510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K163133

B. Purpose for Submission:

New device

C. Measurand:

IgG autoantibodies specific for mitochondrial proteins
IgA/IgG/IgM autoantibodies specific for mitochondrial proteins

D. Type of Test:

Immunoassay, qualitative and semi-quantitative

E. Applicant:

IMMCO Diagnostics Inc.

F. Proprietary and Established Names:

ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgG ELISA

ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM 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, indirect immunofluorescent, antigen, control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended uses:

   ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgG ELISA:
   An enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of anti-mitochondria IgG antibodies in human serum to aid in the diagnosis of primary biliary cirrhosis (PBC) in conjunction with other laboratory tests and clinical findings.

   ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:
   An enzyme linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of anti-mitochondria antibodies (AMA) in human serum to aid in the diagnosis of primary biliary cirrhosis (PBC) in conjunction with other laboratory tests and clinical findings.

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statements:

   For prescription use only

4. Special instrument requirements:

   An ELISA microplate reader capable of reading absorbance values at 450 nm. If a dual wavelength microplate reader is available, the reference filter should be set at 600–650 nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of 12 x 8-antigen coated microwell strips, negative control (1x 1.75 mL), positive control (1x 1.75 mL), five assay calibrators (5x 1.75 mL), horseradish peroxidase anti-human IgA/IgG/IgM or anti-human IgG conjugate (1x 15 mL), TMB enzyme substrate (1x 15 mL), stop solution (1x 15 mL), wash buffer (2 vials) and diluent (1x 60 mL). The results are read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL) and reported as positive or negative.

J. Substantial Equivalence Information:

1. Predicate device name (Predicate 510(k) number):
   INOVA QUANTA Lite™ M2 EP (MIT3) ELISA (K052262)
   Trinity Captia™ Mitochondria IgA/IgG/IgM Screen (K982121)

2. Comparison with predicate:

Similarities		
Item	**New Device** ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA	**Predicate** INOVA QUANTA Lite™ M2 EP (MIT3) ELISA
Intended Use	An enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of anti-mitochondria IgG antibodies in human serum to aid in the diagnosis of primary biliary cirrhosis (PBC) in conjunction with other laboratory tests and clinical findings.	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.
Assay Type	ELISA	Same
Capture Antigen	Recombinant BCOADC, PDC, and OGDC	Same
Sample Type	Serum	Same
Assay Format	Semi-quantitative and qualitative	Same
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgG	Same
Enzyme Substrate	Tetramethylbenzidene (TMB)	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Signal/Wavelength	Optical density/ 450nm	Same
Instrumentation	Microwell plate reader	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA	**Predicate** INOVA QUANTA Lite™ M2 EP (MIT3) ELISA
Calibrators	Set of five: Values in EU/mL: 160, 80 40, 20, 1	One calibrator Value in U/mL
Linear Range	3.3 EU/mL–160 EU/mL	Not specified
Limit of Detection	3.3 EU/mL	Not specified

Differences		
Item	**New Device** ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA	**Predicate** INOVA QUANTA Lite™ M2 EP (MIT3) ELISA
Sample dilution	1:101	1:51
Cutoff	20 EU/mL	20 Units
Results Interpretation	Negative: < 20 EU/mL Indeterminate: 20–25 EU/mL Positive: > 25 UE/mL	Negative: ≤ 20 EU/mL Equivocal: 20.1–24.9 EU/mL Positive: ≥ 25 UE/mL

Similarities		
Item	**New Device** ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA	**Predicate** Trinity Captia Mitochondria IgA/IgG/IgM Screen
Intended Use	An enzyme linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of anti-mitochondria antibodies (AMA) in human serum to aid in the diagnosis of primary biliary cirrhosis (PBC) in conjunction with other laboratory tests and clinical findings.	On the IFU form: Intended for the in vitro determination of mitochondrial (M2) antibodies. Anti-M2 assays are used as an aid in the diagnosis of Primary Biliary Cirrhosis. In the Package Insert: The Trinity Biotech Captia™ Mitochondria Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection and semi-quantitation of antibodies to mitochondria in human sera. The assay is to be used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of primary biliary cirrhosis. For in vitro diagnostic use. High complexity test.
Assay Type	ELISA	Same
Sample Type	Serum	Same
Assay Format	Semi-quantitative and qualitative	Same
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgA/IgG/IgM	Same

