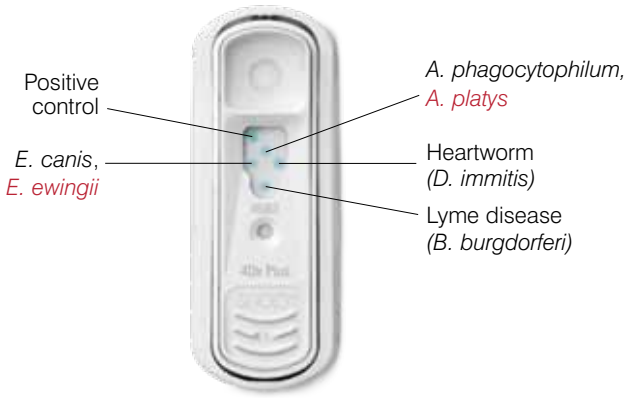
IDEXX SNAP® 4Dx® Plus Test—Expanded capability, same great performance

Introduction

There is mounting evidence of emerging tick vectors and vector-borne diseases spreading throughout North America. To help veterinarians better guard their patients against these threats, IDEXX Laboratories now offers the SNAP® 4Dx® Plus Test. The new test expands the utility of the widely adopted SNAP 4Dx Test by adding antibody detection for two additional tick-borne pathogens (*Ehrlichia ewingii* and *Anaplasma platys*) while providing the same reliable results. The widely expanding *Amblyomma americanum* (the lone star tick) transmits two species of *Ehrlichia* to dogs and people, *E. ewingii* and *Ehrlichia chaffeensis. A. platys*, closely related to *Anaplasma phagocytophilum*, is widely believed to be transmitted to dogs by the brown dog tick. Both vectors and pathogens are found in the southern region of the United States, making the SNAP 4Dx Plus Test the most complete choice for an annual screen.

The SNAP 4Dx Plus ELISA can be used to detect the antigen of *Dirofilaria immitis* (the parasite that causes heartworm) and antibodies against *A. phagocytophilum, A. platys, Borrelia burgdorferi, Ehrlichia canis* and *E. ewingii* in a single blood, plasma or serum sample.

The addition of *E. ewingii* and *A. platys* antibody detection enables veterinarians to screen their canine patients for exposure to six pathogens using only a few drops of blood. Equally important, the test can reveal exposure to as many as four different tick vectors, thus aiding in the diagnosis of a more comprehensive list of tick-transmitted diseases. This is especially helpful when any single species of tick may be capable of transmitting multiple infectious organisms.



Study 1: Validation of the SNAP 4Dx Plus Test

To determine the diagnostic performance of the test, each of the assays on the SNAP 4Dx Plus Test was compared to conventional reference testing results in a blinded and randomized study.[1]

Study Design:

Approximately 1,200 reference method-confirmed positive and negative canine samples were tested on the SNAP 4Dx Plus Test. The SNAP 4Dx Plus results were compared to the reference method results.

Results:

Experimental results are shown in Table 1.

Table 1: Comparison of SNAP 4Dx Plus Test results with conventional reference testing results

Comparison Test	Sample Size SNAP 4Dx Plus/Reference Test + / +	– / +	+ / –	– / –	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit
Heartworm[a,b]	94	1	2	269	366	Serum/Plasma	Sen., 98.9% (95% CL 94.3%–99.8%) Spec., 99.3% (95% CL 97.3%–99.8%)
A. phagocytophilum[c]	123	13	15	235	386	Serum/Plasma	Sen., 90.4% (95% CL 84.3%–94.3%) Spec., 94.0% (95% CL 90.3%–96.3%)
A. platys[c]	102	21	15	235	373	Serum/Plasma	Sen., 82.9% (95% CL 75.3%–88.6% Spec., 94.0% (95% CL 90.3%–96.3%)
B. burgdorferi[d]	112	7	10	246	375	Serum/Plasma	Sen., 94.1% (95% CL 88.4%–97.1%) Spec., 96.1% (95% CL 93.0%–97.9%)
E. canis[e]	131	3	18	217	369	Serum/Plasma	Sen., 97.8% (95% CL 93.6%–99.2%) Spec., 92.3% (95% CL 88.2%–95.1%)
E. ewingii[f]	109	4	10	154	277	Serum/Plasma	Sen., 96.5% (95% CL 91.3%–98.6%) Spec., 93.9% (95% CL 89.1%–96.7%)

[a]Necropsy, [b]PetChek® Heartworm PF Antigen Test, [c]*A. phagocytophilum* IFA, [d]*B. burgdorferi* IFA, [e]*E. canis* IFA, [f]*E. ewingii* ELISA

Note: The C_6 peptide used for *B. burgdorferi* antibody detection is highly specific and capable of distinguishing antibodies associated with infection from those of vaccination.[2] Nine of the 10 samples that had antibodies to *B. burgdorferi* on the SNAP 4Dx Plus Test but were negative on Lyme IFA had sufficient volume for retesting on Lyme Western blot. Seven of the 9 samples (78%) proved to be positive (infected) based upon Western blot results.

