISA. We suspect that GlpQ specificity may need further examination.

A limitation of our study is that we analyzed a limited number of specimens with confirmed BMD. This was due to the fact that BMD diagnosis is rare and results in a paucity of well-characterized BMD specimens. Also the timing of the convalescent serum samples post treatment might have impacted our findings. Nevertheless, we observed a similar pattern of reactivity from all samples and anticipate that we identified the major reactive *B. miyamotoi* peptides. Although at present the TBD-Serochip is not yet employed for patient serodiagnosis, these peptides can be ported to other serologic platforms that are typically used in clinical laboratories. We utilized a similar approach for

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385 the development of a diagnostic assay for Zika virus (52). We anticipate that the panel of
386 peptides we identified in this work can build the foundation of future studies examining
387 the utility of these epitopes for the specific diagnosis of BMD.
388
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396
397 **Disclosures:**
398 Drs. Tokarz, Mishra, and Lipkin are listed as inventors in patent application No.
399 62/848,701, that covers the diagnostic peptides identified on the TBD-Serochip.
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401 Systems Biology, Rarecyte, Inc., and Quidel Corporation. He owns equity in
402 Abbott/AbbVie; has been an expert witness in malpractice cases involving Lyme disease
403 and babesiosis; and is an unpaid board member of the American Lyme Disease
404 Foundation.
405 Dr. Marques is a coinventor on U.S. patent 8,926,98, which uses the Luciferase
406 Immunoprecipitation System to evaluate antibody responses to the synthetic VOVO
407 polypeptide, derived from VlsE and OspC antigens. Dr. Marques is an unpaid scientific
408 board member of the Global Lyme Alliance and the American Lyme Disease
409 Foundation.
410

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412 policies of the Department of Health and Human Services, nor does mention of trade
413 names, commercial products, or organizations imply endorsement by the U.S.
414 Government.
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418 Figure Legends
419
420 **Figure 1**. **Identification of reactive epitopes within the conserved region of Vsps**.
421 Panel A displays the alignment of five *B. miyamotoi* Vsp homologs. Regions of homology
422 are shown in black. The numbers on top of the alignment denote the relative aa position
423 of each protein relative to Vsp1. The location of the consensus reactive epitope VS-C-1
424 and its aa sequence are indicated. Panels B and C show IgM reactivity plots of Vsp1
425 (accession number AGS80212) and Vsp4 (accession number ALU64345), respectively.
426 Numbers 1 to 7 represent the BMD samples. Reactivity to control sera (from a healthy
427 individual and various stages of Lyme disease) are shown on the right. The Y axis
428 represents the location of 12-mer peptides positioned along the contiguous protein
429 sequence of Vsp1 and Vsp4. Immunoreactivity with the 12-mer peptides is indicated in
430 green, with darker color corresponding to increasing reactivity. The location of VS-C-1
431 (samples 2, 4, 5, 6 and 7) and its corresponding 12-mer peptide sequences are
432 indicated in the bracket on the left in panels B and C.
433
434 **Figure 2**. **Conservation of the VS-C-1 peptide in *B. miyamotoi***. Shown is the aa
435 alignment of all Vsp sequences deposited in Genbank as of June 28, 2019. Accession

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436 numbers are indicated on the left. Location of the VS-C-1 peptide is shown in red. The
437 numbers on top denote the aa position within Vsp1.
438
439 **Figure 3. Vsp diversity and reactivity to the Vsp-C-1 epitope.** Each panel represents
440 the reactivity of the seven tested sera (labeled 1-7) to the peptides from Vsp homologs
441 present on the TBD-Serochip. The variant Vsp 12-mer peptide sequences are shown on
442 the left; accession numbers are indicated on the right. Amino acid differences to the
443 consensus sequence (based on Vsp1) are displayed in red. Immunoreactivity to the 12-
444 mer peptides is indicated in green with increasing signal intensity displayed from light to
445 dark.
446
447 **Figure 4. Locations of major immunogenic linear peptides in Vsp and OspC**. The N
448 terminal portions of Vsp1 and OspC were aligned to contrast the reactive portions of
449 OspC (in blue) and Vsp (in red).
450
451 **Figure 5. Identification of reactive peptides within Vlp δ.** Shown is the IgM and IgG
452 reactivity map displaying reactive 12-mer peptides (in green) of Vlp 15/16 (accession
453 number ALM31565). Numbers 1 through 7 represent the 7 BMD sera. Reactivity to
454 control sera (healthy individual from a Lyme endemic area and a patient with Lyme
455 disease at convalescence) are shown on the right. The numbers on the Y-axis represent
456 the aa location of the 12-mer peptides positioned along the contiguous protein sequence
457 of Vlp 15/16. Regions with immunoreactive 12-mer peptides are indicated in green with
458 increasing signal intensity displayed from light to dark. The asterisks indicate major
459 reactive epitopes; * Vlpδ-C-1, ** Vlpδ-C-2, *** Vlpδ-V-1, **** C6-like.
460

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Figure 6. Identification of reactive peptides within Vlp γ. Shown is the IgM and IgG reactivity map displaying reactive 12-mer peptides (in green) of Vlp5 (accession number AHH06031). Numbers 1 through 7 represent the 7 BMD sera. Reactivity to control sera (healthy individual from a Lyme endemic area and a patient with Lyme disease at convalescence) are shown on the right. The numbers on the Y-axis represent the aa location of the 12-mer peptides positioned along the contiguous protein sequence of Vlp 5. Regions with immunoreactive 12-mer peptides are indicated in green with increasing signal intensity displayed from light to dark. The asterisks indicate major reactive epitopes; * Vlpγ-C-3, ** Vlpγ-V-2, *** Vlpγ-V-1

Figure 7. **Sequence heterogeneity does not impact the reactivity to Vlpδ-V-1.** Panel A - alignment of *B. miyamotoi* Vlp homologs with *B. burgdorferi* VlsE. The position of Vlpδ-V-1 reactive region is indicated in red, and the *B. burgdorferi* C6 epitope in blue. Panel B - Signal intensity of the 12-mer peptides representing Vlpδ-V-1 sequence variants. * - maximum intensity.

Figure 8. Immunoreactivity comparison of the *B. burgdorferi* C6 and the C6-like peptide of *B. miyamotoi*. Panel A displays the reactivity maps of the 12-mer peptides that constitute the 26 aa C6-like region of *B. miyamotoi*. Panel A shows the corresponding C6 12-mer peptides from *B. burgdorferi*. The individual 12-mer peptides are indicated on the Y axis. Regions with immunoreactive 12-mer peptides are displayed in green with increasing signal intensity displayed from light to dark.

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Figure 9. Reactivity to FlaB. Reactivity plots from a convalescent-phase Lyme disease serum (panel A) and BMD serum (Panel B). The reactive regions (in green) are indicated on the contiguous protein sequence of *B. burgdorferi* (accession number AAB36994-left) and *B. miyamotoi* (accession number AAT99442-right). Panel C shows a close up of the primary reactive region, located between 190-220 on both antigens. Panel D displays an alignment of the primary reactive region, with the *Borrelia* specific epitope indicated in red.

BIBILOGRAPHY

1. **Platonov AE, Karan LS, Kolyasnikova NM, Makhneva NA, Toporkova MG, Maleev VV, Fish D, Krause PJ.** 2011. Humans infected with relapsing fever spirochete Borrelia miyamotoi, Russia. Emerg Infect Dis **17:**1816-1823.

2. **Sato K, Takano A, Konnai S, Nakao M, Ito T, Koyama K, Kaneko M, Ohnishi M, Kawabata H.** 2014. Human infections with Borrelia miyamotoi, Japan. Emerg Infect Dis **20:**1391-1393.

3. **Molloy PJ, Telford SR, 3rd, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, Hewins ME, Goethert HK, Berardi VP.** 2015. Borrelia miyamotoi Disease in the Northeastern United States: A Case Series. Ann Intern Med **163:**91-98.

4. **Cochez C, Heyman P, Heylen D, Fonville M, Hengeveld P, Takken W, Simons L, Sprong H.** 2015. The Presence of Borrelia miyamotoi, A Relapsing Fever Spirochaete, in Questing Ixodes ricinus in Belgium and in The Netherlands. Zoonoses Public Health **62:**331-333.

