510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k132195

B. Purpose for Submission:

New assay

C. Measurand:

Anti-PLA2R antibodies

D. Type of Test:

Qualitative or semi-quantitative enzyme immunoassay

E. Applicant:

EUROIMMUN US INC

F. Proprietary and Established Names:

EUROIMMUN Anti-PLA2R ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5780 Anti-phospholipase A2 receptor immunological test system

2. Classification:

   Class II

3. Product code:

   PGV-Anti-phospholipase A2 receptor

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The EUROIMMUN Anti-PLA2R ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against phospholipase A2 receptor (PLA2R) in human serum. It is used as an aid in the diagnosis of primary membranous glomerulonephritis (pMGN), in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Microwell plate reader capable of measuring OD at 450nm and 620nm for dual wavelength readings.

I. Device Description:

The EUROIMMUN Anti-PLA2R ELISA (IgG) consists of a microwell ELISA plate coated with PLA2R antigen, five calibrators, positive and negative controls, peroxidase-labeled 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):

   EUROIMMUN Anti-PLA2R IFA (IgG)

2. Predicate 510(k) number(s):

   k132379

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of IgG antibodies against PLA2R	Same
Sample	Human Serum	Same
Controls	Two controls: positive and negative	Same
Reagent preparation	All reagents are ready to use, except for the wash buffer.	Same

Differences		
Item	Device	Predicate
Technology	ELISA	IFA/BIOCHIP TITER PLANE
Antigen	Recombinant PLA_2R (type M)	PLA_2R transfected cells and control-transfected cells
Calibrators and controls	Five calibrators: 2, 20, 100, 500 and 1500 RU/ml; Two controls: positive and negative	Two Controls
Conjugate	Anti-human IgG labeled with horseradish peroxidase	Fluorescein-labeled anti-human IgG
Assay range	2 - 1500 RU/ml	Not applicable
Borderline	≥14 – <20 RU/ml; ≥0.7 – <1.0 (ratio)	No borderline
Cutoff level	20 RU/ml or 1.0 (ratio)	1:10 dilution
Assay format	Qualitative or semi-quantitative (using either all calibrators or the cut-off calibrator only)	Qualitative
Procedure	ELISA: Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with conjugate, wash step, incubation with substrate, addition of stop solution, photometric reading	IFA: Sample incubation with tissues/cells, followed by a wash step, incubation with conjugate, wash step, embedding, fluorescence microscopy reading
Reagents	96 well microplate, 5, Sample buffer, Wash buffer (10x concentrate), Substrate solution (TMB), Stop solution (0.5 M sulphuric	BIOCHIP slides, Salt for PBS pH 7.2, Tween 20, Embedding medium, Cover glasses

Differences		
Item	Device	Predicate
	acid)	
Sample dilution	1:101 in sample buffer	1:10

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

CLSI EP17-A1: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

Patient samples are diluted 1:101 in sample buffer, then 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 30 minute incubation at room temperature, the microtiter wells are again washed three 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:

1. Repeatability

Repeatability was investigated using samples with values at different points on the calibration curve. Within-run, between-run, between-day and total standard deviations (SD) and coefficients of variation (CV) were calculated based on 150 determinations per sample performed in six different runs on three different days (with two runs per day and 25 replicates per run) according to the package insert with the same lot and by the same technician. EUROIMMUN's acceptance criterion was that the CV's show results below 12% for positive and borderline samples. Their acceptance criterion for the ratio-based results was that all qualitative results (positive, borderline, negative) of the samples be in line with the expected result. The following results were obtained:

		Semi-quantitative: RU/mL							
		Within-Run		Within-Run		Between-Lot		Total	
Sample	Mean (RU/mL)	SD	%	SD	% CV	SD	% CV	SD	% CV
1	3	0.28	9.2	0.38	12.9	0.64	22.4	0.43	14.8
2	17	0.57	3.3	1.04	6.4	1.25	7.5	0.95	5.7
3	22	0.87	3.9	1.56	7.3	1.08	5.1	1.17	5.4
4	24	0.75	3.2	1.60	6.7	1.51	6.4	1.28	5.4
5	884	69.95	7.9	72.49	8.3	66.51	7.6	69.65	7.9
6	1356	65.65	4.8	33.59	2.5	45.60	3.4	48.28	3.6

