Anti-LKM1 Antibodies
Enzyme Immunoassay Test Kit

REF **7052**

Enzyme immunoassay for the quantitative measurement of anti-LKM1antibodies in human serum

October 2014

BIOMERICA

I. INTENDED USE

The Biomerica anti-LKM1 (Liver Kidney Microsome Type 1) antibodies kit is a quantitative enzyme immunoassay for the detection of autoantibodies to LKM1 in human serum. This assay is intended FOR *IN VITRO* DIAGNOSTIC USE ONLY.

II. SUMMARY AND EXPLANATION

Autoimmune hepatitis (AIH) is a chronic progressive liver disease of unknown origin. After exclusion of alcoholic, drug-induced or viral liver disease, a diagnosis of AIH can be established based on epidemiological, histological and biological findings and on a positive response to immunosuppressive therapy.[1,2] Several autoantibodies can be observed in AIH and, although it is not recommended to subdivide patients with AIH based on their autoantibody profile, it is commonly admitted that type 1 autoimmune hepatitis is associated with various anti-nuclear antibodies (ANA) and/or with anti-smooth muscle antibodies (ASMA) reacting with actin cables.[3,4] In contrast, type 2 AIH is characterised by the absence of ANA and ASMA and the presence of antibodies to liver/kidney microsome type 1 (anti-LKM1) and liver cytosol type 1 (anti-LC1). Other autoimmune markers, such as anti-soluble liver antigen /liver pancreas (anti-SLA/LP) have been proposed in the putative type 3 AIH.[5,6]

Three types of anti-LKM autoantibodies have been characterized, the serological marker for type 2 AIH being the LKM type 1 autoantibody first described in 1973 by Rizetto using the immunofluorescence method on rodent liver and kidney sections.[7] Later on, Zanger et al. showed the identity of the LKM1 antigen with the polymorphic cytochrome P-450 db1.[8] Finally, Marms et al. demonstrated that anti-LKM1 autoantibodies recognize a short linear sequence of the recombinant antigen cytochronic mono-oxygenase CYP2D6 (P4502D6).[9]

Type 2 AIH is more common in Europe and in South American countries than in the US for unknown reasons. Genetic factors are probably involved and the disease has been associated with the HLA DRB*07 and DRB1*03 alleles.[10]
In its classical form, type 2 AIH predominantly affects females rather than males (gender ratio 8:1) and most cases occur between the ages of 2 and 14 years.[11]

Prednisone in combination with azathioprine as a corticosteroid-sparing agent is the mainstream treatment for AIH(2). Despite the usual severity of symptoms at diagnosis, most patients respond well to conventional therapy and can avoid a liver transplant.

Most patients will enter into remission, but relapse after withdrawal of therapy occurs in 60 to 80% of children[12] and is also common in adults. Although it appears that the concentration of anti -LKM1 antibodies parallels changes in disease activity,[11] the current consensus is to use histological findings or other biological indicators (aminotransferase, bilirubin) to assess a state of remission.[2] Therefore, the main clinical application of anti-LKM1 testing remains, together with ANA and ASMA testing, the frontline laboratory work up of chronic or acute hepatitis of unknown cause.[13]

Low concentrations of anti-LKM1 autoantibodies can be found in patients with hepatitis C. In Europe, it has been shown that these antibodies react to different epitopes on the recombinant CYP2D6 molecules, compared to antibodies found in type 2 AIH.[14] From an epidemiological standpoint, these patients are quite different from the type 2 AIH group - they are older, predominantly male and with true HCV infection. Screening for ANA, ASMA and anti-LKM1 in HCV patients is now recommended before initiating alpha interferon therapy. While immunosuppressive therapy for AIH is not harmful, interferons may exacerbate the overlapping autoimmune hepatitis.[15,16]

Finally, it should be noted that when AIH is part of an autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED), a rare genetic disease with a Mendelian pattern of inheritance, anti-LKM autoantibodies are detectable by immunofluorescence but not by this ELISA method as they recognize different hepatic cytochromes.[17]

III. PRINCIPLE OF THE TEST

The Biomerica anti-LKM1 Kit is an enzyme immunoassay: polystyrene wells are coated with human recombinant P450 2D6 cytochrome. Serum to be tested is incubated in the well. Anti-LKM1 antibodies, if present, are bound to the P450 2D6 cytochrome-coated solid phase. After washing, rabbit anti-human IgG conjugated with horseradish peroxidase is added. At the end of a second incubation, unbound conjugate is removed by washing. When enzyme substrate is added, a blue color develops if anti-LKM1 antibodies are present in the well. 1N sulfuric acid is added to stop the enzyme reaction and the absorbance of standards, controls or samples are measured using a plate reader with wavelength set at 450 nm.