Enzyme Substrate	Tetramethylbenzidene	Same
Instrumentation	Microwell plate reader	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA	**Predicate** Trinity Captia Mitochondria IgA/IgG/IgM Screen
Calibrators	Set of five: Values in EU/mL: 160, 80 40, 20, 1	One
Capture Antigen	Recombinant BCOADC, PDC, and OGDC	Native immunoreactive domain of E2-PDC
Linear Range	3.3 EU/mL–160 EU/mL	Not specified
Limit of Detection	3.3 EU/mL	Not specified
Sample dilution	1:101	1:21
Results Interpretation	Negative: < 20 EU/mL Indeterminate: 20–25 EU/mL Positive: > 25 UE/mL	Index Value: ≤ 0.90 Negative 0.91–1.09 Equivocal ≥ 1.10 Positive

K. Standard/Guidance Document Referenced:

CLSI guideline EP05-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline Second Edition"

CLSI guideline EP06-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"

CLSI guideline EP07-A2, "Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition"

CLSI guideline EP09-A2, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline."

CLSI guideline EP12-A2, "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline–Second Edition"

CLSI guideline EP17-A2, "Evaluation of Detection Capability for Clinical. Laboratory Measurement Procedures; Approved Guideline–Second Edition"

L. Test Principle:

Mitochondrial antigen mixture composing of recombinant the E2 subunit of the branched-chain 2-oxoacid dehydrogenase complex (BCOADC-E2), the E2 component of pyruvate dehydrogenase complex (PDC-E2) and the E2 subunit of the 2-oxoglutarate dehydrogenase complex (OGDC-E2) is bound to the wells of a polystyrene microwell plate followed by blocking of the unreacted sites to reduce non-specific binding. Controls, calibrators and diluted patient sera are added to separate wells, allowing any anti-mitochondria antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. After washing away any unbound conjugate, specific enzyme substrate (TMB) is then added to the wells. After stopping the enzymatic reaction, the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL).

Semi-quantitative results are determined from a series of five calibrators (160 EU/mL, 80 EU/mL, 40 EU/mL, 20 EU/mL, and 1 EU/mL). Values less than 20 EU/mL are considered negative results while values greater than 25 EU/mL are considered positive; results between 20 EU/mL and 25 EU/mL are considered 'indeterminate/borderline'. The sponsor states the following recommendations in the Package Insert: "*Indeterminate/borderline results should be retested and evaluated along with other laboratory methods*".

Qualitative results are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values greater than or equal to 20 EU/mL are considered positive and <20 EU/mL values are considered negative.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*
   Semi-Quantitative Precision:
   Precision performance was evaluated in accordance with CLSI guideline EP05-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline". A panel consisting of seven patient sera with levels of AMA antibodies that cover the analytical measuring range was assayed in duplicate, twice a day, for 20 days with one reagent lot (a total of 80 replicates per sample). Testing was performed by two operators using different equipment sets. The first operator performed the assay with a multi-channel pipettor, microplate washer and microplate reader. The second operator used different instruments with the same type of equipment. All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below for each assay:

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

Specimen	Mean (EU/mL)	Within-Run (Repeatability)		Between-Run		Between-Day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	13.8	0.8	5.8	1.1	7.9	0.6	4.2	1.4	9.8
2	16.0	0.7	4.1	1.0	6.0	1.2	7.7	1.6	10.0
3	19.7	0.7	3.7	1.3	6.7	0.4	2.0	1.5	7.6
4	23.6	0.7	3.1	1.1	4.5	0.6	2.3	1.3	5.5
5	78.1	3.2	4.1	6.4	8.2	1.9	2.5	7.2	9.2
6	111.2	4.5	4.0	7.8	7.0	1.1	6.9	9.0	8.1
7	159.9	4.9	3.0	8.8	5.5	2.7	1.7	10.0	6.3

