**Indications:** Test system for the in vitro determination of antibodies against mitochondrial antigens M2 in human serum or plasma for the diagnosis of the following diseases: primary biliary liver cirrhosis (PBC), overlap syndrome with autoimmune hepatitis.

**Clinical significance:** PBC is an immune-mediated chronic inflammatory cholestatic liver disease. The disease is characterized by female predominance (>90%) with most cases observed between the ages of 40 and 60 years. PBC incidence in different parts of the world is estimated to be 4 to 31 cases/million per year.

PBC is marked by lymphocellular infiltration around the small intra-hepatic biliary ducts (bile canaliculi) and the build-up of bile (cholestasis). 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 obstructive jaundice develops after a varying period of time.

The diagnosis of PBC includes liver function tests (determination of alkaline phosphatase, aspartate transaminase and alanine transaminase), the determination of serum lipids, screening for AMA and anti-nuclear antibodies (ANA) and the differentiation from other chronic inflammatory diseases of the liver such as chronic viral hepatitis, autoimmune hepatitis or primary sclerosing cholangitis.

**Application of the Anti-M2-3E ELISA:** The detection of anti-mitochondrial antibodies (AMA) is of great importance in the diagnosis of PBC. So far four of different AMA types (antibodies against the antigens M2, M4, M8 and M9) have been detected in the serum of PBC patients.

Antibodies against M2 antigens are the most sensitive and specific diagnostic marker. They are found in up to 94% of all PBC patients. High-titre 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 and immunopathological disorders not affecting the liver primarily, such as progressive systemic sclerosis, Sjögren’s syndrome and systemic lupus erythematosus.

The molecular target antigens of autoantibodies against M2 have been identified as members of the 2-oxoacid dehydrogenase complex of enzymes within the mitochondrial respiratory chain, including the E2 subunit of the branched-chain 2-oxoacid dehydrogenase (COADH), the E2 subunit of the pyruvate dehydrogenase (PDH), the E2 subunit of the 2-oxoglutarate dehydrogenase (OGDH), E1β subunits of PDH and E3 binding protein (protein X). Among these components, E2 component of PDH is the most autoantigen of PBC since the serum samples of the majority of patients (80-90%) contain PDH-E2 specific AMA. In addition to PDH-E2, approximately 60% of PBC patients are also reactive with COADH-E2. 4-13% of sera from patients with PBC only recognize COADH-E2 but not PDH-E2. The E2 component of OGDH-E2 is recognized in 30 to 80% of sera from patients with PBC. The immunodominant epitopes of COADH-E2, PDH-E2 and OGDH-E2 are lipoyl binding domains, but antibodies against them do not cross-react. The three lipoyl binding domains of COADH-E2, PDH-E2 and OGDH-E2 were fused by EUROIMMUN using recombinant techniques to give the artificial protein BPO which contains all relevant epitopes. This designer protein (produced in E.coli) mixed with native M2 (porcine pyruvate dehydrogenase) in a monospecific test system for the determination of antibodies against M2 increases the sensitivity compared to a system based on native M2 alone.
Test characteristics

Anti-M2-3E ELISA (IgG)

Clinical sensitivity and specificity: Sera from 170 PBC patients, a control panel of 589 patients with other autoimmune diseases and 400 healthy blood donors were investigated using the EUROIMMUN Anti-M2-3E ELISA. The sensitivity of the ELISA for PBC was 92.9%, with a specificity of 97.8%.

Comparison of the ELISA with the Anti-M2 ELISA: 170 sera from PBC patients were incubated with the Anti-M2-3E ELISA and the Anti-M2 ELISA. The sensitivity of the Anti-M2 3E ELISA was 92.9% and is therefore significantly higher than the sensitivity measured for the Anti-M2 ELISA (79.4%). The specificity of the test systems determined using 989 control samples was 97.8% (Anti-M2-3E ELISA) and 99.5% (Anti-M2 ELISA).

Correlation of the ELISA and IIFT (substrate: rat kidney): The sensitivity determined with 170 sera from PBC patients amounted to 92.9% for the Anti-M2-3E ELISA and 88.8% for IIFT. The specificities calculated for both test systems based on 188 hepatitis virus sera and 49 autoimmune hepatitis sera were comparable (ELISA: 98.4%, IIFT: 97.8%).

Reference range: Levels of anti-M2 antibodies were analysed in 400 sera from healthy blood donors of between 18 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 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.

ROC analysis: In an analysis of 170 samples from PBC patients and 989 control sera the following results were achieved:

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different run tests.

Technical data:

Antigen: Mixture from bovine PDH and the recombinant fusion protein BPO produced in E. coli, which comprises the immunogenic domains of the E2 subunits of PDH, BCOADH and OGDH.

Calibration: Quantitative, in relative units per milliliter (RU/ml).

Sample dilution: Serum or plasma; 1:101 in sample buffer.

Reagents: Ready for use. Exception: wash buffer (10x). Colour-coded solutions, largely exchangeable with those of other EUROIMMUN ELISA.

Test procedure: 30 min / 30 min / 15 min. Room temperature. Fully automatatable.

Measurement: 450nm. Reference wavelength between 620nm and 650nm.

Kit format: 96 single break-off wells, incl. all necessary reagents.

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