EUROLINE AMA Profile (IgGM)

- **Detection of autoantibodies against the liver antigens AMA-M2, -M2-3E, -M4 and -M9**
- **Supports the diagnosis of primary biliary cholangitis (PBC)**
- **Increased sensitivity due to the use of the recombinant fusion protein M2-3E**

Technical data

Antigens	**AMA-M2**: native M2 antigen, main component: E2 subunit (74 kDa) of pyruvate dehydrogenase complex (PDH); **M2-3E (BPO)**: recombinant fusion protein of the immunogenic domains of the E2 subunits of the three enzyme complexes of the M2 antigen: branched-chain 2-oxoacid dehydrogenase (**B**COADH), **P**DH and 2-oxoglutarate dehydrogenase (**O**GDH); **M4**: native M4 antigen, sulfite oxidase; **M9**: native M9 antigen, glycogen phosphorylase (phosphorylase a)
Sample dilution	Serum or plasma, 1 : 101 in sample buffer
Test procedure	30 min / 30 min / 10 min (sample / conjugate / substrate incubation), room temperature, fully automatable
Test kit format	16 or 64 membrane strips; kit includes all necessary reagents
Automation	Compatible with the EUROBlotOne or EUROBlotMaster from EUROIMMUN; the evaluation is performed using the EUROLineScan software.
Order number	**DL 1620-1601-1 O (16 strips) or DL 1620-6401-1 O (64 strips)**

Clinical significance

Primary biliary cholangitis (PBC) is a chronic non-suppurative destructive cholangitis with progressive inflammatory destruction of the small bile ducts. Symptoms of the disease are jaundice, itching, fatigue and abdominal pain. With progressing PBC, severe hyperbilirubinaemia and portal hypertonia as well as ascites, oesophageal varices and encephalopathy may develop. In the final stage of PBC, which is decompensated liver cirrhosis, only liver transplantation will save the patient's life. As many as 75 % of PBC patients can thus be successfully treated; the remaining patients will experience a slowly progressing relapse. With 80 %, PBC primarily affects women between 40 and 60 years of age and has a prevalence of approx. 1.9 to 40.2 cases per 100,000 inhabitants.

Autoantibodies can be detected already several years before the onset of PBC. More than 90 % of PBC patients are positive for anti-mitochondrial antibodies (AMA), particularly for those directed against the antigens M2, M4, M8 and M9. The most important subtype in PBC diagnostics are anti-M2 antibodies. The immunogenic domains of the M2 antigen mainly comprise the E2 subunits of the branched-chain 2-oxoacid dehydrogenase (BCOADC-E2), pyruvate dehydrogenase (PDH-E2) and 2-oxoglutarate dehydrogenase (OGDC-E2). A correlation between AMA titers and the clinical course or therapy success could not be confirmed by the majority of studies.

Diagnosis of PBC can be secured if two of the following three criteria are present: (a) AMA titer higher than or equal to 1 : 40, (b) indication of non-suppurative cholangitis and destruction of the intra-hepatic bile ducts, (c) unexplained increased values of alkaline phosphatase over a period of more than six months. Moreover, PBC patients often show increased concentrations of aminotransferases and immunoglobulins, primarily IgM. In the case of negative AMA tests, additional determination of PBC-specific anti-nuclear antibodies (ANA) against the nuclear membrane proteins sp100 and PML as well as the nuclear membrane component gp210 is recommended. The occurrence of these antibodies is therefore often associated with a severe course.

In up to 84 % of cases, PBC occurs together with other autoimmune diseases, e.g. Sjögren's syndrome, collagenoses, Hashimoto's thyroiditis, glomerulonephritis, systemic sclerosis, coeliac disease and ulcerative colitis. 2 to 19 % of PBC patients show characteristics of AIH (AIH-PBC overlap syndrome). In these patients, autoantibodies against SLA/LP, double-stranded DNA and smooth muscle may also be present.

