

Performance of the Abaxis VetScan Canine Lyme Rapid Test for Lyme Disease

Rajesh Mehra, Ph.D. – Abaxis, Inc.

Introduction

Lyme disease, also known as Lyme Borreliosis, is now reported to be the most common tick-borne disease in the USA and Europe (1-5). Lyme disease in its various manifestations has been diagnosed in human, dog, equine and bovine patients (1-5). Deer, white-footed mice, chipmunks and shrews are the main reservoirs of this disease (1-5), while deer support the tick vector populations that help spread the disease. Descriptions of Lyme disease in the USA are somewhat recent, although similar disorders, especially affecting skin, were described in Europe in the late nineteenth century (6). Lyme arthritis was first described by Dr. Allen Steere (7) in the Old Lyme area in Connecticut in the mid-1970's. The disease was originally thought to be a variant of juvenile arthritis. However, Dr. W. Burgdorfer in the early 1980's isolated the bacteria that caused the disease found in the town of Old Lyme, Connecticut (8). The spirochete bacterium isolated by Dr. Burgdorfer belonged to the genus *Borrelia*. A number of species belonging to the *Borrelia* genus are now known to be involved in the pathogenesis of Lyme disease. Some of the notable species include: *B. burgdorferi*, *B. garinii*, *B. afzelii*, *B. lusitanae*, *B. valaisiana*, *spielmanii* and *B. bavariensis* (1-5). It appears that *B. burgdorferi* is the sole causative agent of Borreliosis in the USA where as *B. garinii* and *B. afzelii* are the most common agents isolated from Lyme patients in Europe. These bacteria are somewhat difficult to culture but now have been studied in substantial detail and their whole genome has been sequenced making it possible to target specific sequences for identification (9).

Although a variety of animal species and humans are infected by *B. burgdorferi* in the USA, dogs and man are the most intensively studied species. Human Lyme disease has generated substantial controversy over the years with respect to the possible chronic nature of the infection and its successful treatment by antibiotics (10). Both the peer-reviewed and popular literature is full of articles supporting and discrediting the notion of chronic infection in man. A recent article from Tulane researchers appears to suggest that *B. burgdorferi* may actually persist for a long time in infected individuals although the disease may actually be in remission (11).

Skin rashes in the form of Erythema migrans (EM) and history of tick bites point to the possibility of Lyme disease in humans. Other clinical presentations such as acrodermatitis chronica atrophicans (ACA), cardiac and neurological symptoms and arthritis are also noted. The

Centers for Disease Control and Prevention (CDC) recommend a two-tier laboratory testing approach in the presence of specific signs and possible exposure to Ixodes tick bites in humans. Laboratory testing is not recommended in the absence of clinical signs, symptoms and/or history of tick bites. Dogs do not present with EM but may have some of the other clinical signs seen in humans. Clinical signs in dogs may include acute, chronic or shifting lameness which may be recurrent, stiffness, sensitivity to touch, respiratory difficulties, fever and lack of appetite and lymphadenopathy. Glomerulonephritis is also seen in canine patients. Cardiac and neurological disease occur at a lower frequency in canine patients.

Laboratory diagnosis of *B. burgdorferi* infection in dogs has generally relied on serological tests (12-14). Liang et al (15) developed an antibody test to a 26 AA peptide in the IR6 region of the VlsE protein of *B. Burgdorferi*. This test was shown to be sensitive and specific for diagnosis of active infection with the bacteria. This test is used in the ELISA-based tests in microwell and lateral flow format now sold by IDEXX. The test is now known in the veterinary industry as C₆. The IDEXX Lyme Quant C₆ has been the only available quantitative test to determine *B. burgdorferi* status in canine patients. Membrane-based ELISA tests using recombinant antigens have been available for use in dogs in Europe. Whole cell-lysate ELISA kits have also been sold. Abaxis has developed a highly sensitive and specific qualitative rapid lateral flow test to offer veterinarians a choice for diagnosing Lyme disease in dogs.

Materials and Methods

A total of 194 dog samples were procured from participating veterinary clinics. These samples were tested at the participating clinic by IDEXX SNAP™ 3Dx or 4Dx and the results were communicated to Abaxis. Some of the samples were also tested at Abaxis with the 3Dx or 4Dx test. All SNAP™ testing was performed according to manufacturer's instructions using kits within their shelf-life. The participating clinics also sent samples to the IDEXX laboratory for quantitative C₆ testing and sent an aliquot to Abaxis for additional testing using the Abaxis Quant Lyme Test, formerly offered by Abaxis Veterinary Reference Laboratories.