IV. KIT COMPONENTS

Kit Components	Description	Symbol
Test Strips	96 microwells coated with human recombinant P450 2D6 cytochrome.	**PLA**
Enzyme Conjugate	One (1) bottle containing 10 ml of Rabbit anti-human IgG conjugated to horseradish peroxidase.	**CONJ ENZ**
Calibrators	One set of five vials (1ml each) containing human anti-LKM1 antibodies in diluted human serum at the nominal concentrations of 0, 2, 6, 20, and 60 U/ml.	**CAL0-4**
Positive Control	One (1) vial containing 1 ml of human amti-LKM1 antibodies in human serum.	**CTRL +**
Sample Diluent	One bottle containing 85ml of Phosphate Buffered Saline (PBS).	**DIL**
Substrate Solution	One (1) bottle containing 20 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	**SUBS TMB**
Wash Concentrate (30X)	One bottle containing 13 ml of concentrated tris buffer and preservative.	**BUF WASH 30X**
Stop Solution	One (1) bottle containing 20 ml of 1N sulfuric acid.	**STOP**

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader with 450nm wave-length capability.
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Micropipettes capable of delivering 10 µL, 100 µL, and 1ml.
- Single or multichannel dispenser, 300 µL.
- Wash Bottle (>500ml).
- Absorbent Paper.
- Timer
- Reagent grade (DI) water.
- Sample Dilution Tubes, polystyrene, 12 x 75 mm.
- Graduated cylinder, 500 ml.

V. WARNINGS AND PRECAUTIONS FOR USERS

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- All reagents are for *In Vitro* Diagnostic Use Only.
- Reagents from different kit lots should not be interchanged.
- Do not use reagents that are beyond their expiration dates.
- All reagents should be at room temperature before using.
- The Substrate reagent (SUBS TMB) should not be exposed to strong light during storage or incubation. Ensure that the reagent is colorless before using.
- Wear gloves when performing the test, and handle specimens and used microwells as if able to transmit infectious agents.
- Unused microwells should be stored in the resealable pouch with dessicant to protect them from moisture.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any sample spills.
- Avoid contact with STOP. Stop solution is 1N sulfuric acid. In case of contact with skin or mucous membranes, flush with water immediately.
  WARNING: This kit contains some reagents made with human serum which have been tested and found to be non-reactive for the presence of HBsAg and antibodies to hepatitis C and to HIV. Since no known test method can offer complete assurance that infectious agents are absent, standards, negative control and patient samples should therefore be handled as though capable of transmitting infection.

VI. SAMPLE COLLECTION AND STORAGE

This kit has been validated with serum samples. If specimens are to be stored, they should be refrigerated at 2 to 8 °C or frozen at - 10 °C or lower for long term storage.

VII. REAGENT PREPARATION AND STORAGE

The following reagent preparation steps are required before running the test:

1. **Wash Buffer.**
   Pour the contents of the Wash Buffer Concentrate (BUF WASH 30X) bottle into a 500 ml graduated cylinder. Adjust to 400 ml with deionized water. Transfer the resulting solution to a suitable plastic bottle, label it "Wash Buffer" and store it at 2 - 8 °C.

2. **Serum sample dilution.**
   Dilute 1:100 each patient serum sample in polystyrene test tubes as follows:
   Add **10 µl** of serum to **1 ml** of Sample Diluent (DIL). Mix thoroughly prior to use.

3. **Antigen coated plate preparation (PLA)**
   The plate consists of 12 breakable strips of 8 wells, each coated with human recombinant P450 2D6 cytochrome. Each serum, control, calibrator or blank will occupy one of these wells.
   Calculate the number of wells to be used (see further). Remove the plate from the plastic bag. Remove the strips by inverting the plate and pressing gently the bottom of wells. Break one strip if necessary. Return the strips and wells not required for the test run to the package. Reseal the bag and store at 2 - 8 °C.

Reagent stability and storage

All kit components should be stored at 2 - 8 °C and are stable to the labeled expiration date.

DO NOT FREEZE KIT COMPONENTS.

VIII. ASSAY PROCEDURE

Assay procedure

1. Allow the reagents to come to room temperature.

2. Place an appropriate number of strips and wells in the secure grid. An example of configuration is presented hereafter.

	1	2	3	4	5	6	7	8	9		10 11	
A	Bl	Bl	S2	S2								
B	0	0	S3	S3								
C	2	2	S4	S4								
D	6	6										
E	20	2 0										
F	60	60										
G	C+	C+										
H	S1	S1										

3. Wash each well by adding **300 l Orkit® Buffer.** Invert the strips over a paper towel and blot dry.

4. **Sample, control and calibrator dispensing**

   - Add 100 µl of Calibrators 0– 60 U/ml and Positive Control (undiluted) into the appropriate wells.
   - Add 100 µl of each 1:100 sample dilution.

