Canine Heartworm Testing – Understanding Low Worm Burden Testing and a Comparison of Tests on the Market

Kenneth Aron, PhD[1]; Rajesh Mehra, PhD[1]; Craig Tockman, DVM[2]; Keith DeJong, DVM, DACVP[2]
[1]*Abaxis, Inc., Research and Development*
[2]*Abaxis, Inc., Professional Services, North American Animal Health*

Background

Heartworm disease caused by infection with *Dirofilaria immitis* has been diagnosed in all 50 states[1,2]. Testing for this parasite is routinely performed during regular checkups and annual visits as well as when clinical signs are present. Veterinarians count on the accuracy and precision of point-of-care antigen tests to provide accurate screening for the healthy patient as well as to assist with diagnosis of the patient suspected of infection when presenting with clinical signs.

Patients with heartworm infection may have as few as one worm or as many as several hundred. Some of the factors that determine this include environment, frequency of exposure, prevalence in the region and frequency of preventive use[1-3]. Higher worm counts usually provide a higher antigen level for the tests to identify. Conversely, it is assumed that low worm counts may not provide sufficient antigen leading to a false negative test. However, worm burden is only one aspect that can lead to false negative results.

Severity of the disease is thought to be directly related to worm burden, length of infection and the response of the infected patient [4]. However, of equal, if not greater, importance is the activity level of the dog. A controlled study showed dogs that were infected by surgical transplantation with 50 heartworms and exercise-restricted took longer to develop clinical disease and developed less pulmonary vascular resistance than dogs with 14 surgically transplanted heartworms that were allowed moderate activity. This outcome was also evident in naturally infected dogs where, again, there was no correlation between the number of heartworms and pulmonary vascular resistance. Both of these scenarios indicate that the host-parasite interaction plays a significant role in the severity of disease[1]. Some patients, such as those not on a regular preventive and living outdoors in highly endemic areas, can have very high numbers of worms at the time of diagnosis. In cases of indoor patients with minimal exposure in areas of low prevalence, worm numbers are likely to be lower. So clearly a veterinarian may see high burden infections, low burden infections and infections at all levels in between.

Even with this knowledge, some practitioners desire to understand the sensitivity and specificity of heartworm tests at low worm burdens to compare available tests. To provide this information in a fair manner, Abaxis has performed a study to understand the test results at multiple levels of infection.

Materials and Methods

Serum from 25 known positive and 24 known negative patients, verified by antigen testing and necropsy, were purchased from the University of Florida College of Veterinary Medicine, Department of Infectious Diseases and Pathology. Each sample was identified with the number of female, gravid female, male, immature and dead worms in each patient. The samples were tested on 4 lateral flow devices according to manufacturer's instructions. The devices used were: IDEXX SNAP® Heartworm RT Test[a] (Serial # 09440-EG652), Heska Solo Step CH[b] (Serial # 204290), Synbiotics Witness® HW[c] (Lot No. 1101463) and Abaxis Vetscan® Canine

Heartworm Rapid Test (Serial # 103144). Number of female worms ranged from 1 to 55 across the 25 patient samples.

Results

All 24 negative patients tested negative on all 4 tests. Since there were no false positives, all tests had 100% specificity. Serum from 9 patients had confirmed infection with 4 or less female worms. Results for this subgroup are shown in Table 1 and complete experimental data is presented in Table 4.

Table 1 – Results of Tests From Patients With 4 or Less Female Worms

4 Female Worms or Less					
	Vetscan	SNAP	Solo Step	Witness	Total Positive
Positive	8	8	7	6	9
False Negative	1	1	2	3	

Sensitivity values for 4 or less female worms are as follows:

Vetscan	89%
SNAP	89%
Solo Step	78%
Witness	67%

Summary data from all 25 known positive patients are shown in Table 2.

Table 2 – Results From All Positive Patients

1-55 Female Worms					
	Vetscan	SNAP	Solo Step	Witness	Total
Positive	23	23	21	20	25
Negative	24	24	24	24	24
False Negative	2	2	4	5	

Sensitivity values for all positive patients are as follows::

Vetscan	92%
SNAP	92%
Solo Step	84%
Witness	80%

Discussion

Antigen tests are highly specific and sensitive in diagnosing heartworm infection. It is important to remember that specificity and sensitivity values are valid only when the test is run according to the manufacturer's instructions, commonly found in the package insert for the product. They are available as point-of-care tests to be used in veterinary facilities. The USDA Center for Veterinary Biologics regulates all in-house heartworm tests. Before any heartworm test can be sold, the performance of the test has to be demonstrated through a series of clinical studies showing acceptable performance in the following areas: accuracy, precision, ruggedness, sensitivity and specificity. The submission data is the basis of the package insert for these tests and tests cannot be sold without meeting the USDA criteria. The Abaxis Vetscan Canine Heartworm Rapid Test meets or exceeds all these USDA standards[5].

Specificity is often perceived to mean accuracy, however, specificity is by definition the measurement of the frequency with which a result will be negative (or normal) in patients without the disease. To clarify, a test with 98% specificity would mean that if 100 negative or normal patients are tested, then the test would in fact show negative or normal in 98 of them, while 2 of the patients would show a false positive. Therefore a test with high specificity rules out the disease with a high level of certainty. Specificity is calculated as True Negatives/ (True Negatives + False Positives).

Sensitivity indicates the frequency of a positive (or abnormal) test in patients that have the disease. Again, to clarify, for a test that has 98% sensitivity and 100 known positive patients are tested, then 2 of the patients will have a false negative result. A test with high sensitivity is an excellent screening test for disease -- there will be false negative results, however, unless its sensitivity value is 100%. Sensitivity is measured as True Positives/ (True Positives + False Negatives).