Quantitative testing (Abaxis Lyme Quant) on the above listed samples was performed using an ELISA protocol previously developed at Abaxis. Proprietary peptides

devised at Abaxis were used in this assay. These synthetic chimera peptides mimic certain antigens found in *B. burgdorferi* and thus are able to interact with antibodies elicited in dogs upon exposure to the spirochete. These Abaxis peptides are coated in the wells of 96-well plates for performing an indirect ELISA. The plates were blocked using a casein-based reagent. Appropriate dilutions of dog samples were allowed to react with the immobilized peptides. The plates were washed to remove un-reacted species and the captured dog IgG molecules were identified using standard technology. Appropriate calibrators were used to define a cut-off that separates patients likely to be clinically ill from exposed patients.

The VetScan® Canine Lyme Rapid Test, recently developed at Abaxis and approved by the Center for Veterinary Biologics, was performed as described in the Package Insert to assess the Lyme status of the samples previously tested by SNAP™ or C₆ Quant™. Briefly, samples suspected of containing antibodies against *B. burgdorferi* were mixed with the conjugate and applied to the sample port followed by a chase buffer. The devices were read at 8-10 minutes. All reagents, devices and ancillary materials used were within their shelf-life.

The data sets obtained from the Abaxis Lyme Quant, VetScan® Canine Lyme Rapid Test, IDEXX SNAP™ and IDEXX C₆ Quant were evaluated by standard comparative methods to evaluate their performance.

Results

Abaxis Lyme Quant vs. IDEXX C₆ Quant Testing: Table I summarizes the comparative data obtained from 194 dog samples tested using the IDEXX C₆ and the Abaxis Lyme Quant test. Of 194 samples, 99 samples tested negative with C₆ test whereas 95 samples tested negative using Abaxis Lyme Quant. Both tests reported 95 of the same samples to be positive. Therefore, when comparing the Abaxis Lyme Quant to the C₆ Quant, the Abaxis Lyme Quant has a sensitivity of 100%. Since the Abaxis Lyme Quant reported 4% of the C₆ negative samples as positive samples, the Abaxis Lyme Quant Test thus offers a 96% specificity as well as a 96% positive and 100% negative predictive value with respect to C₆ Quant.

Table I

		C ₆		
		+	-	
Lyme Quant	+	95	4	N = 194
	-	0	95	
	Sensitivity	100%		
	Specificity	96% (correlating to C ₆ > 29)		
	PPV	96%		
	NPV	100%		

IDEXX SNAP vs. IDEXX C₆ Quant: The data shown in Table II shows that 76 of the samples testing positive by SNAP™ actually reported negative by C₆. Consequently, the specificity of SNAP™ vs. C₆ is 23% and the positive predictive value is 56%.

Table II

		C ₆		
		+	-	
SNAP	+	95	76	N = 194
	-	0	23	
	Sensitivity	100%		
	Specificity	23% (correlating to C ₆ > 29)		
	PPV	56%		
	NPV	100%		

VetScan® Canine Lyme Rapid Test vs. Abaxis Lyme Quant: The final set of data reported in this study shows the relationship between the VetScan® Canine Lyme Rapid Test and the Abaxis Lyme Quant Test (Table III). Of the 111 samples testing positive with the VetScan® Rapid Test, 99 were deemed positive by the Lyme Quant. Thus the specificity of the Abaxis Rapid Test is 87% with respect to the Lyme Quant. This compares with 23% specificity of SNAP™ vs. C₆ Quant (Table II). In addition, the VetScan® Rapid Test offers 89% positive predictive value and 100% negative predictive value.

Table III

		Lyme Quant		
		+	-	
VetScan Rapid	+	99	12	N = 194
	-	0	83	
	Sensitivity	100%		
	Specificity	87% (correlating to Lyme Quant value of > 39)		
	PPV	89%		
	NPV	100%		

Discussion

In general veterinary practice, Lyme testing is performed not only when clinical signs indicate the need, but also as a routine screening test during well patient visits, especially in endemic areas. It has been suggested (16) that the samples found negative by SNAP™ should not be tested further. However, it is recommended that the positive samples should undergo C₆ testing (16). This additional test may be declined by the pet owner for any number of reasons. When the test is declined, the clinician then has the choice of using the in-house test as a diagnostic tool and initiating treatment, or may determine to observe the patient until clinical signs occur. However, if there are a high number of non-clinical, positive results associated with the in-house test, this may cause either significant stress for the owner and veterinarian in waiting for signs to occur, potential unnecessary medication and cost if treatment is chosen, or could cause additional stress and cost to send in a confirmatory quantitative test to the commercial lab.

Until now, veterinarians have had limited diagnostic methods for detecting and treating Lyme disease in dogs.

