An OspA Antigen-Capture Enzyme-Linked Immunosorbent Assay for Detecting North American Isolates of *Borrelia burgdorferi* in Larval and Nymphal *Ixodes dammini*

THOMAS R. BURKOT,1,* ROBERT A. WIRTZ,2 BENJAMIN LUFT,3 AND JOSEPH PIESMAN3

Division of Vector-Borne Infectious Diseases, Centers for Disease Control, P.O. Box 2087, Fort Collins, Colorado 80522;2 Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C. 20370-5100; and Department of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794

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An antigen-capture enzyme-linked immunosorbent assay (ELISA) was developed for detecting North American isolates of *Borrelia burgdorferi* in larval, nymphal, and adult ticks. The assay uses an anti-OspA monoclonal antibody (H5332) for antigen capture and biotin-labelled polyclonal sera with streptavidin-horseradish peroxidase for signal generation. The assay recognized 15 of 15 North American *B. burgdorferi* isolates and did not cross-react with spirochete antigens of *Borrelia hermsii*, *Borrelia turicatae*, *Borrelia coriaceae*, or *Borrelia parkeri*, or with tick antigens of *Ixodes dammini*, *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes cookei*, *Ixodes angustus*, or *Amblyomma americanum*. The assay, with a sensitivity of less than 15 spirochetes, can detect infections in larval, nymphal, and adult ticks. In addition to fresh ticks, *Borrelia burgdorferi* infections in ticks stored frozen, dried, or in 70% ethanol can be determined with the assay.

Present methods of determining *Borrelia burgdorferi* infection rates in ticks include dissecting individual ticks for dark-field examination with subsequent examination by immunofluorescent antibody assays (FA) (10, 25, 26). Spirochetes may also be cultured from ticks in BSKII media (3) or analyzed by polymerase chain reaction (18, 22, 27). The labor intensiveness of individual tick dissections and subsequent culturing of tick extracts limits the number of ticks which can be processed by dark-field examination, FA, and culture. The problems of false negatives in dark-field examination (24) and false positives in both FA (23) and polymerase chain reaction tests (22) complicate large-scale applications of these methods for determining *B. burgdorferi* infection rates in ticks.

Sensitive, specific, and inexpensive antigen-capture enzyme-linked immunosorbent assays (ELISAs) have been developed for other arthropod-borne pathogens, including viruses (33) and protozoa (11, 36). Human pathogen detection in arthropods by ELISA offers a number of advantages over other methods, including low cost, long shelf life of reagents, ease of standardization, the ability to quantitate results, the ability of arthropods to be stored under a variety of conditions, and the capacity to test pools of arthropods. While generally not as sensitive as nucleic-acid-based assays, ELISAs can detect as few as 10 malaria sporozoites (11, 36) or 62 pg of virus antigen (33). In this study, a sandwich antigen-capture ELISA for determining infections of North American *B. burgdorferi* strains in larval, nymphal, and adult ticks is described.

MATERIALS AND METHODS

The antigen-capture ELISA was optimized by varying a number of test parameters. Both polystyrene (Immurex II) and polystyrene plates (DynaTech catalog no. 2597 and Costar catalog no. 2797) were evaluated. Blocking buffers and diluents tested in phosphate-buffered saline (PBS) included bovine serum albumin (BSA; 1, 1.5, 2.5, and 5%), casein (0.5 and 1%), 0.5% boiled casein, 0.5% boiled casein with 0.05% Tween 20, powdered skim milk (2.5 and 5%) with or without 0.05% Tween 20, and mouse sera (10, 5, 2.5, 1.25, and 0.6%). Monoclonal antibodies tested in the antigen-capture ELISA included antibodies against the 31-kDa OspA (184.1 [17] and H5332 [7]), the 34-kDa OspB (H6831) (6), the 41-kDa flagellin (H9724) (4), and a 66-kDa molecule (149.1) (20). *Borrelia* antigens were extracted with Nonidet P-40 (NP-40), sodium dodecyl sulfate, Triton X-100, and Tween 20 at initial concentrations of 2, 0.5, 0.25, and 0.12%.

Rabbit polyclonal antisera and mouse hyperimmune ascites against *B. burgdorferi* were raised by repeated needle inoculation of BSKII medium-cultured Gullfjord strain and B31 spirochetes, respectively. Polyclonal antibodies were protein A purified prior to biotin labelling as described in the manufacturer's recommendations (Amersham). Biotin-labelled antibodies were tested with both streptavidin-horseradish peroxidase conjugates and streptavidin-alkaline phosphatase conjugates. 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate(6)] (ABTS) and tetramethylbenzoini (TMB) (Kirkegaard & Perry Laboratories) were used as substrates for horseradish peroxidase, and Sigma 104-105 was used as a substrate for alkaline phosphatase.

