EUROIMMUN Medizinische Labordiagnostika AG

# Autoimmune liver diseases

## Serological diagnostics of autoimmune hepatitis, primary biliary cholangitis and primary sclerosing cholangitis

- IIFT Liver Mosaics
- Anti-M2-3E ELISA, Anti-LKM-1 ELISA, Anti-LC-1 ELISA, Anti-SLA/LP ELISA
- EUROLINE Autoimmune Liver Diseases
- EUROASSAY Liver Profile (Anti-M2, -LKM-1, -LC-1, -SLA/LP)

# Autoimmune liver diseases

Autoimmune liver diseases mainly include three different disease images: autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). Out of these three diseases, AIH is the most frequent, with a prevalence of 17 cases per 100,000 persons, followed by PBC and PSC. AIH and PBC mainly occur in women during menopause, AIH can also affect children and young adults. PSC mainly affects men from 20 to 40 years of age. In most cases, the three diseases can be reliably distinguished by serological analysis. However, overlap syndromes may occur in which patients present symptoms of two autoimmune liver diseases.

The detection of specific autoantibodies allows precise differentiation between autoimmune liver diseases and infectious, toxic and other forms of hepatitis. AIH is often associated with chronic inflammatory rheumatic systemic diseases such as rheumatoid arthritis, Sjögren's syndrome and systemic lupus erythematosus. The majority of patients respond excellently to anti-inflammatory or immunosuppressive treatment, which should be initiated promptly after the diagnosis is made.

## Autoimmune hepatitis (AIH)

AIH is a progressive chronic form of hepatitis which occurs in phases and leads to destruction of the hepatocytes. The aetiology of the disease has not yet been clarified. However, a connection with infections with hepatitis, measles, cytomegalo- or Epstein-Barr viruses have been discussed. While some patients show no to only mild symptoms, fulminant liver failure can also occur. The most frequent complaints encompass stomach ache, pruritus, nausea, anorexia and general malaise.

In laboratory diagnostic analyses, AIH patients show elevated levels of transaminases and bilirubin as well as an increase in the total IgG titer. In at least 80% of AIH patients, autoantibodies are also detected. These allow distinction of two AIH types (see table). Delimitation from other chronic forms of hepatitis and consequently a clear diagnosis of AIH is essential since untreated AIH is associated with a five-year mortality rate of 50%.

| **Autoimmune hepatitis** | |
|---|---|
| Associated autoantibodies | Prevalence |
| Type 1 AIH | |
| ASMA type F-actin | 70–80% |
| ANA | 70–80% |
| SLA/LP | ~20% |
| Type 2 AIH | |
| LKM-1 | 1–3% |
| LC-1 | 1–3% |
| SLA/LP | ~20% |

## Primary biliary cholangitis (PBC)

PBC is characterised by progressive inflammation-mediated destruction of the small intrahepatic bile ducts which leads to cholestasis and fibrosis. Liver cirrhosis and liver failure are possible consequences of the disease. The symptoms are very similar to those of AIH. With PBC, the laboratory analyses yield an increase in cholestatic enzymes, elevated immunoglobulin values (mainly IgM) and the presence of different autoantibodies.

| **Primary biliary cholangitis** | |
|---|---|
| Associated autoantibodies | Prevalence |
| AMA-M2 | 90–95% |
| ANA (nuclear dots): PML, Sp100 | 25–40% |
| ANA (nuclear envelope): gp210, p62 | 25–40% |

## Primary sclerosing cholangitis (PSC)

PSC is a chronic fibrosing inflammation of the intra- and extrahepatic bile ducts. Over the course of disease, liver cirrhosis develops, requiring liver transplant within 7 to 12 years. Around 60 to 80% of PSC patients also suffer from the chronic inflammatory bowel disease ulcerative colitis. Atypical (p)ANCA (DNA ANCA) are characteristic markers of both diseases, but do not represent a diagnostic proof. Approximately 5% of the patients show clinical, laboratory and histological signs which can be assigned to PSC, but have an inconspicuous cholangiogram (small-duct PSC).