One of the common in-house tests and its corresponding send-in laboratory method have been the only technology available. However, the Abaxis VetScan Canine Lyme Rapid Test now offers both a cost-effective and time saving option. As shown in this report, the Abaxis VetScan Canine Lyme Rapid Test reports antibody levels more consistent with active disease and yet is highly sensitive and excludes exposed non-clinical animals.

Conclusions

This study demonstrates that the Abaxis VetScan® Canine Lyme Rapid Test is a reliable, cost-effective and time saving point-of-care test. The majority of the patients testing positive by the Lyme Rapid Test are confirmed infected based on quantitative testing. These patients can be appropriately treated and monitored by retesting with the Vetscan Rapid Test.

Citations

- ¹ Rizzoli A, Haufler H, Carpi G, Vourc H G, Neteler M, Rosa R. Lyme borreliosis in Europe. *Euro Surveill*. 2011 Jul 7;16(27). pii: 19906. 21794218.
- ² Elbaum-Garfinkle S. Close to home: a history of Yale and Lyme disease. *Yale J Biol Med*. 2011 Jun;84(2):103-8.
- ³ Coumou J, van der Poll T, Speelman P, Hovius JW. Tired of Lyme borreliosis. *Lyme borreliosis in the Netherlands*. *Neth J Med*. 2011 Mar;69(3):101-11
- ⁴ Shen AK, Mead PS, Beard CB. The Lyme disease vaccine--a public health perspective. *Clin Infect Dis*. 2011 Feb;52 Suppl 3:s247-52
- ⁵ Kugeler KJ, Griffith KS, Gould LH, Kochanek K, Delorey MJ, Biggerstaff BJ, Mead PS. A review of death certificates listing Lyme disease as a cause of death in the United States. *Clin Infect Dis*. 2011 Feb 1;52(3):364-7. Epub 2010 Dec 28.
- ⁶ Piesman J, Gern L. Lyme borreliosis in Europe and North America. *Parasitology*. 2004;129 Suppl:S191-220.
- ⁷ Steere AC, Malawista SE, Snyderman DR, Shope RE, Andiman WA, Ross MR, Steele FM. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. *Arthritis Rheum*. 1977 Jan-Feb;20(1):7-17.
- ⁸ Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease--a tick-borne spirochetosis? *Science*. 1982 Jun 18;216(4552):1317-9.
- ⁹ Barbour AG, Jasinskas A, Kayala MA, Davies DH, Steere AC, Baldi P, Felgner PL. A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with *Borrelia burgdorferi*. *Infect Immun*. 2008 Aug;76(8):3374-89. Epub 2008 May 12.
- ¹⁰ Stricker RB, Johnson L. Lyme disease: the next decade. *Infect Drug Resist*. 2011;4:1-9. Epub 2011 Jan 7.
- ¹¹ Embers ME, Barthold SW, Borda JT, Bowers L, Doyle L, Hodzic E, Jacobs MB, Hasenkampf NR, Martin DS, Narasimhan S, Phillippi-Falkenstein KM, Purcell JE, Ratterree MS, Philipp MT. Persistence of *Borrelia burgdorferi* in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection. *PLoS One*. 2012;7(1):e29914. Epub 2012 Jan 11.
- ¹² Krupka I, Knauer J, Lorentzen L, O'Connor TP, Saucier J, Straubinger RK. *Borrelia burgdorferi* sensu lato species in Europe induce diverse immune responses against C₆ peptides in infected mice. *Clin Vaccine Immunol*. 2009 Nov;16(11):1546-62. Epub 2009 Sep 2.
- ¹³ Ivanova L, Christova I, Neves V, Aroso M, Meirelles L, Brisson D, Gomes-Solecki M. Comprehensive seroprofiling of sixteen *B. burgdorferi* OspC: implications for Lyme disease diagnostics design. *Clin Immunol*. 2009 Sep;132(3):393-400. Epub 2009 Jul 2.
- ¹⁴ Gerber B, Haug K, Eichenberger S, Reusch CE, Wittenbrink MM. Follow-up of Bernese Mountain dogs and other dogs with serologically diagnosed *Borrelia burgdorferi* infection: what happens to seropositive animals? *BMC Vet Res*. 2009 May 8;5:18.
- ¹⁵ Liang, F.T., Jacobson, R.H., Straubinger, R.K., Grooters, A., Philipp, M. T. Characterization of a *Borrelia burgdorferi* VlsE Invariable Region Useful in Canine Lyme Disease Serodiagnosis by Enzyme-Linked Immunosorbent Assay *J.Clin. Microbiol*. 2000 Nov 38: 4160-4166.
- ¹⁶ http://www.idexx.com/pubwebresources/pdf/en_us/smallanimal/reference-laboratories/lyme-quant-c6-white-paper.pdf