Autoimmune hepatitis (AIH)
Primary biliary cholangitis (PBC)

Enzyme immunoassays for the diagnostics of autoimmune liver diseases

MICROBLOT-ARRAY kits are optimized and validated for the detection of IgG antibodies in human serum and plasma

IVD CE 2265

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Autoimmune liver disease (AILD) is a chronic disease caused by an immune-mediated inflammatory response in genetically susceptible individuals. It is a group of complex and serious diseases affecting the immune system that lead to inflammation and damage to liver tissue. It is common that AILD is accompanied by other autoimmune diseases affecting other organs in susceptible individuals. The AILD group includes autoimmune hepatitis types 1 and 2 (AIH1 and AIH2), along with the less common autoimmune hepatitis type 3 (AIH3), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC).

Autoimmune hepatitis (AIH)

AIH type 1 is an autoimmune disease characterised by chronic inflammation of the liver. It is the predominant type of AIH and most commonly affects women between the ages of 15 and 40 but can also occur in men and other age groups. In addition to antinuclear antibodies (ANA), the main antigenic markers include anti-SLA/LP or anti-SMA antibodies.

AIH type 2 is a less common form, occurring mainly in children and adolescents, the main markers are anti--LKM-1 and anti-LC-1 antibodies.

AIH type 3 is a rare form, characterized by the presence of antibodies to SLA/LP. Clinically and therapeutically, it does not differ much from AIH1.

Primary biliary cholangitis (PBC)

PBC is characterized by autoimmune-mediated destruction of inflamed bile ducts, predominantly affecting the small intrahepatic bile ducts. PBC primarily affects middle-aged women.

Key antigenic markers include anti-mitochondrial antibodies (AMA), particularly M2 and 3E (BPO) antibodies. In cases where patients test negative for AMA autoantibodies, positivity for autoantibodies targeting antinuclear antibodies (ANA) such as anti-gp210, anit-Sp100 or anti-Ro52 antibodies.

Primary sclerosing cholangitis (PSC)

PSC is autoimmune inflammation of the intrahepatic and extrahepatic bile ducts. A similar course of the disease without the autoantibodies can be induced by viruses, termed secondary cholangitis. The disease affects mostly men, especially under the age of 40. In addition to ANA and p-ANCA antibodies, anti-gp210 antibodies are important markers.

Most PSC patients are at risk of AIH and up to 30% of patients have autoimmune gastrointestinal diseases (ulcerative colitis or Crohn's disease).

Diagnostics of autoimmune liver diseases

The diagnosis of AILD is complex including clinical, biochemical, histological, and serological examination together with the exclusion of other possible causes of liver damage (viral hepatitis, alcoholic, or toxic damage).

The detection of autoantibodies plays a key role in the diagnostics of AILD, particularly in AIH and PBC. Diagnostic based on the determination of antibodies to AILD using IIF, ELISA, CLIA and immunoblot technologies are nowadays considered state-of-the-art.

