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	24 Tests	Complete Test kit

IVD

Please read the instructions carefully before testing.

Intended Use

IMTEC-Liver-LIA S is an indirect membrane based enzyme immunoassay for the qualitative measurement of IgG class antibodies against AMA M2, Sp100, LKM1, gp210, LC1 and SLA in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of autoimmune liver diseases.

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 antigens (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 samples autoantibodies present in the sample will bind to the antigens on the strip. For the detection of the bound antibodies a secondary horseradish peroxidase (HRP)-labelled anti-human IgG antibody is used. After addition of the substrate and stop solution the appearance of brown lines indicate the existence of (auto) antibodies against the respective antigen.

Kit Content

STRIP	24	**Test Strips** (brown colour coding) coated with antigen (see table), ready for use
DIL LIA	3 Bottles	**Powder** for the preparation of 30 ml dilution buffer (blue cap)
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) concentrate (20x) for 1 l buffer
CON	29 ml	**Conjugate Solution** (white cap) anti-human-IgG conjugate, ready for use
SUB LIA	30 ml	**Substrate Solution** (black cap), ready for use colourless to bluish 3,3', 5,5'-tetramethylbenzidin 1.2 mmol/l hydrogen peroxide 2.4 mmol/l
STOP LIA	26 ml	**Stop Solution** (red cap) sulphuric acid, ready for use 0.1 mol/l
	2 pcs.	**Incubation Tray**
	1 pc. each	**Scoring sheet, Tweezers, bonding sheet,** transparent **Evaluation Template**

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.

Stability

When stored at 2...8°C unopened vials are stable until the expiry date.

After reconstitution, DIL LIA and WASH and opened CON are stable for 6 weeks at 2...8°C.

Store SUB LIA protected from light.

Precautions ⚠

DIL LIA, WASH 20x WB03 and SUB LIA may be interchanged between lots and LIA 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.

Do not use polystyrene vessels for handling of CON.

Any crystallised salt of WASH 20x inside the bottle must be resolved before use.

Do not dry STRIP during the incubation steps.

Do not touch STRIP with fingers, use tweezers.

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

Specimen, Controls

Serum and plasma with the anticoagulants citrate or EDTA.

Do not use highly lipemic, hemolysed or icteric specimens.

Undiluted specimens may be stored at 2...8°C for 5 days, or for one year at -20°C**. Freeze and thaw once only.** Thawed specimen should be carefully homogenised. Eliminate particulate matter by centrifugation or filtration.

Reagent Preparation

Bring all reagents to **room temperature** (15...25°C) before use.

Reagents not in use should always be stored at 2...8°C.

Washing Buffer Solution WASH

Dilute 1 part WASH 20x with 19 parts distilled water.

Dilution Buffer Solution DIL LIA

Dissolve the content of one bottle DIL LIA with 30 ml of WASH and agitate well.

Procedure

Wash Procedure

The wash procedure is critical. Insufficient washing will result in poor precision or falsely high band intensity.

W1: Remove liquids completely.

W2: Add WASH and incubate for 5 min with gentle agitation.

W3: After washing, remove remaining liquid.

Pipetting Scheme

Follow the procedure exactly as described. Pay particular attention to the washing procedure!	
⚠ Reagents and specimens should be at room temperature before use. **⚠ Use rocking shaker during all incubation steps.**	
Sample Preparation: Dilute specimen 1:101 with reconstituted DIL LIA (10 µl serum + 1 ml DIL LIA) 1 ml is needed for each well.	
Step 1	***Well [ml]***
Insert STRIP into the incubation tray colour coding facing up	--
WASH to wet the membrane	1
Incubate 1 min. at room temperature	
Remove WASH	
Step 2	
Diluted samples	1
Incubate 30 min. at room temperature	
Wash 3 times as described (see W1 - W3)	
WASH	1
Step 3	
CON	1
Incubate 30 min. at room temperature	
Wash 3 times as described (see W1 - W3)	
WASH	1
Step 4	
SUB LIA	1
Incubate 10 min. at room temperature	
Remove SUB LIA	
Step 5	
Add distilled water	1
Incubate 1 min. at room temperature	
Remove destilled water	
STOP LIA	1
Incubate 5 min. at room temperature	
Remove STOP LIA	
Dry STRIP thoroughly	

Automation

The IMTEC-Liver-LIA S may be processed with suitable automated Blot analyzers. Applications have to be validated in prior to diagnostic use. For automated interpretation of LIA strips use HumaScan (REF ITC02851).

Test Validation

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

- Function control is visible.
- Cut-off control is 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. In the case of an equivocal result the test should be repeated with a new sample.

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.

Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

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

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

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

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Note

The handling should always be in compliance with common GLP requirements (*)! The validation criteria must be met!

(*This includes: Proper caps being replaced on the vials and firmly tightened / Remove only reagents required for a run from stock solutions if they could come into contact with other contaminating solutions like patient specimens etc. / Stock solutions always returned to 2...8°C when not in use.)

Safety Notes

STOP **Warning!**

· Hazard statements

H315 Causes skin irritation.
H319 Causes serious eye irritation.

DIL LIA WASH CON STOP

· Precautionary statements

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

STRIP SUB LIA

· Precautionary statements

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P337+P313 If eye irritation persists: Get medical advice/attention.

P401 Store in accordance with local/regional/national/international regulations.

P410 Protect from sunlight.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

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