The optimized protocol for the antigen-capture ELISA is as follows. Wells of polyvinyl chloride plates (Costar no. 2797) were coated with 50 μl of protein A-purified monoclonal antibody H5332 at a concentration of 10 μg/ml in PBS, pH 7.4. After an overnight incubation at 4°C, wells were washed three times with PBS-0.05% Tween 20 (PBST) and then blocked with 200 μl of 2.5% skim milk in PBST per well for 1 h at room temperature (RT).

Strains of *Borrelia* spp. tested in the assay were grown in BSKII medium and washed three times with PBS prior to the determination of spirochete numbers. Spirochetes were enumerated by using standard procedures of the Division of Vector-Borne Infectious Diseases, Centers for Disease Con-
trol (18). Briefly, spirochetes were quantitated by counting 10 × 400 fields by dark-field microscopy (24). The conversion to spirochete concentrations was enabled by use of a method from a previous study in which dark-field microscopy counts were compared with simultaneous counts with a Petroff-Hauser counting chamber. The accuracy of this method of quantitating spirochetes has been confirmed by limiting dilution experiments to clone B. burgdorferi.

**Borrelia** spp. to be tested in the assay were first diluted in 0.25% NP-40 in PBS (NP-40–PBS) for 10 min prior to a fivefold addition of 1% BSA in PBS (BSA-PBS). Larval and nymphal tick lysates were homogenized in 20 μl of NP-40–PBS in microtubes (Bio-Rad) by using a pestle shaped to fit the tube and attached to a power drill. Homogenization was facilitated by the addition of a minute amount of sterile sand to the microtube. Nymphal and larval homogenates were then diluted to 150 μl with BSA-PBS. Adult ticks were homogenized in 50 μl of NP-40–PBS as described above, and 250 μl of BSA-PBS was added.

After blocking, wells were washed as described above and 50 μl of the test samples was added to individual wells for 2 h at RT. Wells were again washed three times with PBST, and 50 μl of biotin-labelled polyclonal rabbit serum at a concentration of 0.25 μg/ml in BSA-PBS with 1% mouse serum was added to each well. After 1 h of incubation at RT, streptavidin-horseradish peroxidase (Amersham) at a 1:2,000 dilution in BSA-PBS was added to each well for 1 h. After washing as described above, 100 μl of ABTS (Kirkegaard & Perry) was added. Plates were read at 405 nm after a 30- to 60-min incubation.

The specificity of the ELISA was evaluated against 24 isolates of *Borrelia* spp., including *B. burgdorferi*, *B. garinii*, *B. hermsii*, *B. turicatae*, *B. coriacae*, and *B. parkeri*. The assay sensitivity was determined against twofold standard dilutions of *Borrelia* spp. The geographic and host origins of isolation and culture passage numbers of these strains are shown in Table 1. Antigen profiles for European, Asian, and selected North American isolates (B31, JD1, DN127, and WI-206) were obtained with the Pro-Blue staining system (Integrated Separation Systems) on 10% polyacrylamide resolving gels with a 5% stacking gel. Proteins of 105 spirochetes per lane were resolved under reducing conditions at 85 V for 120 min prior to staining. Mini-immunoblots of 105 spirochetes were resolved under conditions described above and blotted onto nitrocellulose at 100 V for 60 min. Immunoblots were blocked with 5% powdered milk in Tris-buffered saline (pH 7.6) (TBS) with 0.1% Tween 20 for 60 min before probing for 90 min with H5332 at a concentration of 3 μg/ml in TBS. After a 60-min incubation period with 0.5 μg of goat antimouse immunoglobulin G (heavy and light chains) (Kirkegaard & Perry) in 3% powdered milk in TBS with 0.06% Tween 20, antigens were visualized by the addition of BCIP (5-bromo-4-chloro-3-indolyl phosphate toluidinium)-nitroblue tetrazolium for 5 min.

