Accuplex® FAQ

Heartworm • Lyme • *Ehrlichia* • *Anaplasma*

GENERAL

What are the clinical signs of Lyme disease in dogs?

Most dogs that are exposed to or infected with Lyme disease no clinical signs. The two most common manifestations of infection with Borrelia burgdorferi in dogs are Lyme polyarthritis and Lyme nephritis. Clinical signs of acute Lyme polyarthritis may include fever, lameness, joint swelling and pain. Lyme nephritis, caused by immune complex glomerulonephritis can occur due to current or previous exposure to Borrelia burgdorferi, and is initially recognized by proteinuria which can then rapidly progress to acute kidney injury and possibly failure.

How is Lyme disease diagnosed?

In accordance with recommendations from the 2018 ACVIM consensus update on Lyme borreliosis in dogs and cats, Accuplex now screens for antibodies against the C6 peptide, the invariable region (IR6) of the surface VlsE, a 35-kDa surface lipoprotein of *Borrelia burgdorferi*. While different proteins may be expressed at various times to evade host immunity, the C6 protein is expressed during natural infection within the host, meaning a positive C6 test result is indicative of infection from *B. burgdorferi* without interference from vaccinal antibodies. This helps facilitate DIVA (Differentiation of Infection from Vaccinal Antibodies). A positive Lyme C6 result identifies natural infection or exposure, but not necessarily illness; around 95%[1] of Lyme C6 positive dogs never have any corresponding clinical signs.

1. Littman MP, Goldstein RE, Labato MA, et al. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. J Vet Intern Med. 2006;20:422–434. https://doi.org/10.1111/j.1939-1676.2006.tb02880.x

How do I treat a Lyme C6 positive dog with clinical signs consistent with Lyme Disease?

When clinical signs are consistent with Lyme disease (polyarthritis or glomerulonephritis), it is recommended to perform, at minimum, a CBC, chemistry profile, and urinalysis. A 30-day course of doxycycline or minocycline (10mg/kg orally 1-2 times daily) is recommended. In the case of Lyme polyarthritis, without proteinuria, monitoring for the development of proteinuria every 3-4 months, over the next year or two, while preventing further tick exposure is recommended. In cases with proteinuria present, at the time of diagnosis, a thorough diagnostic workup of the patient is recommended, including further evaluation for potential co-infections. Diagnostic work up and treatment should be consistent with the **2018 ACVIM Consensus Update on Lyme Borreliosis in dogs and cats** and **IRIS GN study group**.

Download the **Accuplex Lyme Algorithm.**

Should I treat an asymptomatic Lyme C6 positive dog?

There is currently no consensus on the treatment of dogs that are asymptomatic but test positive for Lyme disease. If a patient has a positive Lyme C6 result on the Accuplex test, per the ACVIM guidelines, Antech recommends a thorough investigation into the presence or absence of underlying clinical signs, a UPC ratio test to assess for the presence of persistent proteinuria, and providing pet owner education on prevention.

Lyme disease is a complex syndrome and clinical research is still necessary to better understand this infectious disease. Currently, only one of the five ACVIM Lyme Consensus panelists recommends a quantitative C6 test for Lyme C6 positive dogs that are otherwise healthy, nonproteinuric, and showing no clinical signs. Regardless of whether the dog is treated or not, it is recommended that you monitor carefully for proteinuria every 3 to 4 months for a year and provide pet owner education about tick prevention and control. It is also important to evaluate for the presence of co-infections in each patient.

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Is there a Lyme PCR test?

PCR is not optimal for detecting *B. burgdorferi*. Because the spirochete is only transiently present in blood samples, you would need to test tissues where you suspect the organism might be located such as synovial fluid. False negative testing would be likely because such low numbers of organisms are present.

A tick was found and removed from a canine patient, when should I submit an Accuplex? Is there follow-up recommended?

If the owner has the tick, it can be submitted for PCR assessment to determine what organisms are present. If the tick has been discarded, a baseline Accuplex should be performed to document current serological status from any previous exposure. This can be performed with or without a Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960), which will evaluate for bacteremia that may occur with acute exposure. If any aspect of PCR panel is positive (bacteremia) then the patient should be treated. If the Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) is negative or was not elected, a second Accuplex is recommended, 4-6 weeks after the first test, to determine whether positive seroconversion from baseline has occurred. Follow up testing will depend on which of the markers have become positive when compared to baseline (see clinical steps document).

