

HOB-LIA-LIVER

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

For Qualitative Analysis of Autoantibodies in Liver Diseases in Human Serum

REF MB00053; Liver-8, 16 Tests

REF MB00050; Liver-4, 16 Tests

INTENDED USE

The **HOB-Liver-LIA** is for the qualitative measurement of IgG class antibodies against AMA M2, CENP-B, sp100, gp210, LKM-1, LC-1, SLA/LP, SS-A/52kDa in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of autoimmune liver disease.

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. Autoimmune hepatitis is a relatively rare disease. It is necessary to distinguish it from viral hepatitis strictly. Because of the improvement of the serological diagnosis ability, the detection rate of the autoimmune hepatitis also increases. One of the causes is the autoimmune response to liver tissue caused by the decline of the autoimmune tolerance. It is not fully understood that whether the autoantibodies generated in this autoimmune response have pathologic correlation. Perhaps, liver cell damage is caused by T cells.

Autoantibodies play an important role in distinguishing viral hepatitis from three different types of autoimmune chronic active hepatitis (AIH). In AIH type I, ANA and anti-smooth muscle antibodies (ASMA) can be detected. In AIH type II, the corresponding antigen is liver and kidney microsomal antigen (LKM - 1). Soluble liver antigen antibody mainly appears in AIH type III.

Autoantibodies which could be detected in the corresponding diseases are as below:

AIH type I: ANA, ASMA

AIH type II: LKM-1, LC-1

AIH type III: SLA/LP

PBC: AMA M2, sp100, gp210, (CENP-B)

Anti-AMA M2 antibody

Anti-mitochondrial antibodies (AMA) directed against the inner and outer mitochondrial membranes are highly specific for PBC. Since 1963, indirect immunofluorescence was used to detect AMA, which is especially combined with epithelial cells of the renal tubular ending. Over the past few years, the research of the target antigens of AMA has acquired a great progress.

Nine antigens with unknown molecular structure are considered to be the target antigens of AMA, which are M1 - M9.

In recent years, many research institutions regard pyruvate dehydrogenase multi-enzyme complex as the main anti-M2 antibody target antigen.

Antibodies directed to the underlying antigen M2 can be detected in about 90% of all patients with PBC, therefore, the antibody has a high diagnostic sensitivity. Under certain

conditions, it is the marker of PBC. In spite of this, the AMA M2 antibodies can be detected in other rheumatic diseases.

Anti-CENP-B antibody

Anti-CENP-B antibody is correlated with the limited type of systemic sclerosis and primary biliary cirrhosis.

Anti-Sp100 antibody

In the deep research of PBC and chronic arthritis, a special kind of fluorescent karyotype performance (i.e., nuclear dots type) can be detected by indirect immunofluorescence method. Those autoantibodies could produce fluorescence with dot shape in the nucleus. These immune fluorescent performances have a clear difference from the spot type of anti-RNP antibody and typical dot type of anti-CENPB antibody. Its target antigen is a kind of protein abstracted from HeLa cell with the electrophoretic migration rate 95-100 kD. According to its specific immunofluorescence karyotype performance (nuclear dots type) and characteristics of electrophoretic migration, this autoantigen is named as "sp100".

Anti-Sp100 antibodies can be detected in 31% of all patients with PBC. In the group of AMA-negative patients with PBC these antibodies are detected at a frequency of 48%. These antibodies are not detectable in other autoimmune liver diseases. Due to their high level of specificity, the detection of anti-Sp100 antibodies has a significant meaning to the diagnosis of PBC.

Besides PBC, anti-Sp100 antibodies are also found in PBC related rheumatic disease, but the frequency is low as 5% for progressive scleroderma and 1.5% for SLE.

Anti-gp210 antibody

In indirect immunofluorescence method, anti-gp210 antibody in Hep-2 cells appears as a typical nuclear peripheral fluorescent karyotype. The corresponding antigen to these antibodies is glycoprotein 210 on the cell membrane.