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

Specimen	Mean (EU/mL)	Within-Run (Repeatability)		Between Run		Between-Day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	11.2	0.3	2.2	0.7	5.8	0.3	2.2	0.7	6.6
2	15.5	0.2	1.2	0.5	3.2	0.3	2.0	0.6	4.0
3	20.3	0.3	1.4	0.7	3.2	0.9	4.4	1.1	5.6
4	24.5	0.5	1.8	1.2	5.0	0.7	3.0	1.5	6.1
5	51.6	0.5	1.1	2.0	3.8	0.2	0.3	2.0	4.0
6	104.7	1.4	1.4	4.2	4.0	2.3	2.2	4.4	4.2
7	145.3	1.4	1.0	4.8	3.3	0.1	0.1	5.0	3.4

Qualitative Reproducibility:

Studies were performed under the guidance of CLSI EP12-A2 "User Protocol for Evaluation of Qualitative Test Performance." Eighty replicates of each patient serum in the negative range, ~20% below cut-off, at cut-off, ~20% above cut-off and in the moderate positive range of the assays were performed to determine intra-assay qualitative reproducibility. Results were calculated using single-point (qualitative) analysis as indicated in the product insert. All values for qualitative agreement were within the manufacturer's pre-determined acceptance limit. Results are summarized below.

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/negative	%
Negative	13.5	80	0/80	100
Cut-off −20%	15.8	80	2/78	98
Cut-off	19.6	80	30/50	63
Cut-off +20%	23.5	80	80/0	100

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/negative	%
Moderate Positive	83.6	80	80/0	100
Moderate to High Positive	101.6	80	80/0	100
High Positive	142.1	80	80/0	100

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/negative	%
Negative	10.5	80	0/80	100
Cut-off −20%	15.2	80	0/80	100
Cut-off	20.2	80	34/46	58
Cut-off +20%	24.2	80	79/1	99
Moderate Positive	56.0	80	80/0	100
Moderate to High Positive	89.1	80	80/0	100
High Positive	118.0	80	80/0	100

Lot-to-lot Reproducibility:
To evaluate lot-to-lot reproducibility, a panel of seven samples with levels of AMA antibodies spanning the assay range was tested in duplicate on three different lots over the course of five days. All %CV values were within the manufacturer's pre-determined acceptance limit. The results are summarized in the table below for each assay:

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

Specimen	Mean (EU/mL)	Within-Run		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	4.1	0.1	1.4%	0.1	1.5%	0.1	1.9%
2	16.2	0.6	3.9%	0.3	2.1%	0.7	4.0%
3	20.5	1.2	6.0%	0.4	1.7%	1.2	5.7%
4	24.2	0.9	3.7%	1.1	4.7%	0.9	3.9%
5	57.1	3.7	6.5%	2.1	3.7%	3.4	5.9%
6	67.2	5.7	8.4%	3.4	5.1%	5.8	8.6%
7	149.0	5.7	3.9%	6.2	4.2%	7.5	5.0%

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

Specimen	Mean (EU/mL)	Within-Run		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	10.7	1.0	9.4%	0.4	3.4%	0.9	8.8%
2	16.6	1.3	7.5%	0.9	5.1%	1.3	7.9%
3	21.5	1.9	8.6%	0.7	3.4%	1.8	8.5%
4	28.3	1.6	5.8%	1.0	3.4%	1.6	5.6%
5	38.3	3.3	8.6%	0.8	2.0%	3.0	7.8%

Specimen	Mean (EU/mL)	Within-Run		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
6	60.7	1.5	2.5%	1.2	2.1%	1.4	2.3%
7	125.3	2.8	2.2%	2.1	1.6%	2.9	2.3%

b. Linearity/assay reportable range:

Linearity and recovery were tested by diluting positive specimens across the measuring range in equidistant dilutions with negative patient sera. The observed values were graphed against the calculated values and a linear regression was performed. Results summarized in the table below were within the manufacturer's pre-determined acceptance criteria.

