

Recombinant Assay for Serodiagnosis of Lyme Disease Regardless of OspA Vaccination Status

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All current seroassays using cultured *Borrelia burgdorferi* as their antigen source have been rendered obsolete by the recombinant OspA Lyme disease vaccine. OspA is the major outer surface protein expressed in cultured *B. burgdorferi*, and any seroassay that uses whole organisms as its antigen source cannot differentiate between subjects who received the vaccine and those who were naturally infected. We developed a new sensitive and specific enzyme-linked immunosorbent assay (ELISA) utilizing recombinant chimeric borrelia proteins devoid of OspA (rNon-OspA) that can be used to detect antibodies to diagnostically important *B. burgdorferi* antigens in both OspA-vaccinated and nonvaccinated individuals. We tested sera from patients with Lyme disease and with conditions associated with false-positive serologies, OspA-vaccinated individuals, and healthy high-risk workers from an area of endemicity and normal sera from individuals from areas of nonendemicity. The rNon-OspA test was compared with two commercially available whole-cell immunoassays. The rNon-OspA assay is as sensitive and specific as the whole-cell assay ($P > 0.05$) for detection of anti-*B. burgdorferi* antibodies. However, the rNon-OspA assay can differentiate between populations comprised of naturally infected and OspA-vaccinated individuals ($P < 0.05$). Our data demonstrate that this new sensitive rNon-OspA ELISA can be used for the laboratory detection of *B. burgdorferi* antibodies regardless of vaccination status and could replace existing serologic assays for Lyme disease.

Lyme disease is a progressive tick-borne infectious disease involving multiple organ systems. The manifestations are frequently protean, and Lyme disease is included in the differential diagnosis of numerous dermatologic, rheumatologic, neurologic, and cardiac conditions. The best clinical marker for the disease is the initial skin lesion, erythema migrans (EM), that occurs in most but not all patients (2).

Lyme disease is the most common vector-borne disease in North America and Europe (17), and its range and incidence are increasing. It is also an emerging problem in northern Asia (12). The spirochete *Borrelia burgdorferi* is the infectious agent responsible for this disease, and it is transmitted to humans via ticks of the genus *Ixodes*. The accurate diagnosis of Lyme disease depends on correlating objective clinical abnormalities with serological evidence of exposure to *B. burgdorferi*.

The laboratory confirmation of Lyme disease relies heavily on enzyme-linked immunosorbent assays (ELISAs) for initial screening of sera (7). Currently, a two-step approach is recommended for the serodiagnosis of *B. burgdorferi* infection involving a first assay, which if positive or equivocal must be followed by a Western blot. The first-step serological tests commonly used to detect antibodies against *B. burgdorferi*, ELISA or immunofluorescence assay, use cultured whole-cell *B. burgdorferi* preparations. OspA is the major protein expressed in cul-

tured organisms. Therefore, any seroassay using cultured organisms as its antigen source contains large amounts of OspA.

With the approval of an OspA vaccine by the Food and Drug Administration in December 1998, large numbers of individuals at risk for Lyme disease are being vaccinated. Because virtually all of the current commonly used ELISAs utilize whole *B. burgdorferi* organisms as their source of antigens, both naturally infected and vaccinated individuals can be expected to yield positive results in these assays. The recombinant OspA vaccine tested provided 49% protection after two doses in the first year and 76% protection after 2 years (three doses) (18). Vaccinated individuals can develop Lyme disease (24). Thus, there is a need for the development of serologic assays to be used for individuals who have received the vaccine.

We have previously developed recombinant chimeric borrelia protein (RCBP)-based assays that can be used to detect antibodies to *B. burgdorferi*. These assays have proven to be more sensitive (6) and more specific (5) than current tests used as the first-tier serodiagnosis of Lyme disease. Because RCBPs are easy to manipulate an ELISA containing a formulation of these antigens containing key *B. burgdorferi* epitopes devoid of OspA (rNon-OspA) was developed to identify serologic evidence of infection in vaccinated and nonvaccinated, naturally infected individuals.