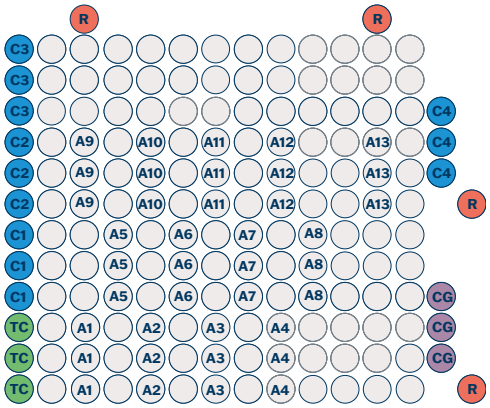
Specific antigens

Disease type	Antigen	Description
Autoimmune hepatitis	**LKM-1**	**Liver Kidney microsomal type 1** – Associated with AIH2 and HCV – In AIH2, lower titers, especially important in pediatric patients
	LC-1	**Liver cytosol-1** – Highly specific for AIH2 (30% of patients) – one of the diagnostic criteria for AIH2 – Associated with higher disease activity
	SLA/LP	**Soluble liver antigen/liver pancreas antigen** – Associated with AIH3 or AIH1 (in about 25% of patients with chronic AIH) – Their presence depends on ethnicity
Primary biliary cholangitis	**ASGPR**	**Asialoglycoprotein receptor** – An important diagnostic marker of PBC – Also present in other liver diseases of viral origin – The level of antibodies correlates with the severity of the disease – Antibodies may disappear during immunosuppressive therapy
	gp210	**Glycoprotein 210** – Associated with nuclear membrane – High specificity for PBC, especially in AMA negative patients (30–50%) – Association with a more severe PBC and a higher risk of developing cirrhosis – May also be associated with PSC
	sp100	**Speckled protein 100 kDa** – Associated with multiple nuclear dots – High specificity for PBC, probable association with progressive PBC and risk of fibrosis – Incidence in 30–50% of AMA negative patients
	PML	**Promyelocytic Leukemia Protein** – Incidence in approximately 12–19% of PBC patients, association with PBC in AMA negative patients (predominantly in coexistence with anti-Sp100)
	Nup62	**Nucleoporin 62** – High specificity for PBC, often simultaneously with anti-gp210 – Association with later stage disease and worse prognosis
	M2	**Intramitochondrial protein** – Binds anti-mitochondrial antibodies (AMA), highly sensitive – Typical for PBC, only in about 5-10% of PBC patients AMA is not formed – Overlapping syndromes with AIH – Rare occurrence in ANA patients (progressive SS, SjS or SLE)
	3E(BPO)	Fusion protein (BCOADC E2 + PDC E2 + OGDC E2)
	OGDC-E2	2-oxo-glutarate dehydrogenase complex
	PDC-E2	Pyruvate dehydrogenase complex
		– M2 subunits – PDC-E2 is the dominant subunit (approx. 85–90% of cases)
	Ro52	**TRIM21** – Probable marker for PBC (occurs in approx. 28% of patients) – Associated with AIH1 (occurrence in approx. 38% of patients) – Diagnostic marker of SLE, SSc, specifically associated with myositis

PBC – Primary biliary cholangitis | **AIH1,2,3** – Autoimmune hepatitis type 1, 2, 3 | **HCV** – Hepatitis C virus | **PSC** – Primary sclerosing cholangitis | **AMA** – Antimitochondrial antibodies

MICROBLOT-ARRAY

Distribution of antigens and control spots



Description of antigens

A1 – LKM-1
A2 – LC-1
A3 – SLA/LP
A4 – ASGPR
A5 – gp210
A6 – Sp100
A7 – PML
A8 – Nup62
A9 – M2
A10 – 3E (BPO)
A11 – OGDC-E2
A12 – PDC-E2
A13 – Ro52

Description of control spots

R – Reference
TC – Test control
CA – Conjugate control IgA
CG – Conjugate control IgG
CM – Conjugate control IgM
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Quick wash using the Universal Solution*
	8.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	9.	Pipette Conjugate 100 µl
	10.	Incubate 30 min. at room temperature
	11.	Quick wash using the Universal Solution*
	12.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	13.	Pipette Substrate solution (BCIP/NBT) 100 µl
	14.	Incubate 15 min. at room temperature
	15.	Quick wash using the distilled water*
	16.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	17.	Dry and evaluate wells

* if using a washer, fill the wells up to the brim and aspirate immediately after filling the last well

