

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k061842

B. Purpose for Submission:

New Device

C. Measurand:

Anti-Mitochondrial (MIT3) Antibodies

Anti-gp210 Antibodies

Anti-sp100 Antibodies

D. Type of Test:

Semi-quantitative ELISA

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA LITE™ PBC Screen IgG/ IgA ELISA

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5090 Antimitochondrial antibody immunological test system

2. Classification:

Class II

3. Product code:

DBM, Antimitochondrial Antibody

NRI, Autoantibodies, Nuclear Pore Glycoprotein (gp210)

NUM Autoantibodies, nuclear body protein, sp100

4. Panel:

Immunology 82

H. Intended Use:

1. Intended use(s):

The QUANTA LITE™ PBC Screen IgG/IgA ELISA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of mitochondrial antibodies, gp210, and sp100 antibodies of the IgG and/or IgA class in human serum. The presence of mitochondrial, gp210, and sp100 antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of primary biliary cirrhosis.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Microplate reader capable of measuring OD at 450 nm (or 620 for dual wavelength readings).

I. Device Description:

Each device contains the following: polystyrene microplate strips with breakaway (12x8) microwells coated with PBC Screen antigen (mixture of purified MIT3, sp100

and gp210 antigens); high positive, low positive, and negative controls (human serum); horseradish peroxidase (HRP) wash concentrate; HRP sample diluent; HRP Anti-human IgG/IgA conjugate (goat); TMB chromogen; and 0.344M sulfuric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
 QUANTA LITE™ M2 EP (MIT3)
 QUANTA LITE™ gp210 ELISA
 QUANTA LITE™ sp100 ELISA
2. Predicate 510(k) number(s):
 k052262 (MIT3)
 k040885 (gp210)
 k050662 (sp100)
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Devices
	QUANTA LITE™ PBC Screen IgG/IgA	Individual devices: QUANTA LITE™ M2 EP (MIT3); QUANTA LITE™ gp210; and QUANTA LITE™ sp100
Intended use	To aid in the diagnosis of Primary Biliary Cirrhosis (PBC).	Same
Technology	ELISA	Same
Assay Format	Semi-quantitative	Same
Platform	96 well microtiter plates	Same
Controls	3 levels: negative, low positive and high positive Pre-diluted human serum. Ready to use.	Same
Sample type and dilution	Serum at 1:101	Same
Sample volume required	5 µL	Same
Wash Concentrate	40X Tris-buffered saline and Tween 20	Same
HRP Sample Diluent	Tris-buffered saline, Tween 20, protein stabilizer, preservative	Same
Substrate	TMB Chromogen	Same
Stop solution	0.344M Sulphuric acid	Same
Incubation times	30-30-30 minutes	Same
OD reading	450 nm (or 620 for dual wavelength readings)	Same
Cut-off	25.0 Units	Same

Differences		
Item	Device	Predicate
Antigen	Mixture of three antigens in one assay: purified recombinant M2 EP MIT3, highly purified synthetic gp210 peptide, and highly purified synthetic sp100 peptide	Individual antigens in three separate assays: purified recombinant M2 EP MIT3; highly purified synthetic gp210 peptide; and highly purified synthetic sp100 peptide
Enzyme-Conjugate	Horseradish Peroxidase, Goat Anti-human IgG/IgA	Horseradish Peroxidase, Goat Anti-human IgG

K. Standard/Guidance Document Referenced (if applicable):

CLSI (NCCLS) H18-A3, Vol. 24(38), 2004: Processing Blood Specimens

CLSI (NCCLS) C24-A2, Vol. 19(5), 2006: Statistical quality control

L. Test Principle:

A mixture containing affinity-purified recombinant antigen MIT3 which includes immunodominant portions of PDC-E2, BCOADC-E2, and OGDC-E2, a purified fragment of the sp100 protein, and a purified fragment of the gp100 protein is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in their native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any mitochondrial antibodies present to bind to the immobilized antigen. Unbound sample antibodies are washed away and an enzyme labeled anti-human IgG/IgA conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG/IgA to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG/IgA, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra-assay precision was determined using seven serum samples, with PBC Screen concentration levels ranged from 13.6 to 61.6 Units/mL. These seven samples were tested five times. The positive samples had $\leq 3.7\%$ CV, and the negative samples had $\leq 2.4\%$ CV as listed below.

Specimen	A	B	C	D	E	F	G
Mean units	61.6	39.8	27.9	13.6	27.5	26.5	23.6
SD	2.3	1.0	0.8	0.3	0.9	0.6	0.5
CV (%)	3.7	2.4	3.0	2.4	3.2	2.2	2.2

The inter-assay precision was determined using eight serum samples in duplicate, assayed twice daily (once in the morning and once in the afternoon) for three days. The positive samples had $\leq 6.1\%$ CV, and the negative samples had $\leq 9.2\%$ CV as listed below.

Specimen	A	B	C	D	E	F	G	H
Mean units	67.5	8.4	29.7	35.2	6.3	31.5	29.8	25.2
SD	1.7	0.3	1.2	0.6	0.6	1.9	1.3	0.25
CV %	2.6	3.6	4.0	1.6	9.2	6.1	4.5	1.0

b. Linearity/assay reportable range:

No claims were made regarding linearity for the assay. It is a semi-quantitative assay with results reported out as negative (0.0 – 20.0 Units), positive (≥ 25 Units), or equivocal (20.1 – 24.9 Units), when results are interpreted by comparison to the low positive control value of 25 Units. Specimens with positive results may be tested for the presence of individual MIT3, gp210, and sp100 antibodies with MIT3, gp100, and sp100 specific ELISA assays.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

There is no recognized standard or reference material for antibodies to the PBC Screen multi-antigen mixture. The positive and negative controls are prepared in house and arbitrary units are assigned during the development process.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Interference by endogenous substances: No data provided. The package insert states that grossly hemolyzed, lipemic, microbially contaminated, heat-treated, or specimens containing visible particulate should be avoided in this assay.

