Performance of the Abaxis VetScan® Canine Flex4 Rapid Test

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Introduction

The purpose of this study is to evaluate the performance of the Abaxis VetScan Flex4 Rapid Test in comparison with the IDEXX SNAP® 4Dx® Plus test, Abaxis in-house ELISA evaluation, and disease-specific immunofluorescent assays (IFA). The ability to detect antigens for Heartworm disease and antibodies for Lyme disease, Ehrlichiosis, and Anaplasmosis at the point-of-care is important to evaluate the ill patient and to screen patients in endemic areas. Often, vector-borne diseases can manifest acutely and with debilitating clinical signs. Diagnosing these diseases quickly allows treatment to commence as soon as possible, decreasing the risk of mortality and morbidity. With animals travelling both regionally and throughout North America, it is important to screen a symptomatic patient for all possible infectious diseases. In some U.S. geographic regions, screening of asymptomatic patients is also recommended by the Companion Animal Parasite Council (CAPC). The Flex4 Rapid Test allows a veterinarian to evaluate Heartworm, Lyme, Ehrlichiosis and Anaplasmosis diseases on a single 4-in-1 patient-side test.

Materials and Methods

103 samples were utilized from banked and private sources throughout the U.S., mostly in Wisconsin, Oklahoma, Minnesota, Arizona, and Pennsylvania. Each sample was run twice on the Flex4 Rapid Test, once on the Vetscan Rapid Single Test respective for each separate disease, and once on the IDEXX SNAP 4Dx Plus test. If the outcome of all of these tests agreed, the outcome was defined as positive or negative and labeled as the true status. If there were any discrepancies, each sample was run an additional time on the Flex4 Rapid Test and IDEXX SNAP 4Dx Plus test for comparison. If there were ongoing discrepancies, the samples were run on an Abaxis ELISA[1] and the disease-specific immunofluorescent assay. The majority outcome was classified as the true status. The following data was collected:

Lyme Evaluation

The Flex4 Rapid Test identified 23/23 Lyme positive patients and 78/80 Lyme negative patients, producing 100% sensitivity/98% specificity. The IDEXX SNAP 4Dx Plus test identified 21/23 Lyme positive patients and 79/80 Lyme negative patients, resulting in 91% sensitivity/99% specificity.

LYME		True Status	
		+	-
FLEX4	+	23	2
	-	0	78

Sensitivity 100%
Specificity 98%

LYME		True Status	
		+	-
4Dx	+	21	1
	-	2	79

Sensitivity 91%
Specificity 99%

Ehrlichiosis Evaluation

The Flex4 Rapid Test, which is licensed by the USDA to identify all three canine infectious *Ehrlichia* species *(E. canis, E. chaffeensis,* and *E. ewingii)*, identified 35/36 *Ehrlichia* positive patients and 65/67 *Ehrlichia* negative patients, resulting in 97% sensitivity/97% specificity. The IDEXX SNAP 4Dx Plus test, which is only licensed to identify two canine infectious *Ehrlichia* species *(E. canis* and *E. ewingii)*, identified 35/36 *Ehrlichia* positive patients and 67/67 *Ehrlichia* negative cases resulting in 97% sensitivity/100% specificity.

EHRLICHIA		True Status	
		+	-
FLEX4	+	35	2
	-	1	65

Sensitivity 97%
Specificity 97%

EHRLICHIA		True Status	
		+	-
4Dx	+	35	0
	-	1	67

Sensitivity 97%
Specificity 100%

Heartworm Evaluation
The Flex4 Rapid Test identified 26/26 Heartworm positive patients and 77/77 Heartworm negative patients, producing 100% sensitivity/100% specificity. In contrast, the IDEXX SNAP 4Dx Plus test identified 24/26 Heartworm positive patients and 77/77 Heartworm negative patients, resulting in 92% sensitivity/100% specificity.