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

Sample	Test Range (EU/ml)	Slope (95% CI)	Y-intercept (95% CI)	R^2	% Recovery
1	3.4 to 57.4	0.99 (0.94 to 1.04)	0.94 (−0.858 to 2.74)	0.997	93% to 102%
2	6.0 to 81.0	1.00 (0.95 to 1.05)	−0.89 (−3.28 to 1.49)	0.9978	100% to 109%
3	54.9 to 162.4	0.96 (0.89 to 1.03)	3.15 (−5.04 to 11.34)	0.995	95% to 106%

ImmuLisa™ Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

Sample	Test Range (EU/ml)	Slope (95% CI)	Y-intercept (95% CI)	R^2	% Recovery
1	5.9 to 31.9	1.01 (0.95 to 1.07)	0.39 (−0.89 to 1.67)	0.997	95% to 98%
2	28.9 to 83.4	1.08 (0.92 to 1.23)	-5.94 (−15.48 to 3.59)	0.979	96% to 113%
3	46.6 to 166.5	0.93 (0.83 to 1.03)	5.68 (−5.48 to 16.84)	0.989	95% to 108%

The linear range was determined to be 3.3–160 EU/mL for the AMA IgG assay and 3.3–160 EU/mL for the AMA IgA/IgG/IgM assay. For test results above 160 EU/mL, the package insert recommends dilution and retesting of the samples.

High dose hook effect: Four high concentrations specimens were serially diluted to assess the presence of any artifactual decrease in assay signal associated with AMA antibody excess (hook effect). No hook effect was demonstrated in sample levels up to 15,356.8 EU/mL in the AMA IgG assay and 17,915.7 EU/mL in the AMA IgA/IgG/IgM assay.

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

i. *Traceability*:

There are currently no recognized international standards for the measurement of AMA antibodies. Calibrator and Control values for both assays are directly traceable to in-house standards.

ii. *Value Assignment:*

Calibrators and positive controls are dilutions of pooled AMA antibody positive sera obtained from various commercial plasma centers. The calibrators and controls are taken from different pooled sera. In order to have traceability with newly made calibrators, each new lot of calibrators is assayed on an already cleared kit and compared to the existing calibrators as reference. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards. Five calibrator levels (Cal A-E) with assigned values from 0–160 EU/mL are included to provide semi-quantitation and must be used with each run. The cut-off calibrator for the qualitative analysis is derived from Cal D. Test results greater than or equal to Cal D are considered positive.

For semi-quantitative determinations the positive controls must give values in the range stated on the vial. The negative control contains negative human serum in buffer. The negative control must be <10 EU/mL.

iii. *Stability:*

Shelf-life Stability:
Accelerated and real-time stability studies for each ImmuLisa Enhanced AMA Antibody ELISAs were conducted on three lots of components/reagents. The accelerated stability study was conducted with materials incubated at 37°C. Data from the accelerated stability study support a shelf-life stability of 18 months. A real-time stability study is on-going and currently supports an 18-month shelf-life stability claim.

Open Kit Stability:
For the open kit stability study, materials in each ImmuLisa Enhanced AMA Antibody ELISA are opened and stored in a dark environment as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Data from the open kit stability study demonstrate that opened reagents are stable at 45 days, but the sponsor chose a more restrictive one month open kit stability claim.

Sample Stability and Storage:
The package insert recommends that sera be assayed soon after separation or stored in aliquots at 2–8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples. It is recommended that frozen specimens be tested within one year.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI EP17-A2. The Limit of Blank (LoB) was determined by assaying a blank sample in 60 replicates. The limit of detection (LoD) was determined by assaying six low negative sera in 10 replicates over three days using two reagent lots of each ImmuLisa Enhanced AMA Antibody ELISAs. The LoB and LoD for the AMA IgG assay was 1.6 and 3.3 EU/mL, respectively. The LoB and LoD for the AMA IgA/IgG/IgM assay was 1.5 and 3.3 EU/mL, respectively.

e. Analytical specificity:

i. Endogenous Interference:

Interference studies were performed according to CLSI EP07-A2 by testing five serum samples with AMA antibody levels corresponding to the negative range, near the assay cut-off, and in the low, moderate and high positive range. Each sample was mixed with known quantities of potentially interfering substances and analyzed in one assay run with two replicates on one kit lot of each assay. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. No significant interference was detected within the manufacturer's pre-determined acceptance criteria for the following substances up to the concentrations listed in the table below:

Potential Interfering Compound	**Test Concentration**
Hemoglobin	2 g/L
Bilirubin	342 µmol/L
Triglycerides	37 mmol/L
Rheumatoid Factor	100 EU/mL
Total cholesterol	13 mmol/L

The 'Limitations of Procedure' section of the Package Insert states "*This test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples*".

ii. Cross-reactivity:

Numerous potentially cross-reactive autoimmune and infectious disease sera were tested for AMA antibody levels. Refer to test results for 898 serum samples from patients in the Non-Target Disease Group in the table presented in the section below on Clinical studies. The number of serum samples tested positive is 12 (0.1%) for AMA IgG and 15 (0.2%) for AMA IgA/IgG/IgM.

f. Assay cut-off:

The assay cut-offs for the ImmuLisa Enhanced AMA Antibody ELISAs were determined by testing specimens from 128 healthy blood donors. Using the 97.5th

percentile value of the results obtained, an arbitrary value of 20 EU/mL was assigned for the cut-off to establish the following result interpretations:

AMA antibody value	Result interpretation
<20 EU/mL	Negative
20–25 EU/mL	Indeterminate (Borderline)
>25 EU/mL	Positive

The cut-off using the 20 EU/mL was validated in a separate study using samples from healthy blood donors and samples from patients with autoimmune, infectious, and other conditions expected to be found in the differential diagnosis of PBC; 2.3% (3/130) and 3.8% (5/130) of the samples from were positive for AMA IgG and AMA IgA/IgG/IgM, respectively.

2. Comparison studies:

a. Method comparison with predicate device:

For analysis of agreement with the predicate device, clinically defined serum samples from the target disease (PBC) and non-target disease group (defined in Section 3.a, below) were tested with both the predicate and the new device. A total of 434 samples were tested by the AMA IgG assay; 154 PBC samples and 289 non-target disease samples were included. A total of 453 samples were tested by the AMA IgA/IgG/IgM assay; 163 PBC samples and 290 non-target disease samples were included. Only specimens in the linear range of both the predicate device and the new device were included in the method comparison. The semi-quantitative results for each assay are summarized below:

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

		Predicate AMA IgG ELISA			
		Pos	Equiv	Neg	Total
ImmuLisa Enhanced AMA IgG	Pos	98	11	14	123
	Equiv	3	0	1	4
	Neg	6	5	296	307
	Total	107	16	311	434

Both assays equivocal samples considered positive:

Positive % Agreement	91.1% 112/123	(95%CI: 84.2 – 95.2)
Negative % Agreement	95.2% 296/311	(95%CI: 92.0 – 97.2)
Overall % Agreement	94.0% 408/434	(95%CI: 91.2 – 96.0)

Both assays equivocal samples considered negative:

Positive % Agreement	91.6% 98/107	(95%CI: 84.2 – 95.8)
Negative % Agreement	92.4% 302/327	(95%CI: 88.8 – 94.9)
Overall % Agreement	92.2% 400/434	(95%CI: 89.1 – 94.4)

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

		Predicate AMA IgA/IgG/IgM ELISA			
		Pos	Equiv	Neg	Total
ImmuLisa Enhanced AMA IgA/IgG/IgM	Pos	91	22	18	131
	Equiv	4	5	5	14
	Neg	3	2	303	308
	Total	98	29	326	453

Both assays equivocal samples considered positive:

Positive % Agreement	96.1%	122/127	(95%CI: 90.6 – 98.5)
Negative % Agreement	92.9%	303/326	(95%CI: 89.5 – 95.4)
Overall % Agreement	93.8%	425/453	(95%CI: 91.1 – 95.8)

Both assays equivocal samples considered negative:

Positive % Agreement	92.9%	91/98	(95%CI: 85.3 – 96.8)
Negative % Agreement	88.7%	315/355	(95%CI: 84.9 – 91.7)
Overall % Agreement	89.6%	406/453	(95%CI: 86.4 – 92.2)

b. Matrix comparison:

Serum is the only matrix indicated for these assays.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 1091 serum samples were included in the clinical validation for the ImmuLisa™ Enhanced AMA Antibody ELISAs. The validation set of samples includes the target disease group consisting of 193 PBC subjects, and a non-target disease group consisting of 898 samples from subjects with autoimmune, infectious, and other conditions expected to be found in the differential diagnosis of PBC. Test results for each individual disease and both disease groups are shown below:

	AMA IgG			AMA IgA/IgG/IgM	
Condition	n	n Pos*	% Pos	n Pos*	% Pos
Target Disease Group					
Primary biliary cirrhosis (PBC)	193	165	85.5	168	87.0
Non-Target Disease Group					
Type 1 Autoimmune hepatitis (AIH)	31	1	3.2	4	12.9

	AMA IgG			AMA IgA/IgG/IgM	
Condition	n	n Pos*	% Pos	n Pos*	% Pos
Type 2 Autoimmune hepatitis (AIH)	7	0	0	0	0
Primary sclerosing cholangitis (PSC)	20	0	0	0	0
Antiphospholipid syndrome	48	0	0	0	0
Celiac disease	99	0	0	0	0
Crohn's disease	25	0	0	0	0
Mixed connective tissue disorder	16	0	0	0	0
Myositis	26	0	0	0	0
Rheumatoid arthritis	106	1	0.9	0	0
Sjogren's syndrome	54	0	0	0	0
Systemic lupus erythematosus	154	0	0	0	0
Systemic sclerosis	26	0	0	1	3.8
Ulcerative colitis	25	0	0	0	0
CMV	20	1	5.0	1	5.0
HepC	74	0	0	0	0
HSV 1	20	0	0	3	15.0
HSV 2	20	1	5.0	0	0
Lyme's disease	16	0	0	0	0
Mononucleosus	20	0	0	3	15.0
Rubella	26	2	7.7	0	0
Syphilis	23	2	8.7	2	8.7
Toxoplasmosis	20	1	5.0	0	0
Alcoholic liver disease	12	0	0	1	8.3
Hepatocellular carcinoma	10	0	0		0
Non-Target Disease Group Total	*898*	*12*	*0.1*	*15*	*0.2*
Total Samples:	1091				
**Borderline samples considered positive.*					

The performance of each ImmuLisa Enhanced AMA Antibody ELISAs was evaluated against the clinical diagnosis of PBC. The clinical sensitivity and specificity were calculated by grouping the assays' indeterminate results with its test negative results, and then sensitivity and specificity were calculated again by grouping the assays' equivocal results with its test positive results:

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgG ELISA:

		Primary Biliary Cirrhosis Diagnosis		
		Positive	Negative	Total
ImmuLisa Enhanced AMA IgG	Positive	162	5	167
	Equivocal	3	4	7
	Negative	28	889	917
	Total	193	898	1091

Equivocal samples considered positive:
Clinical Sensitivity: 85.5% 165/193 (95%CI: 79.5 – 90.0)
Clinical Specificity: 99.0% 889/898 (95%CI: 98.0 – 99.5)

Equivocal samples considered negative:
Clinical Sensitivity 83.9% 162/193 (95%CI: 77.8 – 88.7)
Clinical Specificity 99.4% 893/898 (95%CI: 98.6 – 99.8)

ImmuLisa Enhanced Mitochondria Antibody (AMA) IgA/IgG/IgM ELISA:

		Primary Biliary Cirrhosis Diagnosis		
		Positive	Negative	Total
ImmuLisa Enhanced AMA IgA/IgG/IgM	Positive	162	8	170
	Equivocal	6	7	13
	Negative	25	883	908
	Total	193	898	1091

Equivocal samples considered positive:
Clinical Sensitivity: 87.0% 168/193 (95%CI: 81.3 – 91.3)
Clinical Specificity: 98.5% 883/193 (95%CI: 97.2 – 99.0)

Equivocal samples considered negative:
Clinical Sensitivity 83.9% 162/193 (95%CI: 77.8 – 88.7)
Clinical Specificity 99.1% 890/898 (95%CI: 98.2 – 99.6)

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

A study of 190 normal, apparently disease-free samples tested with the ImmuLisa Enhanced AMA Antibody ELISAs yielded three (1.6%) borderline or positive results for the AMA IgG assay and three (1.6%) borderline results for the AMA IgA/IgG/IgM assay.

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.