

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

A. 510(k) Number:

K092736

B. Purpose for Submission:

New device

C. Analyte:

Anti-M2-3E

D. Type of Test:

Semi-quantitative and qualitative, ELISA

E. Applicant:

Euroimmun US Inc.

F. Proprietary and Established Names:

EUROIMMUN Anti-M2-3E ELISA (IgG)

G. Regulatory Information:

1. Regulation section:
21CFR§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 use:
The EUROIMMUN Anti-M2-3E ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against the mitochondrial antigens M2 in human serum and plasma. It is used as an aid in the diagnosis of primary biliary cirrhosis (PBC), in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
Microwell plate reader capable of measuring OD at 450nm and at 620nm for dual wavelength readings.

I. Device Description:

The EUROIMMUN Anti-M2-3E ELISA (IgG) consists of a microwell ELISA plate coated with M2-3E antigen, 3 calibrators, positive and negative control, peroxidase-labelled rabbit anti-human IgG conjugate, sample buffer, wash buffer concentrate, TMB chromogen/substrate solution and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
Quanta Lite M2 EP (MIT3) ELISA (k052262)

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EUROIMMUN Anti-M2-3E ELISA (IgG)	Quanta Lite M2 EP (MIT3) ELISA
Intended use	Detection of IgG antibodies to mitochondrial antigens as an aid in diagnosis of primary biliary cirrhosis (PBC).	Detection of IgG antibodies to mitochondrial antigens as an aid in diagnosis of primary biliary cirrhosis (PBC).
Technology	ELISA	Same
Assay platform	96-well microtiter plates	Same
Calibration	Relative units	Same
Conjugate	Anti-human IgG labeled with horseradish peroxidase	Same
Substrate	TMB	Same
Stop solution	Sulfuric acid	Same
Reagent preparation	All reagents, calibrators and controls are ready to use, except for the wash buffer.	Same
Procedure	Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with an anti-human IgG enzyme conjugate; wash step, incubation with substrate; then the addition of a stop solution and reading at 450nm.	Same

Differences		
Item	Device	Predicate
Assay format	Qualitative or semi-quantitative (using either the 3 calibrators or 1 calibrator only)	Semi-quantitative
Antigen	Mixture of pyruvate dehydrogenase (isolated from porcine heart) and a recombinant fusion protein. The recombinant protein was produced in E.coli and comprises the immunogenic domains of the E2 subunits from branched-chain 2-oxo-	Affinity purified recombinant M2 EP MIT3

Differences		
Item	Device	Predicate
	acid dehydrogenase (BCOADH), pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase (OGDH), together called BPO.	
Source of conjugate	Rabbit	Goat
Calibrators	3 calibrators 2, 20 and 200 RU/ml	None, the low positive control is used for single-point calibration
Controls	2 controls 1 positive, 1 negative	3 controls 1 high positive, 1 low positive, 1 negative
Samples	Serum or plasma 1:101 dilution	Serum 1:101 dilution
Reported units	RU/mL or Ratio	Units
Cut Off level	20 RU/mL	25 Units

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

None referenced

L. Test Principle:

Patient samples are diluted 1:101 in sample buffer, 100 µL of each diluted patient sample and pre-diluted controls and calibrators are added to the antigen coated microtiter wells and incubated for 30 minutes at room temperature. After incubation the microtiter well strips are washed with wash buffer to remove unbound antibodies and 100 µL of the anti-human IgG enzyme conjugate reagent is added to each microtiter well. After an additional 30-minutes incubation at room temperature, the microtiter wells are again washed 3 times with 300 µL of wash buffer to remove any unbound enzyme conjugate and 100 µL of the chromogen substrate is added. The strips are incubated for 15 minutes at room temperature and 100 µL stop solution is added. The microtiter plates are placed in an ELISA reader and read at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using sera with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days. All CVs were < 12%. The following results were obtained:

Sample #	Intra-assay		Inter-assay	
	Mean value (RU/mL)	CV (%)	Mean value (RU/mL)	CV (%)
1	5.0	5.9	4.9	8.0
2	5.5	6.2	5.6	11.9
3	10	10.5	10	8.4
4	16	4.3	18	10.5
5	19	3.7	25	9.3
6	22	5.5	27	7.2
7	30	3.4	39	6.5
8	45	3.1	43	10.3
9	70	2.3	67	8.6
10	101	2.2	80	7.9
11	101	2.3	103	6.0
12	144	1.6	158	3.0

The precision data for all 12 samples was analyzed qualitatively to generate a summary of the qualitative reproducibility of the assay. The table below shows a summary of these data are shown for one low negative sample, one high negative, one low positive, and one high positive sample.

