

# Performance Comparison of SNAP<sup>®</sup> 4Dx<sup>®</sup> Plus and AccuPlex<sup>®</sup>4 for the Detection of Antibodies to *Borrelia burgdorferi* and *Anaplasma phagocytophilum*

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## ABSTRACT

The purpose of this study was to compare the performance of two commonly used vector-borne disease screening tests, SNAP<sup>®</sup> 4Dx<sup>®</sup> Plus (IDEXX Laboratories, Inc.) and AccuPlex<sup>®</sup>4 (Antech Diagnostics, Inc.), for the detection of antibodies to *B. burgdorferi* and *Anaplasma* spp on a broad population of canine samples. Four hundred and sixty-four samples were tested on SNAP 4Dx Plus and AccuPlex4. Percent agreement between the two tests for *B burgdorferi* and *Anaplasma* spp was 86% and 81%,

respectively. Comparison of SNAP 4Dx Plus and AccuPlex4 to Lyme Western blot demonstrated a significant difference in performance; SNAP 4Dx Plus was 98.5% sensitive and 95.7% specific vs AccuPlex4 which was 78.5% sensitive and 72.9% specific. Performance of SNAP 4Dx Plus and AccuPlex4 compared to *A phagocytophilum* IFA resulted in a sensitivity and specificity of 91.7% and 88.7% for the SNAP 4Dx Plus test and 75.0% and 82.6% for the AccuPlex4 test. The reproducibility of SNAP 4Dx Plus was 96% for *B. burgdorferi* and 95% for *Anaplasma* compared to AccuPlex4 which had reproducibility of 89% and 79%, respectively. In this direct comparison, the SNAP 4Dx Plus test demonstrated better accuracy

and reproducibility than the AccuPlex4 test for the detection of antibodies to *B. burgdorferi* and *Anaplasma* spp.

## INTRODUCTION

While screening for heartworm disease in dogs remains a mainstay of preventive veterinary care, interest in assessing the risk of exposure to other vector-borne infections is growing.<sup>1</sup> In fact, the frequency of exposure to tick-borne infections like *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu stricto, and *Ehrlichia canis* may exceed the frequency of heartworm disease in many areas of the United States. For instance, dogs in the Northeast and upper Midwest are often 10 times more likely to have antibodies to *A. phagocytophilum* and/or *B. burgdorferi* than they are to test positive for *Dirofilaria immitis* antigen.<sup>2</sup> Over the last 10 years, assessing the seroprevalence of *A. phagocytophilum* and *B. burgdorferi* in dogs has been facilitated by the availability of in-clinic screening tests for these pathogens based on p44 and C6 antibody testing (SNAP<sup>®</sup>3Dx, SNAP<sup>®</sup>4Dx<sup>®</sup> and SNAP<sup>®</sup>4Dx<sup>®</sup> Plus, IDEXX Laboratories, Inc, Westbrook, ME). Studies using these screening tests have demonstrated the sensitivity, specificity, and accuracy of the diagnostic analytes.<sup>3-7</sup> Additional screening tests<sup>a</sup> have recently become available for use by veterinarians, although published field studies regarding their performance are lacking. The purpose of this study was to compare the performance of two commonly used vector-borne disease screening tests, SNAP<sup>®</sup> 4Dx<sup>®</sup> Plus, and AccuPlex<sup>®</sup>4 (Antech Diagnostics, Inc), for the detection of antibodies to *B. burgdorferi* and *Anaplasma* spp on a broad population of canine samples.

## MATERIALS AND METHODS

### Samples

All samples tested in the study were derived from a repository of frozen canine serum samples (IDEXX Laboratories), which were maintained as aliquots to ensure sample

integrity. Samples with sufficient volume were selected based upon three criteria that included basic categories considered to be important for the comparison:

- First, approximately 100 samples had to be from purpose-bred, research dogs with no history of tick exposure.
- Second, at least 100 of the samples selected for testing had to have an *A. phagocytophilum* IFA performed.
- Third, at least 100 of the samples selected for testing had to have a Lyme Western blot performed with the original blot available for evaluation.

A total of 464 canine serum samples were selected for testing as part of this study. A total of 99 samples came from purpose-bred, research dogs that were housed in bioresearch facilities according to each institution's IACUC protocol. None of these dogs were exposed to ticks at any time, and this population served as a negative control group for the testing. The remaining 365 samples were from field dogs. These samples originated predominantly from the United States and represented a broad geography including regions where *B. burgdorferi* and *A. phagocytophilum* are considered endemic.

