

Heartworm testing validation: Bionote USA Antigen Rapid CHW Ag Test Kit 2.0 (November 2020)

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Objective:

The objective of this project was to validate the **Bionote USA Antigen Rapid CHW Ag Test Kit 2.0** and determine the sensitivity and specificity of this test. This was performed using canine blood serum or whole blood from heartworm (*Dirofilaria immitis*) negative and positive dogs. Heartworm status had been previously determined by necropsy.

Methods:

Canine Sera

Archived canine sera (N=125) and whole blood (N=5) was used for the Bionote CHW Test validation. This sera had been frozen (-80°C) and determined positive or negative for *Dirofilaria immitis* by necropsy. Each sample had a recorded count of male and female adult worms, determination of microfilaremia and prior antigen test results. Sera samples were chosen based on adult worm counts. Each serum sample was removed from the frozen archive and aliquoted as one 500 µl sample and relabeled in a blinded fashion.

Sera Revalidation

Archived canine sera was revalidated using the DiroCHEK® Canine Heartworm Antigen Test Kit (N=125) (Lot#429013). Each test was performed according to the manufacturer’s instructions. Approximately 0.05mL of heartworm status defined serum (Table 1) was used to verify the antigen status of each archived sample (N=125). The results of each sample were compared to previous results taken when serum was fresh, and collected the day of necropsy.

Bionote CHW Validation

Each Bionote CHW test was performed according to the manufacturer’s instructions. Approximately 0.08 mL of revalidated heartworm status defined dog serum (Table 1) was used to validate the Bionote Canine Heartworm Test Kit (N=125). Each serum sample was subjected to the Bionote CHW test once.

Antigen results determined to be “weak” positive by Bionote Canine Heartworm Test Kit via serum (N=8) were reevaluated by the Bionote CHW test using whole blood (WB) (Table 1).

Archived canine sera with 6+ male/female heartworms (N=5) was mixed at a 1:1 dilution with packed anti-coagulated red blood cells (RBC) and first revalidated using the DiroCHEK® Canine Heartworm Antigen Test Kit and validated using Bionote CHW test (Table 1).

Table 1. Description of Heartworm Sera and Whole Blood Samples as Defined by Adult Counts

Heartworm Necropsy Status	Number of Male and/or Female Heartworms	Number of Samples	Number of Whole Blood Samples
Negative	0	75	-
Positive	Low 1-5	10	4
Positive	Medium/High 6+	40	4
Positive	6+ at 1:1 packed RBC dilution	5	-

Data was manually recorded and then placed in an Excel spreadsheet. Each test was analyzed against necropsy status as the “gold standard”. Sensitivity and specificity was calculated as follows:

Test Result	Known Infection Status	
	Positive	Negative
Positive	a	b
Negative	c	d

$\% \text{sensitivity} = a / (a+c) \times 100$
 $\% \text{specificity} = d / (b+d) \times 100$

Results:

Antigen Test Results

Necropsy Results	Bionote USA Antigen Rapid CHW Ag Test Kit 2.0	
	Positive	Negative
Negative – 0 HW (N=75)	0	75
Positive – 1-5 male/female HW (N=10)	10	0
Positive – 6+ male/female HW (N=40)	40	0
Positive – 6+ male/female HW +RBCs (N=5)	5	0
Positive “weak” – 1+ male/female HW (N=8) WB	8	0

Overall Results

Necropsy Results	Bionote USA Antigen Rapid CHW Ag Test Kit 2.0	
	Positive	Negative
Negative – 0 HW (N=75)	0	75
Positive – male/female HW (N=50)	50	0

Test Result	Known Infection Status (necropsy)	
	Positive	Negative
Positive	50	0
Negative	0	75

Overall %sensitivity = $50 / (50+0) \times 100 = 100\%$

Overall %specificity = $75 / (0+75) \times 100 = 100\%$

% Sensitivity by Groups:

- Positive female only HW (1-5) (N=5) = 100%
- Positive male/female HW (1-5) (N=5) = 100%
- Positive male/female HW (6+) (N=40) = 100%
- Positive – 6+ male/female HW +RBCs (N=5) = 100%
- Positive “weak” – 1+ male/female HW WB (N=8) = 100%

Conclusions:

The Bionote CHW test was able to detect true negatives; all 75 necropsy negative samples were identified, giving a specificity of 100%. Overall, the sensitivity for the Bionote CHW test was 100%.

Of the 40 positive sera samples from dogs with 6+ male and/or female heartworms present at necropsy, the Bionote CHW test was able to correctly identify 40 as positive. Of the 10 samples containing 1-5 male and/or female heartworms, all Bionote CHW test results were in agreement with DiroChek® antigen test and necropsy results. There were eight (N=8) of the positive serum samples in the tested group that were weakly positive with the Bionote CHW test, giving a very faint line in the test column. These were retested using whole blood from the same animal (per Materials 3.2) and they were positive with the Bionote CHW test.

Five (N=5) whole blood samples were additionally selected, apart from the original sample cohort, and mixed with an equal volume (1mL:1mL) of packed anti-coagulated RBCs and tested using the DiroCHEK® Canine Heartworm Antigen Test and Bionote CHW antigen test. All five samples were positive and represented infections of 8+ heartworms.