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5. **Kadkhoda K, Dumouchel C, Brancato J, Gretchen A, Krause PJ.** 2017. Human seroprevalence of Borrelia miyamotoi in Manitoba, Canada, in 2011-2014: a cross-sectional study. CMAJ Open **5:**E690-E693.

6. **Fukunaga M, Takahashi Y, Tsuruta Y, Matsushita O, Ralph D, McClelland M, Nakao M.** 1995. Genetic and phenotypic analysis of Borrelia miyamotoi sp. nov., isolated from the ixodid tick Ixodes persulcatus, the vector for Lyme disease in Japan. Int J Syst Bacteriol **45:**804-810.

7. **Scoles GA, Papero M, Beati L, Fish D.** 2001. A relapsing fever group spirochete transmitted by Ixodes scapularis ticks. Vector Borne Zoonotic Dis **1:**21-34.

8. **Mun J, Eisen RJ, Eisen L, Lane RS.** 2006. Detection of a Borrelia miyamotoi sensu lato relapsing-fever group spirochete from Ixodes pacificus in California. J Med Entomol **43:**120-123.

9. **Kiewra D, Stanczak J, Richter M.** 2014. Ixodes ricinus ticks (Acari, Ixodidae) as a vector of Borrelia burgdorferi sensu lato and Borrelia miyamotoi in Lower Silesia, Poland--preliminary study. Ticks Tick Borne Dis **5:**892-897.

10. **Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP.** 1982. Lyme disease-a tick-borne spirochetosis? Science **216:**1317-1319.

11. **Tokarz R, Jain K, Bennett A, Briese T, Lipkin WI.** 2010. Assessment of polymicrobial infections in ticks in New York state. Vector Borne Zoonotic Dis **10:**217-221.

12. **Lee X, Coyle DR, Johnson DK, Murphy MW, McGeehin MA, Murphy RJ, Raffa KF, Paskewitz SM.** 2014. Prevalence of Borrelia burgdorferi and Anaplasma phagocytophilum in Ixodes scapularis (Acari: Ixodidae) nymphs collected in managed red pine forests in Wisconsin. J Med Entomol **51:**694-701.

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13. **Venclikova K, Betasova L, Sikutova S, Jedlickova P, Hubalek Z, Rudolf I.** 2014. Human pathogenic borreliae in Ixodes ricinus ticks in natural and urban ecosystem (Czech Republic). Acta Parasitol **59:**717-720.

14. **Graham CB, Pilgard MA, Maes SE, Hojgaard A, Eisen RJ.** 2016. Paired real-time PCR assays for detection of Borrelia miyamotoi in North American Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae). Ticks Tick Borne Dis **7:**1230-1235.

15. **Tokarz R, Tagliafierro T, Cucura DM, Rochlin I, Sameroff S, Lipkin WI.** 2017. Detection of Anaplasma phagocytophilum, Babesia microti, Borrelia burgdorferi, Borrelia miyamotoi, and Powassan Virus in Ticks by a Multiplex Real-Time Reverse Transcription-PCR Assay. mSphere **2**.

16. **Oda R, Kutsuna S, Sekikawa Y, Hongo I, Sato K, Ohnishi M, Kawabata H.** 2017. The first case of imported Borrelia miyamotoi disease concurrent with Lyme disease. J Infect Chemother **23:**333-335.

17. **Krause PJ, Fish D, Narasimhan S, Barbour AG.** 2015. Borrelia miyamotoi infection in nature and in humans. Clin Microbiol Infect **21:**631-639.

18. **Telford SR, 3rd, Goethert HK, Molloy PJ, Berardi VP, Chowdri HR, Gugliotta JL, Lepore TJ.** 2015. Borrelia miyamotoi Disease: Neither Lyme Disease Nor Relapsing Fever. Clin Lab Med **35:**867-882.

19. **Wagemakers A, Staarink PJ, Sprong H, Hovius JW.** 2015. Borrelia miyamotoi: a widespread tick-borne relapsing fever spirochete. Trends Parasitol **31:**260-269.

20. **Molloy PJ, Weeks KE, Todd B, Wormser GP.** 2018. Seroreactivity to the C6 Peptide in Borrelia miyamotoi Infections Occurring in the Northeastern United States. Clin Infect Dis **66:**1407-1410.

21. **Wormser GP, Shapiro ED, Fish D.** 2019. Borrelia miyamotoi: An Emerging Tick-Borne Pathogen. Am J Med **132:**136-137.

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561 22. **Nelder MP, Russell CB, Sheehan NJ, Sander B, Moore S, Li Y, Johnson S,**
562 **Patel SN, Sider D.** 2016. Human pathogens associated with the blacklegged tick
563 Ixodes scapularis: a systematic review. Parasit Vectors **9:**265.
564 23. **Lommano E, Bertaiola L, Dupasquier C, Gern L.** 2012. Infections and
565 coinfections of questing Ixodes ricinus ticks by emerging zoonotic pathogens in
566 Western Switzerland. Appl Environ Microbiol **78:**4606-4612.
567 24. **Diuk-Wasser MA, Vannier E, Krause PJ.** 2016. Coinfection by Ixodes Tick-
568 Borne Pathogens: Ecological, Epidemiological, and Clinical Consequences.
569 Trends Parasitol **32:**30-42.
570 25. **Krause PJ, Narasimhan S, Wormser GP, Barbour AG, Platonov AE,**
571 **Brancato J, Lepore T, Dardick K, Mamula M, Rollend L, Steeves TK, Diuk-**
572 **Wasser M, Usmani-Brown S, Williamson P, Sarksyan DS, Fikrig E, Fish D,**
573 **Tick Borne Diseases G.** 2014. Borrelia miyamotoi sensu lato seroreactivity and
574 seroprevalence in the northeastern United States. Emerg Infect Dis **20:**1183-
575 1190.
576 26. **Sudhindra P, Wang G, Schriefer ME, McKenna D, Zhuge J, Krause PJ,**
577 **Marques AR, Wormser GP.** 2016. Insights into Borrelia miyamotoi infection from
578 an untreated case demonstrating relapsing fever, monocytosis and a positive C6
579 Lyme serology. Diagn Microbiol Infect Dis **86:**93-96.
580 27. **Jahfari S, Sarksyan DS, Kolyasnikova NM, Hovius JW, Sprong H, Platonov**
581 **AE.** 2017. Evaluation of a serological test for the diagnosis of Borrelia miyamotoi
582 disease in Europe. J Microbiol Methods **136:**11-16.
583 28. **Schwan TG, Schrumpf ME, Hinnebusch BJ, Anderson DE, Jr., Konkel ME.**
584 1996. GlpQ: an antigen for serological discrimination between relapsing fever
585 and Lyme borreliosis. J Clin Microbiol **34:**2483-2492.

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29. **Halperin T, Orr N, Cohen R, Hasin T, Davidovitch N, Klement E, Kayouf R, Baneth G, Cohen D, Yavzori M.** 2006. Detection of relapsing fever in human blood samples from Israel using PCR targeting the glycerophosphodiester phosphodiesterase (GlpQ) gene. Acta Trop **98:**189-195.

30. **Bacon RM, Pilgard MA, Johnson BJ, Raffel SJ, Schwan TG.** 2004. Glycerophosphodiester phosphodiesterase gene (glpQ) of Borrelia lonestari identified as a target for differentiating Borrelia species associated with hard ticks (Acari:Ixodidae). J Clin Microbiol **42:**2326-2328.

31. **Hamase A, Takahashi Y, Nohgi K, Fukunaga M.** 1996. Homology of variable major protein genes between Borrelia hermsii and Borrelia miyamotoi. FEMS Microbiol Lett **140:**131-137.

32. **Wagemakers A, Koetsveld J, Narasimhan S, Wickel M, Deponte K, Bleijlevens B, Jahfari S, Sprong H, Karan LS, Sarksyan DS, van der Poll T, Bockenstedt LK, Bins AD, Platonov AE, Fikrig E, Hovius JW.** 2016. Variable Major Proteins as Targets for Specific Antibodies against Borrelia miyamotoi. J Immunol **196:**4185-4195.

33. **Stone BL, Brissette CA.** 2017. Host Immune Evasion by Lyme and Relapsing Fever Borreliae: Findings to Lead Future Studies for Borrelia miyamotoi. Front Immunol **8:**12.