MATERIALS AND METHODS

Chimera construction, protein expression, and immunoblot characterization.

(i) **Chimera construction.** Several sets of RCBPs have been developed to be used in serologic assays for Lyme disease (5, 6). Of these, the recombinant chimeras

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OspB-OspC-Fla (strain B31) and OspC-p93 (strain B31) were applied to this new assay. In addition, we generated two new chimeras using the four outer surface protein C types linked to *B. burgdorferi* invasiveness: OspC1, OspC2, OspC10, and OspC12 (15). Portions of the open reading frames of the different OspC DNAs were cloned sequentially into the expression vector pET9c on the *NdeI*-*Bam*HI sites, using linkers between the sequences in order to produce recombinant fusion proteins. The chimeras OspC1-OspC10 and OspC2-OspC12 were generated. *Escherichia coli*, strain DH5 α , was transformed with the plasmid containing the chimeras, the antibiotic-resistant colonies were isolated, and the purified DNA was characterized via restriction pattern analysis.

(ii) Protein expression and immunoblot characterization. *E. coli* [strain BL21(DE3)/pLysS or strain B834(DE3)] was transformed with the plasmid containing the respective RCBP, grown in 10 ml of Luria-Bertani medium (containing, per liter, 5 g of NaCl, 10 g of tryptone, 5 g of yeast extract, 25 mg of chloramphenicol, and 50 mg of kanamycin) at 37°C, with shaking. When the optical density at 600 nm reached 0.3 to 0.4, protein expression was induced by adding IPTG (isopropyl- β -D-thiogalactopyranoside) to a final concentration of 0.5 mM and cells were grown for an additional 3 h. The cultures were harvested by centrifugation at 3,000 rpm (Eppendorf 5403 centrifuge) at 4°C for 5 min, the cells were resuspended in 200 μ l of 20 mM NaPO₄, pH 7.7, and the crude extracts were stored at -20°C overnight. Once thawed, the RCBP crude extracts were incubated with DNase (20 μ g/ml) in the presence of 5 mM MgCl₂ at room temperature for 30 min and spun at 14,000 rpm (Eppendorf 5417C centrifuge) for 5 min, and 5 μ l of the protein sample supernatant was loaded on a sodium dodecyl sulfate-polyacrylamide gel which was either stained with Coomassie blue or used for immunoblotting. The primary antibody used for the immunoblot was either a monoclonal antibody for one of the proteins in the chimera or a polyclonal serum from an EM-characterized Lyme disease patient. The secondary antibody used was, respectively, alkaline phosphatase-labeled anti-mouse immunoglobulin G (IgG) or anti-human IgA-IgG-IgM.

Protein purification. Crude extracts of each RCBP to be purified were prepared according to the method of Studier et al. (20). The resulting pooled soluble fraction was applied to an anion exchange column (Q Sepharose Fast Flow; Pharmacia) equilibrated with either 20 mM Tris-HCl, pH 7.5, or 20 mM diethanolamine, pH 9.1, depending on the protein being purified. The bound protein was eluted using increasing concentrations of NaCl. The fractions containing the chimeric protein were pooled and concentrated by ultrafiltration using a stirred flow cell (Amicon) with a 30- or 50-kDa cutoff membrane (depending on the protein purified). The protein was dialyzed against 20 mM NaPO₄, pH 7.8, containing 250 mM NaCl. The protein solution was loaded onto an Ni²⁺ metal affinity column (Chelating Sepharose Fast Flow; Pharmacia) equilibrated with 20 mM NaPO₄, pH 7.8, containing 250 mM NaCl. The bound protein was eluted using an increasing concentration of imidazole, and the fractions containing the chimeric protein were concentrated by ultrafiltration (Amicon). The protein concentration was then determined by the measurement of the absorbance shift when Coomassie brilliant blue G-250 reacted with protein (Bio-Rad) and stored at -70°C. Protein purity was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the reactivity was checked by immunoblotting. The yield of purified protein depends on the chimera being purified and ranges between 15 and 60 mg per liter of induced culture.

