



## Lyme Disease Multiplex Testing for Dogs

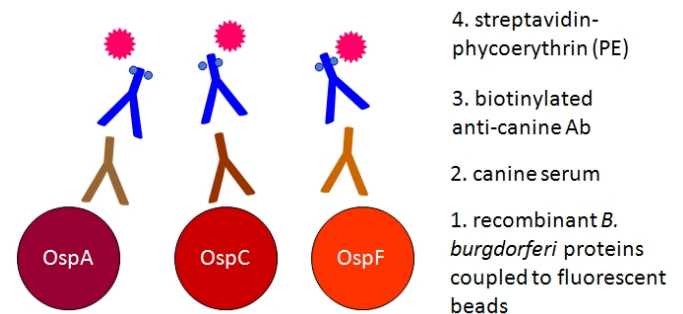
### Background on Lyme disease and Lyme diagnostics in dogs

Lyme disease is induced by the spirochete *B. burgdorferi*. Spirochetes are transmitted to dogs by infected ticks. Similar to humans, dogs are incidental, dead-end hosts for *B. burgdorferi*<sup>1</sup>. Typical clinical signs in dogs are fever, acute arthritis, arthralgia, lameness, and glomerulopathy<sup>2-4</sup>. Clinical signs of lameness often develop 2-5 months after infection. *B. burgdorferi* can persist for at least 1 year in clinically recovered, untreated dogs<sup>3</sup>. In a report from 1992, clinical signs of Lyme disease were estimated to occur in 5-10% of seropositive animals<sup>5</sup>. This percentage likely underestimates the current Lyme disease prevalence for dogs in endemic areas due to a higher awareness of the disease in owners and veterinarians and improved diagnostic methods.

Serum antibodies to different antigens of *B. burgdorferi* are commonly used to identify dogs that were exposed to the pathogen and to diagnose Lyme disease in dogs with compatible clinical signs<sup>2,6-8</sup>. Former ELISA based diagnostic tests identified antibodies as early as 4-6 weeks after infection<sup>3</sup>. The new Lyme Multiplex assay can identify antibodies to *B. burgdorferi* by 3 weeks after infection<sup>9</sup>. High antibody levels were found in serum of experimentally infected dogs for at least 17 months<sup>3</sup> and are likely maintained for much longer times, i.e. as long as *B. burgdorferi* persists in the dog.

### How does the new multiplex test work?

The new Lyme multiplex assay was developed at the Animal Health Diagnostic Center at Cornell University. It detects antibodies to three *B. burgdorferi* antigens in canine serum (Fig. 1). The multiplex test is based on fluorescent bead technology that allows the simultaneous measurement of antibodies to different *B. burgdorferi* antigens in a single sample<sup>10,11</sup>.

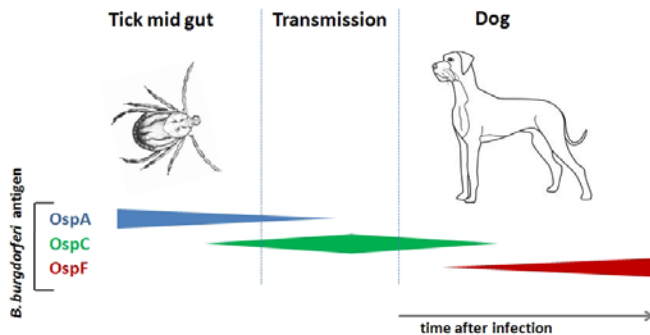


**Figure 1:** Lyme multiplex assay for detection of antibodies to *B. burgdorferi* in canine serum. Osp-antigen-specific antibodies in serum bind to the multiplex beads, are detected by a fluorescent conjugate and are evaluated in a multiplex reader. The assay values are expressed as median fluorescent intensities (MFI).

### Which *B. burgdorferi* antigens are used and how is the test interpreted?

The new multiplex test is based on three different outer surface proteins (Osp) of *B. burgdorferi*. Various research studies have

shown that Osp antigen expression changes on the bacterial surface in response to tick feeding and again after infection of a warm-blooded host, such as dogs, horses, or humans (Fig. 2). In response to infection, dogs develop antibodies to these Osp proteins and testing for antibodies to specific Osp antigens can assist in the diagnosis of infection and Lyme disease.