34. **Barbour AG, Restrepo BI.** 2000. Antigenic variation in vector-borne pathogens. Emerg Infect Dis **6:**449-457.

35. **Hayes LJ, Wright DJ, Archard LC.** 1988. Segmented arrangement of Borrelia duttonii DNA and location of variant surface antigen genes. J Gen Microbiol **134:**1785-1793.

36. **Barbour AG.** 1990. Antigenic variation of a relapsing fever Borrelia species. Annu Rev Microbiol **44:**155-171.

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37. **Carter CJ, Bergstrom S, Norris SJ, Barbour AG.** 1994. A family of surface-exposed proteins of 20 kilodaltons in the genus Borrelia. Infect Immun **62:**2792-2799.

38. **Barbour AG.** 2016. Multiple and Diverse vsp and vlp Sequences in Borrelia miyamotoi, a Hard Tick-Borne Zoonotic Pathogen. PLoS One **11:**e0146283.

39. **Cadavid D, Pennington PM, Kerentseva TA, Bergstrom S, Barbour AG.** 1997. Immunologic and genetic analyses of VmpA of a neurotropic strain of Borrelia turicatae. Infect Immun **65:**3352-3360.

40. **Tokarz R, Mishra N, Tagliafierro T, Sameroff S, Caciula A, Chauhan L, Patel J, Sullivan E, Gucwa A, Fallon B, Golightly M, Molins C, Schriefer M, Marques A, Briese T, Lipkin WI.** 2018. A multiplex serologic platform for diagnosis of tick-borne diseases. Sci Rep **8:**3158.

41. **Kumru OS, Schulze RJ, Rodnin MV, Ladokhin AS, Zuckert WR.** 2011. Surface localization determinants of Borrelia OspC/Vsp family lipoproteins. J Bacteriol **193:**2814-2825.

42. **Zuckert WR, Kerentseva TA, Lawson CL, Barbour AG.** 2001. Structural conservation of neurotropism-associated VspA within the variable Borrelia Vsp-OspC lipoprotein family. J Biol Chem **276:**457-463.

43. **Barbour AG, Hayes SF, Heiland RA, Schrumpf ME, Tessier SL.** 1986. A Borrelia-specific monoclonal antibody binds to a flagellar epitope. Infect Immun **52:**549-554.

44. **Dressler F, Whalen JA, Reinhardt BN, Steere AC.** 1993. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis **167:**392-400.

45. **Luft BJ, Dunn JJ, Dattwyler RJ, Gorgone G, Gorevic PD, Schubach WH.** 1993. Cross-reactive antigenic domains of the flagellin protein of Borrelia burgdorferi. Res Microbiol **144:**251-257.

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638 46. **Moore A, Nelson C, Molins C, Mead P, Schriefer M.** 2016. Current Guidelines,
639 Common Clinical Pitfalls, and Future Directions for Laboratory Diagnosis of Lyme
640 Disease, United States. Emerg Infect Dis **22**.
641 47. **Marques AR.** 2015. Laboratory diagnosis of Lyme disease: advances and
642 challenges. Infect Dis Clin North Am **29:**295-307.
643 48. **Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, Drexler NA, Dumler JS,**
644 **Folk SM, Kato CY, Lash RR, Levin ML, Massung RF, Nadelman RB,**
645 **Nicholson WL, Paddock CD, Pritt BS, Traeger MS.** 2016. Diagnosis and
646 Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever
647 and Other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis -
648 United States. MMWR Recomm Rep **65:**1-44.
649 49. **Krause PJ, Carroll M, Fedorova N, Brancato J, Dumouchel C, Akosa F,**
650 **Narasimhan S, Fikrig E, Lane RS.** 2018. Human Borrelia miyamotoi infection in
651 California: Serodiagnosis is complicated by multiple endemic Borrelia species.
652 PLoS One **13:**e0191725.
653 50. **Barbour AG, Bundoc V.** 2001. In vitro and in vivo neutralization of the relapsing
654 fever agent Borrelia hermsii with serotype-specific immunoglobulin M antibodies.
655 Infect Immun **69:**1009-1015.
656 51. **Connolly SE, Benach JL.** 2001. Cutting edge: the spirochetemia of murine
657 relapsing fever is cleared by complement-independent bactericidal antibodies. J
658 Immunol **167:**3029-3032.
659 52. **Mishra N, Caciula A, Price A, Thakkar R, Ng J, Chauhan LV, Jain K, Che X,**
660 **Espinosa DA, Montoya Cruz M, Balmaseda A, Sullivan EH, Patel JJ, Jarman**
661 **RG, Rakeman JL, Egan CT, Reusken C, Koopmans MPG, Harris E, Tokarz**
662 **R, Briese T, Lipkin WI.** 2018. Diagnosis of Zika Virus Infection by Peptide Array
663 and Enzyme-Linked Immunosorbent Assay. MBio **9**.

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Table 1. List of *B. miyamotoi* sequences used to design 12-mer peptides for the TBD-Serochip

Vmp antigens	**Non-Vmp antigens**
Vsps **13 complete:** AGS80212, AHH0975, AHH0976, AHH05982, AHH06030, AJA67245, ALM21566, ALN43422, ALN43425, ALU64345, ALZ4557, WP_02444404, WP_044003917 **4 partial:** AHH05655, WP_025444233, WP_025444484, WP_025444486	**GlpQ**: ACT35309, AHM02192, AIK22387, ALU84911, ALU84913, WP_0209954631, WP_025443886 **FhbA:** AGS80215, WP_025444356 **ipA:** WP_025444145, WP_025444146 **P66:** AAC09059, AAC09061, AAC09063, ACC09064, AAR17598, AAV52738, AAV52742, AAV52744, AHH04809, AHH04811, WP_002557191, WP_002658111, WP_002662685, WP_002665258, WP_020954965, WP_024705620, WP_031541601, WP_031557381 **OppA2:** ACC46283, ACC34274, ADQ44805, AHH05084, WP_020954717, WP_020954718 **FlgG:** AGT27718, WP_020954670, WP_020955136, WP_02443450, WP_02443451, WP_043867901 **FlaB:** AAB37005, AAL99374, AAR00324, AAT99442, AGC773382, AMB26827, ACO38653, AHI10982, AIK22386, AML32120, ACP11868, BAS29553, CAA49308, CAA49322, CAA49315, CAA49318, CAA34735, CAA4931, CAA53011, CAT00959, CAT00959, CAT00967, NP_212282, WP_002556748, WP_020954538, WP_020954528, **FliL:** WP_020954670
Vlps **33 complete:** AGS80213, AHH05904, ALM13565, ALM31567-ALM31568, ALN43424, ALU64347-ALU64349, ALU64350-ALU64352, ALZ45558, AHH05899, AHH06031,WP_02444234-WP_025444237, WP_025444399-WP_02444401, WP_02444405, WP_025444408, WP_025444446, WP_025444448, WP_025444450-WP_025444452, WP_025444482-WP_04403984, WP_051480340 **26 partial:** AGS80214, AHH05967, ALN43420, ALN43421, ALN43423, ALU64346, ALZ45552, AMW90882, AHH05648, AHH05649, AHH05654, AHH05892, AHH05998, AHHH05903, AHHH05905, AHH05966, AHH05968, AHH05969, AHH05976, AHH05977, AHH05979-AHH05981, AHH06035, WP_051480326, WP_051480341	

Table 2. BMD samples tested on the TBD-Serochip

Samples	BMD Diagnosis (acute stage)
BMD-1	PCR
BMD-2	PCR
BMD-3*	PCR
BMD-4	PCR
BMD-5*	PCR
BMD-6	PCR and GlpQ ELISA (IgM & IgG)
BMD-7	GlpQ ELISA (IgM & IgG)

*previous diagnosis of Lyme disease

Table 3. Major immunoreactive linear regions of *B. miyamotoi* Vmps identified with the TBD-Serochip.

