

QUESTIONS RELATED TO INTERPRETATION OF THE IDEXX SNAP 4DX

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Veterinarians play a central role in the diagnosis, treatment and prevention of tick-transmitted infectious diseases of companion animals. Veterinarians also play an increasingly important role in advising the public as to the zoonotic potential of organisms that are transmitted from ticks to pets or to their owners.

The SNAP 4DX test detects *Dirofilaria immitis* antigen, antibodies directed against *Borrelia burgdorferi* C-6 peptide, antibodies to two synthetic *Ehrlichia canis* immunodominant proteins and antibodies to *Anaplasma phagocytophilum* immunodominant peptides.

***Borrelia burgdorferi*:**

Clinical Application: The C-6 peptide of *B. burgdorferi* is a highly specific diagnostic peptide that is used to detect *B. burgdorferi* antibodies in dog sera. Infection with closely related bacteria or prior vaccination with a “Lyme Disease” vaccine will **not** result in antibodies to *B. burgdorferi* C-6 peptide. However, when used as an annual screening test, occasional false positive test results should be anticipated. To date, all C-6 peptide positive test results confirmed by the Vector Borne Diseases Diagnostic Laboratory at NCSU-CVM have been in dogs with a travel (and tick exposure) history north of Richmond, Virginia. Although *B. burgdorferi* transmission may be occurring in focal sites in the southeastern United States, to date, we have not confirmed a C-6 SNAP positive test result in a dog indigenous to North Carolina. (See the reference citation below) Questionable *B. burgdorferi* positive test results (i.e. any C-6 peptide positive dog that lacks a travel history outside of North Carolina) should be further examined by Western immunoblot at Cornell University. We would be very interested hearing about any C-6/WB positive dogs that have never traveled outside North Carolina.

Treatment: Based upon current data, I do not recommend treatment of C-6 antibody positive dogs, unless there is concurrent clinical disease. (See the ACVIM Consensus Statement on Lyme Disease in Dogs) To date, Lyme disease in dogs is characterized by lameness, which can be intermittent and self-limiting without antibiotic therapy. Less well documented abnormalities include encephalitis, myocarditis and acute renal failure (particularly in Labrador and Golden Retrievers). Although antibiotic treatment can enhance the resolution of lameness, no antibiotic has been proven to be curative. If we can not cure the infection, treatment of a healthy dog is more difficult to justify.

***Ehrlichia canis*:**

Clinical Application: The SNAP 4DX test detects antibodies to two synthetic *E. canis* immunodominant proteins. Due to serological cross reactivity, the test will be positive in dogs previously exposed to *E. canis* or *E. chaffeensis*. Dogs infected with *Ehrlichia ewingii* or *Anaplasma phagocytophilum* (previously *Ehrlichia equi*) do not produce antibodies that cross react with these *E. canis* peptides. Therefore a dog can have chronic ehrlichiosis, due to *E. ewingii* infection, which will not be detected by reactivity to the *E. canis* peptides in the 4DX test.

The SNAP 4DX test is intended to be used as a screening test, not a diagnostic test. For this reason, the test does not detect low *E. canis* antibody titers (i.e. most IFA titers below 1:256 will result in a negative test result). When used as a screening test, statistics dictate that occasional false positive test results should be anticipated. False positive results can be clarified by IFA and PCR testing. Failure to provide a specific antibody titer, (an indication of antibody concentration in the dog's serum) is one disadvantage of the *E. canis* component of the 4DX test as compared to IFA testing. Some veterinarians elect to confirm all *E. canis* SNAP positive results by performing an IFA titer and PCR for the detection of *E. canis* or other *Ehrlichia* species DNA (this confirms active infection).

Treatment: Following introduction of the SNAP test, the VBDDL participated in a collaborative study with IDEXX laboratories to determine the extent to which *E. canis* SNAP positive, healthy dogs are IFA seroreactive and actively infected based upon PCR testing. In 86 *E. canis* SNAP+ samples submitted by regional veterinary hospitals from untreated dogs, 58% of the dogs were thrombocytopenic, 99% IFA seroreactive and 14% PCR +. It does appear that some dogs, naturally infected with *E. canis*, are capable of mounting an effective immunological response that eliminates the organism. These dogs would mount an antibody response detectable by SNAP or IFA, but have normal hematological findings and would be PCR negative. (**Caution:** A negative PCR result can support immunological or therapeutic elimination of an infection, but can never completely confirm that an individual animal is not infected with a given organism). When veterinarians tested sick dogs, the presence of antibody appeared to correlate with active infection, regardless of the level of the antibody titer. This may not be true in the healthy dog population. If a positive *E. canis* SNAP result is obtained on a healthy dog, I would recommend examination of a complete blood count prior to treatment. If the dog is anemic, neutropenic, thrombocytopenic or hyperglobulinemic, then treatment with doxycycline 5 mg/kg every 12 hours for 4 weeks (See ACVIM Consensus Statement on Canine Ehrlichiosis) would be recommended. If the complete blood count values are within normal reference ranges, treatment may not be indicated. Alternatively, the SNAP test result can be confirmed by IFA testing and the current infection status of the dog can be determined by PCR testing. PCR detects *Ehrlichia* genus DNA and therefore will detect all known *Ehrlichia* species. In our laboratory the design of this PCR test allows for the detection of all *Anaplasma* species, as well as all *Ehrlichia* species. If the genus PCR is positive, the dog is actively infected and treatment would be indicated.

