Diagnostic Testing for Canine Lyme Disease: A New Two-Tiered Approach for Effective Management

Canine Lyme disease continues to spread across the country and there is great interest in managing this often subclinical disease. IDEXX's new diagnostic test for canine Lyme disease allows you to identify infection and use valuable, measurable information to decide on a therapeutic plan and to monitor your treatment choice.

The new Lyme Quantitative C_6 Antibody Test is a reference laboratory ELISA test based on measurement of antibody to the C_6 antigen, the same unique antigen used in the SNAP® 3Dx® in-house screening test. The combination of these assays provides you with the latest diagnostic tools for a new two-tiered approach to managing Lyme disease: an in-house screen with a follow-up quantitative assessment.

Background

Since 1995, the Centers for Disease Control and Prevention (CDC) has recommended that serological testing for Lyme disease consist of a two-tiered approach—an initial screening with an enzyme-linked immunoassay (ELISA) or an indirect fluorescent antibody (IFA) test, followed by a Western blot to corroborate a positive or suspect test result.[1] However, this approach has limitations.

Limits of the Traditional Approach

The traditional approach is limited because these tests measure antibodies to the whole *Borrelia burgdorferi* organism (called whole-cell antibodies). Whole-cell antibodies elevate after exposure or vaccination, and often remain elevated after successful treatment. Traditionally, dogs are only tested after clinical signs are evident. The lack of early clinical signs, such as erythema migrans, in infected dogs places greater importance on the laboratory method used when diagnosing canine Lyme disease.[2] With recent research showing that subclinical infections of canine Lyme disease are more prevalent than once thought, the diagnostic approach to these cases should be reexamined.[3]

In addition, the traditional whole-cell antibody tests often yield false-positive test results due to cross-reaction with autoimmune antibodies or infection from other tick-borne diseases such as rickettsiosis, babesiosis and ehrlichiosis.[4] Also, current Lyme vaccines complicate the diagnostic usefulness of these tests and, in many cases, render them uninformative. For example, IFA and whole-cell ELISA tests cannot distinguish natural exposure from vaccine. And, although the Western blot has been considered a useful tool to help distinguish exposure from vaccination, new research suggests that interpretation can be challenging.[5] Furthermore, the Western blot is technique-dependent, expensive and time-consuming.

The traditional tests are also limited because they cannot be used to determine treatment protocol and cannot measure response to treatment. Whole-cell antigens of the *Borrelia burgdorferi* spirochete are assumed to be rapidly sequestered by the follicular dendritic cells (FDCs), resulting in long-lived B cell memory. Therefore, dogs tested for whole-cell antibodies rarely have a significant decrease in antibody, even as successful treatment lowers the *Borrelia burgdorferi* spirochete numbers in the body.

The Benefits of C_6 Technology

With the discovery of the C_6 antigen and the dog's unique antibody response to it, IDEXX developed the SNAP® 3Dx® test as a screening tool. C_6 is a synthetic peptide derived from VlsE, an outer-surface immunodominant portion of *Borrelia burgdorferi.* Research into the antibody response to C_6 at Tulane University and to VlsE at the University of Texas identified unique properties that are beneficial for Lyme diagnostics.

Tulane University scientists discovered that the anti-C_6 antibody response (often referred to as the C_6 antibody) is more sensitive than whole-cell antigen in early infection, detectable as early as three weeks post-exposure.[6] Antibody to the C_6 antigen is also highly specific for *B. burgdorferi* infection. Dogs with leptospirosis, Rocky Mountain spotted fever, babesiosis, ehrlichiosis and heartworm disease did not have antibodies to C_6. Nor were antibodies to C_6 produced in response to immunization with currently available canine Lyme vaccines.[6, 7]

The benefits of C_6 diagnostics are not confined to the veterinary field. Because of its sensitivity, specificity and the fact that it does not cross-react with Lyme vaccinations, C_6 antibody is presently the major diagnostic test for Lyme disease in the human field.[8, 9] In fact, recent research indicates that the C_6 ELISA alone is comparable or superior to the traditional two-tiered testing method of IFA and Western blot, and that decreases in the titer of antibodies against C_6 can indicate a successful therapeutic outcome for Lyme patients.[10, 11, 12, 13, 14] These are major breakthroughs in the diagnosis and treatment of Lyme disease.

