Detection of antibodies against Leishmania infantum, L. chagasi and L. donovani
High specificity due to the use of a recombinant antigen
Efficient automation solutions available

Antigen
Recombinant antigen of the Leishmania donovani complex

Calibration
Semiquantitative:
Calculation of a ratio from the extinction of the sample and the extinction of the calibrator

Result interpretation
EUROIMMUN recommends interpreting results as follows:
Ratio ≥ 0.8:  negative
Ratio ≥0.8 to < 1.1:  borderline
Ratio ≥1.1:  positive

Sample dilution
Canine serum or plasma, 1:101 in sample buffer

Reagents
Ready for use, with the exception of the wash buffer (10 x), colour-coded solutions

Test procedure
30 min (37 °C) / 30 min (37 °C) / 15 min (room temperature), fully automatable

Measurement
450 nm, reference wavelength between 620 nm and 650 nm

Test kit format
96 break-off wells; kit includes all necessary reagents

Order no.
EI 2232-9601 GC

Leishmaniasis is a zoonotic infection that is caused by protozoa of the Leishmania genus. These monacellular parasites are transmitted to humans or animals via the bite of female sandflies of the genera Phlebotomus (Africa, Asia, Europe) or Lutzomyia (Central and South America).

Up to now, canine leishmaniasis has been considered as an imported disease, brought from travelling abroad. However, the vector is further spreading to central and northern Europe, benefiting from the increasing climate change. Canine leishmaniasis is endemic in Mediterranean countries, where it is assumed that 50% to 80% of dogs are infected with Leishmania. Leishmaniasis in humans causes around 40,000 deaths worldwide, with one to two million new infections every year. Dogs are the main reservoir. Due to the zoonotic potential, infected dogs are a major problem in veterinary and human medicine. However, Leishmania infection is not synonymous with canine leishmaniasis. Less than 10% of infected dogs show clinical symptoms. Certain dog breeds, such as Boxer, Cocker Spaniel, Rottweiler and German Shepherd, and the age of the dog are associated with the manifestation of leishmaniasis.

Leishmaniasis infection is characterised by long incubation periods (months to years). The various zymodemes of the individual Leishmania species can cause different clinical manifestations. In canine leishmaniasis it is often impossible to discriminate between the visceral and cutaneous form because visceral leishmaniasis is also often accompanied by skin changes. Symptoms include fever, weight loss, anorexia, various skin changes (e.g. alopecia, dermatitis, hyperkeratoses, paw pad fissures), eye problems (e.g. uveitis, keratoconjunctivitis, loss of eyesight) and e.g. glomerulonephritis, hepato- and splenomegaly, diseases of the musculoskeletal system (e.g. due to polyarthritis) and haemogram changes (e.g. hyperglobulinaemia, hypoalbuminaemia, proteinuria). Clinical symptoms of canine leishmaniasis can improve or even subside with chemotherapy. However, relapses are possible since the treatment does not allow complete elimination of the parasite. A vaccine for dogs is available. Vaccination is highly recommended before travel to endemic areas.
Direct detection of Leishmania is possible, for instance, via cytological smears from lymph node aspirate, conjunctiva smears or histopathological tissue samples. For a quick laboratory diagnosis of canine leishmaniasis, serological antibody detection is the method of choice. The antigen originates from a protein that is conserved in the Leishmania donovani complex (L. chagasi, L. infantum and L. donovani). Thus, the Anti-Leishmania ELISA Dog (IgG) is suited for the detection of leishmaniasis in both Europe and America.

Reproducibility

The reproducibility was investigated by determining the intra- and inter-assay coefficients of variation using three sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on four determinations performed in six different test runs.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Mean value (ratio)</th>
<th>CV (%)</th>
<th>Mean value (ratio)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1</td>
<td>2.8</td>
<td>1.2</td>
<td>5.4</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>2.4</td>
<td>1.7</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>4.9</td>
<td>3.7</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Cross reactivity

A cohort of 11 sera from dogs that were negative for Leishmania in the IIFT and positive for either Hepatozoon canis (6 sera) or Babesia canis (5 sera) were investigated using the Anti-Leishmania ELISA Dog (IgG). A negative result was obtained for all 11 samples. There were no cross reactions with samples positive for Hepatozoon canis or Babesia canis. Antibodies against Trypanosoma cruzi, Ehrlichia canis, Toxoplasma gondii and Neospora caninum may also show cross reactivity in serological tests for canine leishmaniasis. This applies particularly to areas that are endemic for these pathogens. In order to minimise cross reactivity, a recombinant antigen is used in this ELISA.

Sensitivity and specificity

The sensitivity and specificity of the ELISA were determined by investigating a total of 1301 canine sera with the EUROIMMUN Anti-Leishmania ELISA Dog (IgG) and by comparing them to the results yielded with a commercial immunofluorescence test or to the expected value, which was given as negative for samples from laboratory dogs and normal dogs from Sweden. The sensitivity amounted to 97 %, with a specificity of 99 %. Borderline results were not included in the calculation.

<table>
<thead>
<tr>
<th>Precharacterisation</th>
<th>n = 1301</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>EUROIMMUN Anti-Leishmania ELISA Dog (IgG)</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Literature

5. Mencke N. Future challenges for parasitology: vector control and ‘One health’ in Europe: the veterinary medicinal view on CVBDs such as tick borreliosis, rickettsiosis and canine leishmaniosis. Vet Parasitol (2013) 256-271