

DETECTION OF LIVER-KIDNEY MICROSOMAL (LKM-1) ANTIBODIES BY A NEW HUMAN CYTOCHROME P4502D6 ELISA

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ABSTRACT

Objective: Evaluate the performance of a newly developed LKM-1 ELISA designed to detect anti-cytochrome P450 2D6 antibodies, a serological marker of autoimmune hepatitis type 2 (AIH-2), in normal and clinically-defined specimens.

Methods: Three laboratories evaluated the performance of the QUANTA Lite™ LKM-1 ELISA on panels of specimens assembled in their laboratory. Specimens were also tested by immunofluorescence (IFA) on rodent-kidney-stomach-liver slides (INOVA Diagnostics).

Results:

Site 1 (INOVA Diagnostics, San Diego, CA): Testing of 194 specimens from normal, healthy individuals with ages ranging from 1 to 75 years old, resulted in a specificity of 100% (194/194). The panel included 43 pediatric specimens. A second panel of 79 specimens, including 8 known healthy controls and 71 specimens submitted for LKM-1 testing at various clinical laboratories was tested by IFA and by the LKM-1 ELISA. The agreement between the ELISA and IFA results (excluding equivocal results) was 93% (66/71). A variety of other autoimmune and liver disease sera were negative.
 Site 2 (Centre Hospitalier de Luxembourg): Eighty four clinically-defined specimens were tested. All 22 patients described as AIH-2 were positive by both the LKM-1 ELISA and by IFA. Specimens from 34 non-AIH-2 patients which were positive for LKM-1 reactivity by IFA and negative for hepatitis C virus (HCV), were all negative by the LKM-1 ELISA.
 Site 3 (University of Iowa): Forty five clinically-defined sera were tested. All AIH-2 specimens (4/4) were positive by both the ELISA and IFA. Of 34 sera representing a variety of other autoimmune and liver diseases, 29 were negative by LKM-1 antibodies by both ELISA and IFA. Three HCV sera were positive by both the LKM-1 ELISA and IFA, one HCV serum was positive by the LKM-1 ELISA only, and one post-obstructive pancreatitis specimen (from autopsy) was positive by both the LKM-1 ELISA and IFA.

Conclusion: In contrast to the IFA procedure currently used for LKM-1 antibody testing, the QUANTA Lite™ LKM-1 ELISA is reproducible, standardized, and is not dependent on subjective interpretation. The availability of the QUANTA Lite™ LKM-1 ELISA will allow more widespread and reproducible testing for autoimmune hepatitis type 2.

Methodology

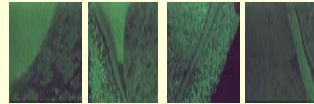
QUANTA Lite™ LKM-1 ELISA

The QUANTA Lite™ LKM-1 ELISA assay uses partially purified full-length recombinant cytochrome P4502D6 antigen bound to the wells of color-coded 96 well polystyrene microwell plates. Patient specimens are run at a 1:101 dilution. The ELISA assays use pre-diluted controls, single point antigen specific calibration, 30 minute room temperature incubations, ready-to-use conjugate, and single vial TMB substrate solution. Results are expressed in arbitrary units.

Normal Range

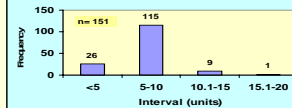
A combined panel of 194 specimens collected from random, asymptomatic, healthy blood donors residing in California and New York was tested with the QUANTA Lite™ LKM-1 ELISA kit to establish a normal range for the assay.

