IMTEC-LIVER-LIA S

Liver-LIA S

Line Immuno Assay (LIA) for the Detection of Autoantibodies in Autoimmune Liver Diseases (IgG)

(AMA M2, Sp100, LKM1, gp210, LC1, SLA)

Package Size

REF	ITC66205	16 Tests	Complete Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

RCNS 20ml **Blocking Reagent,** BUF WASH 10x **WB03,** SUBS TMB **TMB LIA and** SOLN STOP **STOP LIA may be interchanged between lots and test kits that share the same reagent designation.**

All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits

Store reagents at 2...8°C.

Intended Use

Autoimmune hepatitis (AIH) types 1-3, primary biliary cirrhosis (PBC), and immune cholangiopathy, regarded as the overlap syndrome between AIH and PBC, are some of the most common autoimmune liver diseases.

Antimitochondrial antibodies (AMA) directed against the inner and outer mitochondrial membranes are highly specific for PBC.

Antibodies directed to the underlying antigen M2 can be detected in about 90% of all patients with PBC.

Anti-Sp100 antibodies can be detected in 31% of all patients with PBC. These antibodies are not detectable in other autoimmune liver diseases. Due to their high level of specificity, anti-Sp100 antibodies are considered to be markers of PBC.

Anti-gp210 antibodies can be detected in approx. 10% of all patients with primary biliary cirrhosis, and they are considered to be highly specific for PBC. In the group of AMA-negative patients these antibodies are detected at a frequency of 21%.

Anti-LKM1 antibodies are regarded as markers of type 2 autoimmune hepatitis. However, they can also be detected in around 7% of patients with chronic hepatitis C and, in very rare cases, in patients with halothane-induced hepatitis.

Anti-LC1 Antibodies (liver-cytosolic-antibodies) are detectable (mainly) in young patients with an AIH type 2. At least 50 – 60 % of patients with anti-LKM1 antibodies show anti-LC1 antibodies as a secondary marker antibody. Nevertheless both antibodies can occur isolated.

Type 3 autoimmune hepatitis is characterised by the occurrence of antibodies to soluble liver proteins (SLA). Anti-LKM1 antibodies are not detectable in this type of hepatitis and in many cases, ANA and liver membrane antibodies also do not occur.

Principle

The test is based on the principle of the line immuno assay (LIA). The antigens are applied as lines on a nitrocellulose membrane:

Antigens	identity
PDH (AMA M2)	native
Sp100	recombinant, patented
LKM1	peptide, patented
gp210	peptide
LC1	recombinant
SLA	recombinant, patented

The nitrocellulose membrane is blocked to prevent unspecific reactions. During incubation of a strip with diluted patient serum autoantibodies present in the sample will bind to the antigens on the strip. For the detection of the antibodies bound to the strip a secondary horseradish peroxidase (HRP)-labelled antibody is used that is directed against human IgG. After addition of a substrate solution the bound autoantibodies are visualised as blue lines (switching to brown after stopping).

Reagents and Contents

STRIP	**16 Test Strips** (brown colour coding) coated with PDH (AMA M2), Sp100, LKM1, gp210, LC1 and SLA, ready to use
RCNS 20ml Blocking Reagent	**3 Bottles Non-Fat dry milk powder** for the preparation of 20 ml incubation buffer
BUF WASH 10x WB03	**50 ml Washing Buffer** (black cap) Concentrate (10x) for about 0.5 l Phosphate buffer pH 6.7 ± 0,2
CONJ a(hum IgG):HRP	**17 ml Conjugate Solution** (white cap) anti-human-IgG HRP conjugate, ready for use
SUBS TMB TMB LIA	**17 ml TMB Solution** pH 5.0 ± 0.2 (black cap) ready for use, colourless to bluish 3,3',5,5'-tetramethylbenzidine 1.2 mmol/l Hydrogen peroxide 2.4 mmol/l
SOLN STOP Stop LIA	**17 ml Stop Solution** (red cap) Sulphuric acid, ready for use 0.1 mol/l
	1 Pc each reference strip (colour copy), tweezers, evaluation template and incubation tray
	3 Pcs labels for incubation buffer

Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens should be handled as potentially infectious. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

SOLN STOP, SUBS TMB can irritate eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

Reagent Preparation

Attention!

