

ABSTRACT

Borrelia burgdorferi (*Bb*) vaccines are commonly administered to prevent *Bb* infection and clinical Lyme disease in endemic areas. Numerous published studies have documented that assays based on *Bb* VlsE derived C6 peptide do not react with sera from vaccinated dogs. However, because of variability in vaccine formulations, study designs and possible variability in interpretation of confirmatory assays, evaluation of samples derived from a controlled experimental vaccination study was warranted.

Twelve purebred specific pathogen-free beagles were utilized. Groups of three dogs were assigned to a vaccine group and administered one of four vaccines [Recombitek™ Lyme (Merial), LymeVax® (Fort Dodge Laboratories), Galaxy® Lyme (Schering-Plough and Nobivac® Lyme (Merck)]. The first three vaccines were administered on weeks 0, 2, 33, 36 and 39 to generate high titer serum. The Nobivac Lyme Vaccine was administered on weeks 0 and 3. Blood samples collected before and after vaccinations at all time points were processed and serum was tested using *Bb* IFA (IDEXX Reference Laboratories), Quant C6™ ELISA (IDEXX Reference Laboratories) and SNAP®4Dx® Plus.

Sera from all dogs had positive IFA titers, ranging from 1:800 five weeks post vaccination to 1:6400 following additional vaccinations. The Lyme Quant C6 Test and the SNAP 4Dx Plus test were negative for all samples from all vaccinated dogs at all time points including at peak IFA titers.

The results of this study document that the *Bb* vaccines studied did not induce antibodies detectable in C6 based immunoassays, even when dogs are hyper-vaccinated.

INTRODUCTION

Introduction of Lyme disease vaccines for use in canines has led to the widespread use of vaccine in Lyme disease-endemic areas of the United States. The SNAP 4Dx Plus Test Kit uses *Borrelia*-specific peptide (C6 peptide) for detection of antibody to *Borrelia burgdorferi*. Numerous published scientific articles have documented that those immunoassays incorporating the C6 peptide as the target diagnostic antigen failed to react with sera from vaccinated dogs.¹⁻⁵

The purpose of the study was to test sera from vaccinated dogs using the C6-peptide-based microtiter format ELISA and SNAP 4Dx Plus test to demonstrate that these tests are non-reactive with samples from dogs receiving repeated vaccinations.

METHODS

Vaccine Study: Experimental vaccinations were performed in collaboration with Covance Research Products (Denver, PA) and Colorado State University using standard vaccination protocols. Groups of 3 dogs each received following vaccines - Recombitek™ Lyme (Merial); LymeVax® (Fort Dodge Laboratories); Galaxy® Lyme (Schering-Plough) and Nobivac® Lyme Vaccine (Merck dog Health). Vaccine was administered on weeks 0, 2, 33, 36 and 39. The Nobivac® Lyme Vaccine (Merck dog Health) was administered on weeks 0 and 3. Blood samples were drawn at weekly intervals, from weeks 0 through week 11.

***B. burgdorferi* IFA and Quantitative C6 ELISA:** An indirect IFA and the Lyme Quant C6® Test were performed on each sample at IDEXX Reference Laboratories.

***B. burgdorferi* Western Blot (WB) Assay:** Samples were sent to University of Connecticut Veterinary Medicine Diagnostic Testing Lab for Lyme Western Blot testing.

SNAP® 4Dx® Plus Test All samples were blinded and run on the SNAP 4x Plus Test Kit and read by one operator visually, per the kit insert protocol.

- Liang FT. 2000; *J Clin Micro* 38(1), 4160-4166.
- Levy S. 2002; *Vet. Ther.* 3(3):308-315.
- Levy, S.A. 2002; *Vet Ther* 3(4):420-424.
- O'Connor TP. 2004; *Clin Diagn Lab Immun* 11(3):458-462.
- Marques AR. 2002; *J Clin Micro* 40(7):2591-2593.

RESULTS

SNAP 4Dx Plus and Quant C6 Assays: In the Recombitek™ Lyme, LymeVax®, Galaxy® and Nobivac® Lyme vaccination studies, sera from vaccinated dogs were tested by the SNAP 4Dx Plus and Quant C6 Assays. All dogs were negative for antibody to *B. burgdorferi* in both assays throughout the study (Tables 1-4).

Immunofluorescence Assay

In the Recombitek™ Lyme, LymeVax®, and Galaxy® Lyme vaccination study, sera from all of the vaccinated dogs had significant IFA titers (individual titers ranged from 1:800 to 1:1600) when tested 5 weeks following the initial vaccination (Tables 1-3). Serum antibody titers decreased 4 to 16 fold (individual titer range 1:100 to 1:400) by week 33. Antibody titers increased substantially following administration of additional doses of vaccine on weeks 33, 36 and 39 and reached their highest levels (individual titer range 1:1600 to 1:6400) one-week following the fifth vaccination (week 40). In the Nobivac® Lyme vaccination study, sera from all of the vaccinated dogs had significant IFA titers (individual titers ranged from 1:1600 to 1:6400) when tested 4 weeks following the vaccination (Table 4).

