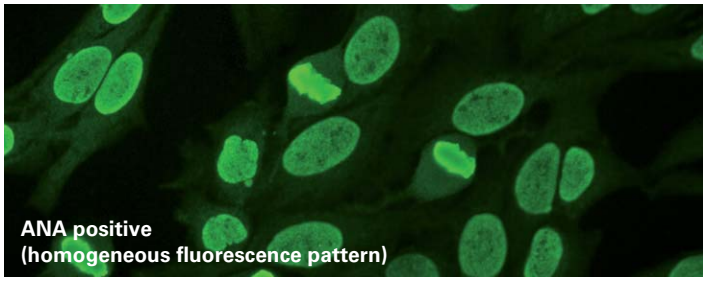
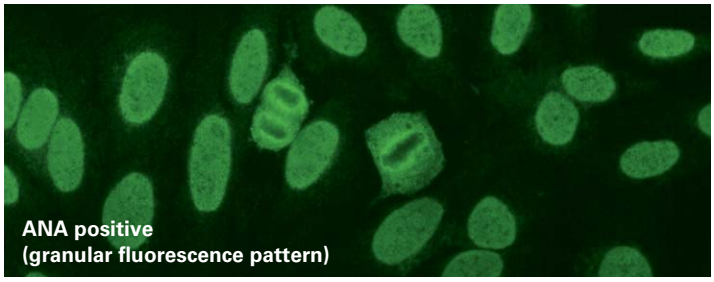
HEp-2 IIFT Dog (IgG)


ANA positive
(homogeneous fluorescence pattern)


ANA positive
(granular fluorescence pattern)

- **First fully validated assay for the detection of anti-nuclear antibodies (ANA) in dogs**
- **Incubation automatable**

Technical data

Antigen substrate	HEp-2 cells
Sample dilution	Canine serum or plasma
	Qualitative evaluation: 1:100
	Semiquantitative evaluation: 1:10 / 100 / 1000 etc.
Reagents	Ready for use, with the exception of the PBS-Tween buffer (for dilutions and washing steps)
Test procedure	30 min (sample) / 30 min (conjugate), room temperature
Microscopy	Objective 40 x
	Light source: EUROIMMUN LED or mercury vapour lamp, 100 W
	Excitation filter: 488 nm, colour separator: 510 nm, blocking filter: 520 nm
Stability	All kit components are stable for at least 18 months from the date of manufacture
Test kit format	10 slides, each containing 5 or 10 test fields, kit includes all necessary reagents
Order no.	**FA 1520-1005 C**
	FA 1520-1010 C

Clinical significance

In human medicine, the detection of autoantibodies against cell nuclei (anti-nuclear antibody, ANA) is an important diagnostic indicator in many autoimmune diseases. Antibodies against nuclear antigens are directed against various cell nuclear components. These encompass nucleic acids, cell nuclear proteins and ribonucleoproteins.

Systemic lupus erythematosus (SLE) and lupus-associated diseases are also found in dogs. Clinical symptoms of SLE in dogs include non-erosive polyarthritis, skin lesions, fever of unknown origin, glomerulonephritis, haemolytic anaemia, thrombocytopenia, polymyositis, pericarditis and neurological manifestations.

Anti-nuclear antibodies occur in almost all dogs with SLE (97 - 100 %), often with high titers. Healthy animals and dogs with infections (e.g. Bartonella vinsonii, Ehrlichia canis or Leishmania infantum) or other diseases can exhibit mainly low titers of anti-nuclear antibodies. In mild courses of discoid or cutaneous lupus erythematosus (skin symptoms without systemic manifestation) the detection of anti-nuclear antibodies is negative in most cases.

In dogs (such as in humans) positive ANA results and/or reversible SLE symptoms may occur after treatment with some drugs such as anticonvulsants (e.g. phenytoin), antiarrhythmics (e.g. procainamide), antihypertensives (e.g. hydralazine), the antimycotic agent griseofulvin and some antibiotics (e.g. tetracyclines). In contrast to humans, dogs with SLE produce primarily antibodies against histones and/or ribonucleoproteins, whereas antibodies against dsDNA and nucleosomes are less frequent.