Immunoassays such as those for canine heartworm antigen are very specific to variations among individuals. Not all individuals have identical immune responses. Likewise, all infections are not identical and may be modulated by the health of the host or a variety of other factors. In addition, whether based on polyclonal or monoclonal technologies, the antibodies for different antigen tests may be specific to different antigens or even different epitopes of a common antigen, and normally differ among different manufacturers[6]. These are only some of the reasons that any test is capable of producing a false positive or false negative heartworm test regardless of the worm burden of the patient. To be led to believe anything else is dishonest at best.

Some companies aggressively market the accuracy of tests at low worm burdens, considered by some to mean 4 female worms or less, to suggest that their tests are more accurate than others, in spite of the fact that many heartworm infections involve much higher worm burdens along with clinical signs. Several papers or studies have been presented to the veterinary community describing these results, with significant skewing of those results towards the publishing company's product[6].
The practitioner should also consider the positive and negative predictive value of each test they perform.

Positive predictive value is the percentage of patients with a positive test that actually have the disease in question (True Positives) and includes either prevalence data or in the clinical setting, the likelihood of the disease based on history, physical examination and clinical signs. Negative predictive value is the percentage of patients who test negative to a disease and are in fact negative (True Negatives), but it also accounts for prevalence data, clinical illness or the lack thereof. Prevalence is the percentage of true positive animals within the tested population. When interpreting the diagnostic result, it is imperative to include either prevalence data for a screening protocol, or history and clinical signs in an ill patient[2,7].

The results of this study show all tests to have excellent specificity. It also clearly shows that all 4 of the tests, while good to excellent at identifying infection at all worm burdens, will generate an occasional false negative result. Practitioners must understand that this is a possibility with all tests and any company claiming to be perfect every time with every test is not being honest with the customer. A veterinarian with a patient exhibiting clinical signs of disease in the face of a negative test should confirm his/her clinical suspicions with a test of a different methodology as recommended by the American Heartworm Society[1]. Conversely, a patient which is not exhibiting clinical signs of disease in the face of a positive test should also be retested for confirmation with a different methodology.

The study also showed that the Abaxis Vetscan Canine Heartworm Rapid Test provided excellent results in comparison to all competitive brands across all worm burdens.

Table 4 – Complete Experimental Data

ID	Worm Female	Gravid	Worm Male	Worm Immature	Worm Dead	Worm Total	Vetscan	SNAP	SoloStep	Witness
2011308	45	Y	25	2	2	70	+	+	+	+
2011315	3	N	3	0	1	6	+	+	+	-
2011332	2	Y	1	0	1	3	+	+	+	+
2011340	4	Y	5	0	0	9	+	+	+	+
2011341	16	Y	12	0	0	28	+	+	+	+
2011342	3	Y	3	0	0	6	-	-	-	+
2011344	8	Y	10	0	0	18	-	-	-	-
2011347	46	Y	30	2	1	76	+	+	+	+
2011354	11	Y	4	0	2	15	+	+	+	+
2011355	33	Y	16	0	2	49	+	+	+	+
2011356	17	Y	26	0	1	43	+	+	+	+
2011357	20	Y	31	0	3	51	+	+	+	+
2011358	15	Y	12	0	1	27	+	+	+	+
2011365	3	Y	2	2	0	5	+	+	+	+
2011366	19	Y	9	0	1	28	+	+	+	+
2011371	15	Y	16	0	1	31	+	+	+	+
2011372	3	Y	1	0	0	4	+	+	+	+
2011373	55	Y	56	0	2	111	+	+	+	+
2011376	3	Y	4	0	2	7	+	+	+	-
2011377	10	N	0	0	1	10	+	+	+	+
2011384	10	Y	9	0	1	19	+	+	-	-
2011388	49	Y	40	2	5	89	+	+	+	+
2011389	2	Y	5	0	1	7	+	+	+	+
2011390	3	Y	7	0	1	10	+	+	-	-
2011391	25	Y	21	0	2	46	+	+	+	+

[a]SNAP RT, IDEXX Laboratories, Portland, ME

[b]Solo Step CH, Heska Corp, Fort Collins, CO

[c]Witness® HW, Synbiotics, Kansas City, MO

[d]Vetscan® Canine Heartworm Rapid Test, Abaxis, Inc., Union City, CA

1. McCall JW, Gerrero J, eds. 2007. Diagnosis, prevention, and management of heartworm (*Dirofilaria immitis*) infection in dogs. Retrieved September 30, 2011, from http://www.heartwormsociety.org/veterinary-resources/Guidelines-Can-HW-Disease.pdf

2. Bowman D, Little SE, Lorentzen L, et al. 2009. Prevalence and geographic distribution of *Dirofiliaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survery. Vet Parasitol 160(1-2):138-148.

3. Kaiser L, Williams JF. 2004. *Dirofilaria immitis*: worm burden and pulmonary artery proliferation in dogs from Michigan (United States). Vet Parasitol 124(1-2):125-129.

4. Atkins CE. Canine heartworm disease. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine. 6th ed. St. Louis, MO. Elsevier Saunders. 2005: 1118-1136.

5. USDA Veterinary Biological Products. 2011. Retrieved October 4, 2011 from http://www.aphis.usda.gov/animal_health/vet_biologics/publications/CurrentProdCodeBook.pdf

6. Courtney CH, Zeng Q. 2001. Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens. Vet Parasitol 96(4):317-322.

7. Erb HN. 2011. Prior probability (the pretest best guess) affects predictive values of diagnostic tests. Vet Clin Path. 40(2): 154-158.