

DETECTION OF MULTIPLE PRIMARY BILIARY CIRRHOSIS-SPECIFIC AUTOANTIBODIES BY A NEW MULTI-ANALYTE, DUAL ISOTYPE PBC SCREEN IgG/IgA ELISA

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Abstract

Objective: To evaluate the performance of a new PBC screening ELISA designed to detect both IgG and IgA antibodies to MIT3 (AMA), gp210, and sp100 antigens in a large cohort of well-characterized specimens.

Method: A combined cohort of 1333 subjects, including 564 individuals with definite PBC and 769 without PBC was evaluated. The PBC group included 120 "AMA-negative" PBC patients. The non-PBC group included sera from healthy controls (520) and individuals with viral hepatitis (149), PSC (48), non-PBC liver disease(23), and other non-liver disease controls(23). All specimens were tested with the Quanta Lite™ PBC Screen IgG/IgA ELISA (INOVA Diagnostics, Inc.) as well as with specific Quanta Lite™ ELISAs for MIT3 IgG, MIT3 IgA, gp210, and sp100. The Quanta Lite™ PBC Screen IgG/IgA ELISA simultaneously tests sera for the presence of IgG and IgA antibodies to the AMA MIT3 recombinant, gp210, and sp100 antigens.

Results: Since one of the goals of new serological tests for PBC is to detect "AMA-negative" PBC patients, our cohort is enriched for these types of specimens. Consequently, sensitivity calculations need to be done both excluding or including these specimens. Overall the PBC Screen interpreted 422 of 444 PBC patients (excluding the AMA-negative patients) as positive, resulting in a sensitivity of 95.0%. Inclusion of AMA-negative PBC patients in the calculation resulted in a sensitivity of 84.2% (475/564). When the same cohorts were tested by the 3 individual ELISA assays, the overall sensitivity for the group excluding AMA-negative specimens was 92.8% (412/444). Inclusion of the AMA-negative specimens yielded a sensitivity of 82.4% (465/564). The PBC Screen and the combined individual assays detected PBC-specific antibodies in 44.1%(53/120) and 43.3% (52/120) respectively of the AMA-negative group. The specificity of the PBC Screen assay was 96.1% (739/769). The mean and median values for the non-PBC specimens were 8.1 and 5.3 units respectively. For comparison, a positive result is greater than or equal to 25 units.

Conclusions: Overall the Quanta Lite™ PBC Screen IgG/IgA ELISA is approximately 2% more sensitive than the combined results of the 3 individual ELISA assays. A positive result can be followed up with testing for the individual antibodies. In addition to confirming the screen results, follow-up testing to specifically assess the presence of anti-gp210 antibodies may be reasonable based on the increasing evidence of the prognostic significance of gp210 antibodies.

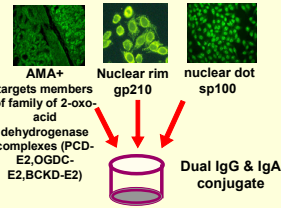
Background

Primary biliary cirrhosis (PBC) is a chronic liver disease characterized by the destruction of the small intrahepatic bile ducts. Progressive duct destruction leads to increasing functional impairment of the liver and, over time, can lead to liver failure and the necessity of liver transplantation.

Serological assays are important aids for recognition and diagnosis of PBC. Anti-mitochondrial antibodies (AMA), detected by immunofluorescence assay (IFA) on kidney/stomach/liver (KSL) or HEP-2 substrates, are the classic serological markers of PBC and can be found in up to 90-95% of PBC patients. IFA is labor intensive and highly dependent on skill and experience of the observer for interpretation of the IFA patterns. Liver disease patients often have antibodies to multiple targets and this can make interpretation difficult and lead to both false positive and negative results. ELISA assays developed using a purified mitochondrial antigen fraction, originally designated "M2" have permitted more sensitive and objective detection of AMA antibodies. More recently Gershwin and Leung created a recombinant fusion protein (MIT3) which includes the immunodominant portions of the 3 primary targets of AMA. This patented antigen has shown to have a higher diagnostic sensitivity than conventional ELISAs for AMA.

Despite the sensitivity of IFA and ELISA assays for AMA, at least 5-10% of PBC patients test negative for AMA. Some of these "AMA-negative" specimens have antibodies to other PBC-specific antigens such as gp210 and sp100. We have previously constructed ELISA assays for detection of AMA (MIT3), gp210, and sp100 antibodies. Optimal detection of PBC-specific antibodies sera would require testing for each of these PBC-specific antibodies individually. Practically however, the costs involved in running 3 tests may discourage this ideal approach and some individuals with PBC may remain unrecognized or present clinicians with diagnostic or therapeutic dilemmas. To address this problem, a new multi-analyte, dual isotype ELISA test which screens for IgG and IgA antibodies to AMA (MIT3), gp210, and sp100 simultaneously in each test sera has been developed. Comparison of the results obtained by this new assay show it as sensitive and specific as testing for AMA (MIT3), gp210, and sp100 antibodies individually. Positive results can be followed-up with testing for individual antibodies. Determination of the antibody specificity responsible for the positive screen test may become increasingly important as clinical significance is ascribed to antibodies, as is the case with gp210 antibodies and their association with more severe liver disease.

