trūRapid™ HW/Lyme Test Product Bulletin

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trūRapid Heartworm/Lyme test demonstrates excellent sensitivity and specificity for heartworm antigen and Lyme C6 antibody screening and detection.

Key Points

- Canine heartworm disease caused by the parasite *Dirofilaria immitis*, is a life-threatening disease.
- The prevalence of heartworm disease is increasing in North America and Europe.
- Lyme disease caused by the spirochete *Borrelia burgdorferi* (Bb) and related Borrelia strains results in lameness, fever, lethargy, anorexia and renal disease in dogs.
- The prevalence and geographic range of Lyme disease is increasing in North America, the United Kingdom (UK) and continental Europe.
- Heartworm antigen and Lyme/Bb C6 antibody assays in the trūRapid test are the recommended tests for screening and diagnosis of heartworm and Lyme disease.
- The trūRapid Heartworm/Lyme test has excellent sensitivity and specificity relative to reference methods and currently marketed commercial heartworm and Lyme tests.

Introduction

Dirofilaria immitis, the heartworm, is a parasitic roundworm that spreads from host to host through the bites of mosquitoes (Bowman D, et al, 2009). The parasite commonly resides in the pulmonary arterial system as well as the heart. A major effect on the health of infected animals is a manifestation of damage to the lung vessels and tissues (Dantas-Torres F, et al, 2013). Diagnosis of *Dirofilaria immitis* infection in dogs is based on many factors, including detection of an adult female worm antigen in serum, plasma, or anticoagulated whole blood. (https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines, 2024).

Lyme disease is a bacterial infection caused by *Borrelia burgdorferi* (Bb) sensu lato complex, bacterial spirochetes transmitted through the bite of an Ixodes sp. tick. In North America, *Borrelia burgdorferi* is the main cause of Lyme disease, while in Europe, *Borrelia afzelii* and *Borrelia garinii* are the primary causes. (Hengge, UR, et al 2003 ; Marques AR, et al 2021). Clinical signs of Lyme disease are most commonly reported in humans and in dogs. Asymptomatic infection is common in many domestic species. (Littman MP, et al, 2006; 2018; Springer A, et al, 2020). Wildlife, especially deer and mice, are reservoir hosts for Bb.

Heartworm

Heartworm Disease, caused by infection from *Dirofilaria immitis*, is a globally dispersed and clinically significant cardiopulmonary disease seen in canids and felids, as well as other genera (e.g. Mustelidae, Otariidae). Endemic areas in the United States have shown up to 45% prevalence, with tropical climates conferring the chance at even higher infection rates (Bowman D, et al, 2009) (Self SW, et al, 2019). Over the last 10 years, heartworm has spread from southern Europe into eastern and northeastern countries. (Morchon R et al, 2022).

Clinical signs of *D. immitis* infection in dogs include respiratory distress, epistaxis, hemoptysis, ascites, exercise intolerance, and anorexia. Despite the chance to observe those severe clinical signs, during early stages of infection and disease, dogs typically present asymptomatically. Progression of disease tends to lead to worsening clinical signs and prognosis (https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines, 2024).

Medical and surgical management of canine heartworm disease can be difficult for a myriad reasons, including *D. immitis* life cycle (Table 1), side effects and efficacy of medical therapies, and clinical presentations in practice. Furthermore, even with complete resolution of the infection, some animals experience long term effects from the damage inflicted by the infection. Prevention and monitoring remain key aspects of eradication programs, regardless of levels of endemicity (https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines, 2024).

Table 1. *Dirofilaria immitis* Life Cycle

D. Immitis Life Stage	L1, L2, L3	L4	S5 (immature adult)	Adult
Stage Complete	10–14 Days	45–65 Days	4–5 Months	6– months (total)
Location	Mosquito	Host tissue	Host bloodstream	Host bloodstream

Point-of-care heartworm antigen tests can detect *D. immitis* in whole blood, serum or plasma, and give veterinary professionals vital tools in diagnosing and monitoring canine heartworm infection and disease. Antigen tests are developed to recognize

antigen of adult female heartworms, which can complicate diagnosis in the presence of low worm burdens, infections with only larvae or immature stages of the worms, or all-male infections. Sensitivity and specificity remain high for antigen tests broadly, with as much as 99% sensitivity and 100% specificity, depending on test type and worm burden (https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines, 2024)

Lyme/Borrelia sp.

