

SOLUBLE LIVER ANTIGEN (SLA) ANTIBODY DETECTION BY ELISA AND MULTIPLEXING TECHNOLOGIES

Zakera Gandhi¹, Ansgar W. Lohse², Johannes Herkel², Gary L. Norman¹, ¹INOVA Diagnostics, San Diego, CA; ²Johannes Gutenberg University, Mainz, Germany.

ABSTRACT

Objective: Develop and evaluate assays for the detection of antibodies to soluble liver antigen (SLA). SLA, also known as liver/pancreas antibody, was found to be 100% specific for autoimmune hepatitis (AIH) in a recent study of 2000 sera collected from individuals with various disease conditions and healthy individuals (Wies et al. Lancet 2000; 355:1510). Although SLA antibodies occur in only about 30% of patients with autoimmune hepatitis, they are found in some individuals with AIH who are negative for other autoantibodies.

Methods: Specimens from patients with autoimmune hepatitis, non-autoimmune liver disease, various autoimmune conditions, as well as specimens from healthy individuals were tested by the INOVA QUANTA Lite™ SLA (Soluble Liver Antigen) ELISA. The SLA ELISA test uses a recombinant SLA antigen. The feasibility of simultaneous detection of antibodies to SLA, LKM-1, and M-2 antibodies was also determined using the QuantaPlex™ test system (Luminex).

Results: Site 1 (San Diego): Testing of 132 specimens from normal, healthy individuals with ages ranging from 5 to 69 years old, resulted in a specificity of 100% (132/132). A second panel, consisting of 10 clinically-defined samples obtained from Dr. Lohse (5 positive and 5 negative), was tested and in complete agreement with the results obtained in the Mainz laboratory by an inhibition ELISA assay and western blot analysis. A panel of 18 specimens positive for various autoimmune or disease antibodies, including GBM, LKM-1, ANA, SLE, M-2 and GPA, were all found to be negative on the SLA ELISA.

The multiplex assay was able to clearly discriminate and identify SLA, LKM-1, and M-2 positive samples and offers a promising new methodology for evaluation of patients with suspected liver disease. Site 2 (Mainz): A panel of 200 sera were tested. These included 32 SLA/LP positive AIH sera, 18 SLA/LP negative AIH sera, 100 sera from viral hepatitis patients, 15 sera from patients with non-AIH liver diseases and sera from 35 patients with various disease conditions. 31 of the 32 SLA positive sera were clearly positive and one was equivocal. Excluding the equivocal result, the sensitivity was 100%.

Conclusions: Our data shows that with the exclusion of the 1 equivocal result, the SLA ELISA test had 100% specificity and 100% sensitivity. No cross-reactivity was seen with sera positive for other autoimmune markers. Availability of assays for detection of SLA antibodies will provide a new marker to assist in the diagnosis of patients with autoimmune hepatitis. Detection of SLA antibodies is especially valuable for testing the 10-15% of AIH patients who are negative for conventional autoantibodies.

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory disorder of unknown etiology characterized by hypergammaglobulinemia, increased prevalence among females, HLA association, and favorable response to immunosuppressive treatment. AIH has been classified into 4 different subgroups based on the status of the circulating antibody.

AIH Type 1: This is the most common type of AIH and is often referred to as "Classic" AIH. The principal antibodies seen are anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) directed against actin. Anti-DNA antibodies are seen in some ANA positive cases and anti-mitochondrial antibodies may be seen in association with ANA or SMA. Antibodies to liver-specific asialoglycoprotein are prevalent, but at present they are not used as a diagnostic tool.

AIH Type 2: This is a rare type of AIH. It is characterized by the presence of antibodies to liver-kidney microsomes (LKM-1 antibodies). The precise target antigen has been identified as cytochrome P4502D6. Antibodies to liver cytosol-1 (LC-1) antigen, recently identified as formiminotransferase cycloaminase, have also been proposed as serological markers for AIH 2.

AIH Type 3: This subgroup has been proposed for patients with antibody to soluble liver antigen (SLA). Whether AIH 3 should be recognized as a separate subgroup or as a subset of AIH remains controversial since patients seropositive for anti-SLA are clinically identical to patients who are anti-SLA negative. It has also been reported that anti-SLA antibodies may be found in conjunction with some of the autoantibodies seen in AIH Type 1.

Antibodies to a soluble liver protein were first identified by Manns in 1987. Subsequently, further studies showed that the molecular target for this antibody is a 50 kd cytosolic protein. Expression cloning experiments (Wies et al., Lancet 2000; 355:1510) identified a 422 amino acid protein as the sole target antigen. Amino acids 371 - 409 were found to be the specific region recognized by the SLA antibody. Though liver cytokeratins 8 and 18 and glutathione-S-transferase (GST) were thought to be the putative target antigens of SLA antibodies, there is increasing evidence that this is not the case and the target antigen of SLA antibody is the UGA-suppressor tRNA associated protein. Antibodies to liver-pancreas (LP) and SLA have similar reactivity to the same target antigen and hence are also described as anti-SLA/LP antibodies. Anti-SLA/LP antibodies are very specific for AIH Type 1. They have been reported to occur in 10 - 30% of patients with AIH. They are critical in identifying the approximately 10% of AIH patients who are negative for the more conventional markers. The findings of SLA antibodies in some cases of cytogenetic hepatitis allows diagnostic clarification of these patients and their subsequent treatment.