| **Primary sclerosing cholangitis** | |
|---|---|
| Associated autoantibodies | Prevalence |
| Atypical pANCA (DNA ANCA) | 42% |

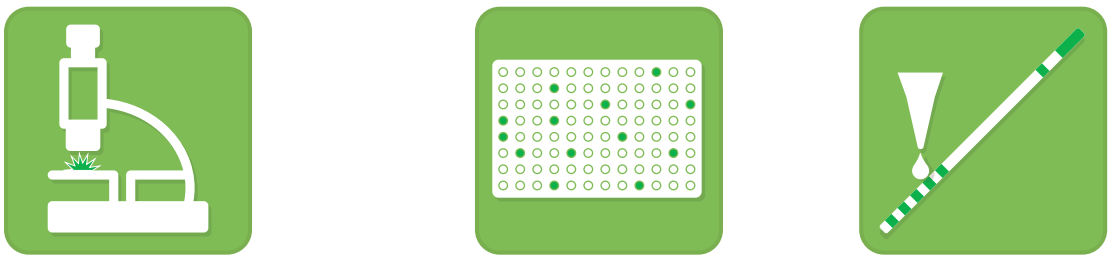
# Diagnostics of autoimmune liver diseases

In suspected cases, abdominal ultrasound is indicated for initial diagnostics.[1] Increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), bilirubin or albumin values can also indicate liver disease.[1] If the clinical suspicion of autoimmune liver disease is substantiated, specific autoantibodies should be determined. Serological diagnostics of AIH, PBC and PSC encompasses:

- Quantification of the total immunoglobulins of classes IgG, IgM and IgA for assessment of the selective increase [1,2]
- Detection of autoantibodies in serum by means of IIFT: [1,2]
  - ANA, ASMA, AMA and LKM-1 on a combination of three rat tissue sections: stomach, liver and kidney
  - PBC-associated ANA against Sp100, gp210 and centromeres by means of HEp-2 cells
  - ASMA against F-actin using VSM47 cells
  - Anti-SLA/LP antibodies using transfected cells
- Monospecific antibody detection in serum by ELISA or immunoblot

Positive serological findings indicating the diagnosis AIH should be verified by liver biopsy. In PBC diagnostics, biopsy is only recommended with unclear findings.[1] The International AIH Group (www.iaihg.org) has developed simplified diagnostic criteria for AIH based on antibody titers, histology and the exclusion of viral hepatitides.[3] For diagnostics of paediatric AIH, a modified IAIHG scoring system should be applied.[1] The AIH-PCB overlap syndrome lacks generally accepted diagnostic criteria.[1]

**Serological test systems for diagnostics of autoimmune liver diseases**



**Indirect immunofluorescence**

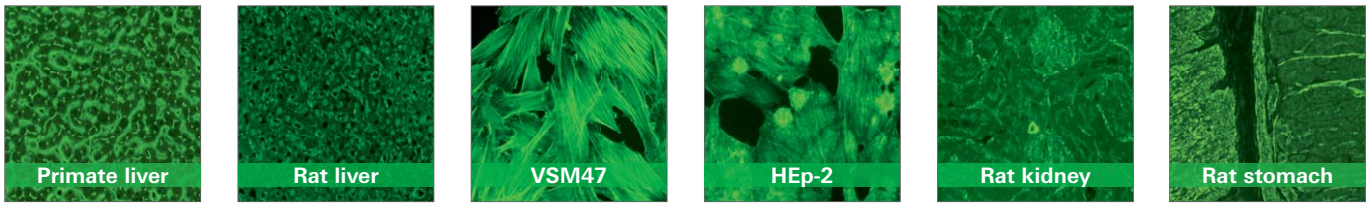
**Monospecific tests, e.g. ELISA or blot**