The prevalence and geographical range of Lyme disease is increasing although precise estimates are difficult due to variation in case definition and surveillance methods. (Rizzoli A, et al, 2011; Dantes – Torres F et al, 2012; Bajer A, et al, 2014; Michelet L, et al, 2014; Bjurman NK, et al, 2016; Cairns V, et al, 2019; Tulloch JSP et al, 2019; Springer A et al, 2020; Marques, AR, et al, 2021; Miro G et al, 2022; Kugeler KJ et al, 2022; Bajer A et al, 2022; https://capcvet.org/; https://www.canada.ca/en/public-health/services/diseases/lyme-disease/surveillance-lyme-disease.html#a1) The Mid-Atlantic, Northeast, Upper Midwest regions of the United States have the greatest prevalence of Lyme disease in the United States. (Kugeler KJ, et al, 2022) In Canada, most cases are reported in Ontario, Quebec and Nova Scotia. (https://www.canada.ca/en/public-health/services/publications/diseases-conditions/lyme-disease-surveillance-canada-annual-edition-2022.html) In the UK, the majority of cases are reported from Scotland, South and Southwest England.(Cairns V, et al, 2019; Tulloch JSP et al, 2019) In continental Europe, the prevalence of Lyme Disease is greatest in Scandinavia, Baltic States, Austria, Czech Republic, Germany and Slovenia. (Marques AR, et al, 2021) Expansion of the range of the Ixodes sp. tick vectors associated with climate change, encroachment of suburban into rural areas and increased human activities in tick environments are important factors in the increased number of Bb infections. (Littman MP, et al, 2018)

Diagnosis of Lyme disease depends on demonstration of Bb antibodies in the blood of dogs with characteristic clinical signs. The clinical signs and syndromes most associated with Lyme disease are lameness, fever, lethargy, anorexia, lymph node enlargement and glomerulonephritis. (Littman MP, 2006, 2018) However, the majority of Bb infections in animals are asymptomatic which presents challenges in confirming a diagnosis. Serology for the Bb C6 antibody is the recommended assay for supporting a diagnosis of Lyme disease and eliminates interference resulting from vaccine associated antibodies.(Littman MP, et al, 2018) The C6 peptide is highly specific and antigenically conserved among various species of *B. burgdorferi* sensu lato complex. The C6 peptide derived from *B. burgdorferi* detects the infection regardless of infecting strains. (Liang FT, et al,. 2000. Tjernberg I,et al 2007; Christova I et al, 2013; Rouhiainen M et al, 2021). Levels of C6 antibody decrease in infected animals during treatment. The trūRapid Heartworm/Lyme test detects the C6 antibody. Routine serologic screening of dogs is recommended in areas endemic for Lyme disease, followed by screening seropositive dogs for proteinuria. (Littman MP, 2018) The presence of Bb antibody indicates tick exposure and a risk for not only Lyme disease but also for a variety of other tick-borne diseases. (Dantes – Torres F, et al, 2012; Michelet L, et al, 2014; Bajer A, et al, 2022; Miro G et al, 2022).

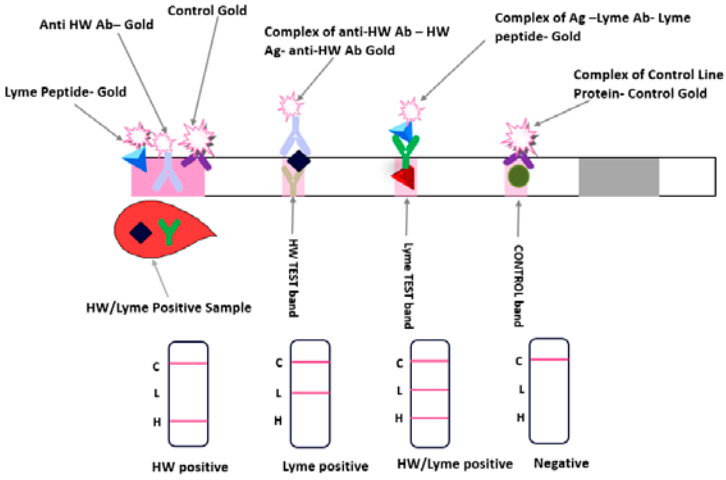
Confirming a diagnosis of Lyme disease in a symptomatic dog requires demonstration of C6 antibody and rule out of other tick-borne diseases or other potential causes of the presenting clinical signs. Initiation of measures to limit tick exposure is warranted in any dog seropositive for Bb.

trūRapid HW/Lyme technology

trūRapid HW/Lyme point of care (POC) test strips are sandwich-immunoassays intended for the visual qualitative detection of both heartworm antigen (*Dirofilaria immitis*) and canine *Borrelia burgdorferi* antibody in canine serum, plasma or anticoagulated whole blood on a single nitrocellulose membrane-based test strip.

The assays are set up as follows: two antibodies are used in the test strip for the detection of the female *D. immitis* antigen. One antibody is coupled on gold nanoparticles and a second, high specificity antibody is immobilized in the heartworm test line area (H). A combination of a protein and a highly purified Lyme C6 peptide are used in the test strip for *Borrelia burgdorferi* antibody detection. The purified peptide is coupled on gold nanoparticles and the protein is immobilized in the Lyme test line area (L). The control gold nanoparticles bind to the antigen, a protein immobilized in the control line area, forming a control line.