	Qualitative: Ratio					
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Mean value (x):	0.20	0.74	0.91	1.00	7.57	8.20
Range of values:	0.16 – 0.25	0.68 – 0.81	0.76 – 1.27	0.83 – 1.17	6.34 – 8.22	7.45 – 8.72
Expected result:	negative	borderline	borderline	positive	positive	positive
% positive:	0%	0%	1%	52%	100%	100%
% borderline	0%	97%	99%	48%	0%	0%
% negative:	100%	3%	0%	0%	0%	0%

2. Lot to Lot

Reproducibility between lots was investigated using samples with values at different points on the calibration curve. Within-run, between-run, between-lot and total SD's and %CV's were calculated based on 18 determinations per sample performed in three different runs on three different days (with three runs per lot and two replicates per run, using three lots). EUROIMMUN's acceptance criterion was that the CV's show results below 12% for positive and borderline samples. Their acceptance criterion for the ratio-based results was that all qualitative results (positive, borderline, negative) of the samples be in line with the expected result. The following results were obtained:

		Semi-quantitative: RU/mL							
		Within-Run		Within-Run		Between-Lot		Total	
Sample	Mean (RU/mL)	SD	%	SD	% CV	SD	% CV	SD	% CV
1	3	0.45	16.4	0.15	5.4	0.33	11.9	0.31	11.2
2	17	1.19	7.2	0.42	2.5	0.69	4.1	0.76	4.6
3	23	1.46	6.5	0.30	1.3	0.99	4.4	0.91	4.0
4	26	1.78	6.7	0.85	3.2	1.37	5.2	1.33	5.0
5	304	15.71	5.2	6.48	2.1	10.61	3.5	10.93	3.6
6	1260	127.19	10.1	35.80	2.8	33.53	2.7	65.51	5.2

	Qualitative: Ratio					
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
Mean value (x):	0.19	0.77	0.99	1.12	5.62	8.43
Range of values:	0.16 – 0.21	0.68 – 0.83	0.91 – 1.12	1.06 – 1.20	5.29 – 6.12	7.71 – 9.04
Expected result:	negative	borderline	borderline	positive	positive	positive
% positive:	0%	0%	28%	100%	100%	100%
% borderline	0%	94%	72%	0%	0%	0%
% negative:	100%	6%	0%	0%	0%	0%

b. Linearity/assay reportable range:

1. Linearity

Five sets of 11-step-wise dilutions were prepared by mixing two low and five high analyte samples for proportional dilutions throughout the claimed ranges. The concentrations ranges tested were from a low concentration of 2 RU/mL to high concentrations of 75 RU/mL, 133 RU/mL, 790 RU/mL, 1100 RU/mL, or 1477 RU/mL. The assay was shown to be linear from 2 to1500 RU/mL.

The regression results from sample sets around the cutoff and throughout the measuring range were:

Concentration range	**Regression and R^2**
2 - 75 RU/mL	$y=0.949x + 1.91$, $R^2= 0.995$
2 - 133 RU/mL	$y=0.94x + 1.221$, $R^2= 0.991$
2-790 RU/mL	$y=1.038x+14.497$, $R^2=0.9843$
2-1100 RU/mL	$y=0.9178x+74.938$, $R^2=0.9846$
2-1642 RU/mL	$y=1.0913x+51.156$, $R^2=0.9713$

Analytical measuring range

The analytical measuring range is 2 to 1500 RU/mL, from the lowest to the highest concentration calibrators.

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

1. Traceability

There is no internationally recognized standard for autoantibodies to PLA2R. Results of this assay are given in arbitrary values (RU/mL or ratio of sample OD to cutoff control OD). The concentration of the calibrators is linked to the concentrations of master reference materials.

2. Calibrators and controls

The calibrators and controls are derived from human serum, purchased from commercial sources. Human originated material is tested and found negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2, diluted to the appropriate concentration and stabilized.. The calibrators are manufactured by dilution of the human serum with stabilizing buffer and adjusted to match the required performance criteria in use with the corresponding assay lot and the corresponding kit controls. The new lot of calibrator is assayed using a reference panel of at least eight positive and two negative reference sera (distributed over the measurement range). By further dilution or spiking with the original serum, the calibrators are adjusted until the following acceptance criteria are met: At least six of the eight positive sera must be found within the established acceptable ranges. The OD of the negative sera must be below the OD of the calibrator. After the adjustment is completed, the components of the new ELISA kit lot are tested together.