Sample #	Intra-assay (n = 20)		Inter-assay (n = 24)	
	Mean value (RU/mL)	% of samples with positive result	Mean value (RU/mL)	% of samples with positive result
Low negative	5.0	0%	4.9	0%
High negative	19	10%	18.2	17%
Low positive	21.8	95%	27	100%
High positive	144	100%	158	100%

The Lot to Lot reproducibility was tested using 3 different kit lots with 8 different serum samples. All CVs were <12%. The following results were obtained:

Sample #	Lot-to-lot	
	Mean value (RU/mL)	CV (%)
1	14	9.6
2	19	7.5
3	26	5.1
4	27	7.0
5	56	4.5
6	90	5.6
7	98	7.9
8	164	5.5

b. *Linearity/assay reportable range:*

The linearity of the test was investigated using serial dilutions of five different patient sera with high antibody concentrations. Each patient serum was diluted up to 1/32 with low-activity serum. The dilutions were measured with the Anti-M2-3E ELISA (IgG) according to the package insert in single determinations and the results calculated in RU/ml. With the exception of two diluted samples, observed/expected (O/E) values in the area of the cut-off were within the specifications ($0.8 > O/E > 1.2$). In one of the cases where O/E was low (0.7) the activity of the sample was close to the Limit of Quantitation.

Dilution	Sample 1			Sample 2			Sample 3		
	Exp. RU/mL	Obs. RU/mL	O/E	Exp. RU/mL	Obs. RU/mL	O/E	Exp. RU/mL	Obs. RU/mL	O/E
1/1	199	199	1.0	161	161	1.0	159	159	1.0
1/2	99	98	1.0	80	110	1.4	79	99	1.2
1/4	49	57	1.2	55	61	1.1	49	53	1.1
1/8	28	26	0.9	31	28	0.9	27	21	0.8
1/16	13	12	0.9	14	11	0.8	10	9	0.9
1/32	6	6	0.9	6	5	0.8	4	3	0.7
	Sample 4			Sample 5					
Dilution	Exp. RU/mL	Obs. RU/mL	O/E	Exp. RU/mL	Obs. RU/mL	O/E			
1/1	140	140	1.0	198	198	1.0			
1/2	70	73	1.0	99	103	1.0			
1/4	36	41	1.1	51	61	1.2			
1/8	21	17	0.8	30	30	1.0			
1/16	9	8	0.9	15	12	0.8			
1/32	4	3	0.8	6	5	0.8			

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

Traceability: There are no reference standards for these analytes.

Stability: Reagents were shown to be stable for up to four weeks under accelerated conditions (room temperature). Original sealed kits were demonstrated to be stable for 12 months when stored at 2-8°C. Opened kits were demonstrated to be stable for up to 12 months when stored at 2-8°C. Reconstituted wash buffer was shown to be stable for up to 28 days.

d. *Detection limit:*

The Limit of Detection was determined to be 0.90 RU/mL based on a formula from the ICH Q2B guideline. The Limit of Quantitation is defined as the lowest calibrator used in the assay (2 RU/mL).

e. *Analytical specificity:*

Cross-reactivity: Cross reactivity was investigated using a panel of 29 sera serologically positive for antibodies against parietal cells (PCA, n = 10), glomerular basement membrane (GBM, n = 10) and liver-kidney microsomes

(LKM, n = 9). All 29 sera were negative in the Anti-M2-3E ELISA (IgG), so no cross reactivity is expected for these three autoantibodies.

Interferences: Interference testing was performed at five different anti-M2-3E levels ranging 4-186 RU/mL. No significant interference was observed for concentrations up to 1000 mg/dL hemoglobin, 2000 mg/dL triglycerides, and 40 mg/dL bilirubin. Recovery values ranged 94-123%. Only one of the five samples showed a recovery value over 110%, and only with triglycerides added.

f. *Assay cut-off:*

The levels of anti-mitochondrial antibodies (IgG) were analyzed with the EUROIMMUN Anti-M2-3E ELISA (IgG) in a panel of 400 apparently healthy blood donors, consisting of 251 men and 149 women with an age range of 19-69 years (average age: 40 years). With a cut-off of 20 RU/mL, all blood donors were found negative.

Lowest value	0.35 RU/mL
Highest value	14.50 RU/mL
Mean value	3.81 RU/mL
Std dev. (SD)	2.944 RU/mL

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 48 samples with anti-M2-3E (IgG) results within the reportable range of the EUROIMMUN Anti-M2-3E ELISA device (2-200 RU/mL) were evaluated and results were compared to the results for the predicate device. The results and the positive and negative agreement between the device and the predicate are shown below.

