Using heat treatment for immune-complex dissociation in challenging heartworm cases

Dirofilaria immitis infections are usually diagnosed based on history, physical examination, and laboratory findings. One of the key components in the diagnosis is the presence of heartworm antigen circulating in the blood of the infected patient. Sometimes, dogs and cats will make a strong immune response that results in antibodies that bind to the heartworm antigen. When these immune complexes form, they can interfere with the detection of the antigen in heartworm tests. Conditions that may favor immune complex formation in dogs include chronic inflammation or the use of "slow kill" to treat a heartworm infection.

Immune-complex dissociation (ICD)

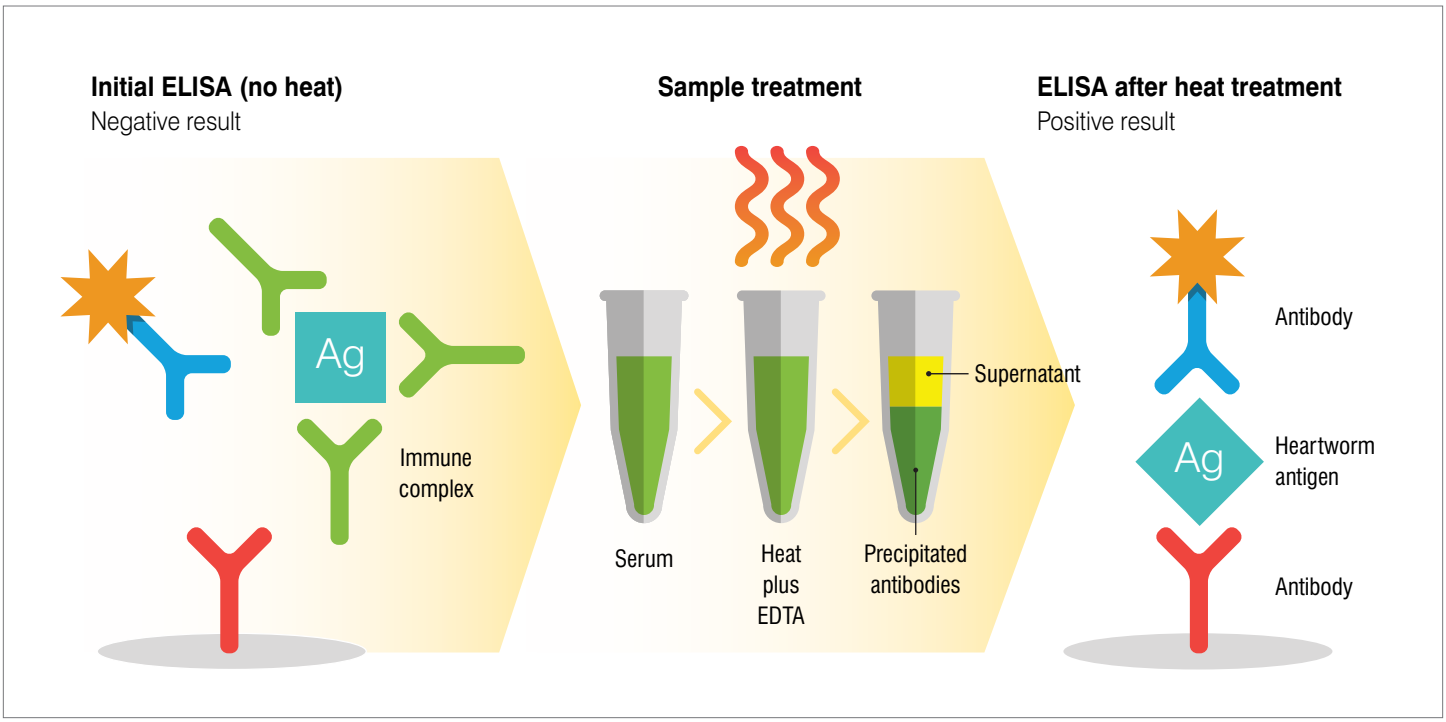
Fortunately, interference from immune complexes does not occur very often in dogs receiving regular veterinary care.[1] However, immune complexing in cats with heartworm infections may occur more frequently than previously recognized.[2] Only in certain circumstances is it necessary to perform a special sample pretreatment step to disrupt these immune complexes and free the heartworm antigen so that it can be detected. This method is referred to as immune-complex dissociation (ICD) and can be performed using several different techniques.