3. Reference panel

The samples used for the reference panel are human sera that are characterized, stabilized, aliquoted and stored frozen until usage. The acceptable range of the reference panel samples is determined as ±3 standard deviations of the mean of 10 runs using different assaylots. The range is set in RU/mL using released lots of the Anti-PLA2R ELISA (IgG). The reference panel is used as a master reference material against which to assign values to new lots.

4. Stability

Three lots of materials were tested with three different concentrations of analyte in real-time and accelerated stability. Claims from real-time stability are below:

a. Shelf-life stability: The sponsor provided data demonstrating the stability of unopened kits for 12 months at 2-8 ^{0}C.

b. Open kit stability: The sponsor provided data demonstrating the stability of the opened kit for 6 months when stored at 2– 8^0C.

c. Reconstituted reagent stability: The sponsor provided data demonstrating that the reconstituted wash buffer is stable for 28 days (4 weeks).

d. Analytical Sensitivity

1. Limit of Blank

The limit of blank (LoB) was determined according to the recommendations of CLSI standard EP17-A. Six negative samples in the range < 2 RU/mL were each run 12 times (four runs x three replicates) for 72 measurements. LoB was calculated according to formula (1) of EP17-A: LoB = $\mu B + 1.645 \times \sigma B$. μB = mean of means of blank sample measurements; σB = mean of standard deviations of blank sample measurements. μB = 1.3 RU/mL; σ_B= 0.3 RU/mL; LoB= 1.8 RU/mL.

2. Limit of Detection

The limit of detection (LoD) was determined according to the recommendations of CLSI standard EP17-A. Nine samples ranging from 2 to 7 RU/mL were each run 12 times (four runs x three replicates) for a total of 108 measurements. LoD was determined according to formula (4) of EP17-A:

LoD = LoB + $1.645 \times \sigma_s$, where σ_s = mean of standard deviations of low positive sample measurements, here σ_s= 0.25 RU/mL; LoD= 2.2 RU/mL.

3. Limit of Quantitation

The limit of quantitation (LoQ) is equal to the LoD, which is 2.2 RU/mL.

e. Analytical specificity:

1. Cross reactivity

Cross reactivity was investigated using a panel of 65 clinically characterized sera positive for thyroiditis (5), systemic lupus erythematosus (5), systemic sclerosis (5), Sjögren's syndrome (5), cANCA (10), pANCA (10), glomerular basement membrane disease (GBM) (10), Hepatitis B surface antigen (10) and rheumatoid arthritis (5). All 65 sera were negative in the Anti-PLA2R ELISA (IgG) assay.

2. Interference

To investigate the influence from hemoglobin, triglycerides and bilirubin, sera with anti-PLA2R concentrations from 12 to 948 RU/mL were spiked with

potential interfering substances and compared with unspiked sera. The concentrations of interferents were: Hemoglobin 0, 250, 500 and 1000 mg/dL; Triglycerides 0, 500, 1000 and 2000 mg/dL: Bilirubin 0, 10, 20 and 40 mg/dL.

EUROIMMUN's acceptance criterion was that all individual recoveries should be within the range of 70 – 130% and the mean of recoveries for each interferent be within the range of 85 – 115%. All recoveries met the acceptance criteria for recovery to 1000 mg/dl for hemoglobin, 2000 mg/dL for triglyceride and 40 mg/dL for bilirubin.

f. Assay cut-off:

1. Semi-quantitative

20 RU/mL; however, the assay has a borderline range.
Results should be interpreted as follows:

<14 RU/mL: negative; ≥14-<20 RU/mL borderline; ≥20 RU/mL positive

2. Ratio-based analysis

Results can be evaluated by calculating a ratio of the OD value of the control or patient sample over the OD value of calibrator 2 (20 U/mL). The ratio is calculated according to the following formula:

$$\frac{\text{OD of the control or patient sample}}{\text{OD of calibrator 2}} = \text{Ratio}$$

Results should be interpreted as follows:

<0.7: negative; ≥0.7 to <1.0: borderline; ≥1.0: positive

2. Comparison studies:

a. Method comparison with predicate device:

The samples from the clinical studies total 560 (275 from pMGN patients, 285 from control groups) were investigated for anti-PLA2R antibodies (IgG) using the two test systems EUROIMMUN Anti-PLA2R IFA and EUROIMMUN Anti-PLA2R ELISA (IgG). Note that the IFA test is a qualitative immunofluorescence test for Anti-PLA2R.

i. Semi-Quantitative (RU/mL):

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) RU/mL	positive	184	1	185
	borderline	6	0	6
	negative	22	347	369
	Total	212	348	560

Semi-quantitative, Borderline counted as Positive:

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) RU/mL	positive	190	1	191
	negative	22	347	369
	Total	212	348	560
Positive Agreement: 184/212 = 86.79%; 95% CI: 81.5–91.0% Negative Agreement: 347/348 = 99.71%; 95% CI: 98.4–100.0%				

Semi-quantitative, Borderline counted as Negative:

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) RU/mL	positive	184	1	185
	negative	28	347	375
	Total	212	348	560
Positive Agreement: 190/212 = 89.62%; 95% CI: 84.7–93.4% Negative Agreement: 347/348 = 99.71%; 95% CI: 98.4–100.0%				

ii. Qualitative (Ratio):

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) Ratio	positive	184	1	185
	borderline	12	1	13
	negative	16	346	362
	Total	212	348	560

Qualitative, Borderline counted as Positive:

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) Ratio	positive	196	2	198
	negative	16	346	362
	Total	212	348	560
Positive Agreement: 184/212 = 86.79%; 95% CI: 88.0–95.6% Negative Agreement: 347/348 = 99.71%; 95% CI: 98.4–100.0%				

Qualitative, Borderline counted as Negative:

		Predicate		
		positive	**negative**	**Total**
Anti-PLA2R ELISA (IgG) Ratio	positive	184	1	185
	negative	28	347	375
	Total	212	348	560
Positive Agreement: 196/212 = 92.45%; 95% CI: 88.0–95.6% Negative Agreement: 347/348 = 99.71%; 95% CI: 98.4–100.0%				

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity

Clinical studies were performed in cooperation with different sites (see below). In total 560 clinically characterized samples (275 from pMGN patients, 285 from control groups) were investigated for anti-PLA2R antibodies (IgG). Diagnosis of pMGN was based on renal biopsy and was considered to be idiopathic/primary when no secondary cause of MN was suspected on the basis of clinical and laboratory criteria. The samples were drawn within eight weeks after biopsy and before treatment; excluding patients who had been or were currently being treated with immunosuppressive drugs. With the EUROIMMUN Anti-PLA2R ELISA (IgG) using the 5-point calibrated analysis and a cut-off of 20 RU/ml, a sensitivity of 66.9% (95% C.I.: 61.0–72.4%) was found in pMGN, which is within the expected range of approximately 70% of anti-PLA2R as reported in the scientific literature. Specificity was 99.6% (95% C.I.: 98.1–100.0%). The results are shown in the tables below. 95% C.I. are calculated by the exact method. The results for the ratio analysis were calculated based on raw OD, not RU/mL:

i. Clinical Sensitivity:

Diagnosis	N (M, W)	Mean age (range, y)	Anti-PLA2R ELISA (IgG)		
			positive	%	95% C.I.
Primary membranous glomerulonephritis (pMGN)	275 (188, 87)	52 (16 – 86)	184; 5 borderline	66.9%	61.0 – 72.4%

ii. Clinical Specificity:

Diagnosis	N (M, W)	Mean age (age range, # unknown)	Anti-PLA2R ELISA (IgG)		
			negative	%	95% C.I.
Secondary membranous glomerulonephritis (sMGN)	68 (35, 33)	51 (19 – 80 , 2)	67	98.5%	92.1 – 100.0%
Non-membranous glomerulonephritides (GN)	63 (32, 30, 1 unk)	44 (16 – 68, 6)	63	100.0%	94.3 – 100.0%
Systemic lupus erythematosus (SLE)	30 (8, 22)	64 (50-86)	30	100.0%	88.4 – 100.0%
Systemic sclerosis (SSc)	30 (6, 24)	52 (30 – 74)	30	100.0%	88.4 – 100.0%
Psoriasis arthritis (PSA)	30 (16, 14)	48 (19 – 73)	30	100.0%	88.4 – 100.0%
Rheumatoid arthritis (RA)	14 (1, 13)	53 (29 – 86)	14	100.0%	76.8 – 100.0%
Thyroiditis	50 (8, 42)	48 (22 – 90)	50	100.0%	92.9 – 100.0%
Total	285		284	99.6%	98.1 – 100.0%

iii. Semi-quantitative Analysis (RU/mL):