Homogenates of uninfected ticks were tested by the

<table>
<thead>
<tr>
<th>Species and strain</th>
<th>Origin, location</th>
<th>Isolated from:</th>
<th>Passage no.</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>B31</td>
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<td>Ixodes dammini</td>
<td>3</td>
</tr>
<tr>
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<td>United States, New York</td>
<td>Ixodes dammini</td>
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<td>United States, New York</td>
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<td>Ixodes dammini</td>
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<tr>
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| Unknown | | | |
| CH2223* | Peoples Republic of China | Ixodes persulcatus | 5 |
| CH2246* | Peoples Republic of China | Ixodes persulcatus | 2 |
| ACA1† | Sweden | Human | 3 |
| IP90° | Russia | Ixodes persulcatus | NA |
| DN127f (clone 9-2) | United States, California | Ixodes pacificus | 52 |

| B. hermsii | United States | | NA |
| B. parkeri | United States | | 4 |
| B. coriacae | United States | | 3 |
| B. turicatae | United States | | 3 |

* NA, not available.
† The precise U.S. geographic location of this isolate is uncertain.
‡ Ribotyping generated a pattern suggestive of *B. garinii*, designated ribotype 3 (28).
§ Ribotyping generated a pattern suggestive of *B. burgdorferi*, designated ribotype 4 (28).
° This isolate was positive by using a flagellin-based polymerase chain reaction which reacts with *B. burgdorferi*, *B. garinii*, and group VS461 (18). However, ribotyping generated a pattern uncharacteristic for these genospecies, designated ribotype 5 (28).
f Genomic fingerprinting was unable to classify this *Borrelia* species (34).
antigen-capture ELISA for false positives. *Ixodes dammini* (a laboratory colony [no. 2324] originally isolated from Massachusetts), *Ixodes scapularis* (F1's from field-collected ticks from North Carolina), *Ixodes pacificus* (F1's from field-collected ticks from Arizona), *Ixodes cookei* (F1's from field-collected ticks from Virginia), *Ixodes angustus* (F1's from field-collected ticks from Washington), and *Amblyomma americanum* (field collected in Missouri) were tested.

*I. dammini* ticks were tested as larvae, nymphs, and adults, either uninfected or infected with the B31 or JD1 strains of *B. burgdorferi*. Ticks were infected by feeding uninfected laboratory colony no. 2324 ticks on specific-pathogen-free Swiss outbred mice from the Centers for Disease Control colony which had been infected with *B. burgdorferi* at least 1 month previously by the bites of infected ticks. Infections in mice were confirmed by BSKII medium culture of ear punch biopsies prior to tick feeding. Live replete and flat nymphs were homogenized immediately or after storage for 11 days at -20°C, in 70% ethanol, or dried at RT.

Positive cutoff values for ticks were determined by taking the mean plus 3 standard deviations of known negative ticks of the same stage as the unknown. Spirochete numbers in ticks were estimated from a regression line by using a square root transformation of a standard curve of known spirochete numbers and the absorbance values for those spirochete numbers. The spirochete numbers in positive ticks (defined as described above from the cutoff value) were calculated by comparing the difference in absorbances for a positive tick and the average of the negative control ticks to the standard curve (after subtracting the absorbance value of the "0" spirochete well from the other values in the curve).

**RESULTS**

Antigen capture using monoclonal antibodies (H5332 and 184.1) directed against the 31-kDa OspA antigen resulted in a more-sensitive ELISA than ELISAs using monoclonal antibodies directed against the 34-kDa OspB (H6831), the 41-kDa flagellin (H9724), or the 66-kDa p60 molecule (149.1) (Fig. 1). Of the two monoclonal antibodies directed against the OspA molecule, monoclonal antibody H5332 gave higher absorbances at lower spirochete densities than monoclonal antibody 184.1 and was therefore used in all subsequent assays.

Among the four ELISA plates tested, the polyvinyl chloride plate produced a slightly more sensitive assay than the polystyrene plate. The Costar no. 2797 polyvinyl chloride plate was selected for subsequent assays on the basis of a marginally greater absorbance when assaying spirochete numbers below 1,000. Among the blocking solutions evaluated, 2.5% milk with 0.05% Tween 20, 0.5% casein, and 1% BSA (all diluted in PBS) gave comparable results. Milk (2.5%) with 0.05% Tween was selected as the blocking reagent on the basis of low cost and ease of buffer preparation. However, 1% BSA in PBS was a superior diluent for spirochete extracts, biotin-labelled antibodies, and streptavidin-horseradish peroxidase. Sodium dodecyl sulfate and NP-40 were equally effective in extracting *B. burgdorferi* antigens at the concentrations tested. NP-40 was used in the following experiments.