The first reported method for disrupting immune complexes was performed using heat and EDTA.[3] This method has proven preferable to other ICD methods for both accuracy and predictability.[4] In this process, heat denatures and precipitates the soluble proteins, including the antibodies. In the presence of EDTA, the supernatant for testing can be cleanly separated from this precipitate following centrifugation.

When to consider ICD

Veterinarians should consider using heat treatment for ICD when the patient's clinical signs, history, physical examination, and/or laboratory findings are inconsistent with the negative results of a heartworm antigen test. Consider using ICD when:

- There are conflicting screening test results, for instance, an antigen-negative and microfilaria-positive result in a dog or an antigen-negative and antibody-positive result in a cat.
- There are chronic inflammatory conditions that stimulate the immune system (e.g., chronic superficial pyoderma, chronic otitis externa, chronic intestinal parasite infections).
- Dogs test negative for antigen but originate from a known heartworm-endemic region and have a history of inconsistent or no heartworm preventive use.
- Cats test negative for antigen but have clinical signs compatible with heartworm disease.
- Dogs are receiving alternative treatment protocol, such as the administration of a macrocyclic lactone and/or doxycycline. The slow death of the parasites may contribute to chronic inflammation and increased antibody production. This in conjunction with a reduction in antigen production could favor the presence of immune complexes.[5]



Benefits of ICD performed at IDEXX Reference Laboratories

While veterinarians may adopt methods that allow them to heat specimens in their practice prior to heartworm testing, there are several advantages to having ICD performed at IDEXX Reference Laboratories:

- The IDEXX microtiter plate ELISA for heartworm antigen has been optimized for use with a spectrophotometer (plate reader) to produce numerical results and a standardized cut-off value.
- Specimen pretreatment conditions have been optimized for time, temperature, and specimen volume. A standardized procedure optimized for the IDEXX microtiter plate ELISA is used to deliver consistent results.
- A positive control is included in each assay to confirm that the specimen pretreatment process has been performed correctly and produces the desired results.
- Both the untreated and the heat/EDTA-treated specimens are tested on the same microtiter plate at the same time, allowing for direct comparison of the test results.
- Results are reported for both the untreated and the heat/EDTA-treated specimens. The IDEXX medical speciality consulting service and diagnostic support veterinarians are available to provide consultation on the results and clinical case.

Ordering information

Test code	Test name and contents
7232	**Heartworm Antigen by ELISA with Heat Treatment—Canine/Feline** Heartworm antigen by ELISA pre- and post-heat treatment **Specimen requirements:** 1 mL serum or EDTA plasma **Turnaround time:** 1–3 working days
72321	**Heartworm Antigen by ELISA with Heat Treatment Add-on—Canine/Feline** Note: May be added on to a negative heartworm test performed at the reference laboratory within 6 days of initial test. **Specimen requirements:** 1 mL serum or EDTA plasma, prior accession number **Turnaround time:** 1–3 working days

Contacting IDEXX

1-888-433-9987

For questions regarding specimen submission or test results, please contact our Laboratory Customer Support Team. For questions regarding individual patient management, please contact our Medical Consulting Services Team.

References

1. Nafe L, Little SE, DeMars P, Baumwart R, Yamanda N, Johnson E. Prevalence of *Dirofilaria immitis* antigen in client-owned pet dogs before and after serum heat [ACVIM Abstract C18]. *J Vet Intern Med*. 2016;30(4):1428.
2. Gruntmeir JM, Adolph CB, Thomas JE, Reichard MV, Blagburn BL, Little SE. Increased detection of *Dirofilaria immitis* antigen in cats after heat pretreatment of samples. *J Feline Med Surg.* 2017;19(10):1013–1016.
3. Weil GJ, Malane MS, Powers KG, Blair LS. Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis*-infected dogs. *J Immunol*. 1985;134(2):1185–1191.
4. Beall MJ, Arguello-Marin A, Drexel J, Liu J, Chandrashekar R, Alleman R. Validation of immune complex dissociation methods for use with heartworm antigen tests. *Parasit Vectors*. In press.
5. Drake J, Gruntmeir J, Merritt H, Allen L, Little SE. False negative antigen tests in dogs infected with heartworm and placed on macrocyclic lactone preventives. *Parasit Vectors*. 2015;8:68.

The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions.

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