Anti-Leishmania ELISA Dog (IgG)

- **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**

Technical data

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 (10x), 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**	

Clinical significance

Leishmaniasis is a zoonotic infection that is caused by protozoa of the Leishmania genus. These monocellular 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.

Leishmania 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.

Diagnostic application

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.

	Intra-assay variation, n = 20		Inter-assay variation, n = 4 x 6	
Serum	Mean value (ratio)	CV (%)	Mean value (ratio)	CV (%)
1	1.1	2.8	1.2	5.4
2	1.6	2.4	1.7	3.8
3	3.7	4.9	3.7	6.5

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 test were determined by investigating a total of 116 canine sera using the EUROIMMUN Anti-Leishmania ELISA Dog (IgG) and comparing the results with a commercial immunofluorescence test or with the expected value, which was given as negative for samples from laboratory and normal dogs from Sweden. The results yielded a sensitivity of 97 % and a specificity of 98 %. Borderline results were not included into the evaluation.

n = 116		Precharacterisation		
		positive	borderline	negative
EUROIMMUN Anti-Leishmania ELISA Dog (IgG)	positive	65	0	1
	borderline	0	1	0
	negative	2	3	44

Literature

1. Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, Alvar J and Boelaert M. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol (2007) 873-882
2. ESCCAP. Möglichkeiten und Grenzen der Leishmaniose-Impfung. www.esccap.de (2013)
3. Greene CE. Infectious Diseases of the Dog and Cat. Elsevier Health Sciences, (2013)
4. Maia C and Cardoso L. Spread of Leishmania infantum in Europe with dog travelling. Vet Parasitol (2015) 2-11
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
6. Miksits K and Hahn H. Parasiten. In: Miksits K and Hahn H Basiswissen Medizinische Mikrobiologie und Infektiologie. Springer-Verlag, 3. Auflage (2004) 249-251
7. Naucke T. Leishmaniose - Einzug in Deutschland. Tierärztliche Umschau (2007) 495-500
8. Noli C and Saridomichelakis MN. An update on the diagnosis and treatment of canine leishmaniosis caused by Leishmania infantum (syn. L. chagasi). Vet J (2014) 425-435
9. Robert-Koch-Institut. Aktuelle Daten und Informationen zu Infektionskrankheiten und Public Health. Epidemiologisches Bulletin (2013) 445
10. Shaw SE, Langton DA and Hillman TJ. Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector? Vet Parasitol (2009) 281-285
11. Solano-Gallego L, Miro G, Koutinas A, Cardoso L, Pennisi MG, Ferrer L, Bourdeau P, Oliva G, Baneth G and The LeishVet G. LeishVet guidelines for the practical management of canine leishmaniosis. Parasit Vectors (2011) 86
12. Sykes JE. Canine and Feline Infectious Diseases. Elsevier Health Sciences, (2013)