At time of presentation, most (>90%) patients with primary biliary cirrhosis are characterized by extremely high titers of anti-M2-3E¹, well above the upper end of the reportable range of the EUROIMMUN assay (200 RU/mL). These samples are not included in the analysis below. Most of the patient samples with anti-M2-3E results in the range 20-200 RU/mL were patients with autoimmune hepatitis/PBC overlap syndrome. In order to cover the assay measuring range, 7 artificial samples (created by mixing high and low samples) were also considered in the analysis below.

Within reportable range (2-200 RU/mL) samples only		Quanta Lite M2 EP (MIT3) ELISA (IgG)		
		Positive	Borderline	Negative
EUROIMMUN Anti-M2-3E ELISA (IgG)	Positive	24	2	9
	Negative	1	1	9

¹ Hohenester, S. (2009) *Semin Immunopathol* 31:283-307; Kaplan, M.M. (2005) *New England J. Med.* 353:1261-1273; Lleo, A. Et al. (2008) *World J. Gastroenterol* 14(21):3328-3337

Borderline samples considered as positive:

Positive agreement (26/28) = 92.9% (95% C.I. = 77.4 – 98.0%)

Negative agreement (9/18) = 50.0% (95% C.I. = 29.0 – 71.0%)

Overall agreement (35/48) = 76.1% (95% C.I. = 62.1 – 86.1%)

Borderline samples considered as negative:

Positive agreement (26/28) = 96.0% (95% C.I. = 80.5 – 99.3%)

Negative agreement (9/18) = 47.6% (95% C.I. = 28.3 – 67.6%)

Overall agreement (35/48) = 73.9% (95% C.I. = 59.7 – 84.4%)

A method comparison analysis with a total of 127 samples from patients with PBC, autoimmune hepatitis (AIH), or PBC/AIH overlap syndrome was also performed, including samples both above and below the reportable range of 2-200 RU/mL for the EUROIMMUN Anti-M2-3E ELISA. The 7 artificial samples were also included in this analysis.

All samples		Quanta Lite M2 EP (MIT3) ELISA (IgG)		
		Positive	Borderline	Negative
EUROIMMUN Anti-M2-3E ELISA (IgG)	Positive	78	2	9
	Negative	1	2	35

Borderline samples considered as positive:

Positive agreement (80/83) = 96.4% (95% C.I. = 89.9 – 98.8%)

Negative agreement (35/44) = 79.6% (95% C.I. = 65.5 – 88.8%)

Overall agreement (115/127) = 90.6% (95% C.I. = 84.2 – 94.5%)

Borderline samples considered as negative:

Positive agreement (78/79) = 98.7% (95% C.I. = 93.2 – 99.8%)

Negative agreement (37/48) = 77.1% (95% C.I. = 63.5 – 86.7%)

Overall agreement (115/127) = 90.6% (95% C.I. = 84.2 – 94.5%)

b. Matrix comparison:

Comparisons between serum and each of EDTA, heparin, and citrated plasma were performed using 21 sample pairs. The samples cover concentrations over the reportable range (4-180 U/mL). Passing-Bablok regression was performed on the data:

	EDTA plasma vs. Serum	Heparin plasma vs. Serum	Citrate plasma vs. Serum
Regression equation	$y = 0.09 + 1.01x$	$y = -0.10 + 0.98x$	$y = 0.56 + 1.02x$
95% C.I. of slope	0.98 – 1.04	0.96 – 1.01	0.98 – 1.06
95% C.I. of intercept	-1.30 – 2.75	-1.32 – 1.11	-1.02 – 3.18
Coefficient of Correlation (R^2)	0.997	0.998	0.996
% BIAS from serum	102%	98%	102%

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:* A total of 1180 clinically characterized samples (251 from PBC patients and 929 from control groups) were investigated for anti-M2-3E (IgG) antibodies. The EUROIMMUN Anti-M2-3E ELISA (IgG) showed a sensitivity for PBC of 92.8% and a specificity of 97.4%. The results are shown in the table below. 95% C.I. are calculated by the exact method.

		Clinical diagnosis of PBC	
		Positive	Negative
EUROIMMUN Anti-M2-3E ELISA (IgG)	Positive	248	24
	Negative	18	905

Sensitivity (248/266) = 93.2% (95% C.I. = 89.5 – 95.9%)

Specificity (905/929) = 97.4% (95% C.I. = 96.2 – 98.3%)

Overall agreement (1153/1195) = 96.5% (95% C.I. = 95.3 – 97.5%)

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

4. Clinical cut-off:

See Assay cut-off.

5. Expected values/Reference range:

The expected value for healthy individuals in the intended use population is negative (≤ 20 RU/mL).

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.