# Serological diagnostics by means of immunofluorescence

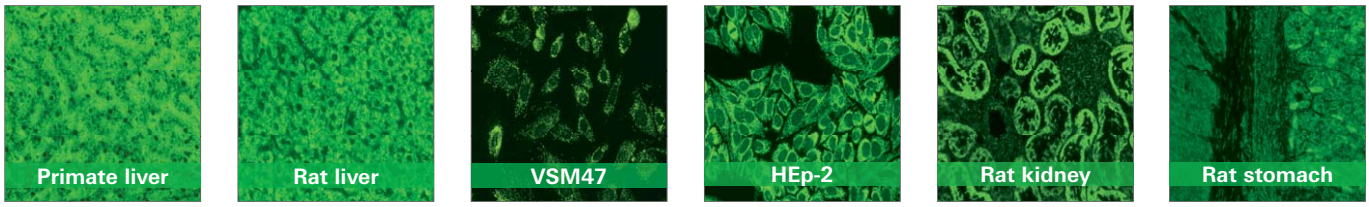
## IIFT Liver Mosaics

The combination of the IIFT substrates HEp-2, kidney, liver and stomach on one BIOCHIP allows simultaneous detection of the antibodies relevant for diagnostics of autoimmune liver diseases. Positive samples show specific fluorescence patterns, e.g. of the cell nuclei and tissue structures.

**Detection of ASMA type F-actin by means of the IIFT Liver Mosaic 8:** The IIFT substrates stomach and VSM47 cells enable simultaneous detection and differentiation of ASMA type F-actin and type non-actin. With frozen sections of rat stomach ASMA show a distinct cytoplasmic fluorescence of the tunica muscularis as well as the lamina muscularis mucosae and the interglandular contractile fibrils of the tunica mucosa. If specific antibodies against F-actin are present, there is a fibrillar fluorescence of the cytoskeleton of the VSM47 cells.




**Detection of AMA and ANA using the IIFT Liver Mosaic 8:** For the detection of AMA, rat kidney is used as the standard substrate. The cytoplasm of the proximal and distal tubule cells shows a granular, basally emphasised fluorescence. The glomeruli are only weakly stained by AMA. AMA show a weaker reaction with liver tissue and lead to a fluorescence of the proximal and distal renal tubules and the parietal cells. Liver tissue and especially HEp-2 cells, however, are particularly suited for ANA detection and differentiation.



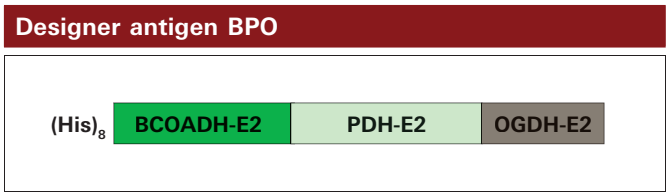

## Suitability of IIFT substrates for the determination of autoantibodies

| Antibodies | Substrate | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | HEp-2 | SLA/LP transf. cells | VSM47 | Rat kidney | Rat stomach | Rat liver | Primate liver | Primate heart |
| AMA M2 | ++ | + | + | ++ | + | + | + | + |
| AMA-M7 | – | – | – | – | – | – | – | ++ |
| AMA-M9 | + | – | – | – | – | – | – | – |
| Nuclear dots | ++ | + | + | + | + | + | ++ | + |
| Nuclear envelope | ++ | + | + | + | + | + | ++ | + |
| Centromeres | ++ | + | + | – | – | – | + | – |
| SS-A/SS-B | ++ | + | + | – | – | – | + | – |
| Scl-70 | ++ | + | + | + | – | – | + | – |
| Nuclear homogeneous | ++ | + | + | + | + | + | ++ | + |
| ASMA type non-actin | – | – | – | + | ++ | + | – | – |
| ASMA type F-actin | – | – | ++ | – | – | – | – | – |
| LKM | – | – | – | ++ | – | ++ | + | – |
| LC-1 | – | – | – | – | – | ++ | – | – |
| SLA/LP | – | ++ | – | – | – | – | – | – |

