

# COMPARATIVE PERFORMANCE OF THE NOVEL, POINT-OF-CARE PLUSLIFE MINI DOCK *DIROFILARIA IMMITIS*/*DIROFILARIA REPENS* DETECTION TEST WITH THE MODIFIED KNOTT'S TEST IN DOGS

G.G. Verocai<sup>1</sup>, T.L. Sanders<sup>1</sup>, M.A. Kelly<sup>1</sup>, A. Tapia<sup>1</sup>, P. Weeraratne<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathobiology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA

Canine heartworm disease caused by *Dirofilaria immitis*, is one of the most important endoparasites of dogs that can result in severe clinical manifestations. The current American Heartworm Society guidelines recommend the concomitant use of an antigen-detection test and a microfilariae detection test (MFDTs) for diagnosing heartworm infection in dogs. The modified Knott's (MK) test is the preferred method for MFDTs to determine morphological characteristics of *D. immitis* microfilariae and related filarial nematodes that may infect dogs, such as *Acanthocheilonema reconditum*. However, MK requires extensive microscopy training and can be time consuming within a clinical setting. RNase HII-assisted amplification (RHAM), a novel isothermal amplification method, has shown promising results for rapid and accurate detection of other pathogens such as SARS-CoV-2. The Pluslife Mini Dock is a point-of-care diagnostic platform that does not require a DNA extraction step, and results may be available within 30 minutes. The aim of this study was to assess the performance of the Pluslife Mini Dock duplex *D. immitis*/*D. repens* assay to the MK in dog blood. Archival, frozen whole blood samples of 250 dogs from shelters located in central Texas were subjected to the Pluslife Mini Dock and compared with the MK test. Overall, 92.1% of results matched between the two tests, but the Pluslife Mini Dock found a higher proportion of *D. immitis* (32.8%; 82/250) positive samples compared to the MK (29.6%; 74/250). There was no statistical significance between tests ( $p=0.099$ ). Cohen's kappa statistic showed near perfect agreement between tests (0.83). Furthermore, the 11 samples in which *A. reconditum* microfilariae were detected did not generate false-positive results. A single sample co-detected *D. immitis* and *D. repens*. Future studies should evaluate fresh blood samples as frozen samples may be subject to DNA degradation, which may negatively impact molecular test results. Our data suggests the Pluslife Mini Dock *D. immitis*/*D. repens* duplex assay provides a novel diagnostic platform and is a suitable option as a point-of-care MFDT.

Word Count: 320/350

## Key Facts:

1. Novel molecular patient-side diagnostic test for *Dirofilaria immitis*
2. A duplex with the ability to detect DNA from *Dirofilaria immitis* and *Dirofilaria repens*
3. Results delivered in just 30 minutes or less
4. Performed comparably to the modified knotts and eliminates need for microscopy training
5. Assay does not cross react with other filarial worms such as *Acanthocheilonema reconditum*