ANTIGEN	EPITOPE DESIGNATION	EPITOPE SEQUENCE	COORDINATES	ACCESSION NUMBER	POSITIVE SERA	
Vsp1	VS-C-1	SKKIKDAVEFAANVKE	52-66	AGS80212	2, 4, 5, 6, 7	IgM
Vsp	VS-C-2	SKKIKDASELMESVKE	49-64	BAA11304	4, 5, 6, 7	IgM
Vsp	VS-C-3	IGKKIKNADELDTVAD	81-96	WP025444465	2, 3, 4, 5, 7	IgM
Vsp2	VS-C-4	DGTLDTLNNKNGSLL	85-88	ALM31566	2, 5, 6, 7	IgM
Vsp3	VS-V-1	ASKAFIDKVKGENASLG	115-131	ALN43425	2, 5, 6	IgM
Vsp4	VS-V-2	TKLEVLEKTAEISNELNGK	104-122	ALU64345	2, 4, 5	IgM
Vsp6S	VS-V-3	ASKAFIDKVKGENASLG	128-144	ALZ45557	2, 5, 6	IgM
Vsp	VS-V-4	DKSSDLGKNDVKDTDAKSAILT	128-149	WP025444486	3, 4, 5	IgM
Vlp δ	Vlpδ-C-1	GCNNGGGEDPQKFLTSI	8-24	ALM31565	1, 2, 3, 4, 5, 6, 7	IgM, IgG
Vlp δ	Vlpδ-C-2	KAETKKEDIGKYFADIEKTMTL	51-60	ALM31565	2, 3, 4, 6, 7	IgM, IgG
Vlp δ	Vlpδ-V-1	TDGIEKAKDAAEIAIAPAV	222-240	ALM31565	1, 2, 3, 4, 5, 6, 7	IgM, IgG
Vlp γ	Vlpγ-C-3	NSNTKKSDVGVYFKKV	47-62	AHH06031	1, 2, 5	IgM, IgG
Vlp γ	Vlpγ-V-2	LKEGKHDAGDDKKASDG	155-171	AHH06031	2, 4, 5, 7	IgM, IgG
Vlp γ	Vlpγ-V-1	VTGADILQAIVKDNGE	214-229	AHH06031	2, 4, 5, 6, 7	IgM, IgG
Vlp α	Vlpα-C2	KTTTKKNDVGVYFNSLG	72-77	AGS80213	2, 5, 7	IgM, IgG
Vlp α	Vlpα-V1	LKFAKGGSDAHLSNSAN	257-262	AGS80213	2, 5, 7	IgM, IgG

Table 4. Reactivity of the C6 peptides in *B. burgdorferi* and *B. miyamotoi*

BMD samples	Prior Lyme disease	Reactivity			
		TBD-Serochip		C6 ELISA	
		B. miyamotoi C6-like	B. burgdorferi C6		Cross reactivity on the C6 ELISA
1	-	-	-	-	-
2	+	+	+	+	+
3	-	-	+	+	-
4	-	-	-	-	-
5	+	+	+	+	-
6	-	+	+	+	+
7	-	+	+	+	+

+ indicates reactivity to the 12-mer peptides within the C6 or C6-like region

Figure 1

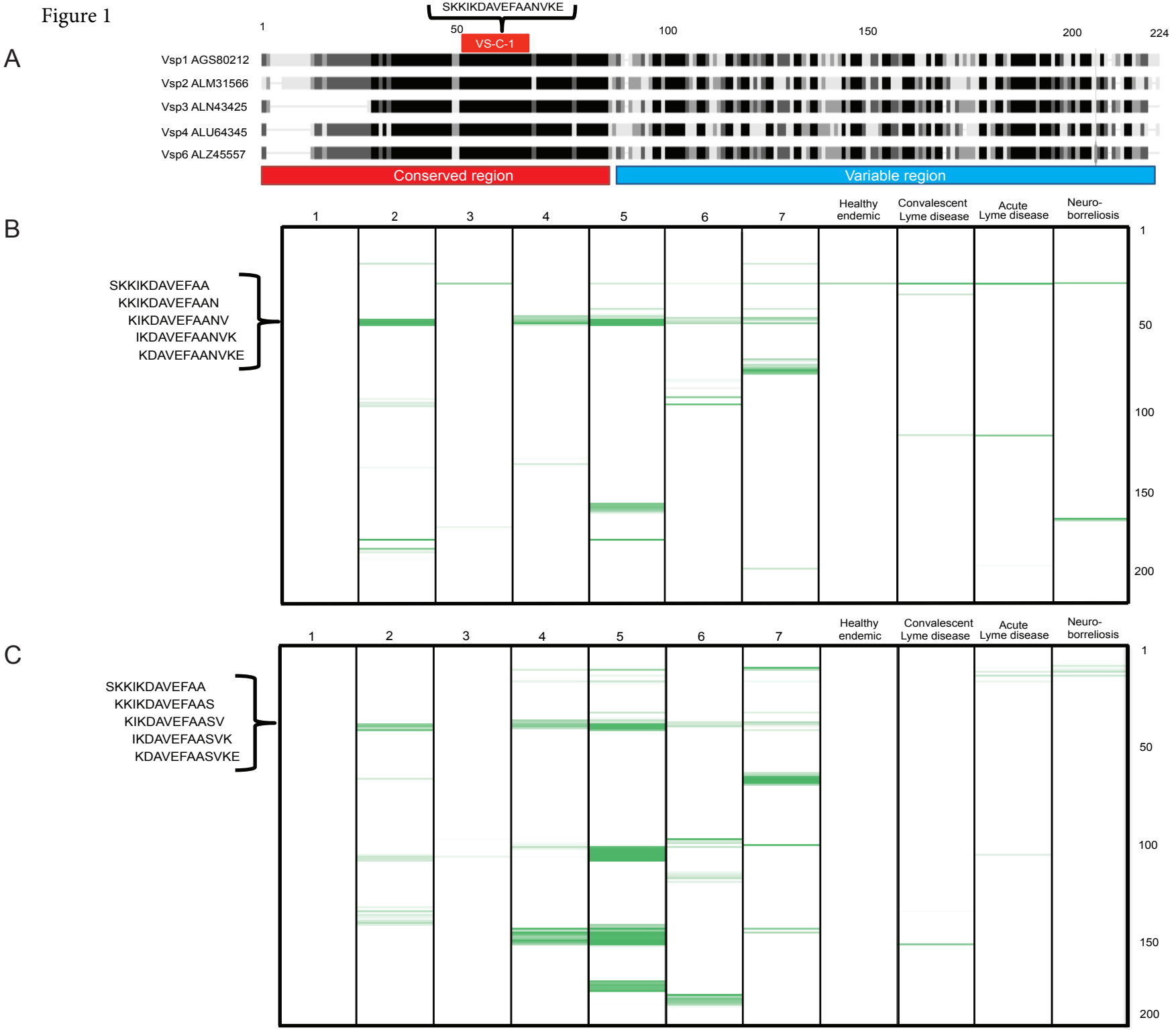

A
SKKIKDAVEFAANVKE
VS-C-1
1
50
100
150
200
224
Vsp1 AGS80212
Vsp2 ALM31566
Vsp3 ALN43425
Vsp4 ALU64345
Vsp6 ALZ45557
Conserved region
Variable region
B
1
2
3
4
5
6
7
Healthy endemic
Convalescent Lyme disease
Acute Lyme disease
Neuro-borreliosis
SKKIKDAVEFAA
KKIKDAVEFAAN
KIKDAVEFAANV
IKDAVEFAANVK
KDAVEFAANVKE
1
50
100
150
200
C
SKKIKDAVEFAA
KKIKDAVEFAAS
KIKDAVEFAASV
IKDAVEFAASVK
KDAVEFAASVKE