ELISA. (i) Immobilization of RCBPs onto ELISA plates (rNon-OspA assay). The recombinant proteins B-C-Fla, C-93, C2-C12, and C1-C10 are combined at 10, 10, 4, and 4 μ M, respectively, in 20 mM sodium phosphate-40 mM NaCl, pH 7.2. Coating buffer (Kirkegaard and Perry Laboratories [KPL]) was diluted 1:10 with water. A solution of purified RCBPs was diluted to a concentration of 2.5 μ g/ml and used to coat commercial microwell plates (MaxiSorp; Nunc). The coating procedure was as follows. One hundred microliters of a solution containing antigen was added to each well, and the microwell plate was incubated overnight at room temperature. The antigen solution was removed from the wells, the plate was washed three times with KPL washing buffer (diluted 1:20), and 300 μ l of blocking solution (diluted 1:20; KPL) was added. Following a 30-min incubation at room temperature, the plates were washed three times as described above and used the same day.

(ii) ELISAs. The standard procedure for the ELISAs was as follows. Serum samples were diluted 1:50 in diluent (diluted 1:20; KPL), and 100 μ l of each sample was added to ELISA plate microwells. Following incubation for 20 min at room temperature, the samples were removed and the plates were washed three times with KPL washing buffer (diluted 1:20). Goat anti-human IgA, IgG, and IgM antibody conjugated to horseradish peroxidase (KPL) was used as secondary antibody. It was diluted at 25 ng/ml in diluent (diluted 1:20; KPL), and 100 μ l of the solution was added to each well. Following incubation for 20 min at room temperature, the plates were washed three times as described above, and 100 μ l of substrate solution (tetramethylbenzidine; KPL) was added to each well.

The plates were incubated for 10 min at room temperature, and 100 μ l of stop solution (H₂SO₄) was added to each well. The absorbance at 450 nm was read on a microplate reader (Dynatech). As negative controls, 10 serum samples from healthy individuals were used. The same negative controls were included in each plate. A sample was considered positive if it produced an average absorbance greater than the mean of the negative controls plus 3 standard deviations.

(iii) Commercial immunoassays. The Lyme disease ELISA used in the comparison experiments done at sites A and C is one of the most commonly used ELISAs in the U.S. market today. It was a gift from Wampole Laboratories (Cranbury, N.J.). The tests are polyvalent and were performed according to the manufacturer's instructions. As for the recombinant assay, values equal to or above 3 standard deviations were considered positive. The assay used by the Centers for Disease Control and Prevention (CDC) (Vidas Lyme immunoassay; Biomerieux) is an automated Lyme disease immunoassay that uses enzyme-linked fluorescence assay technology, associating enzyme marking and fluorescence measurement (23).

(iv) Serum panels and monoclonal antibodies. The recombinant Non-OspA assay developed was tested in three Lyme disease reference laboratories, sites A, B, and C, which used three different serum banks (Table 1). Sera obtained from 395 individuals were used in these studies. The Lyme Disease Center at SUNY Stony Brook, site A, has conducted multicenter clinical trials that resulted in the accumulation of a large bank of sera from patients who participated in these studies. Sera obtained from 173 individuals were used in this site for the preliminary evaluation of sensitivity and specificity of the rNon-OspA ELISA. All serum samples were obtained from physician-characterized individual patients. These serum panels as well as a healthy control panel from an area of endemicity have been kindly made available to test the assay herein described. Potentially cross-reactive sera, such as those from patients with syphilis and autoimmune diseases, were purchased from Bioreclamation, Inc. The CDC site, site B, used 120 sera from individuals with Lyme disease and potentially cross-reactive diseases to test this assay. The Westchester Medical Center, New York Medical College site, site C, used 102 sera from individuals with Lyme disease, OspA-vaccinated individuals, and placebo-vaccinated individuals. "OspA vaccinated" refers to the sera obtained from individuals vaccinated with recombinant OspA. "Placebo vaccinated" refers to the sera obtained from individuals vaccinated with saline solution only. Neither the patient nor the normal healthy control sera used to evaluate the recombinant ELISA were prescreened; they were randomly blinded before being run.