Allow the testkit and all its components to reach room temperature before use! Used bottles should be closed carefully and stored at 2...8°C. Store SUBS TMB protected from light.

Do not use polystyrene vessels for handling of CONJ a(hum IgG):HRP.

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part BUF WASH 10x with 9 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

Incubation Buffer Solution DIL

Pour 20 ml WASH into one bottle RCNS 20ml and mix well. DIL is stable for 6 weeks at 2...8°C.

Specimen

Patient sera

Use samples freshly collected or freeze samples at –20°C. **Freeze and thaw once only**. Do not use serum samples inactivated by heat treatment at 56°C.

Dilute sera 1:101 with DIL (add 10 µl serum to 1 ml DIL).

Procedure

Attention! Do not let STRIP **dry during the incubation steps.**

Do not touch STRIP **with fingers, use tweezers.**

Remove diluted sera completely after incubation of STRIP **to avoid cross contamination.**

All details are valid per STRIP **or patient sample**

- Put STRIP into the incubation tray, the reference line and colour coding facing up. Moisten STRIP in 1 ml DIL to wet the membrane. Remove the DIL afterwards.
- Pipette **1 ml** of **diluted patient serum** and incubate for **30 min.** at room temperature with gentle agitation.

- **Suck off the diluted sera completely.**
- **Wash** STRIP using **1.5 ml** WASH **for 5 min.** with gentle agitation. **Suck off** WASH **after every washing step. Repeat** procedure **3 times.**
- Pipette **1 ml** CONJ a(hum IgG):HRP and incubate **30 min.** at room temperature with gentle agitation.
- **Suck off** CONJ a(hum IgG):HRP.
- **Wash** STRIP using **1.5 ml** WASH **for 5 min.** with gentle agitation. **Suck off** WASH **after every washing step. Repeat** procedure **3 times.**
- Add **1 ml** SUBS TMB and incubate for **10 min..**
- Suck off SUBS TMB.
- Wash with **1.5 ml distilled water** (with gentle agitation).
- Suck off distilled water.
- Pipette **1 ml** SOLN STOP and incubate developed STRIP for **5 min.** with gentle agitation to terminate the enzymatic reaction.
- Dry STRIP between filter paper.

Validation of the Test

The test results are valid provided the following criteria are met for each STRIP:

- A normal test run is indicated by a visible function control.
- The cut-off control must be visible.
- Intensity function control > intensity cut-off control

Interpretation of Results

Fix STRIP onto scoring sheet and align the reference line of the STRIP with the reference line on the scoring sheet.

Align the dotted reference line of the evaluation template with the reference line of the STRIP.

The interpretation of the test results takes place exclusively on basis of the respective cut-off control regarded for each STRIP:

The test result is **negative**, if no band is to be recognised or if the band exhibits a smaller intensity in comparison to the cut-off control.

The test is **equivocal**, if the intensity of the band and the intensity of the cut-off control do not significantly differ.

The test result is **positive**, if a band exhibits a stronger staining in comparison to the cut-off control.

Record the respective test results on the scoring sheet for future reference.

Remarks

In the case of an equivocal result the test should be repeated with a new serum sample.

As example each test kit contains a colour copy with all bands provable in the test.

The colour coding attached above the reference line serves the identification of the available IMTEC-LIA-tests:

colour coding	IMTEC-LIA-test
yellow	ANA-LIA
pink	ANA/dsDNA-LIA
blue	Myositis-LIA
green	Celiac Disease-LIA
brown	Liver-LIA S
purple	Vasculitis-LIA
black	Gastro-LIA

Limitations

The intensity of the band colour does not necessarily correlate with antibody titres as obtained with other reference methodologies. Sera from apparent normal blood donors may contain autoantibodies.

In Hepatitis C Virus (HCV) infected subjects elevated autoantibody levels have been reported. In such cases further confirmatory investigations are recommended.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/la-66205.pdf or
www.human-de.com/data/gb/vr/la-66205.pdf

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