Typical IFA Patterns on KSL Slides

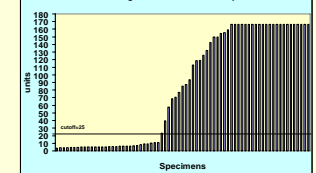


LKM-1+ LKM-1+/GPA+ LKM-1 neg-/AMA+ LKM-1 neg-

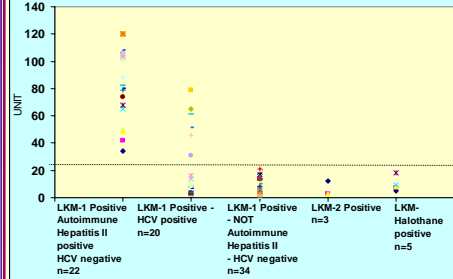
Distribution of Values on Specimens from Healthy Normal Individuals



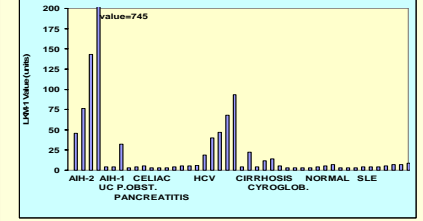
In-House Testing of Normal and LKM-1 Specimens



Evaluation at Centre Hospitalier de Luxembourg - Dr. R. Humbel



QUANTA Lite™ LKM-1 ELISA - External Clinical Study - Dr. J. Goeken, MD, U.Iowa



Comparison of QUANTA Lite™ LKM-1 ELISA and LKM-1 IFA Results

n=79		LKM-1 IFA		
		POS (46)	EQ (8)	NEG (25)
QUANTA LITE™	POS (43)	41	1*	1
LKM-1 ELISA	EQ (0)	0	0*	0
	NEG (36)	5	7**	24

Overall agreement = 91.5% (65/71) (equivocal results excluded)

* one laboratory interpreted this specimen as equivocal, one lab as 2+

** 3 specimens interpreted as 2+, but not clear LKM-1 by external reference lab

INTRODUCTION

Autoimmune hepatitis (AIH) is a heterogeneous disease of unknown etiology¹. Autoimmune hepatitis, type 1 (lupoid AIH) is the more common type of AIH and is characterized by reactivity to antinuclear (ANA), smooth muscle (SMA), and actin antigens. Autoimmune hepatitis, type 2 is characterized by the presence of liver/kidney microsome (LKM-1) antibodies detected on rodent kidney and liver tissue sections by indirect immunofluorescence (IFA). The LKM-1 reactivity is characterized by staining of the hepatocyte cytoplasm and the proximal, but not the distal kidney tubules. Patients with AIH-type 2 (AIH-2) disease tend to be young, female, have severe disease, have a good response to immunosuppressive therapy, and are hepatitis C virus (HCV) negative¹⁻³. The major target antigen of LKM-1 antibodies is cytochrome P450 2D6, a microsomal protein found in the endoplasmic reticulum^{4,5}. Studies have localized the major autoepitopes within the P450 2D6 protein to 4 linear epitopes. Although most patients recognize the sequence extending from amino acids 257 to 269, other patients recognized one or more additional sequences⁶.

LKM-1 antibodies have been reported in 0-8% of patients with chronic HCV infection^{2,3,6,11}. The LKM-1 IFA pattern seen in these HCV patients is indistinguishable from the LKM-1 pattern seen in AIH-2. The actual epitopes recognized by sera from HCV patients however, are different than those recognized by AIH-2 and they appear to be more heterogeneous^{3,6,11,12}. Patients who are HCV positive and LKM-1 positive can present a difficult situation for physicians since the treatments for HCV and AIH-2 differ dramatically. For HCV, alpha-interferon may be used whereas immunosuppression would be considered for AIH-2. Interferon may cause a worsening of AIH, while immunosuppression can be harmful for HCV infected patients¹³⁻¹⁵.

In addition to LKM-1 antibodies, antibodies to cytochrome P450 2C9 (LKM-2) associated with tienilic acid induced hepatitis, and antibodies to uridine diphosphate glucuronosyl transferases (LKM-3) which have been associated with hepatitis D infection, have been identified³. Antibodies to soluble liver antigen (SLA) or liver-pancreas antigen (LP) are reportedly found in some AIH patients who are negative for other autoantibodies and may describe another subgroup of AIH¹⁶.