Monoclonal antibodies directed against *B. burgdorferi* OspB, OspC, and p93 were obtained as described previously (8, 22).

(v) Statistical analysis. McNemar's exact test for correlated proportions (14) was used to evaluate the statistical significance of the results because the same serum was used for both tests, giving a paired-sample design. The *P* value was calculated for each panel. McNemar's test evaluates whether discordant results (i.e., positive in rNon-OspA assay and negative by the other assay or vice versa) are evenly distributed (null hypothesis) or biased one way or the other. *P* values of <0.05 are considered statistically significant.

RESULTS

Study I: comparison of the recombinant Non-OspA and whole-cell sonicate (WCS) ELISAs for sensitivity and specificity (performed at site A). A bank of 173 individual serum samples was used to do the preliminary evaluation of the recombinant Non-OspA ELISA at site A. The tests were done side by side in duplicate.

Sera from 114 Lyme disease patients were used to test the sensitivity of the rNon-OspA assay in comparison with the most commonly used WCS ELISA in detecting anti-*B. burgdorferi* antibodies. The results are given in Table 2. Of a panel of 67 acute-phase sera from patients presenting with EM, 31 were positive by the rNon-OspA assay (46%) and 29 were positive by the WCS assay (43%). Of a panel of 18 serum samples from EM patients, convalescent at 3 weeks postpresentation, 15 were positive by the rNon-OspA assay (83%) and 13 were positive by the WCS assay (72%). Of a panel of 29 serum samples from patients with disseminated Lyme disease, 21 were positive by the rNon-OspA assay (72%) and 20 were positive by the WCS assay (69%). Overall, at site A, the sen-

TABLE 1. Summary of data on serum banks

Site ^a	Serum panel	No. of serum samples
A, SUNY SB (n = 173)	Lyme disease	
	Acute-phase sera from patients presenting with EM	67
	EM convalescent-phase sera at 3 wk postpresentation, <i>B. burgdorferi</i> culture positive	18
	Sera from patients with disseminated Lyme disease (large joint arthritis or neurologic manifestations)	29
	Other	
	Syphilis	19
B, CDC (n = 120)	Immunopathological disorders (RA and SLE) ^b	30
	Healthy individuals from area of endemicity	10
	Lyme disease	
	Early Lyme disease and <i>B. burgdorferi</i> culture positive	65
	Disseminated Lyme disease (Lyme arthritis, PCR positive)	10
	Other	
C, NYMC (n = 102)	Syphilis	6
	Autoimmune diseases	20
	Healthy individuals from an area of nonendemicity	19
	First test	
	Early Lyme disease with EM and <i>B. burgdorferi</i> culture positive	33
	Follow-up, 1 wk postpresentation	28
	Follow-up, 3 wk postpresentation	26
	Second test	
	EM, culture positive, 3 wk postpresentation ^c	20
	OspA vaccinated	40
Placebo vaccinated	20	
Healthy individuals from area of endemicity	9	

^a n, number of individuals tested. Abbreviations: SUNY SB, Lyme Disease Center, State University of New York at Stony Brook; NYMC, Westchester Medical Center, New York Medical College.

^b Abbreviations: RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

^c From the panel previously used.

sitivity of the rNon-OspA assay was equivalent to the sensitivity of the WCS assay ($P > 0.05$).

We tested sera from individuals with diseases associated with serological responses that are known to produce cross-reactivity in currently used tests and sera from healthy individuals to assess the specificity of the rNon-OspA assay (Table 2). Of a panel of 19 sera from syphilis-infected patients, 16 were negative by the rNon-OspA assay and 18 were negative by the WCS assay. Of a panel of 30 autoimmune-patient sera, 26 were negative by the rNon-OspA assay and 30 were negative by the WCS assay. Of a panel of 10 healthy-donor sera, all were negative by both assays. Overall, at site A, the specificity of the WCS assay was better than the specificity of the recombinant rNon-OspA assay, but not significantly so ($P > 0.05$).

