Anti-M2-3E ELISA (IgG)

- **Quantitative serological test system to support diagnosis of primary biliary cholangitis (PBC)**
- **Highest sensitivity and specificity owing to the combination of native and recombinant M2 proteins**
- **Monospecific test system with high diagnostic significance**

Technical data

Antigen	Mixture of bovine PDH and the recombinant fusion protein BPO, which encompasses the immunogenic domains of the E2 subunits of BCOADH, PDH and OGDH (expression system: *E. coli*)
Calibration	Quantitative, in relative units per milliliter (RU/ml) with a cut-off of 20 RU/ml <20 RU/ml: negative ≥20 RU/ml positive
Sample dilution	Serum or plasma, 1:101 in sample buffer
Reagents	Ready for use, with the exception of the wash buffer (10x)
Test procedure	30 min / 30 min / 15 min, room temperature, fully automatable
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Kit format	96 single break-off wells; kit includes all necessary reagents
Order no.	**EA 1622-9601 G**

Clinical significance

Primary biliary cholangitis (PBC, previously: primary biliary liver cirrhosis) is an immune-mediated chronic inflammatory cholestatic liver disease. The disease is characterised by female predominance (>90%) with most cases observed between the ages of 40 and 60. PBC incidence in different parts of the world is estimated to be 4 to 31 cases/million per year. PBC is marked by infiltration of the small intra-hepatic biliary ducts (bile canaliculi) with lymphocytes and the build-up of bile (cholestasis). The clinical picture of the disease often begins with unspecific, very varying general symptoms, such as itching (pruritus), fatigue and pain in the upper right region of the abdomen. An icterus develops after a varying period of time. The diagnosis of PBC includes liver function tests (determination of alkaline phosphatase [ALP], aspartate transaminase [AST] and alanine transaminase [ALT]), determination of serum lipids, screening for AMA and anti-nuclear antibodies (ANA) and differentiation from other chronic inflammatory liver diseases like chronic viral hepatitis, autoimmune hepatitis or primary sclerosing cholangitis.

Diagnostic application

The detection of anti-mitochondrial antibodies (AMA) is of great importance in the diagnosis of PBC. So far four of nine different AMA types (antibodies against the antigens M2, M4, M8 and M9) have been detected in the serum of PBC patients. Anti-M2 antibodies are the most sensitive and specific diagnostic marker. They are found in up to 95% of all PBC patients. High-titer anti-M2 antibody seropositivity is an important tool in the diagnosis of PBC and a very powerful predictor of a future development of PBC in patients without significant liver function disorders or symptoms suggestive of cholestatic diseases. Antibodies against M2 can also be detected in other diseases overlapping with PBC, such as autoimmune hepatitis, or mixed forms thereof and in immunopathological disorders not affecting the liver primarily, such as progressive systemic sclerosis, Sjögren's syndrome and systemic lupus erythematosus.

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Linearity

The linearity of the Anti-M2-3E ELISA (IgG) was determined by performing 4 serial dilutions of different serum samples. The Anti-M2-3E ELISA (IgG) is linear in at least the tested concentration range of 3 RU/ml to 199 RU/ml.

Reference range

Levels of anti-M2 antibodies were analysed in 400 sera from healthy blood donors of between 19 and 68 years of age (149 women, 251 men) using the EUROIMMUN Anti-M2-3E ELISA. The mean concentration of antibodies against M2 was 3.9 RU/ml (± 3.2 RU/ml standard deviation) and the values ranged from 0.2 to 15.9 RU/ml. With a cut-off of 20 RU/ml, no blood donor was anti-M2 positive.

Blood donors (n = 400)			
Percentile	95.0%	98.0%	99.0%
Cut-off (RU/ml)	10.1	12.6	13.5

Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using four samples. 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 (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	45	3.1	39	6.5
2	70	2.3	67	8.6
3	101	2.2	103	6.0
4	144	1.6	158	3.0

Sensitivity and specificity:

A total of 1395 clinically characterised samples were analysed with the EUROIMMUN Anti-M2-3E ELISA (IgG) (251 from patients with PBC, 15 from patients with PBC/AIH overlap, and 1129 from the control panel). The sensitivity of the ELISA for PBC was 93.2%, with a specificity of 97.9%. AMA are also detectable in other diseases overlapping with PBC, e.g. autoimmune hepatitis, systemic sclerosis, systemic lupus erythematosus and Sjögren's syndrome.

Panel	Anti-M2-3E ELISA (IgG)	
	n	positive (%)
PBC	251	233 (92.8%)
PBC/AIH overlap syndrome	15	15 (100.0%)
Sensitivity for PBC	266	248 (93.2%)
Autoimmune hepatitis	131	6 (4.6%)
Viral hepatitis	239	0 (0%)
Primary sclerosing cholangitis	19	0 (0%)
Systemic lupus erythematosus	100	8 (8.0%)
Sjögren's syndrome	120	8 (6.7%)
Rheumatoid arthritis	120	2 (1.7%)
Blood donors without symptoms	400	0 (0.0%)
Specificity for PBC	1129	24 (97.9%)

In a ROC analysis of the results (AUC: 0.966) from 170 patients with PBC and 989 control samples, the following values were determined:

Cut-off	Specificity	Sensitivity
13.7 RU/ml	95%	94%
19.6 RU/ml	98%	93%
26.5 RU/ml	99%	92%

Literature

1. Bowlus et al. **The diagnosis of primary biliary cirrhosis.** Autoimmun Rev. 2014;13(4-5):441-444.
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4. Huang. **Recent advances in the diagnosis and treatment of primary biliary cholangitis.** World J Hepatol. 2016;8(33):1419-1441.

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