Western Blot assay: The Western Blot results at week 5 for sera from dogs immunized with the whole bacterin vaccines (LymeVax®, Galaxy® Lyme) had prominent bands at positions corresponding to *B. burgdorferi* proteins with molecular weights of 31,000 (OspA) and 34,000 (OspB). Sera from dogs immunized with the recombinant OspA vaccine (Recombitek™ Lyme) had a single prominent WB band corresponding to the *B. burgdorferi*-specific Osp A protein (31 kDa). In the Nobivac® Lyme vaccination study, sera from all of the vaccinated dogs induced antibody responses to both OspA and OspC. *B. burgdorferi* proteins, the latter not commonly associated with other commercial Lyme vaccines.

Table 1. Assay results for dogs repeatedly vaccinated with LymeVax® (Fort Dodge)

Sample Time Point	SNAP 4Dx Plus	IFA Titer (1:___)	Western Blot ¶	Quant C6
Prebleed	NEG	All < 100	NEG	NEG
Week 5	NEG	1600, 1600, 800	Osp A OspB	NEG
Week 9	NEG	400, 400, 100	Osp A OspB	NEG
Week 12	NEG	400, 200, 100	Osp A OspB	NEG
Week 17	NEG	400, 200, 100	Osp A OspB	NEG
Week 33	NEG	400, 400, 100	Osp A OspB	NEG
Week 34	NEG	1600, 1600, 1600	Osp A OspB	NEG
Week 38	NEG	3200, 1600, 1600	Osp A OspB	NEG
Week 40	NEG	6400, 1600, 1600	Osp A OspB	NEG
Week 43	NEG	3200, 1600, 1600	Osp A OspB	NEG

¶Major vaccine induced antigen response

Table 2. Assay results for dogs repeatedly vaccinated with Galaxy® Lyme(Schering-Plough)

Sample Time Point	SNAP 4Dx Plus	IFA Titer (1:___)	Western Blot¶	Quant C6
Prebleed	NT,neg,neg	All < 100	NEG	NEG
Week 5	NEG	1600, 1600, 1600	Osp A OspB	NEG
Week 9	NEG	400, 400, 400	Osp A OspB	NEG
Week 12	NEG	400, 400, 200	Osp A OspB	NEG
Week 17	NEG	400, 400, 100	Osp A OspB	NEG
Week 33	NEG	400, 100, 100	Osp A OspB	NEG
Week 34	NEG	3200, 1600, 1600	Osp A OspB	NEG
Week 38	NEG	3200, 3200, NT	Osp A OspB	NEG
Week 40	NEG	6400, 6400, 3200	Osp A OspB	NEG
Week 43	NEG	3200, 3200, 3200	Osp A OspB	NEG

*NT = not tested, due to lack of sample volume
¶Major vaccine induced antigen response

RESULTS-CONTD.

Table 3. Assay results for dogs repeatedly vaccinated with Recombitek™ Lyme (MERIAL)

Sample Time Point	SNAP 4Dx Plus	IFA Titer (1:___)	Western Blot¶	Quant C6
Prebleed	NEG	All < 100	NEG	NEG
Week 5	NEG	800, 800, 800	Osp A	NEG
Week 9	NEG	100, 100, 100	Osp A	NEG
Week 12	NEG	100, <100, <100	Osp A	NEG
Week 17	NEG	All < 100	Osp A	NEG
Week 33	NEG	100, 100, 100	Osp A	NEG
Week 34	NEG	1600, 1600, 800	Osp A	NEG
Week 38	NEG	3200, 3200, 1600	Osp A	NEG
Week 40	NEG	3200, 3200, 3200	Osp A	NEG
Week 43	NEG	1600, 1600, 800	Osp A	NEG

¶Major vaccine induced antigen response

Table 4. Assay results for dogs repeatedly vaccinated with Nobivac® Lyme (Merck)

Sample Time Point	SNAP 4Dx Plus	IFA Titer (1:___)	Western Blot¶	Quant C6
Prebleed	NEG	All < 100	NEG	NEG
Week 1	NEG	<100, 100, <100	Osp A OsPC	NEG
Week 2	NEG	100, 400, 800	Osp A OsPC	NEG
Week 3	NEG	200, 200, 1600	Osp A OsPC	NEG
Week 4	NEG	1600, 6400, 3200	Osp A OsPC	NEG
Week 5	NEG	1600, 6400, 1600	Osp A OsPC	NEG
Week 6	NEG	800, 3200, 1600	Osp A OsPC	NEG
Week 7	NEG	800, 3200, 1600	Osp A OsPC	NEG
Week 8	NEG	400, 1600, 800	Osp A OsPC	NEG
Week 9	NEG	800, 800, 400	Osp A OsPC	NEG
Week 10	NEG	200, 800, 200	Osp A OsPC	NEG
Week 11	NEG	100, 400, 200	Osp A OsPC	NEG

¶Major vaccine induced antigen response

SUMMARY AND CONCLUSIONS

The results of the experimental vaccination study demonstrate the absence of reactivity of C6 based immunoassays with serum antibodies from Lyme-vaccinated dogs known to be free of *B. burgdorferi* infection.

The SNAP 4Dx Plus Test Kit *B. burgdorferi* assay detects antibody induced as a result of natural infection with the organism and not following REPEATED immunization with the following commercial vaccines: Recombitek™ Lyme ; LymeVax® ; Galaxy® Lyme; and Nobivac® Lyme.

Conflict of Interest: Stillman, Thatcher, Beall, O'Connor and Chandrashekar are IDEXX employees. Drs. Lappin and Goldstein consult for IDEXX Laboratories.