



PRE-TREATMENT OF SAMPLES TO IMPROVE DETECTION OF HEARTWORM ANTIGEN

WHY?

- Some dogs and cats with circulating antigen of *Dirofilaria immitis* test false-negative on heartworm antigen tests. Pre-treatment of samples prior to running the test [allows detection of antigen](#) in many of these pets.
- Historically, pre-treatment of samples prior to heartworm testing was [routinely practiced](#).
- Recent publications document that [samples from 5-10% of dogs in animal shelters in the southern United States](#), and [more than 50% of dogs on slow kill](#), may test false negative despite the presence of antigen in the sample regardless of the testing platform used. [Blocked antigen is also common in feline samples](#).
- Results have been confirmed by necropsy identification of heartworms in both [dogs](#) and [cats](#).

WHAT CAUSES THIS?

- In some patients, antigen is present but masked, preventing detection. Historically, this block was [attributed to immune complex formation](#) and heat or chemical pre-treatment was recommended to destroy the complexes, allowing the antigen to be detected.
- Recently, heat treatment has been shown to reveal the presence of heartworm antigen in dogs and cats confirmed to be infected by other means, including necropsy, echocardiography, or detection of *D. immitis* microfilaria confirmed by morphology and PCR.

WHICH PETS SHOULD I CONSIDER TESTING BY PRE-TREATING THE SAMPLE?

- Pre-treatment heartworm testing is recommended for both canine and feline patients suspected to have heartworm despite testing negative on any standard heartworm antigen test.
- Blocked heartworm antigen should be considered in any dog or cat that presents with coughing, murmur, or radiographic evidence leading the veterinarian to suspect heartworm disease but that tests negative. Microfilaria are present in blood from many but not all of these dogs; microfilaria are rarely detected in cats.
- Samples from dogs not on preventive in an endemic area, those with an inconsistent history of preventive use, and heartworm-infected dogs that have been managed with “slow kill” should be particularly suspect.

HOW CAN I GET A TEST RUN? (submission form available at [link](#))

- Submit a sample to [OADDL](#) and request “blocked heartworm antigen” (\$30, 1.0 mL serum or plasma).
- For cats, a combination of feline heartworm antibody and blocked antigen is available (\$30 total, 1.0 mL serum).
- For dogs, a combination of blocked heartworm antigen and microfilaria testing is available (\$30 total, 2.0 mL whole blood EDTA/purple top).

WHAT ABOUT FALSE POSITIVES?

- False positive results are considered rare but can occur with any heartworm antigen test. The positive predictive value of a test improves in a population likely to harbor heartworm (e.g. dogs not on preventive in an endemic area, or dogs with clinical signs of heartworm disease.)
- Known causes of false positive heartworm antigen tests include infection with [Spirocerca lupi](#) and [Angiostrongylus vasorum](#). Other related nematodes (e.g. other *Dirofilaria* spp. of wildlife, *Dracunculus insignis*, *Onchocerca* spp.) are also thought to induce false positive antigen test results. Importantly, these false positives have been documented without any heat treatment of the sample.
- For a dog in an endemic area that has not been on heartworm prevention, the most likely explanation for a positive antigen test before or after heat treatment of a sample is heartworm infection. However, all positive antigen tests should be interpreted by the attending veterinarian considering the overall history and clinical signs and confirmed with a second, different test prior to starting any treatment.

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