Veterinary Lyme Disease Testing at CVMDL

- Types of tests offered
- Comparison of Western blot vs Multiplex recombinant antigen bead assay for diagnosis of Lyme disease

Types of tests offered, and their uses

*Borrelia burgdorferi* Elisa (dog, horse) or IFA (cattle, sheep, goat, deer, llama, cat, others)

We offer a whole cell Elisa (horse, dog or IFA in other species) that is a screening test for antibodies against *B. burgdorferi*. Each patient serum sample is serially diluted, and results are expressed as an antibody titer, giving a quantitative result. Using a whole cell Elisa screening test allows detection of multiple antibodies produced by the patient against *B. burgdorferi*, as opposed to detection of antibodies to a few specific recombinant antigens. We feel that this broader approach is helpful in preventing false negative results. Limitations: *Borrelia burgdorferi* vaccination will result in increased titers, which can be distinguished from natural infection by Western blot. Low positive titers may reflect cross-reactivity with other spirochetal organisms, which can also be distinguished by Western blot. This Elisa is not used in known *Borrelia burgdorferi* vaccinated animals (those samples should go directly to Western blot). (Specimen types: serum, CSF, joint fluid)

*Borrelia burgdorferi* Western blot (immunoblot) (dog, horse, cattle, sheep, goat, deer, llama, cat, others)

The Western blot is a qualitative test, which detects antibodies produced by the patient to the many antigenic proteins of *B. burgdorferi*. Pattern analysis of the antibodies produced can rule out cross-reactivity, indicate common vaccine responses, and give an indication of stage of infection (early, ongoing, chronic), particularly if serial samples are submitted. Serial samples may also be helpful in following progression of infection, and response to treatment. (Specimen types: serum, CSF, joint fluid)

*Borrelia burgdorferi* PCR (ticks, tissues, fluids, all species) Polymerase Chain Reaction (PCR) detects *Borrelia burgdorferi* DNA in specimens. Since *B. burgdorferi* are only rarely present in the bloodstream (usually prior to development of clinical signs), this test is not generally used for blood. The test may be useful for tissues, such as synovial tissue biopsies from affected joints, renal/other biopsies from suspect animals, or necropsy specimens. Urine, synovial fluid and CSF may also be tested by PCR in combination with serological testing. We offer PCR testing of ticks for *Borrelia burgdorferi* and other infectious agents (see below).

Serology and/or PCR for other tick-borne diseases (*Anaplasma phagocytophila*, *Babesia microti*, etc) We offer serology for *Anaplasma phagocytophila* in multiple species (specimen type: serum). We also offer PCR for *Anaplasma phagocytophila* in blood, and PCR for *A. phagocytophila* and *Babesia microti* in ticks.
Important differences between the Lyme Multiplex (recombinant antigen bead) assay for *Borrelia burgdorferi* and the Elisa/Western blot (traditional “gold standard”) combination testing

The whole cell Elisa screening assay, with subsequent Western blot testing on positive samples, provides the broadest view of antibodies produced by the patient against the many proteins of *B. burgdorferi*. At CVMDL, a skilled technician reviews each Western blot. Unusual blots are also reviewed by the Section Head. While certainly more labor intensive, this technique demonstrates all antibodies produced against *B. burgdorferi*, allowing for individual variation in responses and the ability to follow an individual’s serological response over time. Animals develop antibodies to additional *B. burgdorferi* proteins sequentially over the course of a persistent infection; so serial samples provide valuable information regarding progression of infection and response to treatment.

The Lyme Multiplex assay currently available in New York measures antibodies to 3 recombinant antigens of *B. burgdorferi* only. Although sensitive and specific (and less labor intensive!), this type of assay cannot provide the breadth of information that a Western blot can provide regarding immunological response to multiple antigens, or monitoring subtle changes in serological response over a time period. This is a useful assay, but it does not provide all of the information currently derived from the combination Elisa/Western blot.

Of particular concern with the current Lyme Multiplex assay, is the reliance on only Osp C antibody response to indicate early infection, and only Osp F antibody response to indicate chronic infection to *B. burgdorferi*. OspC is known to show genetic variation, so the binding of antibodies produced by an animal during the course of natural infection to the recombinant antigen may also vary. Most of the data regarding both Osp C and Osp F with respect to chronicity of disease has been derived from human and rodent studies; species variation is a concern. Additionally, the Lyme Multiplex assay uses antibodies to OspA as a singular indicator of previous *B. burgdorferi* vaccination. However, many naturally infected horses develop OspA antibodies, and horses persistently infected with *B. burgdorferi* for years sometimes develop a robust OspA response that resembles the wide Osp A vaccine response visible on Western blots. Interpretation of the Western blot of such a horse (which will also have a large assortment of antibodies to the many different antigens of *B. burgdorferi*) along with the clinical history will provide the best diagnostic information for the clinician. This information simply cannot be obtained by examining antibodies to only 3 recombinant antigens.

Finally, both the Lyme Multiplex and the Western blot assays must consider the new generation of canine Lyme vaccines containing multiple antigens of *B. burgdorferi* (including Osp C as well as the traditional Osp A vaccine antigen). The possibility of incorrect interpretation exists with both tests, particularly if specific vaccination history is unavailable. The Western blot provides more information that may make laboratory interpretation easier.

*Sandy Bushmich, MS, DVM  
Professor, Pathobiology and Veterinary Science, University of Connecticut  
Director, Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL)  
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