When the sample, followed by the sample buffer, is put into the



sample wells, it will be absorbed into the absorbing pad of the test strip. The fluid mixes with the gold labelled antibodies and peptides in the conjugate pad. Due to the capillary action of the test strip, the fluid will run up over the test strip, crossing the two test line regions (H and L), then subsequently the control line region. The control line should always appear, signifying correct functioning of the test

If the patient sample contains the antigen of even a single adult female heartworm, a line will appear in the heartworm test line region (H). This happens by building a sandwich between the gold labelled antibodies from the conjugate pad, the antigen from the sample, and the immobilized antibody of the heartworm test line region. Even a faint line is considered positive for heartworm infection.

If no antigen is in the sample, the gold labelled antibodies cannot connect to the immobilized antibodies in the heartworm test line region, and therefore no heartworm test line appears.

If the patient sample contains the *Borrelia burgdorferi* C6 antibody, a line will appear in the Lyme test line region (L). This happens by building a sandwich between the gold labelled peptide from the conjugate pad, the Lyme antibody from the sample, and the immobilized protein of the Lyme test line region. If the test is positive for *Borrelia burgdorferi* C6 antibodies, it means that the animal has antibodies from natural exposure to *Borrelia burgdorferi* (Littman MP, et al, 2018).

If no *Borrelia burgdorferi* C6 antibody is in the sample, the gold labelled peptide cannot connect to the immobilized protein in the Lyme test line region and therefore no Lyme test line appears.

Material and Methods

trūRapid HW/Lyme test strips were tested in this performance evaluation. A total of 270 and 128 samples from naturally infected dogs were tested in two comparison studies comparing the trūRapid HW/Lyme results to other methods. The heartworm performance data was generated by testing samples using a commercial enzyme linked immunosorbent assay (ELISA) and a rapid antigen test. The comparisons for Lyme were generated by testing against an indirect fluorescent antibody assay (IFA) and a rapid antibody test.

Results

Sensitivity and Specificity

Study 1: The sensitivity and specificity of the test kit were evaluated by testing a total of 270 dog serum samples with trūRapid HW/Lyme tests. Sensitivity and specificity were determined comparing DiroCHEK® HW Antigen test, as shown with a 2x2 analysis table (Table 1 and 2). All data is from naturally infected animals.

Table 1: Comparison of heartworm test results - trūRapid versus DiroCheck

trūRapid Heartworm Test	DiroChek® Heartworm Antigen Test		Total
	Detected	Undetected	
Detected	150	2	152
Undetected	0	118	118
Total	150	120	270
Sensitivity	100% (95% CI: 97.6%–100%)		
Sensitivity	98.3% (95% CI: 94.1%–99.8%)		

Table 2: Comparison of Lyme test results—trūRapid versus IFA

trūRapid Lyme Test	Lyme IFA[1]		
	Detected	Undetected	Total
Detected	103	3	106
Undetected	1	163	164
Total	104	166	270
Sensitivity	99.0% (95% CI: 94.8%–100%)		
Sensitivity	98.2% (95% CI: 94.8%–99.6%)		

[1] VMRD - SLD-IFA-LD test.

Study 2: The performance of the trūRapid HW/Lyme test kit was evaluated by testing a total of 128 dog serum samples for both heartworm and Lyme. For heartworm, an additional 150 positive samples were tested. The sensitivity and specificity were compared with IDEXX SNAP 4DX®, with a 2x2 analysis table. All data is from naturally infected animals.

Table 3: Comparison of Heartworm test results - trūRapid HW/Lyme test versus SNAP 4Dx

trūRapid HW/Lyme Test (Heartworm results)	IDEXX SNAP 4DX (Heartworm Results)		
	Detected	Undetected	Total
Detected	149	1	150
Undetected	1	127	128
Total	150	128	278
Sensitivity	99.3% (95% CI: 96.3%–100%)		
Sensitivity	99.2% (95% CI: 95.7%–100%)		

Table 4: Comparison of Lyme test results - trūRapid HW/Lyme test versus SNAP 4Dx

trūRapid HW/Lyme Test (Lyme results)	IDEXX SNAP 4DX (Lyme Results)		
	Detected	Undetected	Total
Detected	49	2	51
Undetected	1	76	77
Total	50	78	128
Sensitivity	98.0% (95% CI: 89.4%–100%)		
Sensitivity	97.4% (95% CI: 91.0%–99.7%)		

Conclusion

The study demonstrates Antech trūRapid HW/Lyme test delivers excellent sensitivity and specificity for heartworm antigen and Lyme C6 antibody when compared to reference methods and in-house, rapid test methods. It is reliable test that provides rapid results for screening and detection.

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