Dirofilaria immitis and D. repens in dog and cat and human infections

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Guideline for the laboratory diagnosis of canine and feline *Dirofilaria* infections

Claudio Genchi, Luigi Venco, Marco Genchi
Microfilariae

*Fresh blood smear (not advised)*

A drop of fresh venous blood is placed on a clean microscopic slide and covered with a coverslip and examined under low microscopic power.

Microfilariae are seen throughout the movement they cause to the blood red cell layer.

*To note that the intensity of microfilaremia is not correlated to the adult worm burden: in general, high microfilaremic dogs harbour few worms.*

When dogs are monthly treated with preventive drugs during heartworm transmission season, re-testing each year before starting again the preventive treatment is advisable.

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**Concentration methods**

Several methods can be used to concentrate circulating microfilariae from the blood. These methods are sensitive and make possible to differentiate microfilariae throughout morphological criteria (see Table 1 and Fig. 1).

Microfilariae can be concentrated by the modified Knott test or by a filter test.

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*Modified Knott test*

One ml of venous blood is mixed with approximately 10 ml of 2% buffered formalin and the mixture is centrifuged for 3-5 minutes at 1500 rpm.

The supernatant is decanted from the centrifuge tube and the sediment is mixed with equal parts of a 1:1000 methylene blue stain. The stained sediment is placed on a slide, covered with a coverslip and examined under a microscope.

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**Advantage**

Rapid and inexpensive

**Disadvantages**

Very low sensitivity, frequent false negative, no species diagnosis (it is not possible to differentiate microfilariae)

Not useful in cats

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*Filter test*

One ml of venous blood with anticoagulant of either EDTA or heparin is added to approximately 10 ml of lysate solution. The mixture is injected through a filter (Millipore) chamber. The filter is removed from the chamber, placed on a glass slide, stained, and examined under a microscope.

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Laboratory diagnosis: Guideline

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Advantage
Rapid and sensitive in dogs
No need for a centrifuge apparatus

Disadvantages
Expensive (tests are sold as kit, Difil Test Evsco); the lysate solution shrinks the microfilariae and new measurement standards are required to differentiate species
Low sensitivity in cats

Histochemical stain

One ml of venous blood collected in EDTA is injected into 10 ml of deionized water and centrifuged at 1500 rpm for 15 minutes. The supernatant is discarded and the sediment placed on a slide and air-dried. The smear is then fixed with absolute acetone, air dried, and covered with acid phosphatase substrate. The substrate needs to be either made fresh, as described by Chalifoux and Hunt (1971), or frozen at -80°C in aliquot portions. After 2 hours at room temperature, the slide is air dried and covered with a coverslip.

- **D. immitis** microfilariae show 2 acid phosphatase activity spots localized around the anal and the excretory pores, respectively.
- **D. repens** microfilariae show only 1 acid phosphatase activity spot localized around the anal pore.
- **Acanthocheilonema spp.** microfilariae show acid phosphatase activity throughout the body.

Peribáñez et al. (2001) have described an alternative method using a commercial kit with similar results.

Advantage
Very specific

Disadvantages
Costly, time consuming, need for a skilled laboratory technician

Table 1. Morphological features of microfilariae\(^1\) from filarial worms of dogs and cats.

<table>
<thead>
<tr>
<th>Species</th>
<th>Length µm</th>
<th>Width µm</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dirofilaria immitis</strong></td>
<td>290-330</td>
<td>5-7</td>
<td>No sheath, cephalic end pointed, tail straight with the end pointed</td>
</tr>
<tr>
<td><strong>Dirofilaria repens</strong></td>
<td>300-360</td>
<td>6-8</td>
<td>No sheath, cephalic end obtuse, tail sharp and filiform often ending as an umbrella handing</td>
</tr>
<tr>
<td><strong>Acanthocheilonema reconditum</strong></td>
<td>260-283</td>
<td>4</td>
<td>No sheath, cephalic end obtuse with a prominent cephalic hook, tail button hooked and curved</td>
</tr>
<tr>
<td><strong>Acanthocheilonema dracunculoides(^2)</strong></td>
<td>190-247</td>
<td>4-6.5</td>
<td>Sheath, cephalic end obtuse, caudal end sharp and extended</td>
</tr>
<tr>
<td><strong>Cercopithifilaria grassii</strong></td>
<td>567</td>
<td>12-25</td>
<td>Sheath, caudal end slightly curved</td>
</tr>
</tbody>
</table>