Some dog breeds seem to be predisposed to develop SLE and lupus-associated diseases. In several studies, the German shepherd was the most frequently affected breed (32 % - 47.6 % of cases). In this breed, only the spotted ANA pattern has been found using HEp-2 cells. Breeds that are more often affected than others also include the Nova Scotia Duck Tolling Retriever, which often presents with immune-mediated rheumatoid disease (IMRD) and steroid responsive meningitis arteritis (SRMA).

Application

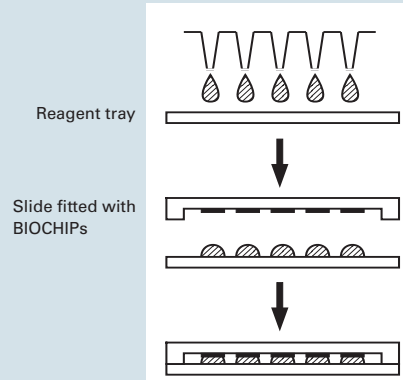
Indirect immunofluorescence based on HEp-2 cells is currently the method of choice for the detection of anti-nuclear antibodies in dogs. HEp-2 cells are in this respect superior to canine cell lines or organ sections. The most frequent pattern observed for ANA in mitotic cells is a granular fluorescence pattern showing a negative chromosomal region (75%) and is seen most often in dogs with diseases of the musculoskeletal system, lethargy and/or fever. The homogeneous pattern with a positive staining of the chromosomal region is less frequent (25%). It is characteristic of animals with systemic manifestations and symptoms such as anaemia, diseases of the musculoskeletal system, fever, skin lesions and polyuria. In both cases, the interphase nuclei can also show a granular or speckled staining. Occasionally other fluorescence patterns such as stained nucleoli, nuclear membrane or spindle apparatus also occur. However, there is no clinical association in dogs known to date.

Test principle and procedure

This test kit is designed exclusively for the in vitro determination of canine antibodies in dog serum or plasma. The determination can be performed qualitatively or semiquantitatively. BIOCHIPs coated with HEp-2 cells are incubated with diluted samples. In the case of positive reactions, specific antibodies of class IgG will bind to the antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-dog antibodies and made visible using the fluorescence microscope.

Slides with EUROIMMUN BIOCHIPs are incubated using the TITERPLANE™ Technique, which enables multiple samples to be incubated next to each other and simultaneously under identical conditions. Incubation of the substrates with the positive and negative controls provided in each kit verifies correct performance of the test and aids evaluation.

Incubation with the TITERPLANE™ Technique



Reference range

Titer <1:100. In a control panel of healthy blood donors (n=24) the ANA prevalence was 0%. In dogs that were positive for Leishmaniasis (n=20) the value was 15%, and in dogs taken to a veterinary practice with various symptoms (n=35) it was 11.4%.

Sensitivity and specificity

Antibodies against cell nuclei were investigated using the EUROIMMUN HEp-2 IIFT Dog (IgG) in 233 sera from dogs with suspected immune-mediated joint disorders and positive predata for ANA (Dr. Helene Hamlin, SLU, Uppsala, Sweden), and in 50 sera from a control panel (healthy dogs, dogs with inflammatory diseases, dogs with suspected immune-mediated joint diseases) with negative predata for ANA. The sensitivity of the test amounted to 99.6% at a specificity of 98.0%.

Sample characterisation	n	Positive results obtained with the EUROIMMUN HEp-2 IIFT Dog (IgG)
Suspected immune-mediated joint disorder, ANA positive	233	232
Sensitivity	233	99.6%
Control panel, ANA negative	50	1
Specificity	50	98.0%

Literature

1. Bremer HD, Lattwein E, Renneker S, Lilliehöök I, Rönnelid J, Hansson-Hamlin H. Identification of specific antinuclear antibodies in dogs using a line immunoassay and enzyme-linked immunosorbent assay. Vet Immunol Immunopathol. (2015)
2. Hansson H, Trowald-Wigh G, Karlsson-Parra A. Detection of antinuclear antibodies by indirect immunofluorescence in dog sera: comparison of rat liver tissue and human epithelial-2 cells as antigenic substrate. J Vet Intern Med 10 (1996) 199-203.
3. Hansson-Hamlin H, Lillienhöök I, Trowald-Wigh G. Subgroups of canine antinuclear antibodies in relation to laboratory and clinical findings in immune-mediated disease. Vet Clin Pathol 35 (2006) 397-404.