++ well suited + suited with limitations – not suited

# Monospecific antibody detection by ELISA or EUROLINE

## Anti-M2-3E ELISA (IgG)




The detection of AMA is of great importance in the diagnosis of PBC. Antibodies against the M2 antigen represent the most sensitive and specific diagnostic marker. The Anti-M2-3E ELISA uses the native PDH complex and the designer antigen BPO as antigen substrate. The combination of artificial polypeptide PBO and native PDH increases the sensitivity by 14% compared to the classic Anti-M2 ELISA. The AMA target antigens belong to the enzyme family of ketoacid dehydrogenase complexes from the mitochondrial respiratory chain. The lipoyl-binding regions (E2) of these enzyme complexes are the main autoantigens in PBC. The recombinant polypeptide His-BPO consists of the E2 subunits of the branched chain keto acid dehydrogenase complex (BCOADH), the pyruvate dehydrogenase (PDH), and the ketoglutarate dehydrogenase (OGDH) and an N-terminal His-tag.

Sera from 251 PBC patients, 15 patients with PBC/AIH overlap syndrome and 1,129 control individuals were investigated with the EUROIMMUN Anti-M2-3E ELISA (IgG). The sensitivity of the Anti-M2-3E ELISA (IgG) for PBC was 93.2% with a specificity of 97.9%.[4]

## Anti-SLA/LP ELISA (IgG)

The ELISA test kit provides a semiquantitative or quantitative in vitro detection of human antibodies of the IgG class against SLA/LP in serum or plasma. Autoantibodies against SLA/LP have the highest diagnostic relevance for AIH.[5] The presence of anti-SLA autoantibodies in AIH patients is of prognostic relevance since they seem to be associated with greater occurrence of relapses, poor prognoses, relapses after liver transplantation and pregnancy complications.[6] In AIH, anti-SLA/LP antibodies usually occur as the only specific antibodies. While their prevalence is only between 10 and 30%, their predictive value is nearly 100%. Basically every positive anti-SLA/LP result confirms an AIH as long as the corresponding clinical symptoms are present. Anti-SLA/LP autoantibodies cannot be reliably detected by IIFT on tissue sections, but only by means of monospecific tests such as ELISA, immunoblot or IIFT with transfected cells.

Sera from 454 AIH patients, 147 patients with other liver diseases and 200 blood donors were investigated using the EUROIMMUN Anti-SLA/LP ELISA (IgG). The specificity of the ELISA was 100%.

## Anti-LC-1 ELISA (IgG)

The Anti-LC-1 ELISA allows semiquantitative determination of anti-LC-1 antibodies. Anti-LC-1 antibodies in AIH can occur independently of or together with other autoantibodies. Especially in cases of anti-LKM-1 negative serology, anti-LC-1 antibodies indicate type 2 AIH.

Sera from 93 AIH patients, 183 patients with other liver diseases including PBC, and 200 blood donors were investigated using the EUROIMMUN Anti-LC-1 ELISA. The prevalence of antibodies against LC-1 in the panel of autoimmune hepatitis amounted to 5.4%, at a specificity of 100%.

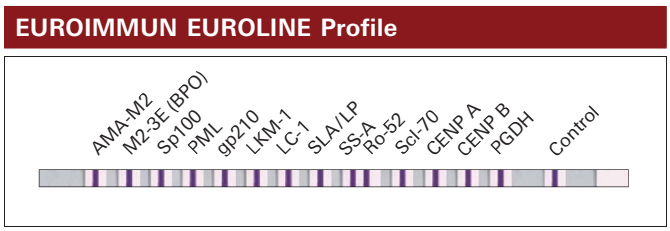
## Anti-LKM-1 ELISA (IgG)