Clinical samples (n = 560)		Clinical diagnosis		
		positive	negative	Total
EUROIMMUN Anti-PLA2R ELISA (IgG) RU/mL	positive	184	1	185
	borderline	5	0	5
	negative	86	284	370
	Total	275	285	560

Borderline samples counted as negative:
Sensitivity 184 / 275 = 66.9%; 95% C.I.: 61.0% – 72.4%
Specificity 283 /285 = 99.6%; 95% C.I.: 98.1% – 100.0%

Borderline samples counted as positive:
Sensitivity 189 / 275 = 68.7%; 95% C.I.: 62.9% – 74.2%
Specificity 284 / 285 = 99.6%; 95% C.I.: 98.1% – 100.0%

iv. Qualitative Analysis (Ratio):

Clinical Samples (n = 560)		**Clinical diagnosis**		
		positive	**negative**	**Total**
EUROIMMUN Anti-PLA2R ELISA (IgG) Ratio	positive	181	1	182
	borderline	9	1	10
	negative	85	283	288
	Total	275	285	560

Borderline samples counted as negative:
Sensitivity 181/ 275 = 65.8%; 95% C.I.: 59.9% – 71.4%
Specificity 283/ 285 = 99.3%; 95% C.I.: 97.5% – 99.9%

Borderline samples counted as positive:
Sensitivity 190/ 275 = 69.1%; 95% C.I.: 63.3% – 74.5%
Specificity 283/ 285 = 99.3%; 95% C.I.: 97.5% – 99.9%

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

Not applicable

4. Clinical cut-off**:**

The assay cut-off is based on a ROC analysis of the OD results of 122 samples from primary membranous glomerulonephritis (pMGN) patients (positive group) and 534 samples from patients with nonmembranous glomerulonephritides (GN), other connective tissue diseases and from normal healthy blood donors (negative group). pMGN diagnosis was based on renal biopsy and the samples were drawn within 8 weeks after biopsy and pre-selected for anti-PLA2R positivity. This population was separate from the samples used in the clinical sensitivity and specificity study. The ROC analysis demonstrated optimal sensitivity (96.7%) and specificity (100.0%) at the OD value of 0.228. The cut-off calibrator 2 was established with a nominal value of 20 RU/mL around this cut-off OD.

5. Expected values/Reference range:

Two studies were performed: the first tested samples from European donors and the second tested samples from US donors.

European donors: The levels of anti-PLA2R antibodies (IgG) were analyzed in a panel of 100 samples from apparently healthy blood donors (83 men and 17 women with an average age of 38 y; age range: 18 – 68 y). There was one positive sample, male, with measurement of 32 RU/ml and a ratio of 1.6.

US donors: The levels of anti-PLA2R antibodies (IgG) were analyzed in a panel of 248 samples from apparently healthy blood donors (151 men, 97 women, mean age 36 y, age range 17 – 50 y). There was one positive sample, The results are shown in the table below. Taken together, these results demonstrate a prevalence of positive results of 0.6% in a normal population.

European Donors		
N	**100**	
Positives	1	
Borderline	0	
Negatives	99	
Prevalence	1.0 %	
	RU/mL	Ratio
Lowest value	0	0.0
Highest value	32	1.6
Mean value	2	0.1
95^{th} percentile	5.05	0.3
Std deviation	3.4	0.18

US Donors		
N	**248**	
Positives	1	
Borderline	0	
Negatives	247	
Prevalence	0.4%	
	RU/mL	Ratio
Lowest value	1	0.0
Highest value	40	1.6
Mean value	2	0.1
95^{th} Percentile	4.65	0.2
Std deviation	2.8	2.8

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.