LKM-1-Ab ELISA

Enzyme immunoassay for the quantitative and qualitative detection of antibodies against liver-kidney microsomes (LKM) in human serum.

REF RE70911

Σ 96

Information: 2-8°C

EU: IVD

U.S.: For research use only. Not for use in diagnostic procedures.
1. Intended Use

**LKM-1-Ab ELISA** is a solid phase enzyme immunoassay employing human recombinant cytochrome p450 IID6 for the quantitative and qualitative detection of antibodies against liver-kidney microsomes (LKM) in human serum. The assay is a tool for the diagnosis of autoimmune hepatitis (AIH).

2. Clinical Application and Principle of the Assay

Autoimmune hepatitis (AIH) is a chronic progressive liver disease of unknown origin that responds well to immunosuppressive therapy, but has a poor prognosis if untreated. Early and accurate diagnosis is therefore of great importance. AIH is characterized by histological features of periportal hepatitis in the absence of viral markers, by hypergammaglobulinemia and, in the majority of patients, by the presence of autoantibodies in serum. Anti-nuclear antibodies (ANA), smooth muscle antibodies (SMA), anti-liver kidney microsomal antibodies (LKM) and antibodies against soluble liver antigen (SLA) are marker autoantibodies for AIH. 52% of AIH patients are positive for ANA and/or SMA, 20% for SLA and 3% for LKM-1. These antibodies are of diagnostic value for AIH but the only autoantibodies highly specific for AIH are SLA. ANA/SMA also occur in 10-15% of patients with viral hepatitis and other immune-mediated diseases. LKM-1 are also associated with hepatitis C.

Three types of LKM antibodies can be distinguished according to the target antigens. LKM-1 antibodies are directed against cytochrome p450 IID6, a 50 kDa cytoplasmic protein found in hepatocytes and renal proximal tubular cells. LKM-2 antibodies are associated with ticrynafen (tienilic acid) -induced hepatitis. The target antigen is cytochrome p450 IIC9, a cytochrome p450 isoenzyme that catalyzes the metabolic oxidation of the drug. LKM-3 antibodies are associated with chronic hepatitis D. The target antigen is UDP-1 glucuronosyl transferase.

LKM-1 associated AIH predominantly occurs in girls between 2 and 14 years of age, thus determination of LKM-1 is very important in pediatrics.

**Principle of the test**

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient’s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.
### 3. Kit Contents

**To be reconstituted:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x Sample Buffer</td>
<td>1 vial</td>
<td>20 ml - 5x concentrated (capped white: yellow solution)</td>
</tr>
<tr>
<td>50x Wash Buffer</td>
<td>1 vial</td>
<td>20 ml - 50x concentrated (capped white: green solution)</td>
</tr>
<tr>
<td>Ready to use:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 vial</td>
<td>1.5 ml (capped green: colorless solution)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 vial</td>
<td>1.5 ml (capped red: yellow solution)</td>
</tr>
<tr>
<td>Cut-off Calibrator</td>
<td>1 vial</td>
<td>1.5 ml (capped blue: yellow solution)</td>
</tr>
<tr>
<td>Calibrators</td>
<td>6 vials</td>
<td>1.5 ml each 0, 3, 10, 30, 100, 300 U/ml (color increasing with concentration: yellow solutions)</td>
</tr>
<tr>
<td>Conjugate</td>
<td>1 vial</td>
<td>15 ml IgG (capped blue: blue solution)</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>1 vial</td>
<td>15 ml (capped black)</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 vial</td>
<td>15 ml (capped white: colorless solution)</td>
</tr>
<tr>
<td>Microtiterplate</td>
<td>12x8</td>
<td>well strips with breakaway microwells</td>
</tr>
</tbody>
</table>

**Material required but not provided:**

- Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-channel pipette or automated system), adsorbent paper.
- Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

### 4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. **Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.**
5. Precautions of Use

5.1 Health hazard data

This product is for in vitro diagnostic use only. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves. WARNING! Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit.
Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 30°C/86°F for automated systems.

Never expose components to higher temperature than 37°C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.
7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:
Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples:
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing:
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:
Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow

For pipetting scheme see Annex: Pipetting scheme.
We recommend pipetting samples and calibrators in duplicate.
Cut-off calibrator should be used for qualitative testing only.

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
8. Quantitative and Qualitative Interpretation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 U/ml</td>
<td>12 - 18 U/ml</td>
<td>&gt;18 U/ml</td>
</tr>
</tbody>
</table>

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgG</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 U/ml</td>
<td>0.046</td>
<td>2.4</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.171</td>
<td>2.6</td>
</tr>
<tr>
<td>10 U/ml</td>
<td>0.372</td>
<td>1.0</td>
</tr>
<tr>
<td>30 U/ml</td>
<td>0.698</td>
<td>3.8</td>
</tr>
<tr>
<td>100 U/ml</td>
<td>1.456</td>
<td>0.4</td>
</tr>
<tr>
<td>300 U/ml</td>
<td>2.396</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Example of calculation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 01</td>
<td>0.533/0.569</td>
<td>0.551</td>
<td>19.8</td>
</tr>
<tr>
<td>P 02</td>
<td>1.156/1.196</td>
<td>1.176</td>
<td>68.7</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Do not use this example for interpreting patients results!