C_6 Method

The Lyme Quantitative C_6 Antibody Test measures the level of C_6 antibody—the unique C_6 antigen is associated with the variable region only in live spirochetes. Research has demonstrated that the C_6 antibody declines rapidly and significantly after effective treatment.

Whole-Cell Diagnostic Methods

Whole-cell diagnostic methods (IFA, KELA, WB) measure the IgG antibodies produced to numerous antigens on the "whole" spirochete. The persistence of these whole-cell antigens produce antibodies that are present and remain elevated even when spirochetes are reduced or eliminated.

Lyme Quantitative C_6 Antibody Test

- Highly specific
- Identifies infection
- Does not cross-react with currently available Lyme vaccines
- C_6 antibodies wane rapidly post-treatment*

IFA and Whole-Cell ELISA

- Nonspecific
- Unable to differentiate infection from exposure to *Borrelia burgdorferi*
- Cross-reacts with vaccine antibodies
- Post-treatment titers typically remain unchanged—uninformative**

Lyme Quantitative C_6 Antibody Test

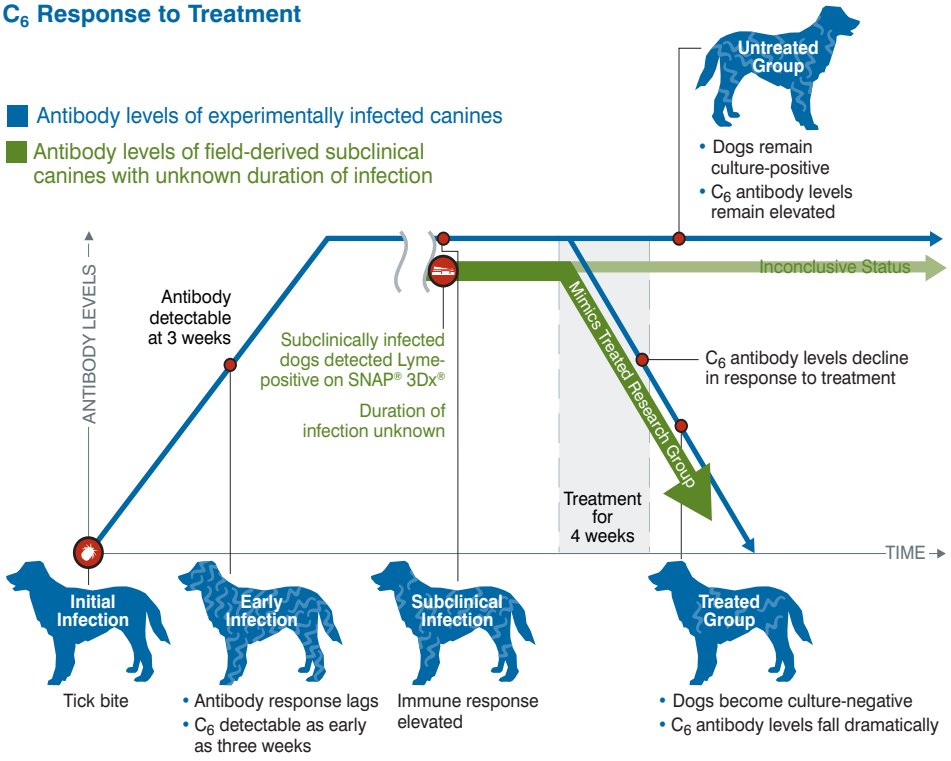
- Quantitative, nonsubjective information
- Does not cross-react with vaccine
- Economical
- Informative post-treatment*

Western Blot

- Subjective and technique-dependent
- May be unreliable in a vaccinated population
- Expensive
- Uninformative as post-treatment test**

*Straubinger RK, Straubinger AF. Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effect of corticosteroids: an experimental study. *Journal of Infectious Diseases*. 2000;181:1069–1081.
**These whole-cell antigens initiate the production of immune system memory cells, so animals continue to have a titer even when spirochete numbers are reduced or eliminated.