\(^1\) microfilariae measure by Knott test
\(^2\) microfilariae from the uterus
Laboratory diagnosis: Guideline

ELISA and immunochromatographic tests for adult female heartworm circulating antigens

Several ELISA and immunochromatographic kits are commercially available to detect the presence of adult female circulating antigens in serum, plasma and whole blood of dogs and cats. Most are very specific, quite sensitive, rapid and easy to be performed. Most are in-clinic test kits for single diagnosis but ELISA plates for multitest are also available. Manufacturers claim for a positive result when 1 adult female worm is infecting the animal, though many factors can affect the sensitivity of the tests such as age of worms, number of female worms versus number of males and the dog size, and reliable and reproducible results can be obtained from 2-3 or more adult female worms. Male worms are not detectable by antigen tests. In dogs, detectable antigenemia develops about 5 to 6.5 months post infection.

Because the clearance of antigens is quite rapid after the death of worms, these techniques can be used to assess the efficacy of an adulticide therapy. However, the newest tests are quite sensitive and for definitive diagnosis to confirm the success of the adulticide therapy dogs have to be retested 5 and 9 months later. If the test at 5 months is negative, testing at 9 months can be avoided.

Because unisex infections consisting of only male worms or symptomatic immature infections are not infrequent in cats, none of the presently available antigen tests can be relied upon to rule out heartworm disease in cats. In cats with heavy infections, detectable antigenemia develops at about 5.5 to 8 months post infection.

To note that semi-quantitative and laboratory ELISA tests (not immunochromatographic tests) have a direct, but imprecise, relationship to the adult female worm burden. The utility of the ELISAs for assessing the degree of parasitism can be limited by the transient increase in antigenemia as a consequence of recent worm death.

When animals are monthly treated with preventive drugs during heartworm transmission season, re-testing each year
before starting the preventive treatment in the following season is advisable.

If the chemoprophylactic treatment is performed with a sustained release drug injection (only for dogs), periodic testing (each 2 or 3 years) will ensure there have been no efficacy breaks (Nelson et al., 2005b).

### Antibody tests

Several antibody test kits are available for the diagnosis of feline heartworm disease. These tests cannot be used in dogs.

Antibody tests have the advantage to being able to detect the exposure of cats to the infection of both male and female adult worms and larvae, and the immune response in detectable as early as 2 months post infection. However, their interpretation is complicate because positive results can be found both in aborted infections (developing larvae are destroyed by the host immune response and will not be able to develop to adult worms) and in patent infections. Furthermore, no proved data is available on whether the antibody level will decrease over the expected two-to-three year lifespan of an adult worm (Nelson et al., 2005a).

### Advantage
- Very specific and sensitive for heartworm diagnosis [gold standard: when positive, the test is the definitive prove of heartworm infection in dogs and cats]

### Disadvantages
- Costly, not available for other filarial infections

### PCR

PCR (polymerase chain reaction) is a sensitive and accurate tool to discriminate microfilariae from the different filarial worms able to infect dogs and cats. Its use is advisable in case of morphological abnormalities of microfilariae, not infrequent in dogs treated incorrectly with preventive drugs or when multiple infections with more than one species of filarial worm makes difficult to differentiate microfilariae (Favia et al., 1996; Mar et al., 2002; Casiraghi et al., 2006; Rishniw et al., 2006).

### Advantage
- Very sensitive
- Able to detect the cat exposure to heartworm infection
- Suitable to asses the infection risk in cats and for epidemiological survey

### Disadvantages
- Costly
- Not fully specific
- Difficult to be interpreted
Guideline for the diagnosis of filarial infections in dogs.

<table>
<thead>
<tr>
<th>Mf Knott</th>
<th>Ag test</th>
<th>Interpretation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Definitive diagnosis of HW</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>ThR can help to manage the disease</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Normal ThR patterns</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Mf Knott</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Low/very low risk of thromboembolic complications</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Histochemical stain or PCR can be used to differentiate Mf</td>
</tr>
</tbody>
</table>

Guidelines for the diagnosis of filarial infections in cats.

<table>
<thead>
<tr>
<th>Mf Knott</th>
<th>Ag test</th>
<th>Ab test</th>
<th>Interpretation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Definitive diagnosis of HW</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Definitive diagnosis of HW</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Filarial infection due to other species than D. immitis</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Definitive diagnosis of HW</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Low adult female worm burden</td>
</tr>
</tbody>
</table>

Mf: microfilariae  HW: heartworm  TR: thoracic radiography  ECHO: echocardiography
References