Study 2: Correlation with the SNAP 4Dx Test

To demonstrate that the SNAP 4Dx Test and SNAP 4Dx Plus Test perform equivalently, the two tests were compared using the same set of canine samples in a blinded and randomized study.[3]

Study Design:

A subset of available canine serum and plasma samples from Study 1 was tested on the SNAP 4Dx Test, and the results were compared to the SNAP 4Dx Plus Test results obtained in Study 1.

Results:

When comparing performance of an assay against itself, 95% agreement is an accepted threshold to demonstrate consistent test performance and repeatability (95% agreement would mean that the same result would be obtained 95 out of 100 times). The overall agreement between the SNAP 4Dx Test and the SNAP 4Dx Plus Test across all test spots for the sample population was 96.4% (656 spot results), reflecting the high degree of consistency between these products. Results by assay spot are shown in Table 2.

Table 2: Comparison of SNAP 4Dx Test and SNAP 4Dx Plus Test results

SNAP 4Dx Test spots	N Sample=173	SNAP 4Dx Plus % agreement
Heartworm positive	34	97.7%
Heartworm negative	139	
A. phagocytophilum positive	52	93.6%
A. phagocytophilum negative	121	
E. canis positive	55	98.8%*
E. canis negative	118	
B. burgdorferi positive	47	96.0%
B. burgdorferi negative	126	

*This value excludes 36 of the 173 samples confirmed as *E. ewingii*-positive by reference method, and thus not capable of being detected positive on the SNAP 4Dx Test.

What to do with an *Anaplasma*- or *Ehrlichia*-positive serologic result on the SNAP 4Dx Plus Test

Using the SNAP 4Dx Plus Test for annual vector-borne disease screening provides valuable medical information about the infectious organisms to which dogs in your practice are being exposed. The *SNAP® 4Dx® Plus Test Clinical Reference Guide* offers an overview of each organism and associated disease(s), including the vector, clinical signs and suggested protocols for dogs with a positive serologic result on the SNAP 4Dx Plus Test.

Summary and Conclusions

The new SNAP 4Dx Plus Test exhibits sensitivity and specificity consistent with the SNAP 4Dx Test while expanding its capabilities to screen for exposure to *E. ewingii* and *A. platys*. The test delivers more comprehensive patient health and disease information to help veterinarians know more about the vector-borne disease risks in their patient population.

Notes

- For dogs with antibodies to *Anaplasma* and/or *Ehrlichia*, it is recommended to perform a CBC with a blood smear. Clinicians should look for cellular inclusions, evidence of thrombocytopenia or other hematologic abnormalities.
- *A. phagocytophilum* IFA (*A. platys* IFA is not available): Dogs with *A. platys* infections may not have antibodies that react with the antigens on the *A. phagocytophilum* IFA, leading to a negative IFA result.
- *E. ewingii* IFA is currently not available. *E. canis* IFA may be used to assess titers, however some dogs with *E. ewingii* infections may not produce antibodies that react with the antigens on an *E. canis* IFA leading to a negative IFA result.[4]
- Tick/Vector PCR panels are available, which detect DNA from the infectious organism in a dog's blood, indicating an active infection. Additional information on PCR testing can be found in the *SNAP 4Dx Plus Test Clinical Reference Guide*.

References

1. Stillman, B.A., Beall, M.J., Monn, M., Liu, J., Thatcher, B., Shields, P., Andrews, B., Little, S.E., Eberts, M. Breitschwerdt, E.B., Chandrashekar, R. (2012) Performance of the new in-clinic SNAP® 4Dx® Plus Test for the detection of Ehrlichia ewingii (granulocytic ehrlichiosis) and Anaplasma platys (thrombocytotropic anaplasmosis) antibodies in dogs. American College of Veterinary Internal Medicine Forum, New Orleans, Louisiana (accepted).
2. Liang FT, Jacobson RH, Straubinger RK, Grooters A, Philipp MT. Characterization of a Borrelia burgdorferi VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J Clin Microbiol.* 2000;38(11):4160–4166.
3. Data on file at IDEXX Laboratories, Inc. Westbrook, Maine USA.
4. Stillman BA, Beall MJ, Shields P, Hegarty B, Breitschwerdt E, Chandrashekar R. Performance comparison of species-specific peptide-based assays with immunofluorescence assays for detection of canine antibodies to *Anaplasma* and *Ehrlichia* spp. [ACVIM Abstract 308]. *J Vet Intern Med.* 2010;25(3):765–766.

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