The *A. phagocytophilum* IFA testing (IDEXX Reference Laboratory), an accepted reference method for the detection of *A. phagocytophilum* antibodies, had been performed on 139 serum samples (82 purpose-bred research dogs and 57 field dogs). The *A. phagocytophilum* IFA was determined to be positive at a titer of 1:200. Lyme Western blots (Borrelia B31 IgG ViraStripe and ViraBlot, Viramed Biotech AG, Munich Germany) were available for 135 samples (31 purpose-bred, research dogs and 104 field dogs). The Western blot is an alternate reference method for the detection of *B. burgdorferi* antibodies.<sup>8-11</sup> The goal was to compare the performance of SNAP 4Dx Plus and AccuPlex4 in a blinded

<sup>a</sup> Canine Multiplex Assay for Lyme Disease, Animal Health Diagnostic Center, Cornell University; AccuPlex<sup>®</sup>4 Test, Antech Diagnostics; VetScan Canine Lyme Rapid Test, Abaxis, Inc.

**Table 1.** Comparison of test results for antibodies to *B. burgdorferi* and *Anaplasma* on SNAP 4Dx Plus and AccuPlex4 in a population of field ( $n=365$ ) and purpose-bred research ( $n=99$ ) dogs.

<i>B. burgdorferi</i>		AccuPlex4		<i>Anaplasma</i>		AccuPlex4	
		Pos	Neg			Pos	Neg
SNAP 4Dx Plus	Pos	87	25	SNAP 4Dx Plus	Pos	114	26
	Neg	40	312		Neg	60	264

fashion to the Western blot results. Western blot testing was optimized for canine serum samples and performed as reported previously.<sup>5</sup> The known canine positive and negative control samples conformed to the criteria established for using Western blot as a diagnostic test.<sup>12</sup> Similar to other studies of canine seroreactivity to *B burgdorferi*, the sensitivity and specificity of this particular brand of Western blot was not determined, but did utilize the same strain of *B burgdorferi* (B31) as a previously available, commercial canine Lyme Western blot.<sup>8,13,14</sup> All blots were available for these samples and independently interpreted by an experienced reader (REG) blind to the results of the vector-borne disease screening tests.

#### Method Comparison Testing

The SNAP 4Dx Plus test is an in-clinic ELISA licensed by the USDA for the detection of *Dirofilaria immitis* antigen and antibodies to *A phagocytophilum*, *A platys*, *E canis*, *E ewingii*, and *B burgdorferi*. The AccuPlex4 test is a reference laboratory test on a BioCD that detects *D immitis* antigen and antibodies to *A phagocytophilum*, *E canis*, and *B burgdorferi*. All serum samples were blinded and tested on SNAP 4Dx Plus at one of two locations (Gainesville, FL or Westbrook, ME) and results recorded. An aliquot of each serum sample was also submitted to

Antech Diagnostic Laboratory according to the laboratory's specifications for AccuPlex4 testing. Results were provided by the laboratory and documented. In addition, reproducibility of SNAP 4Dx Plus and AccuPlex4 was assessed by repeated testing (twice) of 56 samples and comparing the *B burgdorferi* and *Anaplasma* results. Individuals performing the SNAP 4Dx Plus test were blind to the results of the AccuPlex4 test.

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#### Analysis

Statistical analysis was performed using standard formulas to calculate percent agreement between the two vector-borne screening tests and sensitivity/specificity relative to the reference method (Microsoft Excel 2007, Microsoft Corporation, Redmond, WA). Exact binomial limits with 95% confidence intervals was used to assess likelihood of a significant difference between the test methods at a  $P < 0.05$ . Additionally, a Chi-square test was used to compare reproducibility between the two screening methods for *B burgdorferi* and *Anaplasma* (GraphPad Prism v.5, GraphPad Software, La Jolla, CA).

#### RESULTS AND DISCUSSION

Four hundred and sixty-four samples were tested on SNAP 4Dx Plus and AccuPlex4 (Table 1). The percent agreement between the two tests for antibodies to *B burgdorferi* and *Anaplasma* was 86% and 81%, respectively. The number of dogs testing antibody positive for a single pathogen, either *B*

**Table 2.** SNAP 4Dx Plus and AccuPlex4 results categorized by samples with antibodies to *B. burgdorferi* only, *Anaplasma* only, *B. burgdorferi* and *Anaplasma*, or negative for antibodies to either agent.