Anti-gp210 antibodies can be detected in approx.10% of all patients with PBC, 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 antibody

In the past, more attention is given to the diagnosis of AIH type II because it has special clinical significance - about 82% of the patients may be converted to liver cirrhosis. AIH type II occurred mainly in young women. It is characterized by acute attack and multiple inflammations. Its characteristic in the serology is hypergammaglobulinemia, and at the same time, anti-LKM antibody is positive in most cases. Cytochrome P450 2D6 (P450 enzyme) are the target antigen of these antibodies. These antibodies called as anti-LKM-1 antibody (anti liver-kidney microsomal antibody).

Anti-LKM-1 antibodies are regarded as markers of autoimmune hepatitis type II. However, they can also be detected in around 7% of patients with hepatitis C and, in very rare cases, in patients with halothane-induced hepatitis. Main immune reaction region of the protein is 33-amino acids fragments. Most of the anti-LKM-1 antibodies in positive serum of autoimmune hepatitis patients can identify the linear epitope of the region.

Anti-LC-1 antibody

The target antigen of anti-LC-1 antibodies (anti liver cytosolic antibodies) is 60kd protein identified as liver enzyme specificity (imine methyltransferase/cyclization deaminase). Anti-LC-1 antibodies and anti-LKM-1 antibodies are highly correlated, which are detectable frequently in young patients with AIH type II. 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.

The correlation of anti-LC-1 antibodies with AIH type II patients is higher than anti-LKM-1 antibodies. Anti-LC-1 antibodies are regarded to be highly specific for this disease, although it could also be detectable in 10% of patients with chronic hepatitis and a limited number of patients with AIH type I.

The research results of indirect immunofluorescence (IIF) show that a third of patients with PBC are positive to AMA and also positive to ANA. Over the past 10 years, the target antigens of multiple ANA antibodies which are already confirmed and specific for PBC include granulocyte leukemia (PML) protein and sp100 (fluorescence performance is nuclear dot type), two components of the nuclear pore complexes (gp210 and p62, of which the fluorescence performances nuclear peripheral type).

Anti-SLA/LP antibody

Anti-SLA/LP antibodies are highly specific for AIH. So far they are not found in other diseases or normal people, therefore it has a high diagnostic value.

Anti-SS-A/52kDa antibody

Anti-SSA antibodies are correlated with various autoimmune diseases. They most commonly appear in Sjogren's syndrome, and also in Systemic Lupus Erythematosus (SLE), and occasionally in chronic active hepatitis.

ASSAY PRINCIPLE

The test is based on the principle of the line immune assay (LIA).

Autoimmune liver diseases related antigens are applied as lines on a nitrocellulose membrane. 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, an alkaline phosphatase labeled anti-human IgG antibody is used. After addition of the substrate solution, the appearance of purple blue lines indicates the existence of (auto) antibodies against the respective antigens.

MATERIALS PROVIDED

- 1, **Test Strips** coated with autoimmune liver diseases related antigens, ready for use
1 x 16 strips for 16T; STRIP
- 2, **Sample Diluent** consisting of TBS buffer (pH7.2±0.2), ready for use
1 x 100mL for 16T; DIL
- 3, **Wash Buffer Concentrate** consisting of a 10x concentrate of TBS buffer (pH7.5±0.2), dilute with distilled or de-ionized (D.I.) water before use
1 x 50mL for 16T; BUF WASH 10x
- 4, **Conjugate Solution** consisting of a 10x concentrate of anti-human-IgG ALP conjugate, diluted with sample diluents before use
1 x 3mL for 16T; CONJ 10X
- 5, **Substrate Solution** consisting of NBT/BCIP, ready for use
1 x 30mL for 16T; SUBS
- 6, **Scoring Sheet**
1pcs for 16T;
- 7, **Incubation Tray**
2pcs for 16T;
- 8, **Instruction for Use**
1pcs for 16T;

LOT Lot Number

IVD For In-Vitro Diagnostic Use



DETAILS OF AUTOANTIGENS COATED ON STRIP FOR DIFFERENT TYPES.

