Anti-LKM-1 ELISA (IgG)

- **Specific serological detection of anti-LKM-1 autoantibodies**
- **Quantitative test system to support the diagnosis of autoimmune hepatitis (AIH)**
- **Suited to supplement the test range for determination of AIH-associated autoantibodies**

Technical data

Antigen	Recombinantly produced cytochrome P450 IID6 (target of anti-LKM-1 antibodies)
Calibration	Quantitative, in relative units per millilitre (RU/ml)
	Calibrator 1: 200 RU/ml
	Calibrator 2: 20 RU/ml
	Calibrator 3: 2 RU/ml
	Recommended upper threshold of the normal range (cut-off value): 20 RU/ml
Sample dilution	Serum or plasma, 1 : 101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x); colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits
Test procedure	30 min / 30 min / 15 min, temperature, 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 number	**EA 1321-9601 G**

Clinical significance

The prevalence of autoimmune hepatitis (AIH) is 10 to 20 cases per 100,000 people and is characterised by a female predominance (> 75 %). The incidence in western Europe is 1.9 cases per 100,000 inhabitants per year. Untreated, AIH soon develops into liver cirrhosis, hepatocellular carcinoma can also develop. With early and consequent life-long therapy based on low-dosed immunosuppressives, 90 % of patients have a normal life expectancy. For around 10 % of patients the last therapeutic option is a liver transplant.

The detection of circulating autoantibodies is of great importance in the diagnostics. Autoantibodies against soluble liver antigen/liver-pancreas antigen (SLA/PA) provide the highest diagnostic accuracy. However, due to their low prevalence, it is often indispensable to investigate further autoantibodies in suspected AIH. These include autoantibodies against cell nuclei (ANA), granulocytes (perinuclear anti-neutrophil cytoplasmic antibodies, p-ANCA), double-stranded DNA (dsDNA), liver-kidney microsomes (LKM-1), cytosolic liver antigen type 1 (LC-1) and smooth muscle (ASMA, with the most important target antigen F-actin). Antibodies against mitochondria (AMA) are also investigated for the exclusion of primary biliary cholangitis (PBC). 10 to 20 % of patients with PBC develop secondary autoimmune hepatitis (overlap syndrome). In these cases, AIH-associated autoantibodies are also often found.

In literature, AIH is sometimes classified according to its antibody status: subtype I (ANA, ASMA), subtype II (anti-LKM-1 antibodies, anti-LC-1 antibodies) and subtype III (anti-SLA/LP antibodies). This classification is probably neither of clinical nor of therapeutic or prognostic significance. Anti-LKM-1 antibodies, with their most important target antigen cytochrome P450 IID6, are only present in about 1 % of adult AIH patients, but are found more frequently in children. Since they generally do not occur in combination with antibodies against SLA/LP, their determination helps to increase the serological detection rate in AIH diagnostics. Autoantibodies against LKM-1 can also be found in 1 bis 2 % of patients with positive hepatitis C serology, however, they usually recognise a different epitope on cytochrome P450 IID6. For differentiation from viral hepatitis, parallel determination of the other AIH-associated autoantibodies is recommended.

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Detection limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest clearly detectable antibody titer. The limit of detection of the Anti-LKM-1 ELISA (IgG) is approximately 1.4 RU/ml.

Reference range

The levels of anti-LKM-1 antibodies (IgG) were determined in a panel of 200 healthy blood donors using the EUROIMMUN ELISA. With a cut-off of 20 RU/ml, 0.5% of the blood donors were anti-LKM-1 positive.

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs were based on 20 measurements and the inter-assay CVs on 4 measurements repeated on 6 different days.

Intra-assay variation, n = 20		
Serum	Mean value (RU/ml)	CV (%)
1	55	3.0
2	96	2.7
3	158	2.3

Inter-assay variation, n = 4 x 6		
Serum	Mean value (RU/ml)	CV (%)
1	58	3.2
2	96	3.7
3	160	2.5

Specificity and sensitivity

18 sera from patients with confirmed AIH and 489 sera from a reference laboratory were analysed using the EUROIMMUN Anti-LKM-1 ELISA (IgG). The EUROIMMUN IIFT with the substrates rat liver and rat kidney was used as the reference method. At a sensitivity of 100%, the specificity was 99.4% for the ELISA compared to the IIFT. One of the sera which was positive in ELISA and negative in IIFT originated from a patient with diagnosed AIH.

Serum panel (n = 507)		EUROIMMUN IIFT (rat liver/rat kidney)	
		positive	negative
Anti-LKM-1 ELISA	positive	27	3
	negative	0	477

Literature

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