We concluded that at site A both assays, rNon-OspA and

WCS, were equally sensitive and specific for detection of anti-*B. burgdorferi* antibodies.

Study II: comparison of the recombinant Non-OspA and Vidas immunoassays for sensitivity and specificity (performed at site B). A bank of 120 individual serum samples was used to do the evaluation of the recombinant Non-OspA ELISA at site B. Sera from 75 Lyme disease patients, sera from 26 patients with potentially cross-reactive conditions, and 19 serum samples from healthy subjects were used to do the comparative studies with the Vidas immunoassay, the whole-cell Lyme disease first-tier test used at this site. The results are represented in Table 3. The rNon-OspA ELISA was more sensitive than the CDC Vidas immunoassay in detecting the presence of anti-*B. burgdorferi* antibodies in the sera from patients with Lyme disease, but not significantly so ($P > 0.05$). The Vidas assay was more specific than the rNon-OspA assay, but not significantly so ($P > 0.05$).

TABLE 2. Study I: comparison of rNon-OspA and WCS ELISAs for sensitivity and specificity (performed at site A)^a

Clinical diagnostic	No. of samples	No. positive/no. negative (%) by ELISA with:	
		rNon-OspA	WCS
EM acute phase (at presentation)	67	31/36 (46)	29/38 (43)
EM convalescent phase (>3 wk post-presentation), culture positive	18	15/3 (83)	13/5 (72)
Disseminated Lyme disease (arthritis, neurologic)	29	21/8 (72)	20/9 (69)
Syphilis	19	3/16 (16)	1/18 (5)
Autoimmune disease (RA, SLE ^b)	30	4/26 (13)	0/30 (0)
Healthy	10	0/10 (0)	0/10 (0)

^a Note: values of ≥ 3 standard deviations were counted as positive; P values were >0.05 for all panels tested by McNemar's exact test for correlated proportions.

^b Abbreviations: RA, rheumatoid arthritis; SLE, systematic lupus erythematosus.

TABLE 3. Study II: comparison of rNon-OspA ELISA and Vidas immunoassay for sensitivity and specificity (performed at site B)^a

Clinical diagnostic	No. of samples	No. positive/no. negative (%) by ELISA with:	
		rNon-OspA	Vidas ^b
Early Lyme disease, culture positive	65	56/9 (86)	56/9 (86)
Disseminated Lyme disease (arthritis, PCR positive)	10	10/0 (100)	8/2 (80)
Syphilis	6	1/5 (17)	3/3 (50)
Autoimmune disease	20	6/14 (30)	1/19 (5)
Healthy	19	2/17 (10)	0/19 (0)

^a Note: values of ≥ 3 standard deviations were counted as positive.

^b CDC 1st tier; $P > 0.05$ for all panels tested by McNemar's exact test for correlated proportions.

TABLE 4. Study III: comparison of rNon-OspA and WCS ELISAs for sensitivity of detection of *B. burgdorferi* antibodies in the early stages of Lyme disease (performed at site C)^a

Lyme disease clinical diagnosis	No. of samples	No. positive (%) by ELISA with:	
		rNon-OspA	WCS
EM at presentation, culture positive	33	17 (52)	18 (55)
Follow-up, 1 wk post-EM, culture positive	28 ^b	22 (79)	23 (82)
Follow-up, 3 wk post-EM, culture positive	26 ^b	18 (69)	19 (73)

^a Note: values of ≥ 3 standard deviations were counted as positive.

^b The difference between these numbers and the original 33 patients enrolled denotes the number of patients that failed to show up for the follow-up studies. A *P* value of >0.05 for all panels tested by McNemar's exact test for correlated proportions.