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient’s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative: \[ \text{OD}_\text{patient} < 0.8 \times \text{OD}_{\text{cut-off}} \]

Equivocal: \[ 0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_\text{patient} \leq 1.2 \times \text{OD}_{\text{cut-off}} \]

Positive: \[ \text{OD}_\text{patient} > 1.2 \times \text{OD}_{\text{cut-off}} \]
9. Technical Data

Sample material: serum
Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time: 90 minutes at 20-32°C/68-89.6°F
Calibration range: 0-300 U/ml
Analytical sensitivity: 1.0 U/ml
Storage: at 2-8°C/35-46°F use original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on LKM-1-Ab ELISA (RE70911) gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity
The microplate is coated with recombinant human cytochrome p450 IID6.
No crossreactivities to other autoantigens have been found. Anti-LKM-1 antibodies show a diagnostic specificity of >99% for autoimmune hepatitis type 2. The diagnostic sensitivity of anti-LKM-1 antibodies for autoimmune hepatitis type 2 is 84%. The data has been acquired with the LKM-1-Ab ELISA (RE70911).

Correlation:

The comparability of performance data was assessed with 54 sera tested with both, incubation steps of 30-15-15 minutes (old test version) and 30-30-30 minutes (new test version).
A linear regression analysis of the two products showed that the two products are equivalent.
Included in these sera are 39 sera close to cut-off.
10.3 Linearity
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>measured concentration (U/ml)</th>
<th>expected concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 / 100</td>
<td>78.9</td>
<td>80.0</td>
<td>98.6</td>
</tr>
<tr>
<td>1</td>
<td>1 / 200</td>
<td>39.8</td>
<td>40.0</td>
<td>99.5</td>
</tr>
<tr>
<td>1</td>
<td>1 / 400</td>
<td>18.9</td>
<td>20.0</td>
<td>94.5</td>
</tr>
<tr>
<td>1</td>
<td>1 / 800</td>
<td>9.6</td>
<td>10.0</td>
<td>96.0</td>
</tr>
<tr>
<td>2</td>
<td>1 / 100</td>
<td>34.2</td>
<td>33.0</td>
<td>103.6</td>
</tr>
<tr>
<td>1</td>
<td>1 / 200</td>
<td>17.2</td>
<td>16.5</td>
<td>104.2</td>
</tr>
<tr>
<td>1</td>
<td>1 / 400</td>
<td>8.1</td>
<td>8.3</td>
<td>97.6</td>
</tr>
<tr>
<td>1</td>
<td>1 / 800</td>
<td>4.0</td>
<td>4.2</td>
<td>95.2</td>
</tr>
</tbody>
</table>

10.4 Precision
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Mean (U/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>210.0</td>
</tr>
<tr>
<td>2</td>
<td>77.5</td>
</tr>
</tbody>
</table>

10.5 Calibration
Due the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature

   *Autoimmune Hepatitis.*

   *Autoimmune Hepatitis.*

   *International Autoimmune Hepatitis Group Report: a review of criteria for diagnosis of autoimmune hepatitis.*
   J Hepatol 31: 929-938.

   *LKM-1 autoantibodies recognize a short linear sequence in P450 IID6, a cytochrome P-450 monoxygenase.*

   *A new anti-liver-kidney microsome antibody (anti-LKM-2) in tienilic acid-induced hepatitis.*

   *Recognition of uridine diphosphate glucuronosyl transferases by LKM-3 antibodies in chronic hepatitis D.*
   Lancet 344:578-81
ANNEX: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:
For **quantitative interpretation** use calibrators to establish a standard curve.
For **qualitative interpretation** use cut-off calibrator.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td></td>
<td>NC</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td></td>
<td>NC</td>
<td>P2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
<td></td>
<td></td>
<td>CC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
<td></td>
<td></td>
<td>CC</td>
<td>P3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>CalC</td>
<td>PC</td>
<td>P3</td>
<td></td>
<td></td>
<td>PC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>CalC</td>
<td>PC</td>
<td>P3</td>
<td></td>
<td></td>
<td>PC</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>CalD</td>
<td>NC</td>
<td>...</td>
<td></td>
<td></td>
<td>P1</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>CalD</td>
<td>NC</td>
<td>...</td>
<td></td>
<td></td>
<td>P1</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PC: positive control
NC: negative control
CC: Cut-off calibrator
P1: patient 1
P2: patient 2
P3: patient 3
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Assay/Test:** ____________________  
**Date/Datum:** __________

**Incubation / Inkub.:** 1._________min   2._________min   3._________min

**Temperature/Temperatur:** __________ °F _________ °C

**Signature/Unterschrift:** ______________

**Name:** ______________________________
LIABILITY: Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2012-01-20