	<i>B. burgdorferi</i>	<i>Anaplasma</i>	<i>B. burgdorferi</i> and <i>Anaplasma</i>	Negative
SNAP 4Dx Plus	50	78	62	274
AccuPlex4	46	93	81	244

**Table 3.** Sensitivity and specificity of SNAP 4Dx Plus and AccuPlex 4 relative to *B. burgdorferi* Western blot and *A. phagocytophilum* IFA. Exact binomial confidence limits are shown in parentheses.

	<i>B. burgdorferi</i> Western blot (n=135)		<i>A. phagocytophilum</i> IFA (n=139)	
	SNAP 4Dx Plus	AccuPlex4	SNAP 4Dx Plus	AccuPlex4
<b>Sensitivity</b> (95% CL)	<b>98.5%</b> (90.8-100.5)	<b>78.5%</b> (66.9-86.8)	<b>91.7%</b> (72.8-98.7)	<b>75.0%</b> (54.7-88.2)
<b>Specificity</b> (95% CL)	<b>95.7%</b> (87.5-99.0)	<b>72.9%</b> (61.3-81.9)	<b>88.7%</b> (81.4-93.4)	<b>82.6%</b> (74.5-88.5)

*burgdorferi* or *Anaplasma*, on each test and the number testing positive for antibodies to both of these pathogens on each test is shown in Table 2. Comparison of SNAP 4Dx Plus and AccuPlex4 to Lyme Western blot demonstrated a significant difference in performance; SNAP 4Dx Plus was 98.5% sensitive and 95.7% specific vs AccuPlex4, which was 78.5% sensitive and 72.9% specific (Table 3).

Compared to SNAP 4Dx Plus, the AccuPlex4 test had more samples that tested positive for *B burgdorferi* infection but could not be confirmed on Western blot as well as samples that tested negative for *B burgdorferi* infection, but were determined to have natural infections by Western blot (Table 4). Performance of SNAP 4Dx Plus and AccuPlex4 compared to *A phagocytophilum* IFA resulted in a sensitivity and specificity of 91.7% and 88.7% for the SNAP 4Dx Plus test and 75.0% and 82.6% for the AccuPlex4 test (Table 3). The AccuPlex4 test had more discordant results with *A phagocytophilum* IFA than did the SNAP 4Dx Plus test (Table 4). Diagnostic accuracy relative to the reference method for Lyme was 97.0% for SNAP 4Dx Plus and 75.6% for AccuPlex4, and for *Anaplasma* was 89.2% for SNAP 4Dx Plus and 81.3% for AccuPlex4.

Testing of purpose-bred, research dogs that had never been exposed to ticks led to the identification of false positive results on AccuPlex4; 4 samples tested positive for antibodies to *A phagocytophilum*, four samples tested positive for antibodies to *B burgdorferi*, and one sample tested positive

for both. Corresponding results on SNAP 4Dx Plus were negative for antibodies to *B burgdorferi* and *Anaplasma*. Four of the *A phagocytophilum*-reactive samples had been tested by *A phagocytophilum* IFA and were found to be negative. Four of the *B burgdorferi*-reactive samples had been tested by Lyme Western blot and were determined to be negative.

Reproducibility of *B burgdorferi* and *Anaplasma* results on SNAP 4Dx Plus and AccuPlex4 was evaluated by testing 56 samples twice on both tests. For the AccuPlex4 test, 6/56 *B burgdorferi* results and 12/56 *A phagocytophilum* results differed between the testing events, representing 16% discordant results. SNAP 4Dx Plus had 2/56 *B. burgdorferi* results and 3/56 *Anaplasma* results that differed between the testing events, indicating significantly fewer (4%) discordant results (Chi square  $p < 0.05$ ). The reproducibility of SNAP 4Dx Plus was 96% for *B burgdorferi* and 95% for *Anaplasma* compared to AccuPlex4, which had reproducibility of 89% and 79%, respectively. There was a statistically significant difference in reproducibility between the tests for *Anaplasma* (Chi square  $p < 0.05$ ).

