

## Confirmatory Testing

- FREE confirmatory testing at HESKA Veterinary Diagnostic Laboratories in Loveland, Colorado.
- A highly accurate reference lab quantitative antigen assay, developed at Heska, is performed.
- Assistance with microfilariae identification is also available if/when needed.
- FREE consultation with Heska's Medical and Technical Consultants.

### Options for Confirmatory Testing

1. If positive heartworm antigen results are obtained using an antigen test kit, check the animal's blood for *D. immitis* microfilariae.
  - Presence of *Dirofilaria immitis* microfilariae and a positive heartworm antigen test confirm the presence of *D. immitis* adult worms.
2. If no *D. immitis* microfilariae are detected, repeat with a test utilizing a different antigen detection method.
  - Even in areas where the prevalence of heartworm infection is high, many (~20%) heartworm infected dogs may not be microfilaremic.<sup>2</sup>
  - Currently available test formats for heartworm antigen testing include:
    - Membrane ELISA
    - Lateral flow immunoassay
    - Microwell ELISA

### Specificity of Canine Heartworm Antigen Test Kits

None of the commercially available antigen test kits are 100% sensitive and specific. These kits should be considered "screening tests" for the presence of *D. immitis* antigens. Any positive result with an antigen test should be confirmed prior to initiating adulticide therapy.<sup>1</sup>

Solo Step® CH is a very specific test. Based on the number of Solo Step CH tests used by our customers and the total number of confirmed false positive Solo Step CH results, the false positive rate is substantially lower than the 1% false positive rate one might expect from a test with 99% specificity.

In monitoring the performance of Solo Step CH since its introduction to the market, both false negative and false positive results from every commercially available heartworm antigen test kit have been identified.<sup>3</sup>

### Some Comments on Confirmatory Testing

1. Positive results on a single heartworm antigen test kit should be confirmed prior to heartworm treatment.<sup>1</sup>
2. An appropriate confirmatory test should use a different antigen detection method than the original screening test.
  - A false positive or false negative result from an individual animal not resulting from technique errors will continue to occur on multiple tests of the same type. In other words, the false result is typically caused by some unique characteristic of that particular dog, as opposed to some defect with the test kit.
    - A false positive may be caused by some component found in the patient's blood, which has the ability to react in a particular test kit's system to give a positive result in the absence of heartworm antigens.
    - Similarly, some component found in the patient's blood may lead to a negative result in the presence of heartworm antigens.
3. When using a microfilariae detection system (Knott's or filtration-type test) as a confirmatory test, keep these points in mind:
  - Presence of microfilariae, but a negative heartworm antigen test can occur in the following situations:
    - Animal with unknown history that is diagnosed heartworm positive at an earlier date, then treated with an adulticide, but not with a microfilaricide would have no adult worms (*i.e.*, no antigen to be detected), but could have detectable microfilariae for 2 to 3 years.
    - Similarly, a patient could have adult worms that die and be left with circulating microfilariae. The microfilariae could be *Dipetalonema reconditum*. This parasite would not produce *D. immitis* antigen.
    - Very young dogs born to a heartworm-positive bitch could be infected transplacentally and be born with circulating microfilariae, but not have adult *D. immitis* and therefore no detectable *D. immitis* antigen.

- Occasionally, microfilariae will be present before detectable levels of antigen have accumulated.<sup>2</sup>
  - Beware of contaminated lysing solution. Cases of false positive tests for microfilariae have been reported due to the presence of microfilariae in the lysing solution rather than the patient's blood sample.
4. When sending a sample to a reference lab for confirmation, note that most reference labs use commercially available microwell antigen test kits.

#### Reference

- <sup>1</sup> Rawlings CA, Calvert CA: Heartworm disease. In Ettinger SJ (ed): Textbook of Veterinary Internal Medicine. 4th ed. Philadelphia, WB Saunders, 1995, pp 1046–1068.
- <sup>2</sup> Knight DH, Doiron DW, Longhofer SL, *et al.*: American Heartworm Society 2002 Guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in dogs. American Heartworm Society, Batavia, IL, 2002.
- <sup>3</sup> Data on file, Heska Corporation.



For further assistance, please call Heska's Technical Support Services at 800.464.3752, option 3.