

Performance of SNAP® 4Dx® Plus and AccuPlex™4 in Dogs with different Heartworm Burdens

Matthew D. Eberts, DVM

Lakeland Veterinary Hospital, 7372 Woida Rd, Baxter, MN 56425, USA

KEY WORDS: Heartworm antigen, SNAP®4Dx®, sensitivity, specificity

ABSTRACT

SNAP® 4Dx® Plus (IDEXX Laboratories, Westbrook, ME) is a commercially available in-office test kit for the simultaneous detection of *Dirofilaria immitis* antigen and antibodies to *Anaplasma phagocytophilum*, *A. platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *E. ewingii* in blood, plasma, or serum of dogs. AccuPlex™4 test (ANTECH Diagnostics®, Irvine, CA) is a recently launched reference laboratory test that detects heartworm antigen and antibodies to Lyme disease, anaplasmosis, and ehrlichiosis in dogs. In this report, we compared the performance of SNAP® 4Dx® Plus and AccuPlex™4 for detection of heartworm antigen in sera from dogs having necropsy-confirmed infections with a broad range of worm burdens. A total of 72 necropsy confirmed samples with a wide range of worm burden and 21 negative samples confirmed negative by PetChek® Heartworm PF Antigen Test (IDEXX Laboratories, Westbrook, ME) were used in this evaluation. Of the 72 necropsy-confirmed samples, SNAP® 4Dx® Plus test detected 65 samples compared to 54 samples detected by AccuPlex™4 assay. The AccuPlex™4 assay

reported a positive result for one antigen negative sample. For the low-worm burden populations, SNAP® 4Dx® Plus detected 82% of dogs with one worm and 100% of dogs with two worms, while AccuPlex™4 only detected 53% of dogs with one worm and 86% of dogs with two worms, respectively. In this study, where a direct comparison of the test methodologies was performed using the same sample set, the results indicate that SNAP® 4Dx® Plus is more sensitive than AccuPlex™4 in detecting heartworm antigen in dogs with low worm burdens.

INTRODUCTION

Canine heartworm (HW) infection caused by *Dirofilaria immitis* has been reported to be widely distributed throughout the United States.^{1,2} The antigen detection test is the preferred diagnostic method for asymptomatic dogs (annual wellness screening) or to verify the presence of suspected HW infection.^{3,4} Antigen tests detect carbohydrate antigens primarily released by the adult female heartworm,⁵ and are widely used with much success to detect canine HW infections. Currently, tests are available both in-clinic as well as at many veterinary diagnostic laboratories.⁴ Most commercial tests will accurately detect infections with one or more mature, adult female heartworms (at least 7 or 8 months old)⁵ generally have

Table 1: Comparative percent sensitivities of SNAP® 4Dx® Plus and AccuPlex™4

	Necropsy POS	PetChek® HW NEG
AccuPlex®4 POS	54	1
AccuPlex®4 NEG	18	20

	Necropsy POS	PetChek® HW NEG
SNAP®4Dx®Plus POS	65	0
SNAP®4Dx®Plus NEG	7	21

comparable sensitivity for the detection of infection when numerous worms are present and releasing higher quantities of antigen.⁶ However, it is likely that many of the well-cared for dogs seen in veterinary practices today may have lower worm burdens and perhaps lower circulating levels of antigen.¹ Recently, the AccuPlex™4 Test (Antech Diagnostics, Irvine, CA) was launched in their laboratory network with claims of 100% detection of 1-2 worm burden infections (AccuPlex™4 Insight, February 2012) compared to other commercially available tests. The present study was designed to directly compare the performance of the HW analytes in SNAP® 4Dx® Plus and AccuPlex™4 using the same well-characterized set of canine samples for both tests.

MATERIALS AND METHODS

Canine Samples and Confirmatory Tests

Canine samples from dogs with known worm burdens and negative samples were kindly provided by IDEXX Laboratories. All samples were part of IDEXX sample library and were stored aliquoted at -20°C. Samples for HW infection used to assess the sensitivity and specificity of the *D. immitis* analyte, were from dogs naturally infected with heartworms and found during necropsy examination of the heart and pulmonary

arteries or were samples tested by a HW antigen test as per manufacturer's instructions (PetChek® Heartworm PF Antigen Test, IDEXX Laboratories, Westbrook, ME). Samples were tested on SNAP® 4Dx® Plus as per the manufacturer's instructions. An aliquot of each sample was shipped to Antech Diagnostic Laboratory as per the laboratory's sample request protocol for testing in AccuPlex™4. The results from this testing was faxed to our hospital. The results were then analyzed and tabulated.