Detection of autoantibodies against LKM is part of routine AIH diagnostics. Antibodies against LKM-1 (target antigen: cytochrome P450 IID6) occur especially in children, while they are only found in 1% of adult AIH patients. Antibodies against LKM-1 are also detectable in 1 to 2% of patients with positive hepatitis C serology. In patients with AIH, autoantibodies against LKM-1 typically do not occur together with antibodies against SLA/LP. Testing both parameters can increase the serological hit rate for AIH. The parallel determination of other AIH-associated autoantibodies, such as ANA, pANCA, ASMA and antibodies against LC-1 and SLA/LP is recommended for differentiation from viral hepatitis. [5]

18 sera from AIH patients and 489 serum samples from a reference laboratory were investigated using the EUROIMMUN Anti-LKM-1 ELISA (IgG) and an IIFT Mosaic with the substrates rat liver and kidney (IgG) as a reference method. The specificity of the ELISA amounted to 99.4% with a sensitivity of 100% with respect to the IIFT.

## Multiparametric EUROLINE Profiles

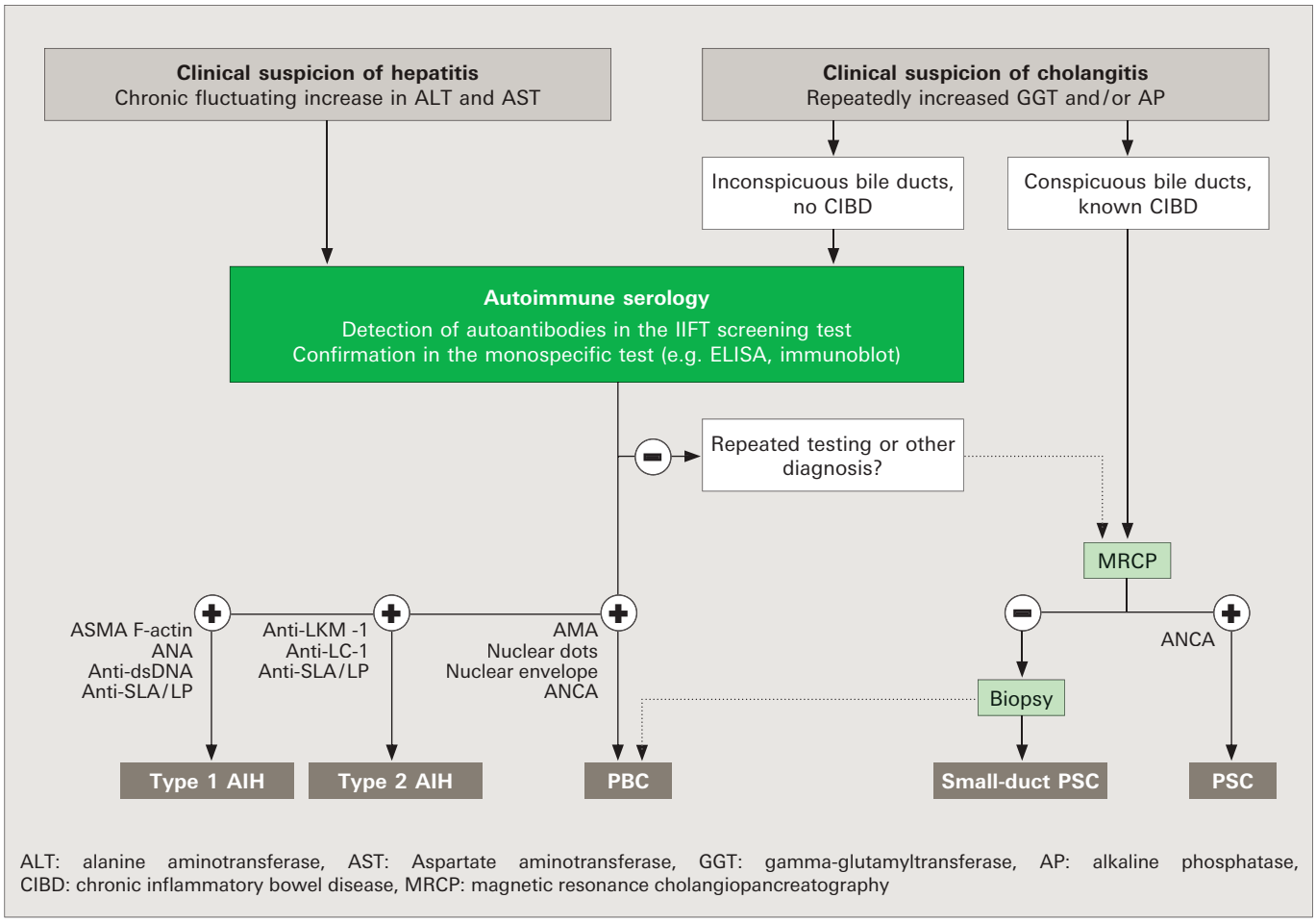
The EUROLINE Profiles for autoimmune liver diseases are qualitative in vitro immunoassays for detection of antibodies against liver antigens in human serum or plasma. They allow simultaneous qualitative determination of several antibodies in one incubation.