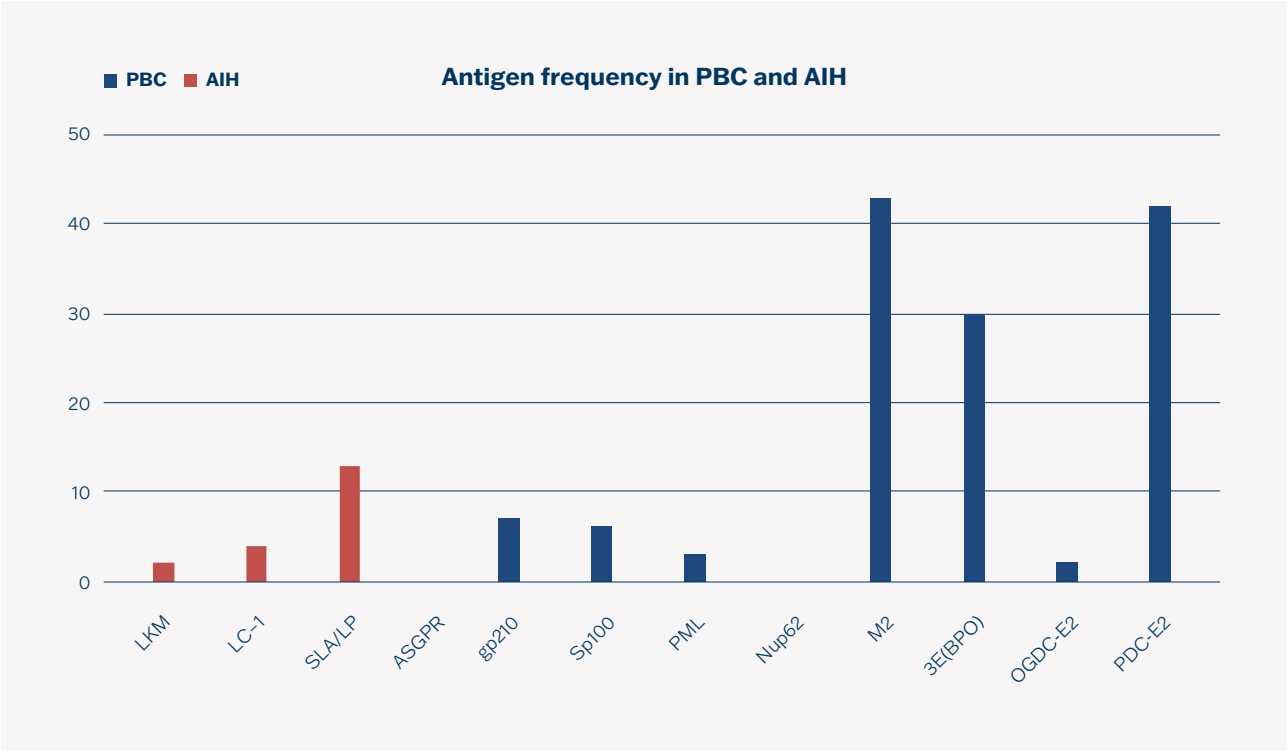
User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Possibility of automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity and specificity

Results of individual antigens in tested panels

Panel	LKM	LC-1	SLA/LP	ASGPR	gp210	Sp100	PML	Nup62	M2	3E(BPO)	OGDC-E2	PDC-E2	Total
PBC (n=44)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	7 16.3%	6 14.0%	3 7.0%	0 0.0%	43 100.0%	30 69.8%	2 4.7%	42 97.7%	44 100.0%
AIH (n=19)	2 10.5%	4 21.1%	13 68.4%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	19 100.0%
Viral hepatitis (n=30)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
RF-positive (n=30)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	2 6.7%	0 0.0%	0 0.0%	3 10.0%	4 13.3%
Donors (n=30)	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%

Antigen frequency in primary biliary cholangitis and autoimmune hepatitis tested on Microblot-Array Liver profile kit



Results of external clinical study for the Microblot-Array Liver profile

	Parameter	Value	95% confidence interval (CI)
Diagnostic sensitivity	ALD (n = 63)	99.9%	94.3 – 100.0%
	PBC (n = 44)	99.9%	92.0 – 100.0%
	AIH (n = 19)	99.9%	82.4 – 100.0%
Diagnostic specificity (n = 60)		99.9%	94.0 – 100.0%
Positive predictive value	ALD (n = 63)	99.9%	94.3 – 100.0%
	PBC (n = 44)	99.9%	92.0 – 100.0%
	AIH (n = 19)	99.9%	82.4 – 100.0%
Negative predictive value	ALD (n = 60)	99.9%	94.0 – 100.0%
	PBC (n = 60)	99.9%	94.0 – 100.0%
	AIH (n = 60)	99.9%	94.0 – 100.0%
Likelihood ratios of the kit	Positive test (LR+)	>100	–
	Negative test (LR-)	<0,0001	–
Comparison with a reference method		96.0%	91.5 – 98.5%

Ordering Information

MICROBLOT-ARRAY

Cat. No	Product	No. of Tests
LKMMA48	Microblot-Array Liver profile	48

TestLine Clinical Diagnostics Ltd.
Krizikova 188/68, 612 00 Brno, Czech Republic
+420 549 121 203
sales@testlinecd.com
www.testlinecd.com

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

V01/2025