The antigen-capture ELISA recognized all North American strains of *B. burgdorferi* tested (Table 1 and Fig. 2). Strain WI-206 gave a diminished signal compared with the other low-passage North American strains. The absorbance for 10³ WI-206 spirochetes was 1.098 compared with absorbances ranging from 2.315 to 3.043 for 10⁴ spirochetes of the other low-passage North American strains. Negative controls averaged 0.136, with a standard deviation of 0.043. However, the ELISA was still sensitive enough to detect *I. dammini* infected with the WI-206 isolate (infected nymphs, x ± SD = 0.462 ± 0.013, n = 2; uninfected nymphs, x ± SD = 0.093 ± 0.043, n = 6).

The reproducibility of the assay is shown in Fig. 3 in which standard curves on 6 consecutive days were assayed by using twofold serial dilutions of from 10,000 to 156 spirochetes of the JD1 strain. The sensitivity of the ELISA using the JD1 strain was less than 156 spirochetes. Absorbance values obtained for wells with 156 spirochetes (mean, 0.214; SD, 0.060) were significantly above absorbances obtained for wells without spirochetes (mean, 0.092; SD, 0.042) after
FIG. 3. Reproducibility of the OspA antigen-capture ELISA. Standard curves consisting of twofold serial dilutions of the JD1 strain of spirochetes (numbering from 10,000 to 156) were assayed on six separate days with a negative control well.

FIG. 4. Specificity of OspA antigen-capture ELISA for *B. burgdorferi* using 10,000 spirochetes as antigen (A, WI-206; B, WI-210; C, JD1; D, NY-944; E, WC-H1; F, HB19; G, GA-747; H, NY14; I, CA5; J, CA4; K, CA-808; L, DN127 (clone 9-2) (does not express OspA as determined by Coomassie blue staining); M, PA606; N, B31; O, PA624; P, PA622; Q, ACA1; R, IP90; S, CH223; T, CH2246; U, B. parkeri; V, B. turicatae; X, B. coriaceae; W, B. hensii).

FIG. 5. Immunoblots of spirochetes (10^5 per lane) probed with H5332. Lanes: 1, B31; 2, WI-206; 3, DN127; 4, CH2246; 5, CH2223; 6, ACA1; 7, IP90).

FIG. 6. *B. burgdorferi* detection in live flat and replete *I. dammini* nymphs or flat nymphs stored for 11 days either frozen, dried at RT, or in 70% ethanol. Ticks that had fed on infected or uninfected mice are represented by filled and open symbols, respectively.

A 30-min substrate incubation (t = 4.98, df = 5, P < 0.005) (Fig. 3). Average absorbances and standard deviations for 10,000, 5,000, 2,500, 1,250, 625, and 312 JD1 spirochetes were 2.535 ± 0.075, 2.253 ± 0.112, 1.803 ± 0.190, 1.203 ± 0.306, 0.644 ± 0.195, and 0.386 ± 0.097, respectively.

The OspA antigen-capture ELISA failed to detect spirochetes of one high-passage *Borrelia* isolate of an unclassified species (34) at concentrations up to 2 × 10^5/ml (Fig. 4). This exception was California strain DN127 (clone 9-2), which was its 52nd culture passage and which was reported to be OspA negative by Coomassie blue staining (8). However, when 10^5 spirochetes per lane were resolved, a weak band at the OspA position was seen with the Pro-Blue staining system (data not shown). Probing of Western blots (immunoblots) with H5332 confirmed low-level expression of OspA as reported previously (8) (Fig. 5).

The assay also did not detect *B. hensii*, *B. parkeri*, *B. coriaceae*, or *B. turicatae*. Although H5332 recognized Swedish strain ACA1, the OspA antigen-capture ELISA did not detect this isolate nor did it recognize *Borrelia* isolates from Russia (IP90) or one of two Chinese isolates (CH2223) (Fig. 4).

The ELISA was able to distinguish *B. burgdorferi*-infected from uninfected *I. dammini* (larvae, nymphs, or adults) when a third of a larval or nymphal homogenate (50 µl of the total homogenate volume of 150 µl) or a sixth of an adult homogenate (50 µl of the total homogenate volume of 300 µl) was used, thereby leaving adequate material for confirmatory testing (Fig. 6 and 7). Flat and replete *B. burgdorferi*-infected *I. dammini* nymphs harbored up to 7,500 and 10,000 *B. burgdorferi* spirochetes, respectively. In addition to detecting *B. burgdorferi* in live ticks, the assay detected *B. burgdorferi* in ticks that had been stored for 11 days either frozen, dried at RT, or in 70% ethanol. The assay did not recognize tick antigens of *I. dammini*, *I. scapularis*, *I. pacificus*, *I. cookei*, *I. angustus*, or *A. americanum*.