HEARTWORM		True Status	
		+	-
FLEX4	+	26	0
	-	0	77

Sensitivity 100%
Specificity 100%

HEARTWORM		True Status	
		+	-
4Dx	+	24	0
	-	2	77

Sensitivity 92%
Specificity 100%

Anaplasmosis Evaluation
The Flex4 Rapid Test identified 27/30 *Anaplasma* positive patients, and 67/73 *Anaplasma* negative patients, resulting in 90% sensitivity/91.8% specificity. In comparison, the IDEXX SNAP 4DX Plus test identified 20/30 *Anaplasma* positive patients and 73/73 *Anaplasma* negative patients, resulting in 67% sensitivity/100% specificity.

ANAPLASMA		True Status	
		+	-
FLEX4	+	27	6
	-	3	67

Sensitivity 90%
Specificity 91.8%

ANAPLASMA		True Status	
		+	-
4Dx	+	20	0
	-	10	73

Sensitivity 67%
Specificity 100%

Due to the significant difference between *Anaplasma* sensitivities in both the Flex4 Rapid Test and the IDEXX SNAP 4Dx Plus test, further IFA testing was completed at Kansas State University Center of Excellence for Vector-Borne Diseases. Of the 103 samples tested, the 19 discrepant samples (that were defined by our discrepancy evaluation stated above) were evaluated by KSU. Of those, 15 samples tested IFA positive. In comparison between the Flex4 Rapid Test and SNAP 4Dx Plus test, the following data was obtained:

KSU IFA Positive sample	FLEX4	4Dx
Positive Samples	12	3
Sensitivity	80%	20%

Discussion
In general, detection of Heartworm, *Ehrlichia*, *Anaplasma*, and Lyme infections are not only evaluated in sick patients, but also used to identify asymptomatic chronically infected canines in endemic regions, as recommended by CAPC. It is important to understand the prevalence of the disease reservoir. Some subclinical infections can cause complications with surgery and certain medications, or silent auto-immune disease processes. Diagnosis and treatment of these diseases is based upon history, clinical signs, vector population, hematologic abnormalities, and serologic findings.

In this study, serological testing compared lateral flow technology to the ELISA test in diagnosing disease states. When there were discrepant results, these values were compared to immunofluorescent antibody tests as the gold standard. Immunofluorescent antibody tests are most commonly used to detect antibodies in serum or other body fluids, most often antibodies specific for an infectious agent or an autoantigen. The IFA is a sensitive test that is often considered the gold standard of infectious disease serology[2].

The data in this study demonstrates that the Flex4 Rapid Test is ideally suited to accurately detect antibodies to Lyme disease, all three species of *Ehrlichia (E. canis, E. chaffeensis,* and *E. ewingii)* and both species of *Anaplasma (A. phagocytophilum* and *A. platys),* as well as the presence of *Dirofilaria immitis* antigen, or canine Heartworm antigen. It shows excellent sensitivity and specificity across all four diseases. On the contrary, the IDEXX SNAP 4Dx Plus test is only USDA-approved to identify *E. canis* and *E. ewingii,* but not *E. chaffeensis*, the pathogen known to be more prevalent than *E. canis*, which could lead to an infected dog being undiagnosed. Furthermore, the data supports that the IDEXX SNAP 4Dx Plus test is inferior in the detection of *Anaplasmosis*, and many dogs can have concurrent infections with Lyme and/or *Ehrlichia*.

Conclusion
The VetScan Canine Flex4 Rapid Test is a reliable, cost-effective and time-saving option to test for common, concurrent tick-borne infections and Heartworm disease. It is both a highly sensitive and specific test to detect the presence of antibodies for three *Ehrlichia* species, two *Anaplasma* species, *Borrelia burgdorferi*, and the *Dirofilaria immitis* antigen. The Flex4 Rapid Test is superior to the IDEXX SNAP 4Dx Plus test in identifying Anaplasmosis and infections produced by all three serotypes of canine Ehrlichiosis. Now, veterinarians have a new, more effective modality to diagnose and treat canines infected with one or more of these diseases.

Bibliography

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