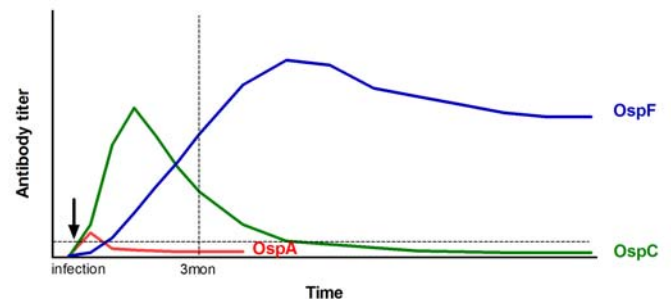


**Figure 2:** *B. burgdorferi* regulates its outer surface protein expression depending on its environment. In the tick gut, OspA is expressed. During tick feeding, the bacteria leave the tick's mid gut and express OspC on their surface. OspC expression is maintained early during infection. In response to the environment in the dog's body, the bacteria again change their surface expression – OspC disappears and OspF is expressed in the chronic infection stage.

Interpretation of Lyme multiplex results<sup>9,10</sup>:

The Lyme multiplex assay is a fully quantitative test. It results in a numeric antibody value for each of the three *B. burgdorferi* antigens tested. An interpretation of each value is submitted with the test report. In addition, the antibody profile gives an advanced interpretation on the infection and vaccination status of the dog. Antibodies to OspA serve as markers for vaccination and those to OspC and OspF as markers for infection (Fig. 3). In infected dogs, quantitative antibody values can then be used to follow-up on treatment success.

1. **OspA** – positive values for antibodies to OspA are typically observed in **vaccinated** dogs. OspA is expressed while *B. burgdorferi* persists in the tick mid-gut and also while the bacteria are cultured in-vitro. During infection of mammalian hosts, the bacteria down-regulate OspA. Therefore, antibodies to OspA are almost undetectable after natural infection in non-vaccinated dogs. Very low positive, transient antibody values to OspA can sometimes be detected 2-3 weeks after infection<sup>9</sup>.
2. **OspC** – has been found to be a valuable indicator for **early infection** with *B. burgdorferi*. Antibodies to OspC can be detected as early as 2-3 weeks after infection. Antibodies to OspC start to decline after 7-11 weeks and become undetectable by 4-5 months after infection<sup>9</sup>.
3. **OspF** – is an indicator of **chronic infection**. Antibodies to OspF are detectable by 5-8 weeks after infection and are maintained at high levels afterwards. Researchers at Cornell observed a very high agreement between antibodies to OspF and C6 as robust markers for infection in dogs in a blinded study<sup>9</sup>. Dogs with positive antibody values to OspF and negative antibody values to OspC have been infected with *B. burgdorferi* for at least 5 months (Fig. 3).



**Figure 3:** Antibody responses to OspA, OspC and OspF in infected dogs. Dogs were not treated and were unvaccinated. Data obtained from Wagner et al. 2012<sup>9</sup>.

### Advantages of the new Lyme Multiplex assay (AHDC, Cornell University)

The new Lyme disease test at the Animal Health Diagnostic Center at Cornell University combines the results previously obtained by ELISA and Western blotting. The advantages of the new Lyme Multiplex test compared to the previous procedure are:

- assay results distinguishing between:
  - (1) early and chronic stages of infection; Antibodies to OspC and OspF serve as markers for infection. Antibody values for OspC and OspF can also identify the stage of infection with *B. burgdorferi* and can determine when the dog was infected. In humans, early infection stages have a better prognosis and increased success rates after antibiotic treatment than chronic infection stages. Similarly, a more rapid decline of antibody levels, indicating successful treatment, was observed in horses that were treated in the early infection stage. It is likely that treatment success rates in dogs are also increased during early infection as *B. burgdorferi* persisted for a shorter period in the host.
  - (2) vaccination and infection; The assay provides a separate quantitative value for antibodies to OspA, as a marker for vaccination, and to determine the dog's vaccination status.
- increased specificity and sensitivity; Improved test performance results in earlier detection of antibodies to *B. burgdorferi* (as early as 2-3 weeks after infection) and a higher accuracy of the Lyme multiplex assay compared to conventional ELISA assays.
- quantitative measurement of individual antigens; The Lyme Multiplex assay has a much broader linear quantification range than ELISA tests. Antibody values are precisely measured in a broad concentration range. Quantification of antibodies and

evaluation of treatment success can be performed based on declining antibody values. Successful antibiotic treatment decreases the bacterial load. The lower or missing antigenic stimulus causes the immune system to produce fewer antibodies which leads to a decrease in serum antibody values. Treatment follow-up can be performed earlier with the Lyme Multiplex assay than with conventional ELISA tests.

The test result provides advanced information beyond any of the currently available Lyme testing methods. The testing allows a better definition of the dog's current infection status and assists in determining treatment options and prognosis. The infection status can be determined in all vaccinated dogs. Interpretation varies slightly depending on the vaccine used.

### Treatment and treatment follow-up by Lyme Multiplex testing

For treatment options and recommendations for infected dogs and those with clinical signs of Lyme disease, we refer to the ACVIM small animal consensus statement on Lyme disease in dogs<sup>3</sup>.