Note:

“Yes” represents that the corresponding autoantigen listed in the below form is coated on that type.

“No” represents that the corresponding autoantigen listed in the below form is not coated on that type.

Autoantigen Coated	HB LIVER08	HB LIVER04
AMA M2	Yes	Yes
CENP-B	Yes	No
sp100	Yes	No
gp210	Yes	No
LKM-1	Yes	Yes
LC-1	Yes	Yes
SLA/LP	Yes	Yes
SS-A/52kd	Yes	No

SHELF LIFE AND STORAGE

Kit is stored at 2-8°C until stated expiration date. Do not freeze any kit component. Bring all the test kit reagents to room temperature (18~25°C) before use. Be careful to avoid the reagents to be polluted which will cause incorrect test results.

The shelf life is 18 months under proper storage conditions. Do not use any kits beyond the stated expiration date.

Kit reagents unsealed should be sealed after use and stored at 2-8°C. Unsealed kit reagents are stable for 2 months.

SPECIMEN COLLECTION

Use fresh patient specimens only or freeze samples at -20°C. Freeze samples only one time prior to use. Do not use 56°C heat inactivated samples.

SAMPLE PREPARATION

Dilute serum samples 1:101 with Sample Diluent. **For example, dilute 20µL of sample in 2mL of Sample Diluent.**

REAGENT PREPARATION AND STORAGE

Attention!

Allow the test kit and all its components to reach room temperature before use.

Used bottles should be closed carefully and stored at 2-8°C.

Unused strips should be sealed into the aluminum pouches together with desiccant.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

Wash Buffer Concentrate

Any crystallized salt inside the bottle must be resolved before use. Dilute 1 part with 9 parts distilled or purified water. Diluted wash buffer is stable for 6 weeks stored at 2-8°C.

RCNS H2O

Conjugate Solution

Pipette certain quantity of Anti-human-IgG ALP Conjugate needed and dilute 1:10 with Sample Diluent. If a test strip needs to be incubated, add 1.35mL of Sample Diluent into 0.15mL of

Anti-human-IgG ALP Conjugate. Diluted Anti-human-IgG ALP Conjugate should be used out within 1 day.

RCNS	DIL
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ASSAY PROCEDURE

Attention!

Do not let test strip dry during the incubation steps.

Do not touch test strip with fingers, use tweezers.

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

Please shake test strips at room temperature with gentle agitation during all incubation steps.

All details are valid per strip or patient sample.

- Put test strip into the incubation tray, the side with color coding faces up. Add 1.5mL of Sample Diluent into each incubation tray, and incubate the test strip for 5 minutes at room temperature with gentle agitation, and then remove it.
- Pipette 1.5mL of diluted patient sample and add it into the incubation tray, incubate for 30 minutes at room temperature with gentle agitation.
- Remove diluted samples completely. Wash test strip 3 times using 1.5mL Wash Buffer for 5 minutes with gentle agitation. Remove Wash Buffer after every washing step.
- Pipette 1.5mL diluted conjugate and incubate for 30 minutes at room temperature with gentle agitation.
- Repeat step 3.
- Remove conjugate. Add 1.5mL Substrate and incubate for 10 minutes at room temperature with gentle agitation.
- Remove Substrate. Wash with 1.5mL distilled or purified water for 1 minute at room temperature with gentle agitation. Repeat this wash step for another 2 times.
- Dry test strip, and stick it onto the Scoring Sheet to save the test results.

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 too.
- Intensity function control > intensity cut-off control.

REFERENCE RANGE

The line immune assay (LIA) is a qualitative test method and no reference range is provided. Proportion for diluting patient samples is 1:101.