Availability of a standardized anti-SLA ELISA provides another serological tool for the identification of patients with AIH. An assay for detection of antibodies will also allow for earlier and accurate identification of a small but critical group of patients suffering from AIH who may otherwise be missed because they lack conventional AIH autoantibodies.

Methodology

QUANTA Lite™ SLA ELISA

The QUANTA Lite™ SLA ELISA assay uses a purified recombinant antigen bound to the wells of color-coded 96 well polystyrene microtiter 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 TMB substrate solution. Results are expressed in arbitrary units.

QUANTA Plex™ Liver Disease Panel

The prototype QUANTA Plex™ assay for liver autoantibodies allows for simultaneous testing of LKM-1, SLA/LP and M-2 antibodies in a single assay.

Antigens bound to different colored microscopic beads are mixed and coated on to wells of a 96 well polystyrene microtiter plate. Prediluted controls, diluted patient samples are added to appropriate wells. A fluorescent probe labelled conjugate is added in the second incubation. Results are reported as fluorescent units and are based on the fluorescent intensity measured for each bead. Semiquantitative results can be obtained for each autoantibody reactivity.

External Clinical Evaluation

QUANTA Lite™ SLA ELISA - Mainz laboratory

| | n | POSITIVE | EQUIVOCAL | NEGATIVE |
|--------------------------|-----|----------|-----------|----------|
| AIH - SLA POSITIVE | 32 | 31 | 1 | 0 |
| AIH - SLA NEGATIVE | 18 | 0 | 0 | 18 |
| VIRAL HEPATITIS | 100 | 0 | 0 | 100 |
| NON-AIH LIVER DISEASE | 15 | 0 | 0 | 15 |
| OTHER DISEASE CONDITIONS | 35 | 0 | 0 | 35 |

Excluding the equivocal result, the sensitivity of this assay for the AIH - SLA positive patient group is 31/31 = 100%

Cross-reactivity Study

QUANTA Lite™ SLA ELISA

| | n | POSITIVE | EQUIVOCAL | NEGATIVE |
|-------|---|----------|-----------|----------|
| GBM | 3 | 0 | 0 | 3 |
| LKM-1 | 3 | 0 | 0 | 3 |
| ANA | 3 | 0 | 0 | 3 |
| SLE | 3 | 0 | 0 | 3 |
| M-2 | 3 | 0 | 0 | 3 |
| GPA | 3 | 0 | 0 | 3 |

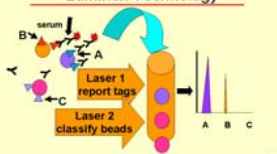
In-house testing of sera positive for various autoimmune conditions

Normal Range

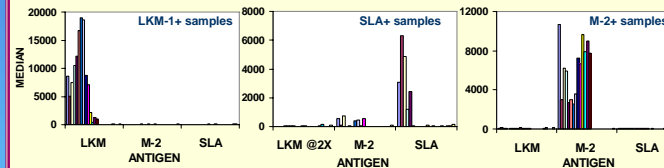
A panel of 132 specimens collected from random, asymptomatic, healthy blood donors (ages 5 - 69) was tested in-house with the QUANTA LITE™ SLA ELISA kit to establish a normal range for the assay.

The specificity of the assay was 100% (132/132) for the panel tested. The values ranged from 0.9 to 5.8 units with a mean value of 2.0 units.

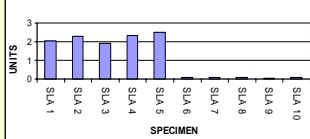
Luminex Technology



Prototype QUANTA Plex™ Liver Disease Panel



SLA CLINICAL PANEL



In-house testing of QUANTA LITE™ SLA ELISA kit on SLA control sera from Dr. Lohse.

Conclusions

1. Anti-SLA antibody is a highly specific diagnostic marker for a small group of AIH type 1 patients that may be negative for other autoantibodies. Detection of SLA antibodies in these patients will help guide earlier diagnosis and treatment of these patients.
2. The specificity of the QUANTA Lite™ SLA ELISA was 100 % on :
 - a) 150 non-AIH patients which included viral hepatitis(100), non-AIH liver disease (15) and other disease conditions (35) tested at Mainz laboratory
 - b) 132 healthy, asymptomatic individuals tested inhouse
 - c) 18 samples seropositive for various autoimmune markers.
3. The QUANTA Lite™ SLA ELISA showed a 100% agreement (with the exclusion of 1 equivocal sample) with results on the 32 AIH - SLA positive samples tested at Mainz Laboratory.
4. The prototype QuantaPlex™ Liver disease multiplex assay showed high specificity and sensitivity on the panel tested. No cross reactivity was seen between the different sera.
5. Availability of commercial, standardised assays for the detection of anti-SLA /LP antibodies in patients serum will allow for more widespread and accurate testing of AIH and aid in identifying the small group of patients who may be negative for other autoantibody markers.