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Lyme disease multiplex Q&A with Dr. Bettina Wagner

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Summer means tick season in many parts of the United States.

Dr. Bettina Wagner, associate professor in the Department of Population Medicine and Diagnostic Sciences and director of serology at the Animal Health Diagnostic Center at Cornell, explains the significance of the Animal Health Diagnostic Center's multiplex assay used for testing for Lyme disease and answers common questions on result interpretation and treatment strategies.



Does Lyme disease treatment with antibiotics affect antibody levels?

Antibiotic treatment affects the pathogen (*B. burgdorferi*). It does not directly influence antibody titers. However, if the treatment is successful and bacteria are removed from the host, the B-cells are not further triggered to produce new antibodies. As a consequence antibodies levels decrease after treatment. Thus, antibody levels are indicators of treatment success (or failure).

How long after treatment should the horse/dog be re-tested?

Antibodies decrease slowly but gradually after the pathogen is cleared from the host's circulation. Re-testing should not be performed too early. For horses/dogs with positive values for antibodies to OspF but negative values for antibodies to OspC (OspC-/OspF+), retesting should be done 3 months after the treatment was started. For horses with a positive OspC antibody value (OspC+/OspF- or OspC+/OspF+) retesting can be done 6-8 weeks after the treatment started. At the time of retesting, a decrease of antibody levels can be expected in successfully treated animals. Serum antibodies may not have completely disappeared at this time. However, they should have dropped by about 50% of the original positive value(s).

For non-vaccinated horses/dogs: If antibodies to Osp A are positive, but antibodies to Osp C and F are equivocal, what is recommended?

Retest in 2-3 weeks. The pattern may indicate an early infection. If the horse/dog is truly infected, antibodies to Osp C and/or OspF will rise during that time.

If an animal were antibody positive for Lyme and treated, then re-tested with no changes in Ab levels, what is recommended?

Antibody titers can decline very slowly in some animals. If the animal shows clinical signs consistent with Lyme Disease re-treating should be considered. It can also be indicated to switch to another antibiotic. If no clinical signs of Lyme disease are observed, different approaches can be tried: (1) A different antibiotic can be used, (2) treatment time can be extended, or (3) the animal is not treated again but its antibody status is monitored every 6-12 months (depending on risk of new infection). If no increase in antibody values occurs for option (3) and the animal is free of clinical signs no further treatment is recommended.

What is MFI?

Median Fluorescent Intensity; The Lyme multiplex assay is based on fluorescent detection technology. The values are expressed as MFIs. The higher the antibody concentration to *B. burgdorferi* is in the sample, the more light is emitted. This is reflected by higher MFI values. Highly positive sera reach values of 20,000 MFI or higher.

Does Lyme Multiplex assay detect IgM or IgG?

The Lyme Multiplex assay detects total antibody levels. The routine test does not distinguish between IgM and IgG. The pattern of antibodies to the different Osp antigens determines early or chronic infection.

Can a tick carry other infectious organisms besides *B. burgdorferi* (Lyme) and cause a double infection?

Yes, it can. The most common other organism is *Anaplasma*.

Does treatment immediately after a tick bite negate signs of early infection?

Not every tick carries *B. burgdorferi*. The infection rate of ticks also varies with geographic region. Therefore, treatment should not be performed based on a tick bite alone. If the tick was found on the animal it can be sent in for *B. burgdorferi* PCR. PCR for *Anaplasma* can be performed simultaneously if requested. If the tick was infected, early treatment can be considered. However, a tick must feed for at least 24 hours on an animal or human for *B. burgdorferi* transmission to occur. Only a serological test will confirm whether transmission of *B. burgdorferi* occurred and the animal was infected. Antibodies can be detected in infected animals as early as 3-4 weeks after infection. Lyme Multiplex testing is recommended after that time. Treatment can be initiated immediately afterwards if antibody levels are positive. Treatment during this early infection phase is generally very effective.

How soon after infection can antibodies be detected with the Lyme Multiplex assay?

As early as 3-4 weeks after infection depending on the tick load, the amount of bacteria per tick and the animal's immune response.

Can an animal get infected despite being vaccinated?

Yes. This has been observed that some animals can still get infected after Lyme vaccination. This can be due to a low immune response of the respective animal and/or a very high exposure rate or Lyme prevalence in the area where the animal lives. A yearly check of the OspA titer is recommended for a vaccinated animal to ensure that the animal responded with high OspA antibodies to vaccination. Please indicate the name of the vaccine and when it has been last applied on the accession form.

Which antibodies does the vaccine from Merial induce?

It is a recombinant vaccine based on Osp A. Vaccinated animals should develop high antibody levels to OspA and maintain negative antibody levels to the remaining two antigens. Infected/non-infected animals can be identified

based on OspC and OspF antibody values. Detection of the early Lyme infection stage is possible after using this vaccine.

Which antibodies does the vaccine from Intervet (Merck) induce?

This vaccine is based on recombinant OspA and OspC and is expected to induce antibodies to both of them. The use of this vaccine interferes with the detection of early Lyme infection in vaccinated animals. However, infected animals can still be identified based on OspF antibody values.

When do clinical signs of Lyme disease occur?

Only 5-10% of infected animals are expected to show clinical signs of Lyme disease. They typically occur at the chronic disease stage and as early as 2-5 months after infection. Clinical signs can also develop much later.

Do antibodies to *B. burgdorferi* determine active infection?

In untreated animals, antibodies to OspC and/or OspF are indicators of infection with *B. burgdorferi*. Many infected animals don't show clinical signs and maintain constant antibody levels to the pathogen. It is believed that many animals can control the persistent infection. However, increasing antibody values indicate re-activation of or re-infection with the pathogen.

In treated animals, antibody levels decrease slowly and are usually still detectable for several months if treatment was successful.

Can we test fluids other than serum?

Yes, CSF samples can be submitted from horses with neurological signs. The CSF sample needs to be submitted together with a serum sample from the same animal and taken at the same time. The relative increase of antibodies in CSF can indicate local production of antibodies in the CNS. Typically, one or two antibody values (out of the three Osp values in the multiplex assay) are increased more than 2-fold if *B. burgdorferi* contributes to the neurologic condition. For other samples, contact the lab first (phone 607.253.3900).

Can an animal on treatment get re-infected?

Yes, infected ticks can also feed on animals while they are treated. In this case, the treatment will likely prevent establishment of persistent infection.

For animals that have not been vaccinated, can Osp A values be positive? How should this be interpreted?

Antibodies to OspA have previously been interpreted as markers for vaccination. Non-vaccinated animals usually have low values for antibodies to OspA, while vaccinated animals develop these antibodies. OspA antibodies have been shown to protect from infection with *B. burgdorferi*.

However, there is accumulating evidence that antibodies to OspA can occur without vaccination. First, residual OspA is present on the bacterial surface at the time of transmission to an animal. This causes a low and transient OspA antibody response early after infection. Second, antibodies to OspA have also been associated with treatment-resistant Lyme arthritis and with development of autoimmunity in human patients. Similarly, OspA antibodies in non-vaccinated dogs or horses can be considered as markers of chronicity and disease severity.

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