

Serology tests

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Serum samples for individual or panel requests are tested by traditional immunofluorescent antibody (IFA)¹ assays using a panel of VBDDL antigens for *Babesia canis*, *B. gibsoni*, *Bartonella henselae*, *B. vinsonii* subspecies *berkhoffii*, *B. koehlerae*, *Ehrlichia canis* and *Rickettsia rickettsia* (for spotted fever group Rickettsia) or by using a commercial ELISA based kit. All IFA antigens are grown in vitro or, in the case of *Babesia canis*, in vivo by personnel in the VBDDL using strains of canine or feline origin. *Dirofilaria immitis* antigen, as well as antibodies to *Anaplasma* spp. (*Anaplasma phagocytophilum*, or *A. platys*), *Ehrlichia* spp. (*Ehrlichia canis*, *E. chaffeensis* or *E. ewingii*) and the C-6 peptide of *Borrelia burgdorferi*, are detected using a commercial in-house ELISA-based kit, Idexx Snap® 4DX², according to manufacturer's instructions.

Interpretation: A titer is defined as diagnostically significant if the titer is $\geq 1:64$ using a dilution range of 1:16 - 1:8192. A titer ≤ 32 would be considered negative. The use of paired acute and convalescent titers to detect increases of titer is critical in acute diseases such as Rocky Mountain spotted fever to accurately distinguish between active infections and persistent antibodies. The endpoint titer of each seropositive sample will be given in test reports. (See separate document covering Snap 4DX Plus for further information.)

NCSU-CVM VBDDL Quality Assurance Statement:

PCR and serological testing performed at the Vector Borne Disease Diagnostic Laboratory at NC State University, College of Veterinary Medicine, conforms to defined testing protocols. EDTA blood and serum banks from experimentally infected animals have been collected for *Rickettsia rickettsii*, *Ehrlichia canis*, *Bartonella henselae* and *B. vinsonii* providing preliminary known controls for assessing the efficiency, sensitivity and specificity of our diagnostic procedures as the first step of the quality control process. Plasmids have been derived from these materials and from naturally infected samples, if experimental samples are unavailable, to produce standardized and reproducible sources of positive controls. Quality assurance for PCR and serology are verified with each testing run by consistent use of positive and negative control samples. Our test methods are classified as "validated for use" by meeting the following AAVLD criteria.

1. Ongoing documentation of internal or inter-laboratory performance using known reference standards for the species and/or diagnostic specimens of interest.
2. Published in a peer-reviewed journal with sufficient documentation to establish diagnostic performance and interpretation of results.
3. Documentation of internal or inter-laboratory comparison to an accepted methodology or protocol.

References

1. Kordick SK, Breitschwerdt EB, Hegarty BC, et al. Coinfection with multiple tick-borne pathogens in a Walker Hound kennel in North Carolina. *J Clin Microbiol* 1999;37:2631-2638.
2. Chandrashekar R, Mainville CA, Beall MJ, O'Connor T, Eberts MD, Alleman AR, Gaunt SD, Breitschwerdt EB. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* and *Dirofilaria immitis* antigen in dogs. *Am J Vet Res* 2010;71:1443-1450.