RESULTS AND DISCUSSION

Ninety three samples were tested by SNAP® 4Dx® Plus and AccuPlex™4. Within this sample set, 21 samples were from dogs testing negative for HW antigen by the PetChek® ELISA and 72 were from necropsy confirmed HW infected dogs. Sixty five of the 72 HW necropsy confirmed samples tested positive by the SNAP® 4Dx® Plus assay compared to 54 samples detected by AccuPlex™4 assay (Table 1). Of the 72 necropsy confirmed samples, 48 samples were derived from low-worm burden population (≤ 2 female worms). Of the 34 samples from dogs with a single adult female worm, 28 samples tested positive by the SNAP® 4Dx® Plus Test while AccuPlex™4 detected only 18 samples.

Table 2: Performance of SNAP® 4Dx® Plus and AccuPlex™4 with samples from dogs with low adult worm burden

	1 adult female worm (N = 34)	Percent Detected	2 adult female worms (N = 14)	Percent Detected
AccuPlex™4	18	53%	12	86%
SNAP®4Dx® Plus	28	82%	14	100%

All 14 samples obtained from dogs with two adult female worms were detected by SNAP® 4Dx® Plus compared to only 12 that were detected by AccuPlex™4 (Table 2). Of the 21 negative samples, one sample was identified as positive by AccuPlex™4, whereas all of the 21 tested negative by SNAP® 4Dx® Plus. The overall sensitivity of the HW analyte in SNAP® 4Dx® and in AccuPlex™4 was 89% and 76%, respectively with greater than 95% specificity in both tests (Table 1). These results are consistent with those of a recent study which reported the sensitivity and specificity of SNAP® 4Dx® Plus to be 98.9% (CI=94.3%-99.8%), and 99.3% (CI=97.3%-99.8%), respectively, for detection of HW antigen.⁷ The overall accuracy of SNAP® 4Dx® Plus and AccuPlex™4 was 93% and 80%, respectively. Studies on the performance of the AccuPlex™4 HW assay have not been published.

In general, commercially available HW antigen tests are sensitive and specific consistent with the findings of this study. To fully appreciate the relative performance of different heartworm antigen tests, it is important to compare their accuracy within the same sample population and include samples that challenge the limits of sensitivity of the assays. Accordingly, this study was designed to evaluate the sensitivity of both tests in dogs with low heartworm burdens, and 48 of the 72 serum samples tested in this study were from dogs with ≤ 2 adult female heartworms only. These samples were selected to ascertain the robustness of the tests to detect low amounts of circulating antigen. As shown in Table 2, the sensitivities of the test kits increased with both tests with increase in HW burden (1 to 2 female worms). Prior studies have reported similar observations.⁸ The diagnostic performance of heartworm antigen tests can vary de-

pending on a number of factors, such as heartworm burden, heartworm sex ratio, and presence, if any of immature or dying heartworms.^{1,3}

In conclusion, in this study we observed that SNAP® 4Dx® Plus was more sensitive than AccuPlex™4 for detection of low HW burden samples. Additional studies are needed to understand the relative performance of these tests with a large sample population derived from heartworm endemic areas.

ACKNOWLEDGEMENTS

We thank IDEXX Laboratories for providing us with the samples tested in this study.

REFERENCES

1. Bowman DD, Atkins CE. Heartworm biology, treatment, and control. *Vet Clin North Am Small Anim Pract* 2009;39:1127-1158.
2. Bowman DD. Introduction: Heartworm. *Top Companion Anim Med* 2011;26:159.
3. McCall JW, Genchi C, Kramer LH, et al. *Adv Parasitol* 2008;66:193-285.
4. Nelson CT, McCall JW, Rubin SB, Buzhardt LF, Dorion DW, Graham W, Longhofer SL, Guerrero J, Robertson-Plouch C, Paul A. Guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in cats. *Vet Parasitol* 2005;24:267-75.
5. Weil GJ. *Dirofilaria immitis*: identification and partial characterization of parasite antigens in the serum of infected dogs. *Exp Parasitol*. 1987;64:244-251.
6. Thilsted JP, Whorton J, Hibbs CM, Jillson GP, Steece R, Stromei M. Comparison of four serotests for the detection of *Dirofilaria immitis* infection in dogs. *Am J Vet Res*. 48:837-841.
7. Stillman BA, Monn M, Liu J, Thatcher B, Foster P, Andrews B, Little S, Eberts M, Breitschwerdt E, Beall M, Chandrashekar R. Performance of a new commercially available in-clinic ELISA for the detection of antibodies to Ehrlichia ewingii (granulocytic ehrlichiosis) and Anaplasma platys (thrombocytotropic anaplasmosis) in dogs. *J Vet Intern Med*. 2012;26:704.
8. Atkins CE. Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. *J Am Vet Med Assoc*. 2003;222:221-223.