

Heartworm



Adult heartworms (*Dirofilaria immitis*) in the pulmonary artery of an infected dog. Such worms cause restrictions of the blood flow, and ultimately lead to congestive heart failure.

Samples

Blood	EDTA-blood as is, purple-top tubes, or EDTA-blood preserved in sample buffer (preferred)
Notes: Send all samples at room temperature, preferably preserved in sample buffer MD Submission form .	

Interpretation of PCR Results

Positive (≥ 10 copies/ml blood)	Infected with multiple adult worms
Negative	<i>Dirofilaria immitis</i> / <i>repens</i> not detectable or in the initial infectious stage; a single adult worm does not release sufficient circulating DNA for PCR detection in 100 μ l blood

Dirofilaria immitis/ *repens*

The nematode parasite *Dirofilaria* (*D.*) *immitis* occurs in many animals including dogs, cats, foxes, wolves, coyotes, ferrets, and occurs rarely in humans ([Theis, 2005](#)). The adult *D. immitis* worms are quite large, measuring up to 10 inches in length, and they typically live in the dog's pulmonary artery and "right" heart. The female worms produce microfilariae that are found in the dog's blood. Demonstration of microfilariae in blood is the primary method of diagnosis. The microfilariae are ingested by a mosquito when it feeds, mature into infective juvenile worms in the vector, and the infection is transmitted to a new host when the mosquito feeds. Many species of mosquitoes serve as vectors of this species ([Nelson et al., 2005](#)). *Dirofilaria repens* may infect dogs causing little disease, but detection is important because of the anthroponozoonotic potential of this parasite that can cause serious disease in humans.

Clinical Signs

Dirofilaria immitis may cause significant pathology in an infected host. Inflammation and thickening of the heart caused by the resident worms result in symptoms such as respiratory insufficiency, chronic cough, and vomiting, and the disease can be fatal. Symptoms in dogs appear when more than 25 worms are present. Worm loads of up to 60 adult worms cause circulatory problems. With 100 or more worms, there is blockage of the pulmonary artery and right side of the heart, accompanied by interference with the action of the heart valves. The right side of the heart becomes dilated and enlarged. Blood backs up in the liver and other parts of the body, causing general congestion and degeneration ([Nelson et al., 2005](#)). Dogs fatigue easily, cough, and appear unthrifty.

Standard Diagnostic Methods

The diagnosis of canine heartworm disease depends upon the accurate patient history, the recognition of varied clinical signs, and other diagnostic procedures including X-ray, angiography and ultrasound, ELISA testing for antigens secreted by female worms, microfilarial detection and differentiation in blood smears, in the worst case scenario, necropsy examination after death ([Nelson et al., 2005](#)). All tests are reliable at high worm loads, but subtle heartworm disease caused by low numbers of adult worms may be difficult to diagnose.

Our Method

The Molecular Diagnostics Laboratory at Auburn University has developed a quantitative PCR approach targeting with highest specificity the 5.8S-ITS2-28S rRNA gene of *D. immitis* and *D. repens*, the two species that are relevant for animals. This PCR aims to detect worm DNA of cells shed by the parasites into the blood stream. The PCR is capable of detecting single worm genome copies in a blood aliquot of 100 μ l used as sample input. In our experience, this sensitivity is sufficient to detect infections by more than one adult worm. For detection of single worms multiple 100 μ l aliquots of a blood sample may need to be tested because of the low numbers of worm cells circulating in blood. An advantage is that DNA may stem from female as well as male worms, while the *Dirofilaria* antigen ELISA test can detect only female worms, since only females produce the antigen. This quick and sensitive diagnostic method can help veterinarians treat dogs and cats timely and efficiently, and to monitor the treatment effectiveness. Most recently, this assay was used to determine the prevalence of heartworm infection, among other blood-borne parasites, in dogs in Nicaragua ([Wei et al. Parasites & Vectors 7, 126, 2014](#)).