Figure 2

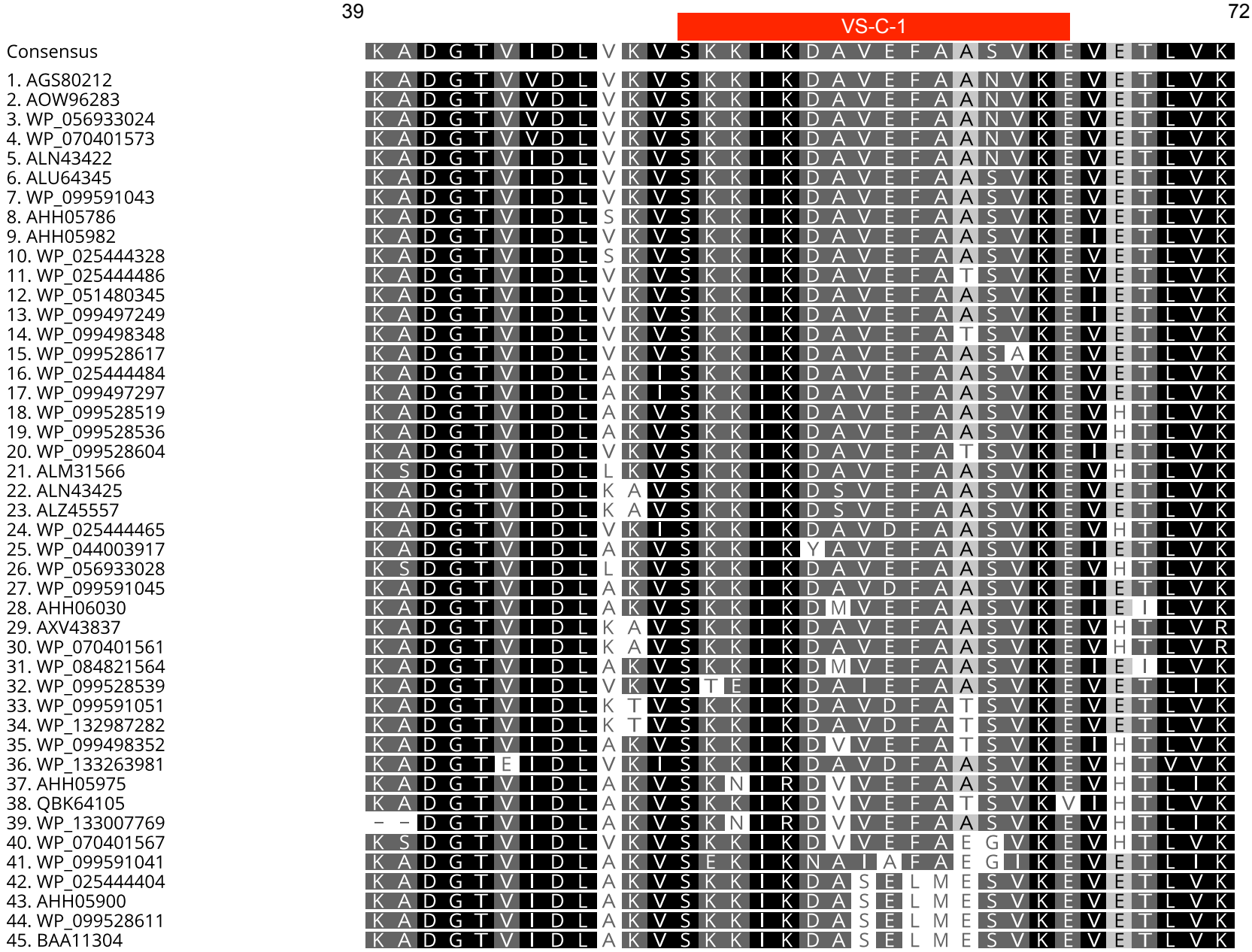

39
72
VS-C-1
Consensus
1. AGS80212
2. AOW96283
3. WP_056933024
4. WP_070401573
5. ALN43422
6. ALU64345
7. WP_099591043
8. AHH05786
9. AHH05982
10. WP_025444328
11. WP_025444486
12. WP_051480345
13. WP_099497249
14. WP_099498348
15. WP_099528617
16. WP_025444484
17. WP_099497297
18. WP_099528519
19. WP_099528536
20. WP_099528604
21. ALM31566
22. ALN43425
23. ALZ45557
24. WP_025444465
25. WP_044003917
26. WP_056933028
27. WP_099591045
28. AHH06030
29. AXV43837
30. WP_070401561
31. WP_084821564
32. WP_099528539
33. WP_099591051
34. WP_132987282
35. WP_099498352
36. WP_133263981
37. AHH05975
38. QBK64105
39. WP_133007769
40. WP_070401567
41. WP_099591041
42. WP_025444404
43. AHH05900
44. WP_099528611
45. BAA11304

Figure 3

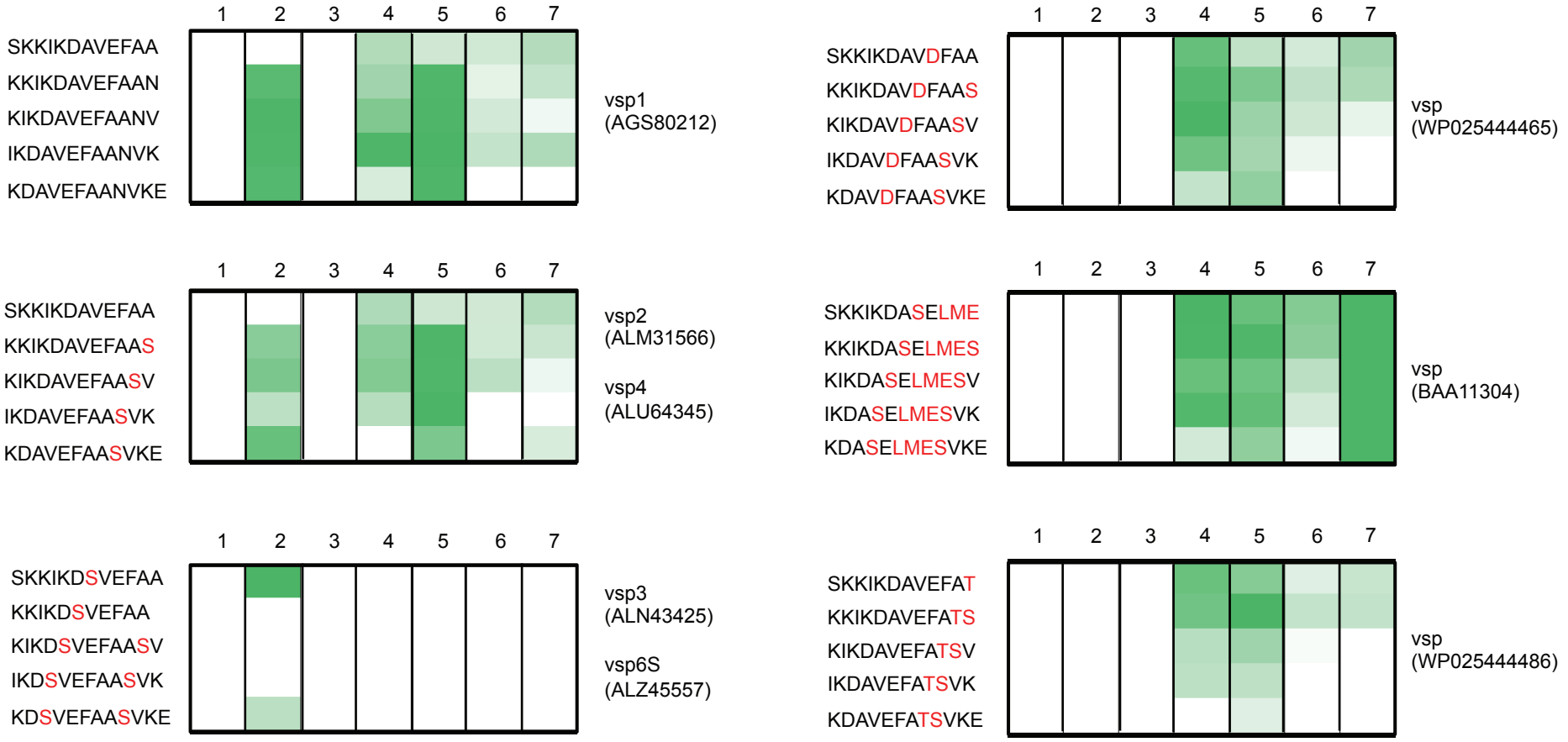

1 2 3 4 5 6 7
SKKIKDAVEFAA
KKIKDAVEFAAN
KIKDAVEFAANV
IKDAVEFAANVK
KDAVEFAANVKE
vsp1
(AGS80212)
SKKIKDAVDFAA
KKIKDAVDFAAS
KIKDAVDFAASV
IKDAVDFAASVK
KDAVDFAASVKE
vsp
(WP025444465)
SKKIKDAVEFAA
KKIKDAVEFAAS
KIKDAVEFAASV
IKDAVEFAASVK
KDAVEFAASVKE
vsp2
(ALM31566)
vsp4
(ALU64345)
SKKIKDASELME
KKIKDASELMES
KIKDASELMESV
IKDASELMESVK
KDASELMESVKE
vsp
(BAA11304)
SKKIKDSVEFAA
KKIKDSVEFAA
KIKDSVEFAASV
IKDSVEFAASVK
KDSVEFAASVKE
vsp3
(ALN43425)
vsp6S
(ALZ45557)
SKKIKDAVEFAT
KKIKDAVEFATS
KIKDAVEFATSV
IKDAVEFATSVK
KDAVEFATSVKE
vsp
(WP025444486)