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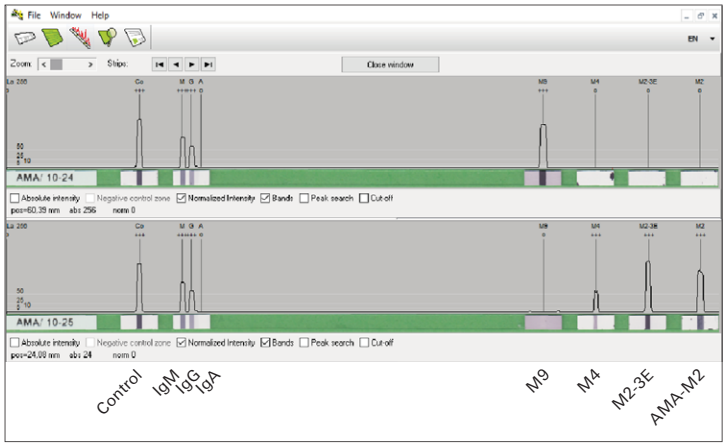
Test principle

The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, diluted patient samples are incubated with the immunoblot strips. In the case of positive samples, specific IgG and IgM antibodies (and IgA) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgGM (enzyme conjugate), which promote a colour reaction upon addition of the substrate solution. Correct performance of all test steps is confirmed by staining of several control bands.

Automated processing

EUROBlotOne is a fully automatic device for the standardised processing of EUROIMMUN line assays (EUROLINE, EUROLINE-WB, Westernblot) – from sample recognition to the final test result. Samples are pipetted by the device and all incubation and washing steps are carried out automatically. Finally the data of the pictures taken by the integrated camera are automatically evaluated and digitally archived by the EUROLineScan software. Alternatively, the immunoblot strips can be incubated by the EUROBlotMaster and scanned using a flatbed scanner. Also in this case, the automatic evaluation is carried out by the EUROLineScan software. The bidirectional communication with a laboratory information management system for import of work lists and export of results is enabled by EUROLineScan or, optionally, the laboratory management software EUROLabOffice 4.0. A separate results sheet can be produced for each sample.



Sensitivity and specificity

Sera from 30 patients with diagnosed PBC and 150 samples from healthy blood donors were investigated for IgG/IgM antibodies against the liver antigens M2, M2-3E, M4 and M9.

In a further study, the sensitivity could be increased by 88% compared to the single substrates, at the same specificity, by combining the antigens M2 and M2-3E.

n = 180	EUROLINE AMA IgG/IgM			
	AMA-M2 positive	anti-M2-3E positive	anti-M4 positive	anti-M9 positive
PBC (30)	25	24	16	5
Blood donors (150)	0	1	0	3
Sensitivity	**83.3%**	**80.0%**	**53.3%**	**16.7%**
Specificity	**100.0%**	**99.3%**	**100.0%**	**98.0%**

198 samples from patients with suspected PBC (precharacterised with a CE-marked reference test) were analysed for IgG and IgM autoantibodies against M2, M4 and M9. Borderline reactions in the reference test were excluded for determination of the positive and negative agreement.

n = 198		CE-marked reference test		
		positive	borderline	negative
EUROLINE AMA IgG/IgM AMA-M2	positive	26	1	3
	negative	0	3	165
EUROLINE AMA IgG/IgM Anti-M4	positive	5	4	1
	negative	0	5	183
EUROLINE AMA IgG/IgM Anti-M9	positive	11	7	0
	negative	2	11	167

n = 198	Positive agreement	Negative agreement
EUROLINE AMA IgG/IgM AMA-M2	100%	98.2%
EUROLINE AMA IgG/IgM Anti-M4	100%	99.5%
EUROLINE AMA IgG/IgM Anti-M9	84.6%	100%

Literature

1. European Association for the Study of the Liver. **EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis.** J Hepatol 67: 145-172 (2017).
2. Strassburg CP et al. **S2k Leitlinie Autoimmune Lebererkrankungen.** AWMF online 21/27 (2017).

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