



Anti-*Anaplasma phagocytophilum* ELISA Dog (IgG)



- Highly specific screening test for the detection of canine anti-*Anaplasma* antibodies
- Based on recombinant antigen
- Fully automatable



Technical data

Antigen	Recombinantly produced and purified <i>Anaplasma phagocytophilum</i> antigen
Calibration	Semiquantitative evaluation using ratio values: Extinction value of the sample over the extinction value 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 220m-9601 GC



Clinical significance

Anaplasmosis is a disease which is transmitted to animals and humans by ticks of the *Ixodes* genus. It is caused by *Anaplasma (A.) phagocytophilum* (formerly: *Ehrlichia phagocytophila*). *A. phagocytophilum* is distributed worldwide, its prevalence depends on the occurrence of the transmitting vectors. The seroprevalence varies greatly between regions and is given as 0 to 56%. There are different names for the clinical image of an infection with *A. phagocytophilum* in dogs: granulocytic ehrlichiosis (obsolete), canine granulocytic anaplasmosis, and, simply, and most frequently used: anaplasmosis.

A. phagocytophilum is a Gram-negative, obligate intra-cellular bacterium which attacks mostly neutrophilic granulocytes, but also, in rare cases, eosinophilic granulocytes. The clinical symptoms of canine anaplasmosis are reduced general condition with fever, weight loss, vomiting, dyspnoea, spleno- and hepatomegaly, lymphadenopathy, oedema of the limbs, leukopenia, anaemia, haemorrhage, polyarthritis, but also symptoms of the CNS as a result of inflammatory processes and bleeding in the meninges. Some dogs are able to eliminate the pathogen, in others the infection is subclinical or chronic. In chronic cases, changing lameness occurs due to polyarthritis and swelling of the joints. Borreliosis should be excluded by differential diagnosis. There are no vaccines available.

Antibodies against *A. phagocytophilum* occur in the serum of specifically infected animals after 7 to 14 days. Different techniques, such as ELISA or indirect immunofluorescence (IIF), are used for the serological detection of antibodies. It should be noted that many dogs that show specific antibodies against *A. phagocytophilum* are not clinically conspicuous. For diagnosis, it is hence necessary to investigate two consecutive blood samples. A twofold titer increase or a seroconversion are diagnostically relevant. If the first blood sample tests negative, a second sample should be examined after two weeks in cases of suspected anaplasmosis since dogs do not produce antibodies in the early phase of infection.



Application

The direct detection of *A. phagocytophilum* by staining or culture is possible, but not conducted routinely since these methods are too complicated for screening diagnostics. PCR is commonly used. The sensitivity of the direct detection depends on the phase of infection as there are "silent phases" when the anaplasmas are virtually not detectable in the blood. It is only in reproductive phases (fever attacks) that a reliable direct detection is possible. It is compulsory to use whole blood for PCR. Therefore, serological detection of antibodies is the method of choice when it comes to laboratory diagnosis of canine granulocytic anaplasmosis. Owing to the use of a specific recombinant antigen, the Anti-Anaplasma phagocytophilum ELISA Dog (IgG) has a high specificity and very high sensitivity.

Principle of the test

The ELISA test kit provides a semiquantitative in vitro assay for canine antibodies of the IgG class against *Anaplasma phagocytophilum* antigens in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with recombinantly produced and purified *Anaplasma phagocytophilum* antigen. In the first reaction step, diluted samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-dog IgG (enzyme conjugate) catalysing a colour reaction.

Reproducibility

The reproducibility was investigated by determining the intra- and inter-assay coefficients of variation (CV) using three sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Serum	Intra-assay variation, n=20		Inter-assay variation, n=4x6	
	Mean value (Ratio)	CV (%)	Mean value (Ratio)	CV (%)
1	1.1	2.2	1.2	5.0
2	1.9	3.5	2.1	4.6
3	3.3	4.9	3.6	5.8

Sensitivity and specificity

59 randomly selected dog sera were investigated using the Anti-Anaplasma phagocytophilum ELISA Dog (IgG) and a commercial immunofluorescence test (IIFT). The test results were compared and showed a sensitivity of 97% and a specificity of 96% (borderline sera were not included in the calculation).

n = 59		Precharacterisation (IIFT)		
		positive	borderline	negative
EUROIMMUN Anti-Anaplasma ELISA Dog (IgG)	positive	33	0	1
	borderline	2	0	1
	negative	1	0	21

Literature

1. Carrade DD, et al. **Canine granulocytic anaplasmosis: a review.** J Vet Intern Med 23(6):1129-1141 (2009).
2. Center for Food Security and Public Health. **Ehrlichiosis and anaplasmosis: Zoonotic species.** College of Veterinary Medicine, Iowa State University (2013).
3. Kohn B, et al. **Infections with Anaplasma phagocytophilum in dogs in Germany.** Res Vet Sci 91(1):71-76 (2011).
4. Tsachev I. **Canine granulocytic anaplasmosis.** Trakia Journal of Sciences 7(1):68-72 (2009).