Crossreactivity study to other autoantibodies was performed. Results were under in section for ‘Clinical specificity’.

f. Assay cut-off:

The cut-off value of 25 units/mL for the PBC Screen IgG/ IgA assay was established from a combined panel of 1012 specimens collected from 520 asymptomatic healthy individuals and 492 patients with a variety of non-PBC diseases, i.e. 29 autoimmune and infectious diseases: 3 anti-Saccharomyces cerevisiae (ASCA), 4 antinuclear antibody (ANA), 3 tissue transglutaminase antibody (tTg), 2 gastric parietal antibody (GPA), 2 glomerular basement membrane antibody (GBM), 7 Helicobacter pylori, and 8 cytomegalovirus (CMV); 213 autoimmune hepatitis (AIH); 48 Primary Sclerosing Cholangitis (PSC); 10 AIH/PSC; 8 autoimmune cholangitis (AIC); 9 cryptogenic hepatitis; 1 drug-induced hepatitis; 11 vanishing bile duct syndrome (VBDS); 71 HBV; 78 HCV; 3 autoimmune hepatitis type 2; 11 alcoholic liver disease. Using the 25 units/mL cut-off, 98.1% (510/520) of the normal samples were

negative. Of the non-PBC samples, 89% (438/492) were negative. If the possible undiagnosed overlap syndromes (AIH/PCS, VBDS, AIC, cryptogenic hepatitis, AIH, and AMA-negative PBC) were excluded from the non-PBC group, 92% (229/249) of the samples were negative.

2. Comparison studies:

a. *Method comparison with predicate:*

A total of 1209 specimens from seven clinical sites, were tested on PBC Screen IgG/IgA ELISA assay and the individual MIT3, gp210 and sp100 ELISA assays. The samples consisted of 440 PBC (426 definite PBC and 14 AIH/PBC overlap); 48 PSC; 149 viral hepatitis (HBV or HCV); 23 non-PBC liver diseases; 29 infectious or other autoimmune diseases, and 520 healthy individuals. Equivocals were excluded from the percent agreement calculations. Results are summarized below.

		Individual QUANTA LITE™ MIT3, gp210, or sp100 ELISA assay			
		Positive	Equivocal*	Negative	Total
QUANTA Lite™ PBC Screen IgG/IgA	Positive	437	6	8	445
	Equivocal*	10	5	8	23
	Negative	2	3	730	732
	Total	439	14	738	1177

*Equivocal results were excluded from the analysis

Positive percent agreement = 99.5% (437/439)

Negative Percent Agreement = 98.9% (730/738)

Overall Agreement =99.2% (1167/1177)

The 14 Equivocal samples with the individual assays had the following diagnosis: 2 normal, 2 AMA+ PBC, 2 PSC, 2 PBC, 3 HBV, 2 HCV, 1 Alc. Liver Dis. The 23 Equivocal samples with the PBS Screen assay had the following diagnosis: 20 non-PBC samples and 3 PBC samples.

b. *Matrix comparison:*

All assay use serum as matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The same samples in the method comparison study were used for the determination of clinical sensitivity and specificity. Equivocal results were included as negative. The sensitivity was 95.9% (422/440) and the specificity was 96.1% (739/769).

N= 1209		Quanta Lite™ PBC Screen IgG/ IgA		
Patient Group	n	Positive	Equivocal*	Negative
PBC	440	422	3	15
Non-PBC (769): healthy controls (520); other disease controls (249)	769	30	20	719
Total	1209	452	23	734

*Equivocal results were included as negative.

Sensitivity: 95.9% (422/440); (95% CI: 93.6% to 97.6%)
Specificity: 96.1% (739/769) (95% CI: 94.5% to 97.4%)

The 20 Equivocal samples with the PBC Screen assay had the following diagnosis: 12 AIH, 2 Alc. Liver Dis, 1 HBV, 2 HCV, 2 PSC, 1 Non-liver disease.

b. Other clinical supportive data (when a. is not applicable):
Not applicable.

4. Clinical cut-off:

Same as assay cut-off.

5. Expected values/Reference range:

Expected values in the normal population should be negative.

A panel of 520 asymptomatic, healthy individuals residing in the USA was tested with the PBC Screen IgG/IgA assay and with the three individual MIT3, gp210, sp100 ELISA assays. Age and gender data were available for 307 specimens and unavailable for the remaining specimens. The samples were from 150 male and 157 female subjects with age ranging from 18-78 years. The average PBC Screen value for this population was 6.6 units with a median value of 4.6 units. The PBC Screen IgG/IgA assay had a specificity of 98.1% (510/520). The 10 positive samples with PBC Screen assay had the following results: one sample had 105 units and showed a classic AMA staining on HEp-2 cells by IFA whereas the other 9 positive had values ranging from 28-46 units. On further testing, one of these 10 specimen did not have sufficient serum, 6 were positive and 1 equivocal with the MIT3 IgG assay, one sample was negative with all three individual assays, and one was negative with the gp210 and sp100 assays but positive with the MIT3 assay only. Since these were donor samples, it is not possible to verify the true clinical status of the reactive specimens.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.