C_6 Response to Treatment



Similar research is being conducted on the canine side. Initial research at Tulane University demonstrates not only the benefits of the higher accuracy in determining infection as compared to traditional methods, but also that measuring C_6 antibody can be useful clinically in measuring response to treatment. C_6 antibody levels rise dramatically after *B. burgdorferi* exposure, and then drop off rapidly after treatment with antibiotics.[15]

This research evaluated 16 dogs experimentally infected with *B. burgdorferi*. The 12 treated dogs showed a dramatic decrease in C_6 antibody post-treatment, and a single treated dog showed minimal arthritis in one joint post-treatment. The four untreated dogs maintained high C_6 antibody levels, and all of them experienced arthritis in numerous joints post-treatment.[15,16]

Similar findings were seen in field-derived samples. Most of the dogs responded with a significant drop of the C_6 antibody. A small fraction were low C_6 antibody-responders with inconclusive status.

These studies showed that quantitative C_6 levels were able to provide a good indication of the infection status. Monitoring these canine Lyme patients following treatment revealed that a drop of 50% or more indicated successful treatment.

Diagnosing Subclinical Lyme Disease Is Important

Recent research at Cornell University demonstrated the progression of Lyme disease in dogs, and highlighted several issues regarding *subclinical* infections. Of 16 dogs infected with Lyme disease, 75% had a three-to-six-day episode of varying degrees of lameness, with the first episode occurring at a median of 71 days post-infection. In all cases, clinical signs resolved without treatment, yet all dogs remained infected as confirmed by culture and PCR.[16] This study indicates that subclinical infections are more common than previously thought and may outnumber clinical infections.

Owners often see episodes of canine lameness similar to those seen in these experimentally infected dogs. However, the lameness may resolve and the owner remains unaware that the dog's lameness was an acute presentation of Lyme disease—the dog is subclinically infected. Research shows that these subclinically infected dogs will display arthritic histopathological changes in the joints.

The Cornell research, mentioned previously, also demonstrated the profound response that antibiotic therapy has in treating Lyme disease. At postmortem, none of the 12 treated dogs had tissue samples that were culture-positive, whereas all four untreated dogs yielded multiple tissue samples that were positive for *B. burgdorferi* by culture. Additionally, when these dogs were immune-suppressed with prednisone, clinical signs returned only in untreated dogs, depicting a viable, yet subclinical infection.[16] This clinical response not only showed the benefit of the antibiotic therapy, but also demonstrated the role the immune system plays in maintaining the subclinical state.

The recognition of the existence of subclinical Lyme disease provides further support for this new two-tiered approach.

A New Two-Tiered Approach to Canine Lyme Testing

With the advent of the SNAP® 3Dx® and the Lyme Quantitative C_6 Antibody Test, we recommend screening dogs and then further characterizing positive dogs by quantifying their C_6 antibody levels. The quantitative C_6 assay provides a two-tiered approach to Lyme disease testing. Veterinarians can screen all dogs for Lyme accurately and economically with the in-house SNAP® 3Dx® and then follow up positive results with the Lyme Quantitative C_6 Antibody Test to accurately measure a dog's antibody level to C_6.

For a dog with clinical signs of Lyme disease, the quantitative C_6 level can provide you with a pretreatment level of C_6 and help gauge the dog's response to treatment with a follow-up level. For a subclinical dog, the quantitative C_6 level can provide you with information on whether treatment is warranted, and, if so, a follow-up test can help measure treatment effectiveness.Test results are provided along with interpretive criteria and recommendations for treatment.

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IDEXX LABORATORIES