Figure 4

Figure 5

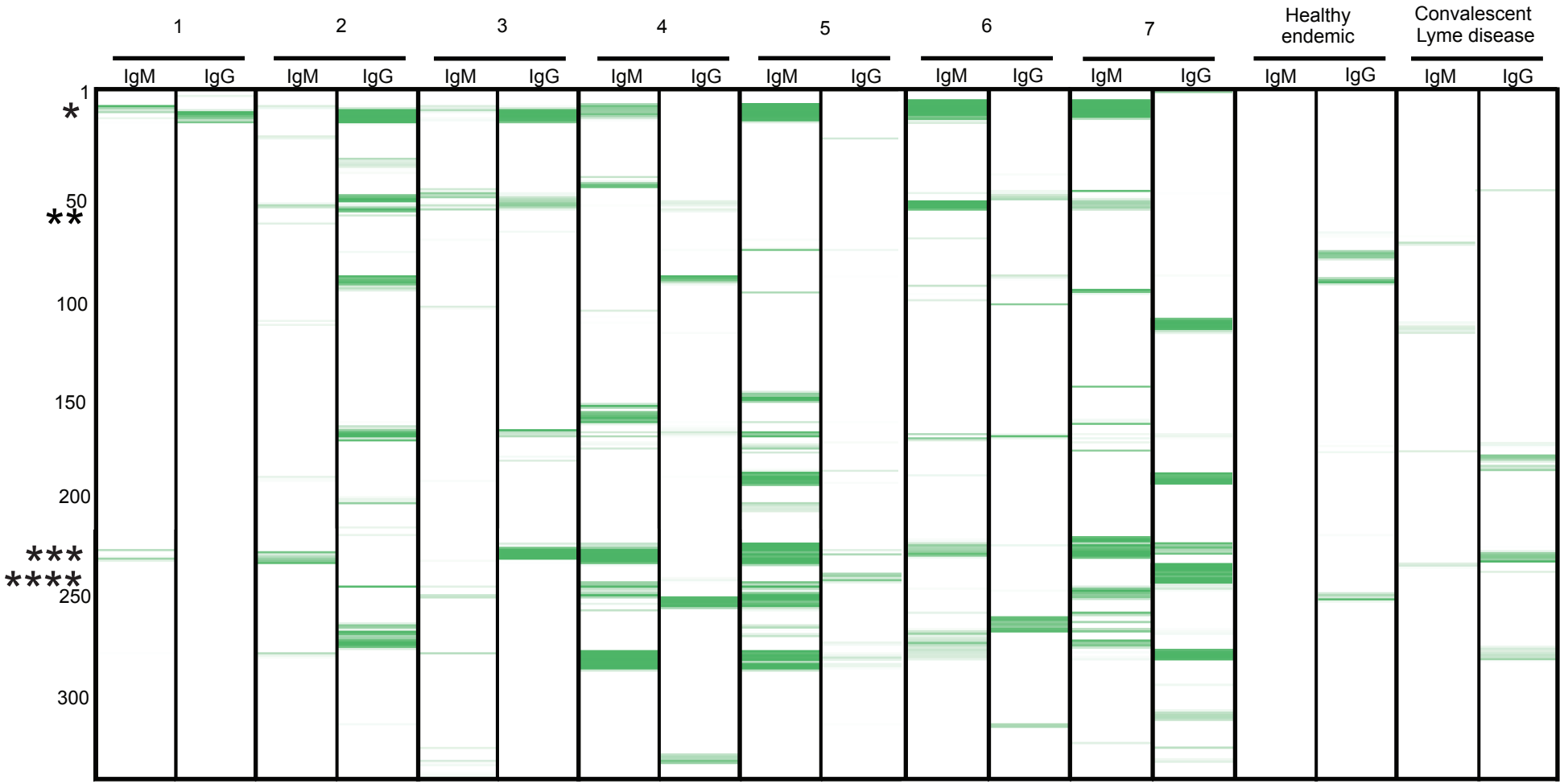

1
2
3
4
5
6
7
Healthy endemic
Convalescent Lyme disease
IgM
IgG
1
50
100
150
200
250
300
*
**
***
****

Figure 6

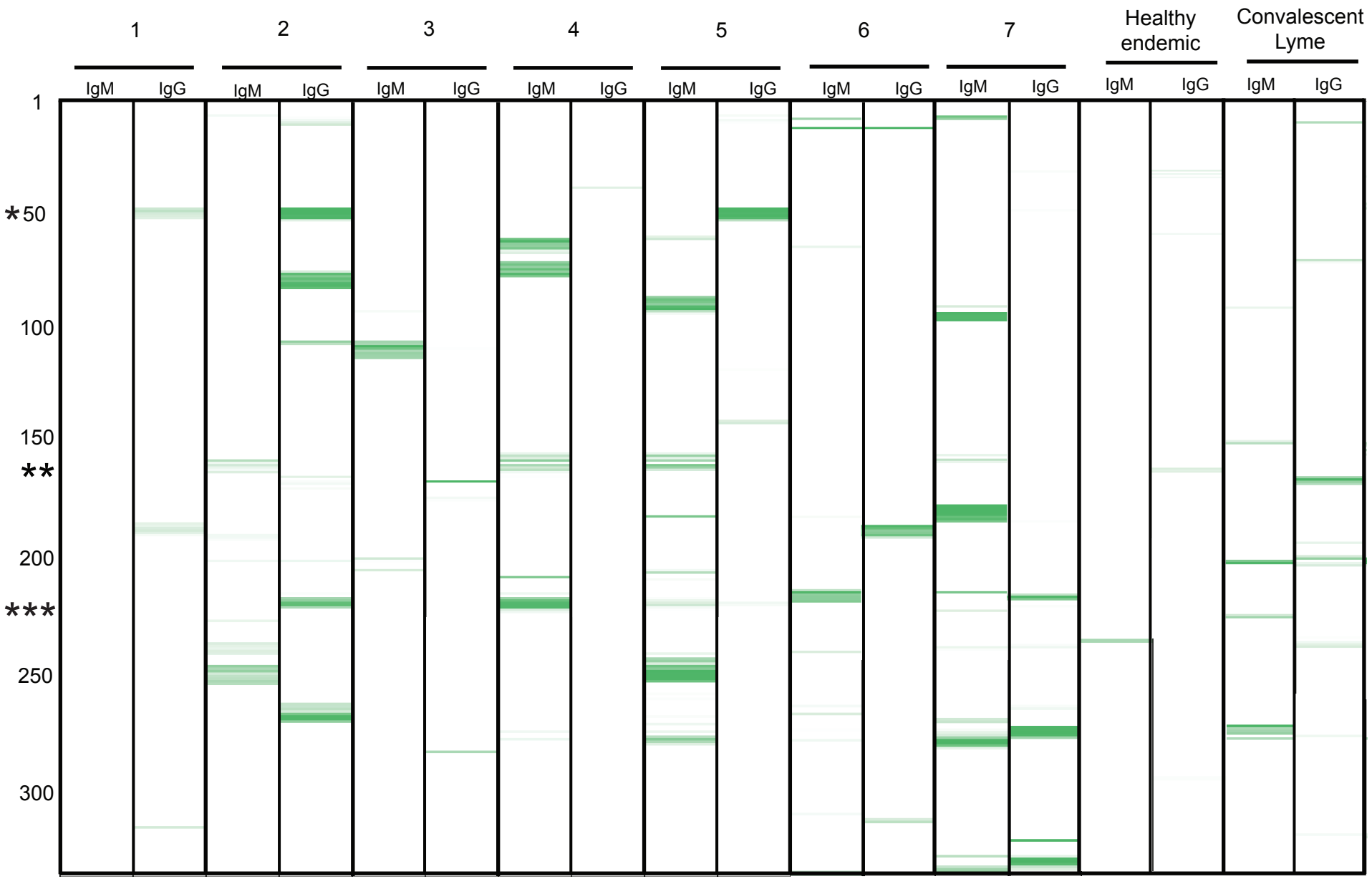

1
2
3
4
5
6
7
Healthy endemic
Convalescent Lyme
IgM
IgG
1
*50
100
150
**
200
***
250
300