We concluded that at site B both assays were about equally sensitive for detection of anti-*B. burgdorferi* antibodies. The Vidas assay was more specific, but not significantly so. However, with a bigger sample size this test seems to show a tendency toward specific significance.

Study III: comparison of the recombinant Non-OspA and WCS ELISAs for sensitivity (performed at site C). A bank of 102 individual serum samples was used to evaluate the recombinant Non-OspA ELISA at site C. A first test was done to determine the sensitivity of the rNon-OspA assay for the early stages of the disease using a panel of 33 culture-positive serum samples obtained from Lyme disease patients with EM at presentation and follow-up sera from the same patients at 1 week ($n = 28$) and 3 weeks ($n = 26$) post-EM presentation. The same WCS assay used at site A was used at this site to do the comparative study. Results are presented in Table 4. Overall, the sensitivity of the rNon-OspA assay was equivalent to the WCS assay ($P > 0.05$).

In a second test, 20 samples from biopsy-positive subjects obtained at 3 weeks post-EM presentation, 40 serum samples from OspA-vaccinated subjects, 20 serum samples from placebo-vaccinated subjects, and 9 healthy serum panels were used. The biopsy-positive serum was obtained from the panel used in the early Lyme follow-up study. These sera were randomly blinded and run side by side on the rNon-OspA and WCS ELISAs. The results are represented in Table 5. The high agreement between the two tests—85% for early Lyme disease patients, 90% for placebo-vaccinated individuals, and 100% for the healthy individuals—indicates that both assays are about equally sensitive and specific for detection of *B. burgdorferi* antibodies ($P > 0.05$). In the OspA-vaccinated panel, the agreement between the two tests is low (10%) due to the high detection of positives by the WCS assay (38 positives) compared to the rNon-OspA assay (3 positives), which indicates that only the rNon-OspA assay is suitable for diagnostic use in an OspA-vaccinated mixed population ($P < 0.05$). As expected, OspA- and placebo-vaccinated individuals revealed the same degrees of incidence of positives as the healthy controls from an area of endemicity.

We concluded that at site C the sensitivity and specificity of the rNon-OspA assay were equivalent to those of the WCS assay, but only the rNon-OspA assay is suitable for detection of Lyme disease in a population comprised of naturally infected individuals as well as vaccinated individuals.

DISCUSSION

In December of 1998, an anti-Lyme disease, recombinant OspA vaccine was approved by the Food and Drug Administration. Although this vaccine was efficacious, some vaccinated individuals became infected after two or three injections of it. In one study, the vaccine was about 76% effective after the third injection (18). In a second study, the vaccine efficacy after the third injection was about 37% (24). Serodiagnostic assays are needed in these cases. Not only does OspA vaccination render the current ELISAs positive, but it also renders Western blots very difficult to interpret because it induces production of antibodies which bind to different borrelia proteins (1, 4, 21). The use of immunoblotting alone without a preceding ELISA will reduce specificity and increase diagnosis costs (1). Even a small reduction in specificity would have a substantial impact on the predictive value of a positive test when testing patients with a low pretest probability of Lyme disease (1, 16).

OspC is a particularly attractive candidate protein for immunodiagnoses. In unfed ticks, spirochetes express OspA but not OspC (19). However, when the tick starts feeding on mammals, OspC synthesis is induced and OspA is repressed. Thus, OspC is the major protein expressed on the surface of *B. burgdorferi* in mammalian infection and it is a logical target for the development of immunodiagnoses. The serotypic variability of OspC has only recently become apparent (15), and the described assays for serodiagnosis of Lyme disease have failed to address this issue (11, 13, 21). Assays containing only one OspC serotype are expected to be less sensitive than assays with multiple OspC serotypes. The diagnostic value of OspC is increased by the fact that it can be used as a way of detecting the early stages of the disease, giving the physician enough treatment time to prevent the more serious neurologic and arthritic symptoms typical of late Lyme disease.