INTERPRETATION OF RESULTS

Score the test results according to the coloring intensity of the strip as negative, equivocal and positive.

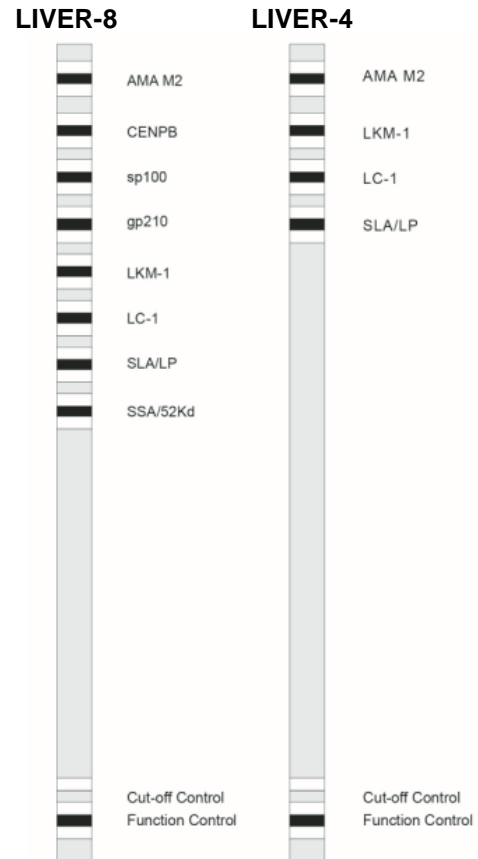
The test result is negative, if no band is to be recognized or if the band exhibits a smaller intensity in comparison to the cut-off control.

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.

Note: Patient samples which are hemolysis with hemoglobin concentration as 5mg/mL, blood-fat with triglyceride concentrations as 20mg/mL and jaundice with bilirubin concentration as 0.4mg/mL have no influence to the test results.

ILLUSTRATION OF THE STRIP



LIMITATIONS

- The intensity of the band color indicating positive results does not necessarily correlate with antibody titers.
- A positive result must be used in associated with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

PERFORMANCE CHARACTERISTICS

- Coincidence rate with negative reference is proved by test as 100%.
- Coincidence rate with positive reference is proved by test as 100%.
- Limit of detection should not be higher than the values as below:

AMA M2	20U/mL
CENP-B	20U/mL
sp100	20U/mL
gp210	20U/mL
LKM-1	20U/mL
LC-1	20U/mL
SLA/LP	20U/mL
SS-A/52kDa	20U/mL

4. Reproducibility

Test the reference reagent for 10 times. Both the test results and coloring are same, which are consistent to corresponding autoantigen type.

5. Inter-lot variation

Test one sample with kits of 3 different lots. Both the test results and coloring are same, which are consistent to corresponding autoantigen type.

6. Stability

The shelf life is 18 months under storage conditions of 2-8°C. Test the kit that is beyond the stated expiry date, the coincidence rate with negative and positive reference, limit of detection and reproducibility should confirm to each formulated performance indicator respectively.














WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. Read the instruction for use carefully before use.
2. Do not use components exceeding the expiry date.
3. Do not combine reagents of other suppliers or kit components of different lots with this kit.
4. 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.
5. All components of this kit should be handled as potentially infectious. Test strips are coated with recombinant and purified native non-anthropogenic extracts from animal tissues, and alkaline phosphatase conjugated goat anti-human IgG.

REFERENCES

Conrad K. et al., Autoantibodies in Systemic Autoimmune Diseases-A Diagnostic Reference; Pabst Science Publisher, Lengerich, Berlin, Riga, Rom, viernheim, Wien, Zagreb, (2002).

SYMBOLS


						
Lot-number	European conformity	Authorized Representative in the European	Sufficient For <n> tests	For In-Vitro Diagnostic use	Temperature Limit	Use before
						
Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when Package is damaged	Do not Re-use	Manufactured by	

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