



Animal Health Diagnostic Center

College of Veterinary Medicine, Cornell University
In Partnership with the NYS Dept of Ag & Markets

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Leptospira Microagglutination Testing at the AHDC

Leptospirosis is a zoonotic disease of worldwide veterinary significance in many animal species. It is caused by infection with antigenically distinct serovars of the spirochete *Leptospira interrogans* sensu lato. Currently, over 250 different leptospiral serovars are known worldwide. These serovars can be further divided into pathogenic, non-pathogenic and opportunistic/possibly pathogenic *Leptospira*. The preferred reservoir host and likely incidental host vary with the serovar as well as the geographic location. Laboratory testing is a critical component to aiding in the clinical diagnosis of this challenging disease.

The microscopic agglutination test (MAT) is the reference test method for the serodiagnosis of leptospirosis both in humans and in animals (World Health Organization (WHO), 2003; World Organisation for Animal Health (OIE), 2008). This test detects antibodies to specific serovars using live leptospiral antigens, and can be performed on serum from any species.

In view of the **serovar specificity** of the MAT, testing should represent the circulating serovars from the geographic area where the animal contracted the infection. The Cornell University Animal Health Diagnostic Center Bacteriology laboratory uses the most prevalent serovars in North America as determined by the National Veterinary Services Laboratory (NVSL; Ames, IA) as live antigens in our leptospiral MAT testing. These antigens include the serovars Pomona, Hardjo, Icterohaemorrhagiae, Grippotyphosa, Canicola, Autumnalis, and Bratislava. The first five (5) listed serovars represent standard antigens tested in our laboratory. Additionally, the last two (2) listed serovars may be requested to encompass all seven (7) antigens. If other serovars not listed are of interest, samples can be referred to the appropriate laboratory for testing.

Lepto MAT Result Interpretation:

The MAT is a qualitative and quantitative test having high diagnostic specificity and relatively low sensitivity. Sera are screened at a 1:100 dilution and those showing agglutination are then serially diluted further to determine a titer endpoint. Very high antibody titers (≥ 1600) are suggestive of recent infection, but paired serum titers produce more reliable prognostic information. A 4-fold rise in titer, or a seroconversion to ≥ 1600 is an indication of a current leptospirosis infection. Multiple low-level titers most likely indicate vaccination reactions, however this test **should not be used to monitor protective vaccination status**. For a more detailed interpretation of your test results, refer to the table provided below.

When testing a single serum sample, the diagnostic significance depends on the magnitude of the titer within the background of cross-reacting or persistent agglutinating antibodies in the population. Testing paired samples at an interval between 2-6 weeks between blood draws has more diagnostic significance than relying on the results from a single sample. This test uses live antigen and this may result in titer differences when retesting previously submitted samples with their convalescent pair. Testing intervals less than 2 or greater than 6 weeks will not be tested in parallel with acutely drawn samples.



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Limitations:

- In up to 40% of healthy horses recently tested by MAT, we have observed persistent low titer reactions (100 to 200) to serovar Icterohemorrhagiae. This could be due to non-specific cross reactions or other unknown reasons that have yet to be identified.
- Hemolyzed and lipemic samples may hinder the ability to interpret the test.
- This test has been validated only for serum. Testing of other sample types, such as plasma or aqueous humor, should be used for research purposes at this time.
- A negative MAT reaction even in serial samples does not rule out the possibility of infection, as the animal may be infected with an alternative serovar that is not included in our panel.
- The MAT may be less sensitive in cases of host-adapted leptospiral infections.

Results	Lepto MAT Interpretation
Acute serum sample	
All serovars negative: <100 titer	A serum dilution antibody titer of <100 is equal to a Neg 1:100. The USDA recognizes this equivalency for regulatory purposes.
Highest titer(s) positive: ≤800 titer	Suggestive of a previous infection or vaccination . Recommend sending another sample within 2 to 6 weeks for paired testing.
Highest titer(s) positive: ≥1600 titer	Suggestive of recent infection in the absence of vaccination . Recommend sending another sample within 2 to 6 weeks for paired testing.
Convalescent Serum (paired) samples	
No change or decrease from acute 100-200 titer	It is unlikely that these results are significant
No change or decrease from acute 400-800 titer	Suggestive of previous infection or vaccination within the past 3 months
No change or decrease from acute ≥1600 titer	Suggestive of recent infection in the absence of vaccination within the past 3 months
4-fold increase from acute <100-200 titer	
Increase to ≥1600 from acute 400-800 titer	
4-fold increase from acute ≥1600	

Additional Leptospira Testing Options:

In addition to MAT serology, *Leptospira* Real-Time PCR testing on blood, urine or ocular fluids may be of diagnostic value in highly suspect clinical cases, especially when the duration of illness is not known. PCR on post-mortem tissues, such as liver or kidney, and abortion tissues, such as placenta, fetal kidney or fetal stomach contents, are recommended for suspect *Leptospira* mortality cases.