PBC Screen ELISA



Methods

QUANTA Lite™ PBC Screen IgG/IgA ELISA

Utilizes a mixture of the recombinant fusion protein MIT3, gp210 peptides, and sp100 peptides

QUANTA Lite™ M2 EP(MIT3) ELISA

recombinant fusion protein MIT3

QUANTA Lite™ sp100 ELISA

sp100 peptide

QUANTA Lite™ gp210 ELISA

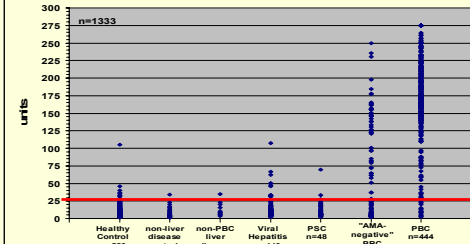
gp210 peptide

Antigens are bound to color-coded 96 microwell polystyrene plates. Patient specimens are run at a 1:101 dilution. All 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 expressed in arbitrary units.

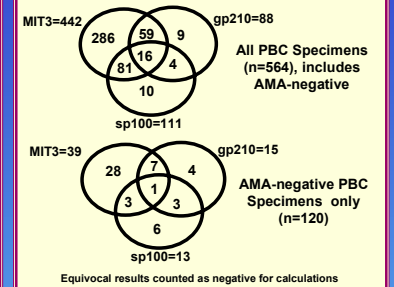
Cutoff Establishment for PBC Screen IgG/IgA ELISA

A panel of 520 specimens collected from healthy individuals and 249 specimens from patients with a variety of non-PBC diseases was tested with the PBC Screen IgG/IgA ELISA kit to establish the cutoff for the assay. The specificity of the assay was 96.1% (739/769) for healthy controls and non-PBC disease sera. Some of the reactive specimens did show AMA reactivity by immunofluorescent examination, so it is possible some might have undiagnosed PBC.

Quanta Lite PBC Screen IgG/IgA ELISA



Overlap of PBC-specific Antibodies



Sensitivity & Specificity

	Combined Individual	PBC Screen
Sensitivity (all PBC, including "AMA-neg", n=564)	82.4%	84.2%
Sensitivity (all PBC, Excluding "AMA-neg", n=444)	92.8%	95.0%
Sensitivity on "AMA-neg" group only, n=120	43.3%	44.1%
Specificity (all non-PBC specimens, n=769)	95.3%	93.5%

Performance of PBC Screen vs Combined Individual Assays

Study Cohort (Includes "AMA-neg." samples)	N=	% Positive on any individual test (MIT3, gp210, sp100)	PBC Screen % positive	% Diff
Vergani	132	75.0%	75.0%	0%
Worman	52	90.4%	92.3%	1.9%
Heathcote	318	82.4%	84.3%	1.9%
Gershwin	55	92.7%	96.4%	3.7%
Overall	557	82.4%	84.0%	1.6%

Study Cohort (Excludes "AMA-neg." samples)	N=	% Positive on any individual test (MIT3, gp210, sp100)	PBC Screen % positive	% Diff
Vergani	74	87.8%	89.2%	1.4%
Worman	52	90.4%	92.3%	1.9%
Heathcote	256	95.3%	96.8%	1.6%
Gershwin	55	92.7%	96.4%	3.7%
Overall	437	93.1%	95.0%	1.9%

Summary and Conclusions

- The new assay detects PBC-specific AMA (MIT3), gp210, and sp100 antibodies of both IgG and IgA subclasses.
- The 95% sensitivity (excluding AMA-negative specimens) of the new screen is slightly greater than that obtained using the combined results of the individual tests (92.8%).
- The specificity of the PBC Screen is 93.4%, slightly lower than the 95.3% of the combined kits.
- Almost 11% of the 120 AMA-negative PBC specimens were only positive for gp210 and/or sp100 antibodies.
- The PBC Screen and the 3 combined individual assays tests found 44.1% and 43.3% respectively of the AMA-negative specimens positive for PBC-specific antibodies.
- The assay provides clinicians with a cost-effective, multi-analyte, dual isotype assay for the detection of serological markers of PBC. This assay should help in the earlier identification, diagnosis, and treatment of PBC patients, especially those negative for conventional markers of PBC.
- The assay is currently under review by the FDA for 510(k) clearance.