Using 1% BSA-PBS as the diluent for the biotin-labelled rabbit polyclonal anti-*B. burgdorferi* serum, the ELISA could distinguish infected engaged ticks from uninfected engaged ticks, although the background absorbances for engorged ticks was higher than those of flat ticks (Fig. 6 and 7). However, the addition of mouse sera to a final concen-
tation of 0.6 to 10% in the BSA-PBS diluent for the biotin-labelled rabbit anti-\textit{B. burgdorferi} sera significantly reduced the absorbance values for spirochete-negative samples without a loss of sensitivity for samples in 15% blood spiked with known numbers of spirochetes (Fig. 8). An examination of the effect of the addition of 1% mouse serum to the BSA-PBS diluent for the biotin-labelled rabbit anti-\textit{B. burgdorferi} sera when assaying fully engorged \textit{I. dammini} nymphs revealed a drop in the absorbance values of known negative ticks but no significant change in the standard curves (Fig. 9). Assaying uninfected adult ticks fully engorged with blood, however, resulted in absorbances significantly above background levels for flat adult ticks.

**DISCUSSION**

Since the original isolation of \textit{B. burgdorferi} from \textit{I. dammini} in Shelter Island, New York (10), the known geographic distribution of Lyme disease has been increasing. Serologic tools including FAs, ELISAs, and Western blots for human case diagnosis of Lyme disease do not correlate well (16). The sensitivities of available serological tests for Lyme disease range from 13 to 73%, with false-positive results in as many as 27% of patients (13). The lack of specificity may be due to antibody cross-reactivity among bacteria (12, 21). The identification of \textit{B. burgdorferi} in ticks is a necessary prerequisite to confirm transmission of Lyme disease in new areas.

As Lyme disease has spread into new geographic areas, novel vectors and reservoirs have been reported, particularly in California (9, 19). Incrimination of new vector species must be confirmed by visual observation of viable spirochetes in field-collected arthropods, and identification must be confirmed by immunological, biochemical, or molecular characterization with documentation of transmission to laboratory hosts. A rapid and inexpensive technique for the preliminary screening of large numbers of field-collected arthropods would facilitate the delineation of new vectors and transmission cycles. Laboratory studies would also benefit from quantitative techniques for \textit{B. burgdorferi}.

Detection of \textit{B. burgdorferi} by the ELISA described in this article is based on recognition of OspA by monoclonal antibody H5332 for antigen capture. In addition to the 15 North American isolates of \textit{B. burgdorferi} that reacted with H5332 in this study, previous studies reported H5332 recognition of 25 of 25 (5) and 6 of 6 (35) North American isolates of \textit{B. burgdorferi}. Signal generation in the OspA antigen-capture ELISA results from recognition of other epitopes on the OspA molecule by biotin-labelled rabbit polyclonal serum together with streptavidin-horseradish peroxidase.

A recent investigation of the taxonomic status of \textit{B. burgdorferi} using rRNA gene restriction patterns, DNA-DNA association, protein electrophoresis, and monoclonal antibody recognition divided the spirochetes formerly known as \textit{B. burgdorferi} into three genospecies: \textit{B. burgdorferi} sensu stricto, \textit{B. garinii}, and group VS461 (2). All North American isolates and some European isolates are now classified as \textit{B. burgdorferi} sensu stricto, with the other
European and Asian isolates being either *B. garinii* or group VS461. Neither of the latter two genospecies react with monoclonal antibodies H3TS (anti-OspA) or H6831 (anti-OspB), and only 4 of 13 *B. garinii* isolates are recognized by H5332 (anti-OspA). All seven strains in the VS461 group tested by Baranton et al. (2) were not recognized by H5332. All *B. burgdorferi* sensu stricto organisms are recognized by H5332, the monoclonal antibody on which the ELISA depends. This study depends for antigen capture ELISA.