Figure 7

A

Vlpδ-V-1 Epitope
C6 Epitope
B. burgdorferi VlsE CAJ41626 EQDGKKPEEAKNPIAAAIGD--KDGGA-EFGQDEMKKDDQIAAAIALRGMAKDGKFAVKDGEKE
δ
B. miyamotoi Vlp 15/16 WP025444401 ADNSKNIEEAKDAASIASAK--KEDNKKEIK-DEAKKDAVIAAGIALRAMAKDGKFAVKSNEEK
B. miyamotoi Vlp D4 ALN43421 D-DSKNIEEAKDAASIASAK--KEDDK-KEIKDEAKKDAVIAAGIALRAMAKGGKFAAKSNE-E
B. miyamotoi Vlp 15/16 WP025444400 S--EAKIETAKDAASIAAAK--VEESK-SL--DVAKQDAVIAAGIALRAMAKDGKFTAKKDE-E
B. miyamotoi Vlp 15/16 WP025444482 T-GEIKIEEAKNAAEIAAAK--AD-SK-DLEIDSAKKDAVIAGGIALRAMAKNGKFAAKNNE-D
γ
B. miyamotoi Vlp AHH06031 ------VKDNGEAIKLASNNAAVA------AVDTNKKDGTIAGGMALRAMAKGGKFAGPSDAAS
B. miyamotoi VlpC3 ALU64351 ------VKDGTE----AAANAAKA----------NAKDATIAGAIALRAMAKDGKFAGPSADAA
α
B. miyamotoi Vlp1 AGS80213 SQESDVALTANADENTTSLKFAKGGSDAHLSNSANPKAAAVAGGIALRSLVKTGKLASGAADNA

B

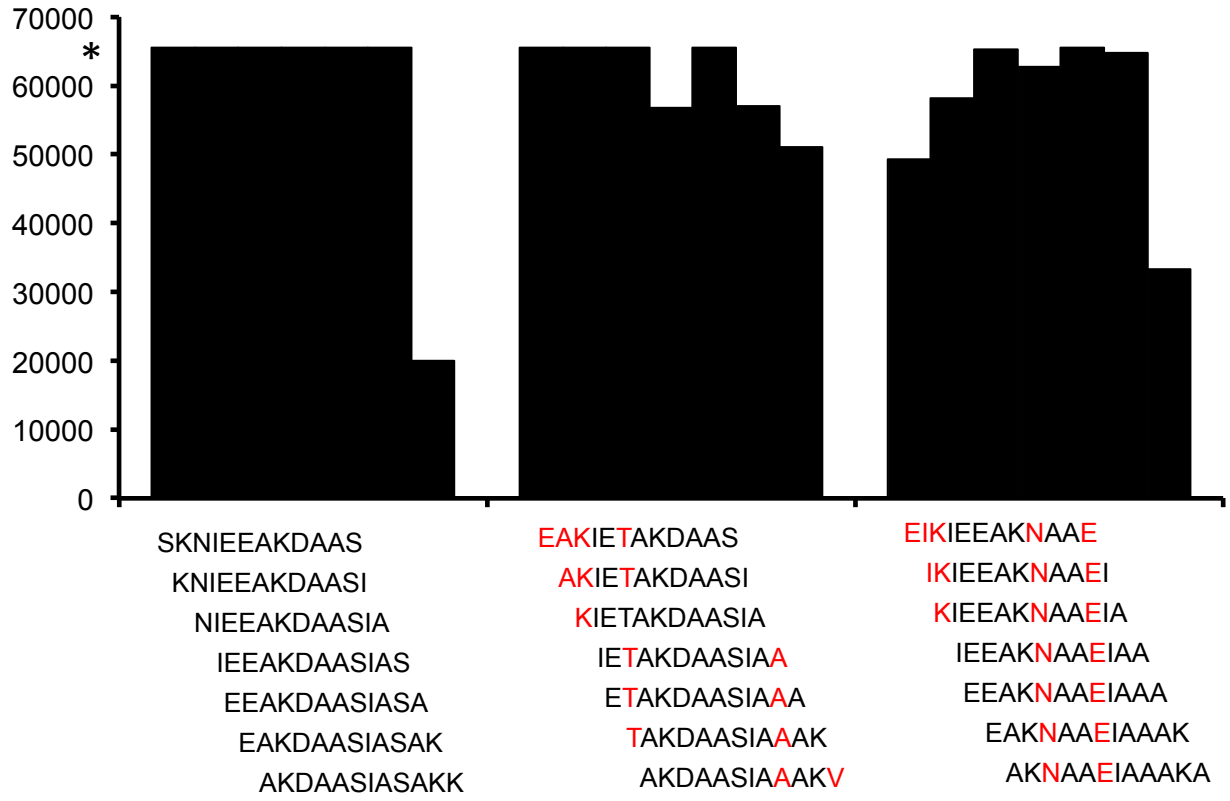
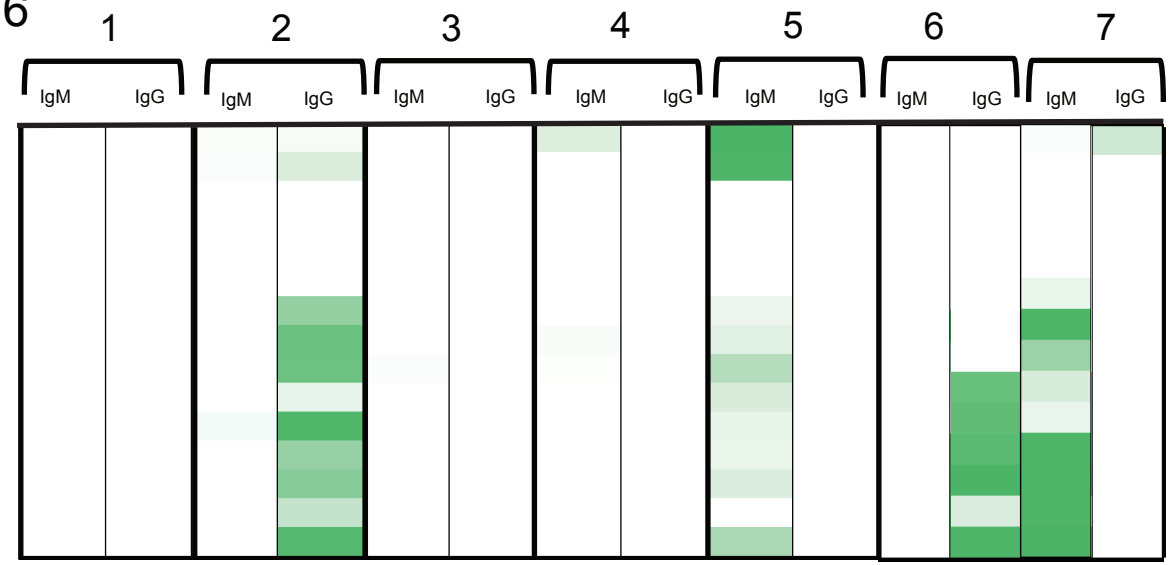

70000
*
60000
50000
40000
30000
20000
10000
0
SKNIEEAKDAAS
KNIEEAKDAASI
NIEEAKDAASIA
IEEAKDAASIAS
EEAKDAASIASA
EAKDAASIASAK
AKDAASIASAKK
EAKIETAKDAAS
AKIETAKDAASI
KIETAKDAASIA
IETAKDAASIAA
ETAKDAASIAAA
TAKDAASIAAAK
AKDAASIAAAKV
EIKIEEAKNAAE
IKIEEAKNAAEI
KIEEAKNAAEIA
IEEAKNAAEIAA
EEAKNAAEIAAA
EAKNAAEIAAAK
AKNAAEIAAAKA