Although our experience to date is limited, some dogs may remain *E. canis* SNAP positive one year after an initial positive SNAP test result and following appropriate treatment with doxycycline. When tested by both IFA and PCR (one year post-treatment) neither antibodies nor DNA was detected suggesting that the infection was therapeutically or immunologically eliminated. This preliminary observation should be considered when repeating an annual test on a dog that was previously *E. canis* positive by SNAP.

Anaplasma phagocytophilum

Anaplasmosis, caused by *A. phagocytophilum*, is characterized by an acute, febrile illness in cats, dogs, horses and human beings. *Anaplasma phagocytophilum* can also infect numerous other wild animal species that serve as reservoir hosts for subsequent transmission by *Ixodes scapularis*, *Ixodes pacificus* and perhaps other tick species.

Clinical Application: The SNAP 4DX test detects antibodies to a synthetic *A. phagocytophilum* immunodominant protein. Due to serological cross reactivity, the test will also be positive in dogs previously exposed to *Anaplasma platys*, the cause of cyclic canine thrombocytopenia. *Anaplasma platys* appears to be transmitted by the brown dog tick, *Rhipicephalus sanguineus*. Based upon PCR testing, canine anaplasmosis is frequently encountered in sick dogs and people in the northeastern, north central and north western United States and southern Canada. Due to transmission by the same tick vector (*I. scapularis* or *I. pacificus*) in the United States, co-infections with *B. burgdorferi* and *A. phagocytophilum* are common in “Lyme-endemic” regions. Dogs infected with *A. phagocytophilum* can develop only mild illness or perhaps no clinically apparent illness at all. Dogs co-infected with *B. burgdorferi* and *A. phagocytophilum* are more likely to develop severe disease signs. Experimentally, dogs can develop chronic *A. phagocytophilum* infection in the absence of clinical signs of disease. The extent to which natural *A. phagocytophilum* infection following tick transmission results in chronic infection or induces chronic disease manifestations is unknown. In contrast, infection with *A. platys* can induce chronic infection, accompanied by a moderate to severe cyclic thrombocytopenia, but generally without accompanying severe clinical manifestations. An *Anaplasma* SNAP+ dog that has resided in the southeastern United States is more likely to have been exposed to *A. platys*, which is a relatively common tick borne infection, as compared to *A. phagocytophilum*, which is infrequently transmitted to cats, dogs, horses or human beings in the southeastern US (similar to data relative to patterns of *B. burgdorferi* transmission).

Treatment: Treatment of healthy *Anaplasma* SNAP+ dogs in Lyme-endemic regions is not currently recommended as there is limited evidence that detection of antibodies correlates with chronic infection due to *A. phagocytophilum*. PCR could be used to establish chronic or recurrent infection due to *A. phagocytophilum*. *Anaplasma* SNAP+ dogs from the southeastern US, Central or South America may be infected with *A. platys*, which would warrant treatment if the dog was thrombocytopenic (remember the decrease in platelet numbers is cyclic) or PCR+. Both infections are thought to respond to doxycycline. The duration of treatment has not been clearly established for either

infection so dogs are generally treated for 4 weeks as proposed for *E. canis* infections. Co-infection with *E. canis* and *A. platys* causes more severe thrombocytopenia and may be more difficult to elicit a cure with doxycycline. The influence of co-infection on treatment outcome is unknown.

In healthy dogs, *B. burgdorferi*, *E. canis* and *A. phagocytophilum* test results should be recorded in the patient record. This information would be useful to the clinician if the dog develops compatible disease manifestations at some future time. For questions related to our testing capabilities, contact the VBDDL at North Carolina State University at 919-513-8279 or by e-mail (Julie_Bradley@ncsu.edu). You can also visit our website at: (www.cvm.ncsu.edu/docs/tickbornediseaselab.html) for diagnostic request forms and other information.

This information is provided to veterinarians as a service of the College of Veterinary Medicine, North Carolina State University.

References:

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