   Each well now contains 100 µl of sample, calibrator or control, except wells 1A and 2A which serve as blanks.

5. Cover and incubate the wells at room temperature (20-25°C) for **90 minutes.**

6. Discard the contents of the wells by gentle inversion and wash each well by adding **300** µl **Wash Buffer.** Invert the strips over absorbent paper and blot. Repeat **two more times.** Avoid air bubbles in wells. Finally, invert strips over absorbent paper and blot dry.

7. Add 100 µl of the Enzyme Conjugate (CONJ ENZ) to all wells, including blank wells. Cover wells and incubate at room temperature for **30 minutes.**

8. Repeat step 6.

9. Set a timer for 30 minutes, add **100** µl of Substrate Solution (SUBC TMB) to the first well as you start the timer, and continue adding substrate in a steady rhythm through to the last well.

10. Cover wells and allow the Substrate to react at room temperature (20-25 °C) for **30 minutes.** A blue color develops.

11. At 30 minutes, add **100** µl of **Stop Solution (STOP)** to each well including blank wells, in the same rhythm and the same order followed in step 9 above. When all the Stop Solution has been dispensed, mix the Stop Solution with Substrate by gently tapping the strips. A color change from blue to yellow is observed.

12. Blank the microtiter reader using wells 1A and 2A and read the absorbance of each well at 450 nm.

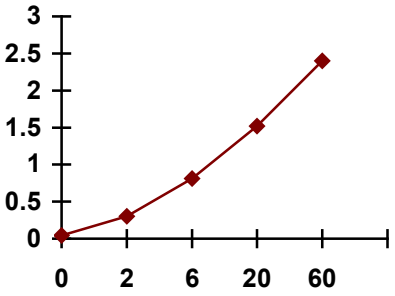
IX. CALCULATION OF RESULTS

Results

- Determine the mean absorbance for each pair of wells (calibrators and samples).
- Plot a curve of absorbance on a semi-log or linear graph paper.
- Read off sample concentrations directly from the curve. **Do not use any correction for dilution factor.**
- Any sample reading greater than the Calibrator 60 U/ml should be further diluted with the Sample Diluent and reassayed.

Example :

Absorbance



Expected values
Samples with serum concentrations above **2 U/ml** are considered positive.

X. QUALITY CONTROL

If the absorbance of the Calibrator 0 U/ml is **> 0.300** at 450 nm, it may indicate a procedural problem and the test should be repeated.

Positive Control > 2 U/ml

XI. PERFORMANCE CHARACTERISTICS

Sensitivity

Minimum detectable dose: 0.2 U/ml

Intra-assay Precision

r = 12	Sample 1	Sample 2	Sample 3
Mean concentration (U/ml)	5.7	47	51
Standard deviation (U/ml)	0.5	3.7	3.7
Coefficient of variation (%)	8.5	7.8	7.2

Interassay Reproductibility

n = 5	Sample 1	Sample 2	Sample 3
Mean concentration (U/ml)	5	12	29
Standard deviation (U/ml)	0.9	1.3	3.2
Coefficient of variation (%)	18	10	11

Linearity

Sample1

Dilution	1/100	1/200	1/400	1/800	1/1600
Expected Concentration U/ml	65	32.5	16.25	8.12	4.06
Measured concentration U/ml	65	31	15.3	9.5	4.9
Recovery%	**100**	**95**	**94**	**116**	**120**

Sample2

Dilution	1/100	1/200	1/400	1/800	1/1600
Expected Concentration U/ml	27	13.5	6.75	3.375	1.688
Measured concentration U/ml	27	13.5	6.5	3.2	1.4
Recovery%	**100**	**100**	**96**	**95**	**83**

Clinical data

In a study of 100 human sera with no known hepatic abnormalities, the following results were obtained with the Biomerica LKM1 Kit:

<0.2 U/ml : 33 samples

0.2 -2 U/ml : 65 samples

2 -4 U/ml : 2 samples

26 human serum samples tested for a suspicion of autoimmune hepatitis were found negative by immunofluorescence and with the LKM1 Orkit® assay.

XII. REFERENCES

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XIII. SYMBOLS

	Storage Temperature
LOT	Lot Code
	Expiration
	Manufacturer
EC REP	Authorized Representative
	Caution, see instructions
IVD	For in vitro diagnostic use
REF	Catalog No.

XIV. ORDERING INFORMATION

ORDERING: Send purchase order to:

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2°C 8°C

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IVD CE

67052-3.doc October 2014

EC REP

according to IVDD 98/79/ EC
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