Why are borderline results reported?

A borderline result indicates that antibody levels are equivocal and are close to the cutoff level for classifying the sample as positive or negative. The frequency of borderline reporting for all 4 organisms evaluated by the Accuplex test are very low. Borderline results should always be interpreted together with the clinical signs exhibited by the patient. Below are guidelines for borderline results with respect to individual organisms.

Heartworm: A borderline positive heartworm result on Accuplex indicates that a low level of antigen has been detected, approximating the cutoff level for the assay. This may represent a very low worm burden, such as 1-2 adult female filarids. All borderline or positive antigen tests should be confirmed with additional testing based on the American Heartworm Society (AHS), such as evaluation for microfiliaria (Knott's assay (U.S. test code T617 • Canada test code CT390)) or a second antigen test.

Lyme: all results are reported as positive or negative, there are no borderline results.

***Ehrlichia*:** If there are no clinical concerns at the time of results, close monitoring of the patient is recommended, with retesting in 2-4 weeks if there is any change in clinical status. If there are non-specific clinical signs, a full minimum database should be considered. Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) may also be submitted to evaluate for coinfections.

***Anaplasma*:** If there are no clinical signs at the time, close monitoring of the patient is rcommended, with retesting in 2-4 weeks if there is any change in clinical status. If acute infection is suspected based on clinical signs (fever, lethargy, and or/anorexia) consider a full minimum database or at minimum a CBC (to evaluate for morulae or thrombocytopenia). If thrombocytopenic consider using Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) to confirm *Anaplasma* infection (and evaluate for coinfections) versus treatment with doxycycline for 30 days. The majority of dogs will show clinical improvement within 1-3 days. If response to doxycycline is suboptimal, further diagnostics are recommended.

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When should a PCR FastPanel test be performed as a follow-up to Accuplex?

Follow-up PCR testing can be helpful in the following four scenarios:

1. A symptomatic dog (clinical signs consistent with possible tick-borne disease) with negative Accuplex results. Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) identifies early-stage *E. canis* and *Anaplasma* spp. infections (prior to seroconversion) and other tick-borne agents not identified by the Accuplex test.

2. Patient is unresponsive to initial treatment for suspected tick-borne infection (based on positive Accuplex or other screening test results). In this scenario, co-infection with another infectious agent should be considered. Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) tests for 15 vector-borne agents. The clinician should ensure that testing is done only after an appropriate antibiotic withdrawal time to maximize validity of the results.

3. In the case of suspected Ehrlichiosis or Anaplasmosis, the Ehrlichiosis / *Anaplasma* PCR panel (U.S. test code T980 • Canada test code CT980) can be performed to confirm current infection. In contrast to serological tests, which detect antibodies from the immune response to an infectious agent, PCR assays detect the presence of a patient's antigens from the infectious agent in the blood of the patient.

4. In the case of confirmed Ehrlichiosis, the Ehrlichiosis / *Anaplasma* PCR panel (U.S. test code T980 • Canada test code CT980) can be run two weeks after antibiotic therapy has been discontinued to confirm that the organism has been eliminated in response to therapy.

LYME

Should a quantitative C6 be performed as a follow up to a positive Lyme disease test result or during and after treatments?

The production of the antibody to C6 has been used to detect natural exposure of dogs to Borrelia burgdorferi but is not considered to predict prevalence or severity of clinical disease nor is it prognostic for disease progression or response to treatment. The quantitative or numerical titer has been shown in the literature to decline after antibiotic treatment for a period of time, but titers can remain high despite treatment and can also decline without antibiotic treatment.[2] No definitive studies or evidence exists that correlate the level of clinical disease with the quantitative C6 level. Decreased C6 levels are associated with more positive clinical outcomes in humans, but this has not been demonstrated in dogs. The ACVIM Lyme Consensus states that "there is insufficient published evidence that higher titers predict illness or are associated with future illness to advocate routine recommendation of this test in healthy dogs." A recommendation to perform a C6 titer or to reevaluate the C6 after appropriate antibiotic treatment is based on the veterinarian's judgment of the perceived value of the results. In determining value, the clinician should consider how the results will change treatment recommendations and how to interpret results in light of clinical status, the risk of re-exposure (dependent on lifestyle and geographic location), and the knowledge that some titers will remain high, while others will decrease, independent of antibiotic therapy.