Figure 8

B. miyamotoi Vlp15/16

A

AKKDAIIAAGIA
KKDAIIAAGIAL
KDAIIAAGIALR
DAIIAAGIALRA
AIIAAGIALRAM
IIAAGIALRAMA
IAAGIALRAMAK
AAGIALRAMAKD
AGIALRAMAKDG
GIALRAMAKDGK
IALRAMAKDGKF
ALRAMAKDGKFI
LRAMAKDGKFIV
RAMAKDGKFIVK
AMAKDGKFIVKD



B. burgdorferi VlsE

B

MKKDDQIAAAIA
KKDDQIAAAIAL
KDDQIAAAIALR
DDQIAAAIALRG
DQIAAAIALRGM
QIAAAIALRGMA
IAAAIALRGMAK
AAAIALRGMAKD
AAIALRGMAKDG
AIALRGMAKDGK
IALRGMAKDGKF
ALRGMAKDGKFA
LRGMAKDGKFAV
RGMAKDGKFAVK
GMAKDGKFAVKD

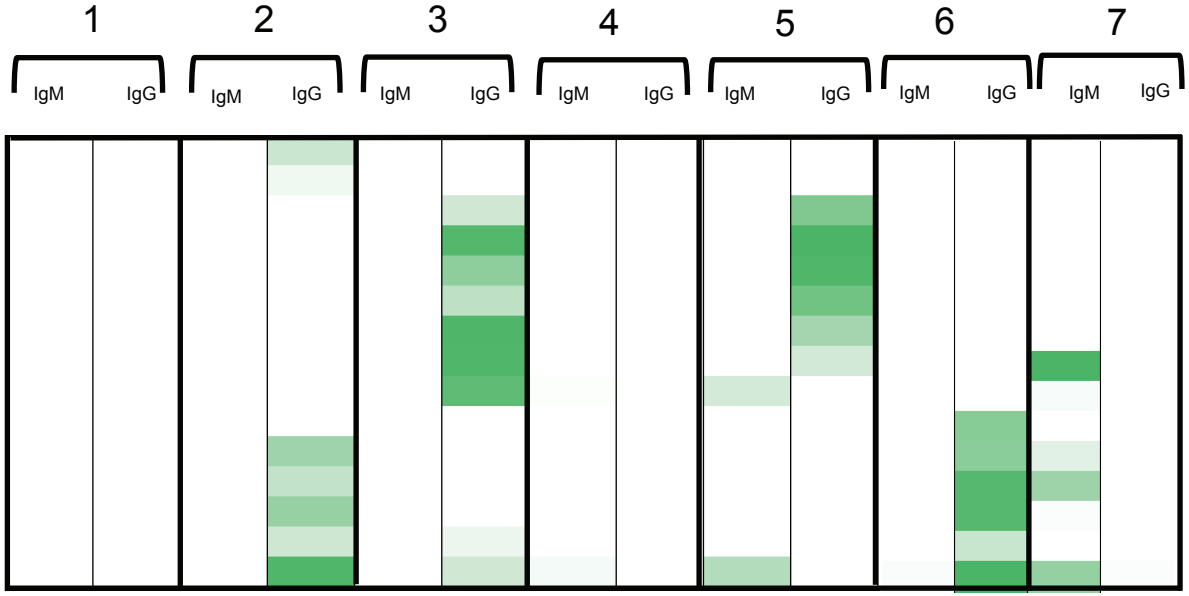
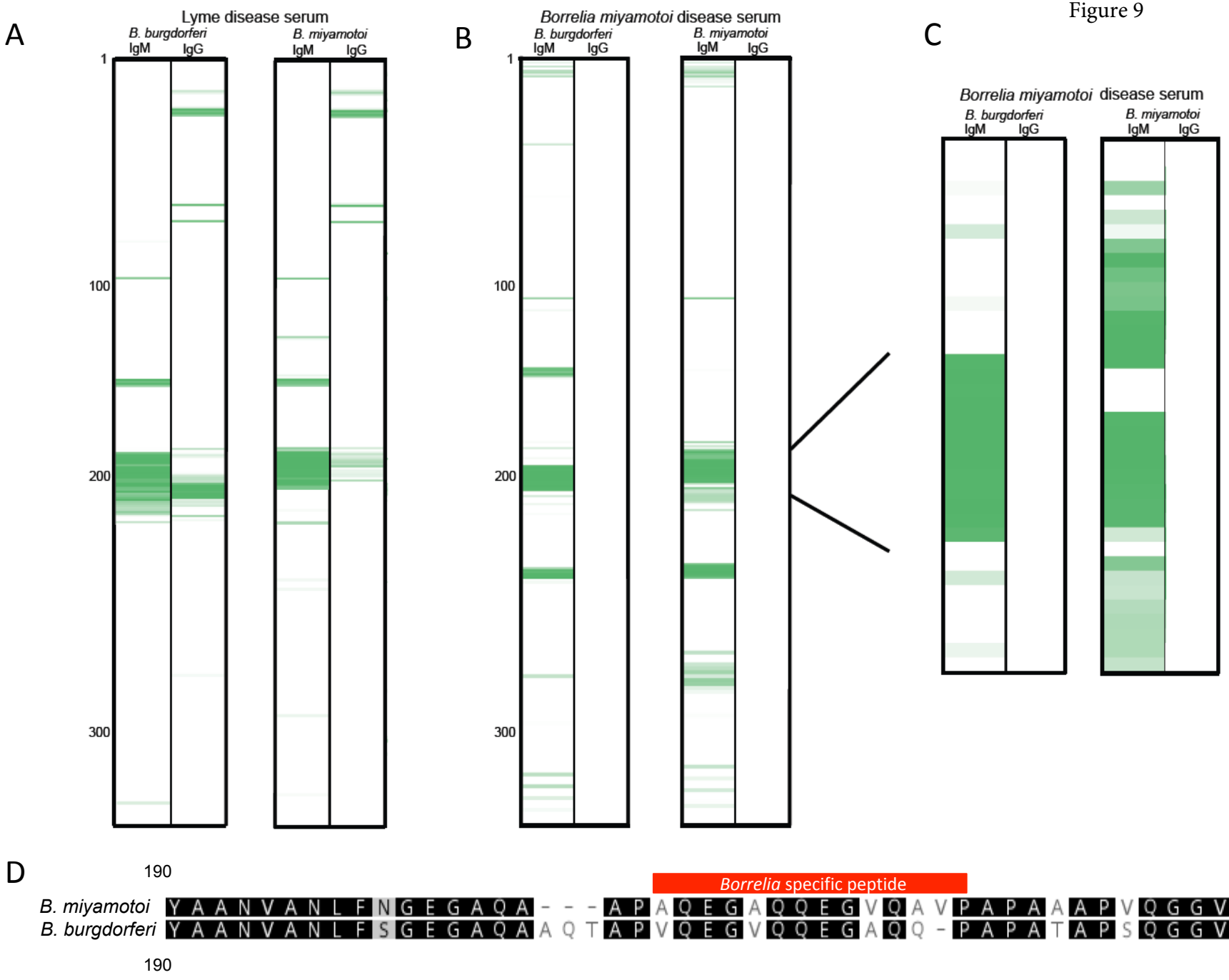


Figure 9


A
Lyme disease serum
B. burgdorferi
IgM
IgG
B. miyamotoi
IgM
IgG
1
100
200
300
B
Borrelia miyamotoi disease serum
B. burgdorferi
IgM
IgG
B. miyamotoi
IgM
IgG
1
100
200
300
C
Borrelia miyamotoi disease serum
B. burgdorferi
IgM
IgG
B. miyamotoi
IgM
IgG
D
190
223
Borrelia specific peptide
B. miyamotoi
Y A A N V A N L F N G E G A Q A - - - A P A Q E G A Q Q E G V Q A V P A P A A A P V Q G G V N S
B. burgdorferi
Y A A N V A N L F S G E G A Q A A Q T A P V Q E G V Q Q E G A Q Q - P A P A T A P S Q G G V N S
190
225