We have previously developed in our laboratory RCBPs that produced two serologic assays, an ELISA and a rapid assay that can be used as the first-tier tests in the screening for Lyme disease (5, 6). In proceeding with this work, we developed a recombinant chimeric assay containing the diagnostically important proteins of *B. burgdorferi* devoid of OspA in an ELISA format. This test, a recombinant Non-OspA assay, provides reasonable coverage of *B. burgdorferi*'s genetic diversity and

TABLE 5. Study III: comparison of rNon-OspA and WCS ELISAs for specificity of detection of *B. burgdorferi* antibodies in OspA-vaccinated individuals (performed at site C)^a

Clinical diagnosis	No. of samples	No. positive/ no. negative by ELISA with:		<i>P</i>	Agreement ^b
		rNon-OspA	WCS		
Lyme disease 3 wk post-EM, culture positive	20	15/5	18/2		17/20 (85)
Placebo vaccinated	20	4/16	2/18		18/20 (90)
OspA vaccinated	40	3/37	38/2	<0.05	4/40 (10)
Healthy	9	0/9	0/9		9/9 (100)

^a Note: Values of ≥ 3 standard deviations were counted as positive. The *P* value was >0.05 for all panels tested except the OspA-vaccinated panel, for which *P* was <0.05 , by McNemar's exact test for correlated proportions.

^b Values are the numbers of samples (negative or positive) for which results of both tests were in agreement/total number of samples. Values in parentheses are percentages.

demonstrates good diagnostic sensitivity and specificity. It is comprised of OspB, Fla, p93, and the four OspC types linked to *B. burgdorferi* invasiveness: OspC1, OspC2, OspC10, and OspC12. In three different Lyme disease reference laboratories, this rNon-OspA assay was tested with several serum panels from patients with early Lyme disease and disseminated Lyme disease, from patients with conditions known to give false-positive serologies, from healthy individuals, and from OspA-vaccinated individuals. A comparison study between the rNon-OspA ELISA and a commonly used whole-cell borrelia ELISA was done to assess their performances on sensitivity and specificity. At the three sites, the rNon-OspA demonstrated the same sensitivity for detection of anti-*B. burgdorferi* antibodies. In addition to the low detection of positives by the rNon-OspA assay in the OspA-vaccinated population, the OspA- and the placebo-vaccinated patient sera yielded similar incidences of positives as would be expected in a healthy population from an area of endemicity, indicating that this assay is suitable for detection of anti-*B. burgdorferi* antibodies resulting from either natural infection or vaccine failure.

Several ELISAs for discrimination of OspA vaccination from spirochete infection have been described (21, 25). These studies describe a test based on an OspA-OspB plasmidless *B. burgdorferi* strain which is a WCS ELISA. Vaccination with OspA induces an antibody response that makes serologic interpretation of infection with *B. burgdorferi* difficult, especially by screening tests based on whole-cell preparations. Assuming a larger sample size, the WCS assay shows a trend to significantly better specificity than the rNon-OspA assay. However, this assay is not suitable for use as a diagnostic tool in a mixed population of naturally infected and vaccinated individuals. On the other hand, the rNon-OspA assay provides significantly increased specificity, 81 to 90%, compared to that of the OspA-B plasmidless whole-cell assay described by Wieneke et al., 33 to 51% (21). Other peptide-based assays have been described that could also prove very useful in the serodiagnosis of Lyme disease in a mixed population of vaccinated and unvaccinated individuals (9, 10).

The current recommendation by the CDC (3) relies on the use of a second-tier, confirmatory test for Lyme disease when the first test yields a positive or equivocal result. This test is usually a Western blot, which is rendered very difficult to interpret in the case of previous OspA vaccination. We developed a sensitive, recombinant Non-OspA ELISA that gives the best specificity compared to similar tests developed with the same purpose. We conclude that this rNon-OspA assay can be useful for the immunologic discrimination of *B. burgdorferi* infection from vaccination with OspA and that it can also suggest when vaccine failure has taken place.

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