Conventional detection of LKM-1 antibodies by IFA on rodent tissue sections is labor intensive and requires highly experienced personnel for the subjective interpretation of the fluorescent patterns observed. LKM-1 reactivity is often difficult to read for even experienced personnel and interpretation can vary significantly from lab to lab. The presence of anti-nuclear antibody (ANA) or anti-mitochondrial antibody (AMA) can contribute to difficulties in interpretation. Even experienced IFA readers sometimes report specimens as "positive, but not clear LKM-1". As a result of the difficulty in interpretation, many laboratories do not offer tests for LKM-1. In the present study, we report on the performance of the new INOVA QUANTA Lite™ LKM-1 ELISA for detection of LKM-1 antibodies.

LKM-1 Cross-reactivity Study

Clinical group (n=129)	n=	Pos	Eq.	Neg
Hepatitis C Virus (HCV)*	40**	5	0	35
LKM-2	3	0	0	3
LKM-halothane	5	0	0	5
Soluble liver antigen (SLA)	5	0	0	5
Autoimmune Hepatitis, type 1	15	0	1	14
Crohn's disease	6	0	0	6
Acute colitis	1	0	0	1
Cirrhosis (one alcoholic, one unsp.)	2	0	0	2
Primary biliary cirrhosis	1	0	0	1
Gryoglobulinemia	3	0	0	3
nonalcoholic steatohepatitis	1	0	0	1
Post-obstructive pancreatitis	1	0	0	1
Celiac disease	1	0	0	1
Scleroderma	1	0	0	1
Addison's disease (adrenal pos.)	1	0	0	1
Islet cell antibody (ICA)	2	0	0	2
glutamic acid decarboxylase (GAD)	2	0	0	2
AMA(8), SMA(3), TPO(9)	20	0	0	20
Sm(1), RNP(1), SS-A(1), SS-B(1), SCL-70, Jo-1(1), Ribosome P(1), Chromatin(1), PCNA(1), ANA/DNA(9), Centromere(1)	19	0	0	19

* HCV positive spec. (unselected for LKM-1 reactivity), LKM-1 IFA pos.

**4/5 are LKM-1 IFA positive

Conclusions

1. Interpretation of LKM-1 IFA patterns on Liver-Kidney-Stomach tissue sections is labor-intensive and requires highly skilled personnel for accurate interpretation of IFA pattern. LKM-1 IFA interpretation is often particularly difficult for even experienced laboratories. This can lead to significant differences in interpretation for the same sample. Autoimmune hepatitis, type 2 is a serious disease and uncertainties in IFA pattern interpretation can complicate patient evaluation.
2. Cytochrome P450 2D6 is the major target antigen of LKM-1 antibodies. The use of human recombinant cytochrome P450 2D6 antigen in an ELISA format offers an objective laboratory test for the presence of LKM-1 antibodies.
3. The specificity of the QUANTA Lite™ LKM-1 ELISA was 100% on a panel of 194 specimens collected from asymptomatic, healthy individuals.
4. The apparent sensitivity of the QUANTA Lite™ LKM-1 ELISA ranged from 89.1 to 100% depending on the clinical panel tested.
5. No cross-reactivity was detected with specimens from patients with a wide variety of autoimmune and liver diseases, although cross-reactivity was seen with some HCV positive specimens.
6. Cross-reactivity resulting from HCV infection was significantly less frequent with the Quanta Lite™ LKM-1 ELISA compared to LKM-1 IFA analysis. Since some cross-reactivity is observed however, specimens positive for LKM-1 antibodies should be tested for HCV infection.
7. The INOVA QUANTA Lite™ LKM-1 ELISA offers a highly reproducible, standardized, automatable assay for detection of LKM-1 antibodies that is not dependent on subjective interpretation. Availability of this assay will allow more widespread and accurate testing for LKM-1 antibodies.