The EUROLINE Profile Autoimmune Liver Diseases encompasses not only the AIH- and PBC specific parameters but also phosphoglycerate dehydrogenase (PGDH). Determination of antibodies against PGDH facilitates the differentiation between AIH and HBV infection. In a study, 31% of tested AIH and 9% of tested PBC patients showed autoantibodies against PGDH in the EUROLINE assay, while none of the HBV patients presented autoantibodies against PGDH.[7]

Villalta and colleagues conducted a study on PBC-specific autoantibody profiles and tested sera from 58 PBC patients, 144 patients with other autoimmune liver diseases, and 67 patients with chronic, non-autoimmune liver diseases using the EUROLINE Autoimmune Liver Diseases Profile 2 and an IIFT Mosaic with HEp-2 cells and rat kidney, liver, and stomach.[8] Due to the high sensitivity and specificity of the test, the authors concluded that the EUROLINE was a well suited assay for confirmation of IIFT results or for evaluation of cases with unclear clinical image and/or serological finding. In specialised reference laboratories where PBC-associated autoantibodies are often detected, the EUROLINE is a good option for primary testing since it allows simultaneous determination of all relevant PBC-specific antibodies and improved and faster definition of AMA subspecificities.

# Serodiagnostic guidelines for autoimmune liver diseases




ALT: alanine aminotransferase, AST: Aspartate aminotransferase, GGT: gamma-glutamyltransferase, AP: alkaline phosphatase, CIBD: chronic inflammatory bowel disease, MRCP: magnetic resonance cholangiopancreatography

## Helpful information for serological diagnostics

- IgG serum levels and anti-actin autoantibody titers can correlate with the activity of AIH. AMA titers, however, do not correlate with the disease activity of PBC.
- Acute/fulminant AIH may lack autoantibodies and IgG increase.
- In the IIFT, sera are usually tested in a 1:100 dilution. Antibody titers up to 1: 80 may occur in adults even without presence of an autoimmune disease. In children and adolescents, however, titers from 1:10 may be clinically relevant.[5]
- Serological AIH diagnostics should include determination of thyroid stimulating hormone (TSH), since AIH is often associated with autoimmune thyroiditis.[1]
- Moreover, AIH is often associated with chronic inflammatory rheumatic systemic diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome).[1]
- A positive AMA finding in the ELISA with negative IIFT is rare, but may be explained by greater sensitivity of the ELISA or detection of infection-associated AMA.
- With suspected PBC, ANCA detection is recommended alongside serological testing for AMA and ANA.