A second study based on ribotyping divided these spirochetes into five groups (28). Ribotypes 1, 3, and 4 corresponded broadly to *B. burgdorferi* sensu stricto, *B. garinii*, and group VS461, respectively, as defined by Baranton et al. (2). However, it must be emphasized that classification of bacterial species based solely on ribotyping (28) or DNA melting temperatures (2) does not give identical results. A bacterial taxonomic study using rRNA sequences did not correlate well with previously published DNA-DNA hybridization studies (15). Isolates of ribotypes 2 and 5, from the United States and Russia, represent groups not seen in the classification system of Baranton et al. (2). The species identification of the Russian isolate (IP90) of ribotype 2, at present, uncertain. The Chinese isolates, CH2223 and CH2246, are ribotype 3, while the isolate from Sweden, ACA1, is ribotype 4. The Swedish and Russian isolates and one of the two Chinese isolates (CH2223) were not recognized by the antigen-capture ELISA.

The antigen-capture ELISA recognized all 15 North American isolates of *B. burgdorferi* tested in this study. Isolate DN127, clone 9-2, which was in its 52nd passage in culture and which was reported to be OspA negative by Coomassie blue staining but weakly positive by Western blot with the anti-OspA monoclonal antibody H5332 (8), was not detectable in the anti-OspA antigen-capture ELISA. Only 10% of DN127 spirochetes reacted with H5332 (8). A clone from this isolate produced by limiting dilution reportedly did not react with H5332 in FA or Western blot analysis (8), although in this study, Western blots of this clone showed weak reactivity with OspA. Genomic fingerprinting classified DN127 as a *Borrelia* sp. but one that is distinct from *B. burgdorferi* (34).

The loss of plasmids during continuous culture of *B. burgdorferi* has been demonstrated (29) and may explain the poor OspA expression of DN127 (the OspA gene is encoded on a 49-kb plasmid). Similarly, the diminished sensitivity of the ELISA for WI-206 could result from the loss of the OspA plasmid by a subpopulation of the WI-206 isolate or by changes in other epitopes on the WI-206 OspA which must be recognized by the rabbit anti-*B. burgdorferi* serum for a positive antigen-capture ELISA result. The strong recognition of WI-206 OspA by H5332 in Western blots suggests that the diminished response in the ELISA results from antigenic changes at epitopes other than the target of H5332.

Another North American strain, 25015, characterized prior to the published description of *B. garinii* and group VS461, is nonpathogenic in mice (1) and, like many *B. garinii* isolates, has a 33-kDa OspA and is not recognized by H5332 (14). While this strain would not be recognized by the OspA antigen-capture ELISA, the identification of this strain is, at this time, uncertain. Recently, Brown and Lane (9) reported that 9% of California isolates lack a 31-kDa OspA.

In addition to the *Borrelia* isolates of ribotypes 4 (ACA1), 5 (IP90), and 3 (CH2223), the OspA antigen-capture ELISA did not recognize *B. hermsii*, *B. parkeri*, *B. coriaceae*, or *B. turicatae* or tick antigens of *I. dammini*, *I. scapularis*, *I. pacificus*, *I. cookei*, *I. angustus*, or *A. americanum*. Further delineation of the species status of the bacterial strains investigated in this study together with testing of additional isolates will be required before the true specificity of this OspA ELISA is known.

At this time, this assay provides a useful tool for laboratory investigations using known strains of *B. burgdorferi*. The low cost of the assay, ease of sample preparation, and quantitative ability of the assay will enable studies on vector competence and growth kinetics of *B. burgdorferi* in vectors. While not as sensitive as polymerase chain reaction analyses, the antigen-capture ELISA is more sensitive than DNA (30) or 23S rRNA hybridization probes (31) (with sensitivities of 5 to 10,000 spirochetes). The OspA antigen-capture ELISA has a biologically relevant sensitivity of less than 156 spirochetes, which is sufficient to detect *B. burgdorferi* infections in larvae, nymphs, and adult ticks.

Until the specificity of the assay is fully characterized, interpretation of assay results on field-collected ticks must be approached with caution. If the specificity of the assay is confirmed on a large number of *B. burgdorferi* isolates, it will provide a useful epidemiological tool for evaluating intervention strategies aimed at disrupting transmission of Lyme disease. The higher background absorbances seen when assaying fully engorged adult ticks may exclude the assay as a tool for determining whether engorged adult ticks are infected with *B. burgdorferi*.

The assay is presently being further characterized with both field-collected and laboratory-infected ticks. Once fully evaluated, the sensitivity, specificity, and low cost of the ELISA together with its compatibility with a variety of tick storage conditions should facilitate the processing of large numbers of ticks for *B. burgdorferi* detection from field-collected ticks in addition to its present use in laboratory studies.

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REFERENCES