For chronic infection stages (OspC-/OspF+): Follow-up testing with the Lyme Multiplex assays can be performed as early as 3 months after starting treatment. It should not be performed earlier to give the antibodies time to decline (half-life of IgG antibodies is 21 days).

For early infection stages (OspC+/OspF+ or OspC+/OspF-): Treatment follow-up can be performed as early as 6 weeks after beginning of the treatment. Early in infection, antibodies have been observed to decline more rapidly. This is likely due to a higher amount of IgM antibodies during early infection and a more rapid decline of IgM compared to IgG.

### Special considerations for vaccinated dogs

The new Lyme multiplex assay can distinguish between vaccinated and infected dogs. All currently available vaccines for dogs induce antibodies to OspA. Therefore, the results on antibodies to OspA should be used to determine the dog's vaccination status. However, some of the available vaccines also induce antibodies to other Osp antigens. To provide our clients with the best interpretation for each animal, we need information on the vaccine used. Please include the name of the vaccine and the date when the dog was last vaccinated on the accession form if a sample of a vaccinated dog is submitted for testing.

### How can the multiplex test be compared to other serological Lyme assays?

Researchers at the Animal Health Diagnostic Center at Cornell University have compared the former ELISA/Western blot procedure and the commonly used C6 assays with the new multiplex test for Lyme disease<sup>9</sup>. Multiplex assay OspF and C6 results highly correlate for the identification of infected or non-infected dogs. Both antibodies to OspF and C6 provide robust markers of infection in dogs. The multiplex assay provides additional information on the dog's infection stage and vaccination status.

### Sample submission

For detection of antibodies to *B. burgdorferi*, 2ml of dog serum needs to be submitted. Serum should be collected in a red top blood tube. The entire red blood tube or isolated serum should be shipped by overnight shipment on an ice pack. For submission forms and shipping address go to the Animal Health Diagnostic Center website (<http://ahdc.vet.cornell.edu>). Samples are tested 3-4 days a week and results are available 2-3 days after the sample arrives at the laboratory. Consultation on the new testing platform or assay interpretation is available by

calling the Serology/Immunology laboratory at the Animal Health Diagnostic Center at Cornell University 607.253.3900.

### References

- 1 Radolf JD, Caimano MJ, Stevenson B, Hu LT. 2012. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochetes. *Nature Rev. Microbiol.* 10, 87-99.
- 2 Levy SA, Magnarelli LA. 1992. Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and the subsequent development of limb/joint borreliosis. *J. Am. Vet. Med. Assoc.* 200, 344-347.
- 3 Appel MJG, Allan S, Jacobson RH, Lauderdale TL, Chang YF, et al. 1993. Experimental Lyme disease in dogs produces arthritis and persistent infection. *J. Infect. Dis.* 167, 651-664.
- 4 Littman MP, Goldstein RE, Labato MA, Lappin WR, Moore GE. 2006. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. *J. Vet. Intern. Med.* 20, 422-434.
- 5 Levy SA, Magnarelli LA. 1992. Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and the subsequent development of limb/joint borreliosis. *J. Am. Vet. Med. Assoc.* 200, 344-347.
- 6 Jacobson RH, Chang YF, Shin SJ, 1996. Lyme disease: laboratory diagnosis of infected and vaccinated symptomatic dogs. *Semin. Vet. Med. Surg. (Small Anim.)* 11, 172-182.
- 7 Wittenbrink MM, Failing K, Krauss H. 1996. Enzyme-linked immunosorbent assay and immunoblot analysis for detection of antibodies to *Borrelia burgdorferi* in dogs. The impact of serum absorption with homologous and heterologous bacteria. *Vet. Microbiol.* 48, 257-268.
- 8 Guerra MA, Walker ED, Kitron U. 2000. Quantitative approach for the serodiagnosis of canine Lyme disease by the immunoblot procedure. *J. Clin. Microbiol.* 38, 2628-2632.
- 9 Wagner B, Freer H, Rollins A, Garcia-Tapia D, Erb HN, et al. 2012. Antibodies to *Borrelia burgdorferi* OspA, OspC, OspF and C6 antigens as markers for early and late infection in dogs. *Clin. Vacc. Immunol.*, PMID:22336289.
- 10 Wagner B, Freer H, Rollins H, Erb H.N. 2011. A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *B. burgdorferi* outer surface proteins in canine serum. *Vet. Immunol. Immunopathol.*, 140, 190-198.
- 11 Wagner B, Freer H, Rollins A, Erb HN, Lu Z, Gröhn Y. 2011. Development of a multiplex assay for detection of antibodies to *Borrelia burgdorferi* in

horses and its validation using Bayesian and conventional statistical methods. *Vet. Immunol. Immunopathol.*, 144: 374-381.

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