2. Littman MP, Gerber B, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med. 2018;32(3):887-903. https://doi.org/10.1111/jvim.15085

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Does a positive C6 result indicate the need for treatment?

A positive C6 result suggests that the organism replicated in the patient at some point. However, post exposure, the patient could have "cleared" the infection (so the C6 antibody is representing a previous C6 exposure), or Lyme could become senescent in the patient (the C6 antibody is in response to the C6 exposure initially), or finally the C6 could indicate ongoing replication/clinical disease. Based on the ACVIM Consensus statement, a positive C6 requires further evaluation of the patient, but does not in itself indicate, a need to treat.

Why are clinical signs of disease, rather than simply seropositive status alone, pivotal in the decision of whether to treat for Lyme disease?

Treatment of non-proteinuric, non-clinical seropositive dogs is not routinely recommended due to concerns about appropriate antibiotic use and potential side effects associated with antibiotics. Additionally, there is a lack of data proving that treatment of healthy dogs is associated with decreased risk of illness, evidence that *Borrelia burgdorferi* may not be cleared from all tissues even with treatment, and that reinfection commonly occurs in dogs in endemic areas.[2]

2. Littman MP, Gerber B, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM consensus update on Lyme borreliosis in dogs and cats. J Vet Intern Med. 2018;32(3):887-903. https://doi.org/10.1111/jvim.15085

EHRLICHIA

A canine patient tested positive for *Ehrlichia* and does not live in an endemic or emerging area. What do I recommend for the owner to do and when? And when would I repeat those tests?

In the case of an *Ehrlichia canis* positive status, the clinician should evaluate travel history and any available prior serology results. *Ehrlichia canis* infection can result in acute illness, subclinical disease, and in rare circumstances, life threatening chronic disease. In an asymptomatic patient a minimum database (CBC, chemistry, urinalysis) could be considered to evaluate for any evidence of subclinical disease. In an ill patient, Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) and a minimum database are recommended to determine the likelihood of *Ehrlichia canis* being the cause of the symptoms. Tick preventive use should be reevaluated and the patient placed on year-round tick control measures. Antibody positivity may be maintained for a prolonged period post exposure so follow up testing via Accuplex should simply occur yearly.

What if ehrlichiosis is still suspected but the Accuplex *E. canis* result is negative?

In the southwestern United States, a strain of *E. canis* has recently been discovered that cannot be identified by Accuplex. If clinical signs or laboratory findings suggestive of ehrlichiosis are present and the Accuplex is negative, an *Ehrlichia* spp. evaluation by IFA or PCR should be considered.

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ANAPLASMA

A dog tested positive for *Anaplasma* last year and this year is negative on annual wellness screening wellness. Should I follow up with any additional tests?

It is likely that the antibody level, from the previous exposure, has waned below the level of detection of the assay. This also suggests that re-exposure has been prevented, possibly due to judicious use of tick preventive strategies. Year round tick prevention should be continued. Yearly evaluation of serological status should be continued to recognize any new exposures and allow for the appropriate follow up.

What should I do with a positive *Anaplasma* result in an asymptomatic patient?

There is currently no consensus as to whether seropositive but clinically normal dogs require treatment. If the patient is asymptomatic, but subclinical infection is a concern consider a full minimum database including a CBC (to evaluate for morulae and thrombocytopenia). If thrombocytopenic consider Antech's Canine Tick Borne PCR Panel (U.S. test code T960 • Canada test code CT960) to confirm *Anaplasma* infection (and evaluate for coinfections) versus treatment with doxycycline for 30 days. A recheck CBC is recommended to document resolution of the thrombocytopenia. Year-round tick control strategies should be implemented or improved in the face of a positive result.