## Order information

| Test system | Test name | Antibodies against | Substrate | Order number |
|---|---|---|---|---|
| **IIFT** | IIFT: Liver Mosaics | Cell nuclei (ANA)<br>Liver antigens, cell nuclei (ANA)<br>LKM + cell nuclei (ANA)<br>LKM + mitochondria (AMA)<br>Smooth muscle<br>Striated muscle<br>F-actin<br>Soluble liver antigen/liver pancreatic antigen (SLA/LP) | HEp-2 cells (human)<br>Liver (monkey)<br>Liver (rat)<br>Kidney (rat)<br>Stomach (rat)<br>M. iliopsoas (monkey)<br>VSM47 cells<br>Transfected cells (EU90) | FA 1300-####-1 to -21<br>FA 1710-####<br>FA 1651-####<br>FA 1302-####-50 |
| | IIFT: HEp-2 /liver | Cell nuclei (ANA)<br>(ANA global testing) | HEp-2 cells (human)<br>Liver (monkey) | FA 1510-####-1 |
| | IIFT: Kidney | Mitochondria (AMA) | Kidney (mouse) | FA 1621-#### |
| | Anti-M2 IIFT | M2 antigen | M2 BIOCHIPs (pig) | FA 1622-#### |
| **ELISA** | Anti-M2-3E ELISA (IgG) | AMA M2 | Antigen-coated microplate wells | EA 1622-9601 G |
| | Anti-LKM-1 ELISA (IgG) | LKM-1 | | EA 1321-9601 G |
| | Anti-LC-1 ELISA (IgG) | LC-1 | | EA 1307-9601 G |
| | Anti-SLA/LP ELISA (IgG) | SLA/LP | | EA 1302-9601 G |
| **EUROLINE** | Autoimmune Liver Diseases 14 Ag | AMA-M2, M2-3E, sp100, PML, gp210, LKM-1, LC-1, SLA/LP, SS-A, Ro-52, Scl-70, CENP A, CENP B, PGDH | Antigen-coated test strips | DL 1300-####-5 G |
| | EUROLINE Autoimmune Liver Diseases 9 Ag plus F-actin | AMA-M2, M2-3E, Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52, F-actin | | DL 1300-####-9 G **NEW!** |
| | AMA Profile (IgGM) | AMA-M2, M2-3E, M4, M9 | | DL 1620-####-1 O |
| **EUROASSAY** | Anti-LKM-1, Anti-SLA/LP | LKM-1, SLA/LP | Antigen-coated test strips | DA 1300-####-1 G |

## References

[1]Strassburg C et al., Leitlinie Autoimmune Lebererkrankungen, AWMF-Reg. Nr. 021-27, Zeitschrift für Gastroenterologie 55(11):1135-1226 (2017)
[2]Vergani D et al., Unusual Suspects in Primary Biliary Cirrhosis, Hepatology 39(1):38-41 (2004)
[3]Hennes E et al., Simplified Criteria for the Diagnosis of Autoimmune Hepatitis, Hepatology 48(1):169-176 (2008)
[4]Dähnrich C et al., New ELISA for detecting primary biliary cirrhosis-specific antimitochondrial antibodies, Clinical Chemistry 55(5):978–85 (2009
[5]Terziroli Beretta-Piccoli B et al., Serology in autoimmune hepatitis: A clinical-practice approach, European Journal of Internal Medicine 48:35-43 (2018)
[6]Efe C et al., Antibodies to soluble liver antigen in patients with various liver diseases: A multicentre study, Liver International 33(2):190-196 (2013)
[7]Xiang D et al., Detection of D-3-phosphoglycerate dehydrogenase autoantibodies in patients with autoimmune hepatitis: Clinical significance evaluation, Hepatology Research 41(9):867-876 (2011)
[8]Villalta D et al., Autoantibody profiling of patients with primary biliary cirrhosis using a multiplexed line-blot assay, Clinica Chimica Acta 438:135-138 (2015)