510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k052262

B. Purpose for Submission:
New Device

C. Measurand:
Anti-Mitochondrial Antibodies

D. Type of Test:
Semi-quantitative ELISA

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA LITE™ M2 EP (MIT3) ELISA

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5090 Antimitochondrial Antibody Immunological Test System
2. Classification:
   II
3. Product code:
   DBM, Antimitochondrial Antibody
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The QUANTA LITE™ M2 EP (MIT3) ELISA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of mitochondria antibodies in human serum. The presence of mitochondria 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 (12-1X8) microwells coated with M2 EP (MIT3) antigen; high positive, low positive, and negative controls (human serum); HRP wash concentrate; HRP sample diluent; HRP Anti-human IgG conjugate (goat); TMB chromogen; and 0.344M sulfuric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   QUANTA LITE™ Mitochondria M2
2. Predicate 510(k) number(s):
   k933180
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
	QUANTA LITE™ M2 EP (MIT3) ELISA	QUANTA LITE™ Mitochondria M2
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
ELISA Negative Control	Pre-diluted human serum. Ready to use.	Same
Sample type and dilution	Serum at 1:101	Same
Sample volume required	5 µL	Same
Enzyme-Conjugate	Horseradish Peroxidase, Goat Anti-human IgG	Same
Substrate	TMB Chromogen	Same
Incubation times	30-30-30 minutes	Same
OD reading	450 nm (or 620 for dual wavelength readings)	Same

Differences		
Item	Device	Predicate
Antigen	Affinity-purified recombinant M2 EP MIT3	Affinity purified Mitochondria M2
High Positive and Low Positive Controls	M2 EP (MIT3) ELISA High and Low positive controls. Pre-diluted. Ready to use.	Mitochondria M2 ELISA High and Low positive controls. Pre-diluted. Ready to use.
Cut-off	25.0 Units	1.3 Units

K. Standard/Guidance Document Referenced (if applicable):
   None referenced.
L. Test Principle:
   The affinity-purified recombinant antigen (MIT3) containing immunodominant portions of PDC-E2, BCOADC-E2, and OGDC-E2 is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any mitochondria antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is

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added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, 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 six serum samples, with M2 EP (MIT3) concentration levels ranged from 7.3 to 168.2 Units/mL. These six samples were tested six times. The positive samples had ≤ 5.7% C.V., and the negative samples had ≤ 13.0% C.V. as listed below.

   Intra-assay Performance of QUANTA Lite^{TM} M2 EP (MIT3) ELISA

	Specimens					
	A	B	C	D	E	F
Mean units	123.8	7.3	55.4	11.8	168.2	8.1
SD	3.46	0.947	3.14	0.957	2.55	0.785
CV %	2.8	13.0	5.7	8.1	1.5	9.7

   The inter-assay precision was measured using five serum samples in duplicate, assayed twice daily for three days. The positive samples had ≤ 2.9% C.V., and the negative samples had ≤ 16.2% C.V. as listed below.

   Inter-assay Performance for QUANTA Lite^{TM} M2 EP (MIT3) ELISA

	HPC	NC	Spec A	Spec B	Spec. C	Spec. D	Spec. E
Mean units	112.7	2.2	110.3	7.8	54.4	11.6	160.3
SD	2.98	0.16	2.56	1.27	1.57	0.61	4.30
CV %	2.6	7.4	2.3	16.2	2.9	5.3	2.7

   b. *Linearity/assay reportable range:*
   Not applicable.

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   There is no reference standard for M2 EP (MIT3). 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, icteric, lipemic, microbially contaminated, or heat-treated samples should be avoided in this assay.

   f. *Assay cut-off:*

The cut-off value of 25 units/mL for the M2 EP (MIT3) assay was established from a combined panel of 683 specimens collected from 520 healthy individuals and 163 patients with a variety of non-PBC diseases, i.e. 31 autoimmune and infectious diseases; 70 autoimmune hepatitis (AIH); 46 primary sclerosing cholangitis (PSC); 8 AIH/PSC, and 8 suspected liver disease. The assay specificity for normal individuals was 98.4% (512/520). The assay specificity for the combined panel of healthy individuals and non-PBC patients was 97.8% (668/683). And the assay specificity is 98.7% (590/598), if the following patient population with suspected liver disease, unconfirmed AIH or AIH/PSC and selected Anti-Mitochondrial Antibody (AMA) negative PBC were excluded.

2. Comparison studies:
   a. *Method comparison with predicate:*
   Testing was performed on 980 diagnosed PBC cohorts from seven clinical sites. The Positive percent agreement was 98.8% (686/694); the Negative Percent Agreement was 40.6% (116/286) and the Overall Agreement was 81.8% (802/980). Although the Negative Percent Agreement is low, the new device with a new recombinant antigen recognizes additional autoantibodies on clinically diagnosed PBC patients. The improved clinical correlation of the new device supports the device effectiveness.

		QUANTA LITE™ Mitochondria M2			
		Positive	Equivocal*	Negative	Total
QUANTA Lite™ M2 EP (MIT3)	Positive	686	0	170	856
	Equivocal	3	0	8	11
	Negative	5	0	108	113
	Total	694	0	286	980

*Equivocal results are considered negative in the analysis

   b. *Matrix comparison:*
   Not applicable.
3. Clinical studies:
   a. *Clinical Sensitivity and Specificity*:
   The clinical sensitivity and specificity study was evaluated on 1578 clinically defined samples from patients with the following diagnosis: 967 PBC; 13 PBC/AIH; 47 PSC; 31 autoimmune and infectious diseases, and 520 healthy individuals. The sensitivity was 87.3% (856/980) and the specificity was 98.7% (590/598).

N=1578		Quanta Lite™ M2 EP(MIT3) ELISA		
Patient Group	n=	positive	equivocal*	negative
PBC(967) or PBC/AIH (13)	980	856	2	122
healthy controls (520); PSC (47), other disease controls (31)	598	8	4	586

*Equivocal results were included as negative.

Sensitivity: 87.3% (856/980) (95% CI: 85.1% to 89.4%)

Specificity: 98.7% (590/598) (95% CI: 97.4% to 99.4%)

  b. Other clinical supportive data (when a. and b. are not applicable):
   Not applicable.
4. <u>Clinical cut-off</u>**:**
 Same as assay cut-off.
5. <u>Expected values/Reference range:</u>
 Expected values in the normal population should be negative.

 A study was performed on a panel of 520 asymptomatic, healthy individuals residing in the USA was tested for both the M2 predicate device and the MIT3 new device. Age and gender data were available for 299 specimens and unavailable for the remaining specimens. The age range was from 18-78 years and included 150 male and 149 female. The average MIT3 value for this population was 7.6 units with a median value of 6.0 units. The MIT3 assay had a specificity of 98.5% (512/520). The M2 predicate had a specificity of 99.2% (566/520). The 8 positive samples with MIT3 assay had the following results: 1 had 26 units, 4 had 30-38 units, 1 had 42 units, 1 had 68.7 units, and 1 had 82.4 units. Two of these samples were also positive with the M2 predicate device. The 8 positive samples were run on the IFA Hep-2 cells and the 2 samples that were positive in both devices showed classic AMA patterns. The other 6 samples showed weak cytoplasmic staining, but not a pattern that would be interpreted as AMA. 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.