



## Lyme Disease Multiplex Testing for Horses

### Background on Lyme disease and Lyme diagnostics

Lyme disease is induced by the spirochete *B. burgdorferi*. The bacteria are transmitted to the host by infected ticks. Not all infected horses develop clinical signs of Lyme disease. If clinical signs occur, they may include chronic weight loss, sporadic lameness, low-grade fever, muscle tenderness, chronically poor performance, swollen joints, arthritis and diverse orthopedic problems<sup>1-4</sup>. In addition, neurological signs such as depression, behavioral changes, dysphagia, head tilt and encephalitis were reported in chronic cases<sup>1,5,6</sup>.

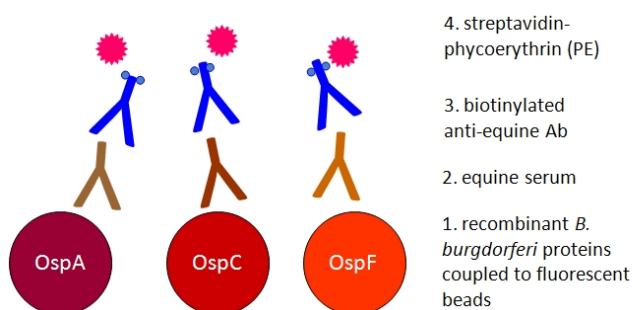
The diagnosis of Lyme disease in horses<sup>7</sup> can be made based on:

- 1) Horses living in an area where *B. burgdorferi* infected ticks are endemic
- 2) Horses that have a history of visiting an area with infected ticks
- 3) Ticks that have been found on the horse
- 4) Clinical signs compatible with Lyme disease
- 5) Ruling out other causes of clinical signs that may have similar signs than those associated with Lyme disease, i.e. orthopedic disease, behavior or training issues.
- 6) A high antibody titer to *B. burgdorferi*

Diagnostic Lyme antibody testing in horse serum has been performed by ELISA followed by a confirmatory Western blot (WB) or by immunofluorescence assays (IFA). These assays detected antibodies as early as 5-6 weeks after infection and resulted in positive antibody titers at around 10-12 weeks in most experimentally infected ponies<sup>8</sup>.

### 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 equine serum. 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>9,10</sup>.

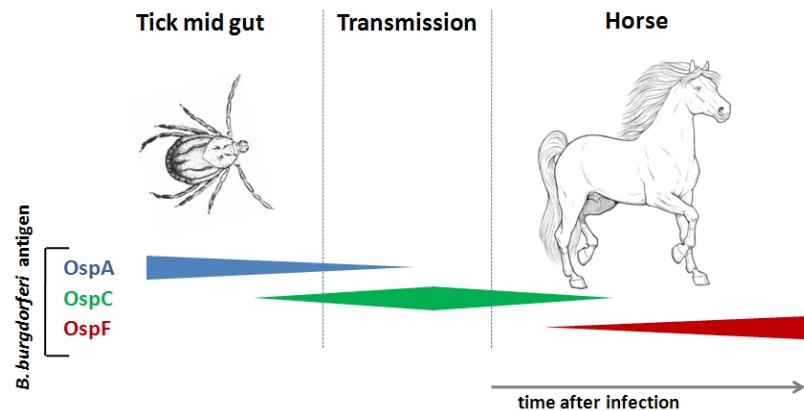


**Figure 1:** Lyme multiplex assay to detect antibodies to *B. burgdorferi* in equine serum. Osp-antigen specific antibodies in serum bind to the multiplex beads and are detected by a fluorescent conjugate. The assay values are expressed as median fluorescent intensities (MFI).

## 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 on the bacterial surface changes in response to tick feeding and after infection of a warm-blooded host, such as dogs, horses, or humans. In response to infection, horses develop antibodies to these Osp proteins and testing for antibodies to specific Osp antigens can assist in the diagnosis of Lyme infection and 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 start to express OspC on their surface. OspC expression is maintained during early infection. In response to the different environment in the horse'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>10</sup>:

1. **OspA** – positive values for antibodies to OspA are typically observed in **vaccinated** animals. 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. Thus, antibodies to OspA are almost undetectable after natural infection in non-vaccinated animals. **In some horses**, however, antibodies to OspA also seem to rise during infection. Thus, the interpretation of results on antibodies to OspA is likely more complex in horses and high OspA antibody titers in unvaccinated horses may also occur during chronic disease. In humans, antibodies to OspA were linked to chronic treatment-resistant Lyme arthritis and autoimmunity related to Lyme disease<sup>11</sup>.
2. **OspC** - was found to be a valuable indicator for **early infection** with *B. burgdorferi*. Antibodies to OspC can be detected 2-3 weeks after infection *B. burgdorferi*. Antibodies to OspC decline after 3-5 months of infection.
3. **OspF** – is an indicator of **chronic infection**. Antibodies to OspF are developed 6-8 weeks after infection and persist afterwards. Researchers at Cornell observed a very high agreement between antibodies to OspF and C6 in horses.

### What are the advantages of the new multiplex test?

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 multiplex test compared to the previous procedure are:

- increased specificity and sensitivity
- quantitative measurement of individual antigens
- improved assay results distinguishing between
  - (1) vaccination and infection
  - (2) early and chronic stages of infection
- sensitive assay to determine treatment success in horses.

The test result provides advanced information beyond any of the current Lyme testing methods. The testing allows a better definition of the horse's current infection status and assists in determining treatment options and treatment success. The infection status can be determined in vaccinated horses.

### Special considerations for vaccinated horses

The new Lyme multiplex assay can distinguish between vaccinated and infected horses. To provide our clients with the best interpretation for each animal, we need information on the vaccine used. This includes the name of the vaccine and when the horse was last vaccinated. Please include this information on the accession form when a sample of a vaccinated horse is submitted for testing.

### Neuroborreliosis in horses

Research at the Animal Health Diagnostic Center has shown that the Lyme multiplex assay can identify antibodies to *B. burgdorferi* in cerebrospinal fluid (CSF) to confirm neuroborreliosis in horses<sup>12</sup> and to distinguish it from other causes of neurologic disease. The diagnosis is based on the identification of antibodies to Osp antigens that are locally produced in neural tissues. This diagnostic approach requires a comparison of serum and CSF fluid from the same horse. For horses with neurological signs, a serum and CSF sample should be obtained at the same time and submitted together.

### 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 current ELISA/Western blot procedure with the new multiplex test for Lyme disease. The table below gives an overview about the properties of the different assays.

**Table 1: Comparison of serological Lyme assays for horses.**

	Sensitivity/ Specificity	Quantitative result	Distinguishes vaccination from infection	Identification of early versus late infection
ELISA (Cornell)	<b>Good</b>	<b>Yes</b>	<b>No</b>	<b>No</b>
WB (Cornell)	<b>Good</b>	<b>No</b>	<b>Yes</b>	<b>No</b>
SNAP 4Dx <sup>®</sup> (IDEXX)	<b>Good</b>	<b>No</b>	<i>Infection only</i>	<b>No</b>
Multiplex test Cornell)	<b>Improved</b>	<b>Yes</b>	<b>Yes</b>	<b>Yes</b>

## Sample submission

For detection of antibodies to *B. burgdorferi* in horses, 2 ml of 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>).

For neurological cases, a CSF sample should be taken at the same time as the serum sample and both samples should be submitted together using the form above.

Samples are tested every day (Mon-Fri) and results are available 2-3 days after the sample arrives at the laboratory. Consultation is available on the new testing platform by calling the Serology/Immunology laboratory at the Animal Health Diagnostic Center at Cornell University 607.253.3900.

## References

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