In this study, results obtained using the SNAP 4Dx Plus test were more accurate and reproducible than those obtained from the AccuPlex4 test when compared to the results from concurrent IFA (*A phagocytophilum*) and Western blot (*B burgdorferi*) assays. Performance of the SNAP 4Dx Plus in this study was similar to published reports of sensitivity and specificity based on earlier

**Table 4.** Comparison of SNAP 4Dx Plus and AccuPlex4 test results to *B. burgdorferi* Western blot and *A. phagocytophilum* (Aph) IFA which are standard reference methods for the detection of antibodies to *B. burgdorferi* and *Anaplasma*, respectively.

<i>B. burgdorferi</i>		Western blot	
		Pos	Neg
SNAP 4Dx Plus	Pos	64	3
	Neg	1	67

<i>B. burgdorferi</i>		Western blot	
		Pos	Neg
Accu-Plex4	Pos	51	19
	Neg	14	51

<i>Anaplasma</i>		Aph IFA	
		Pos	Neg
SNAP 4Dx Plus	Pos	22	13
	Neg	2	102

<i>Anaplasma</i>		Aph IFA	
		Pos	Neg
Accu-Plex4	Pos	18	20
	Neg	6	95

(SNAP 3Dx, SNAP 4Dx) and current versions of this assay.<sup>5,6,13,15,16</sup> This included the performance of SNAP 4Dx Plus relative to the human Lyme Western blot used in this study, which was optimized for canine samples. The commercially available, human Lyme Western blot kits are constructed of whole cell lysate or purified diagnostic antigens from the *Borrelia* organism that are separated by SDS PAGE gel and transferred to a nitrocellulose membrane. As the membrane itself is not necessarily specific for human or canine samples, it can be optimized for canine samples using an anti-canine secondary antibody. Additionally, the blots used in this study are FDA approved for use in human diagnostics, and have been reported to be of acceptable diagnostic quality.<sup>17</sup> If there had been any bias in selecting this Western blot as a reference test, the bias would be equal for both vector-borne screening tests, since the results of both platforms were compared in a blinded fashion to the results from the Western blot.

Several factors may influence the ability of a serological assay to accurately detect antibodies against a specific agent. In an acutely ill patient, there may be a lag between the development of clinical signs and the onset of detectable circulating antibodies. However, when circulating antibodies are present (as determined by IFA and Western blot), the SNAP 4Dx Plus was much more reliable in detecting those antibodies

than was the AccuPlex4 test.

The SNAP 4Dx Plus test had fewer false positives than did the AccuPlex4 when testing samples from purpose-bred, research dogs with no history of tick exposure. The specificity of a test may be influenced by the antigens that are incorporated into the assay. The antigens used to detect antibodies to *B. burgdorferi* and *A. phagocytophilum* in the SNAP 4Dx Plus test are immunodominant peptides derived from well-characterized surface proteins of these organisms.<sup>6</sup> Specifically, the test uses the C6 peptide derived from the VlsE protein of *B. burgdorferi* and the major surface protein p44 of *A. phagocytophilum*.<sup>18,19</sup>

In the United States, dogs residing in Lyme endemic areas are frequently vaccinated for *B. burgdorferi*. The C6 peptide on the SNAP 4Dx Plus test does not detect antibodies generated in response to commercial *B. burgdorferi* vaccines.<sup>13,18,20</sup> Performance of the AccuPlex4 test for detecting antibodies to *B. burgdorferi*, including performance in *B. burgdorferi* vaccinated dogs, and *A. phagocytophilum*, has not been published. Test specificity is particularly important when used for screening of clinically healthy canine populations because the results guide the decision making process in the absence of clinical signs. False positive results could lead to erroneous decisions regarding treatment options, vaccination, and/or more

costly, additional diagnostic testing.

Accuracy of vector-borne screening tests is not only important for the individual patient, but also has broader implications and value as an epidemiological tool. A nationwide analysis of the frequency of *B burgdorferi* antibody positive dogs found that dogs are a sentinel for the risk of human infection.<sup>21</sup> Screening dogs for antibodies to *B burgdorferi* and *A phagocytophilum* can alert family members to the risk of exposure for themselves when they or their pets engage in outdoor activity during times of the year when ticks are active. Veterinarians play a significant role as public health advocates by discussing tick exposure, tick prevention, and prevention of transmission of tick-borne pathogens. Reliable screening tests are critical in enabling veterinarians to accurately perform in this capacity.

In conclusion, clinically-significant differences between the SNAP 4Dx Plus and AccuPlex4 tests were recognized in this direct comparison. The SNAP 4Dx Plus test had significantly better sensitivity and specificity, fewer false positive results, and better test-to-test reproducibility.

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