EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
EUROIMMUN ANTI-PLA2R IFA
DECISION SUMMARY

A. 510(k) Number:

K132379 (DEN140002)

B. Purpose for Submission:

De novo request for evaluation of automatic class III designation for the EUROIMMUN Anti-PLA2R IFA

C. Measurand:

Autoantibodies against phospholipase A2 receptor (PLA2R)

D. Type of Test:

Qualitative, Indirect Immunofluorescent Antibody Assay (IFA)

E. Applicant:

EUROIMMUN US Inc.

F. Proprietary and Established Names:

EUROIMMUN Anti-PLA2R IFA

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5780

2. Classification:

   Class II

3. Product code:

   PGV

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The EUROIMMUN Anti-PLA2R IFA is intended for the qualitative 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):

   Prescription use only

4. Special instrument requirements:

   Fluorescent microscope equipped with a 488 nm excitation filter; 510 nm color separator; and 520 nm blocking filter with a 100W mercury vapor lamp light source or LED bluelight.

I. Device Description:

The kit (for 50 determinations) contains an instruction booklet and the following materials:

- Slides, each containing 5x2 BIOCHIPs coated with PLA2R-transfected and control-transfected cells
- Fluorescein-labelled anti-human IgG (goat), ready for use (1 x 1.5 ml);
- Positive control: anti-PLA2R, ready for use (1 x 0.1 ml)
- Negative control: autoantibody negative, ready for use (1 x 0.1 ml)
- Salt for PBS pH 7.2 (x2)
- Tween 20 (2 x 2.0 ml)
- Embedding medium, ready for use (1 x 3.0 ml)
- Cover glasses (62 mm x 23 mm) (x12)

The EUROIMMUN IFA is an assay for standardized detection of anti-PLA2R antibodies utilized in laboratories familiar with indirect immunofluorescence. The test kit consists of slides, which contain BIOCHIPs coated with PLA2R transfected cells and control-transfected cells, fluorescein-labelled anti-human IgG (goat), a positive control for anti-PLA2R, a negative control, a salt for preparation of PBS, Tween 20, embedding medium, cover glasses and an instruction booklet.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Not applicable

2. Comparison with predicate:

   Not applicable

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

None

L. Test Principle:

Patient samples are diluted 1:10 in PBS-Tween, 30 µL of each diluted patient sample are added to each reaction field of the reagent tray. Reactions are started by fitting the BIOCHIP slides containing the sections from the substrate (PLA2R transfected cells and control-transfected cells) into the corresponding recesses of the reagent tray and incubated for 30 minutes at room temperature. Specific antibodies attach to the PLA2R antigens. After incubation the BIOCHIP slides are washed with PBS-Tween to remove unbound antibodies. In the meantime, 25 µL of fluorescein–labeled anti–human IgG are added to each reaction field of a clean reagent tray and the BIOCHIP slides placed into the recesses of the tray. After 30 minutes incubation at room temperature, the BIOCHIPs are again washed with PBS-Tween to remove any unbound fluorescein-labeled reagent. 10 µL of embedding medium are placed for each reaction field on a cover glass and the BIOCHIP slides, with the BIOCHIPs facing downwards, placed onto the prepared cover glass. Fluorescence is read with a fluorescence microscope.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   For validation purposes, a scoring of results expressed in fluorescence intensity levels is used internally. The fluorescence intensity level is the intensity of the specific fluorescence expressed as a numeric value. These values can vary from "0" (no specific fluorescence) to "5" (positive reaction, very strong specific reaction visible). The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	**Evaluation**
0	No specific fluorescence
1	Positive reaction, very weak specific reaction visible
2	Positive reaction, weak specific reaction visible
3	Positive reaction, specific reaction well visible
4	Positive reaction, strong specific reaction visible
5	Positive reaction, very strong specific reaction visible

Sponsor stated that although customers may decide to use a scoring for evaluation, it is not routinely used for reporting the results to the physicians. The reporting is done either qualitatively or by adding the endpoint titer to the qualitative result, as suggested in the recommendations of the interpretation of results section in the package insert. The sponsor suggests each laboratory establish its own screening dilution and titration scheme based on its population and instrumentation.

Acceptance criterion for analytical performance validation study for EUROIMMUN Anti-PLA2R IFA is that all qualitative results of positive samples be positive and not exceed the acceptable deviation of fluorescence intensity of ±1 intensity level, and results of negative samples be negative.

a. Precision/Reproducibility:

Intra-assay reproducibility was determined by ten-fold repeated measurements of six characterized positive and negative serum samples. Ten determinations were performed for each sample. The results are shown in the following table:

Sample	# 1	# 2	# 3	# 4	# 5	# 6
Mean (intensity)	0	0	1.8	2.4	3	3.9
Expected	Negative	Negative	Positive	Positive	Positive	Positive
% positive	0%	0%	100%	100%	100%	100%
% negative	100%	100%	0%	0%	0%	0%

Inter-assay reproducibility was determined by repeated measurements of six characterized positive and negative serum samples. For each sample, ten determinations were performed using two slides per run at five different times. The results were shown in the following table:

Sample	# 1	# 2	# 3	# 4	# 5	# 6
Mean (intensity)	0	0	1	2	3	4
Expected	Negative	Negative	Positive	Positive	Positive	Positive
% positive	0%	0%	100%	100%	100%	100%
% negative	100%	100%	0%	0%	0%	0%

Lot-to-lot reproducibility was determined by measurements of two sets (six samples per set) of characterized positive and negative serum samples. One set of samples (two negatives and four positives with fluorescence intensity levels of 1, 2, 3, and 4) were tested with 3 different slide lots with one run per slide. Another set of samples (two negatives and four positives with fluorescence intensity levels of 2 and 3 were tested with 3 lots of reagents composed of entirely different manufacturing runs of materials. The variation in fluorescence intensity was within +1 intensity level unit

for positives. All negative samples were tested as negative with intensity level at 0.

Site-to-site reproducibility was determined by testing seven characterized positive and negative serum samples at three different sites. At each site, each sample was tested by different operators with two runs per day for five days. Each result was evaluated by two different technicians independently. The results are summarized in the following table:

Sample	# 1	# 2	# 3	# 4	# 5	# 6	#7
Mean (intensity)	0	0	1	1.95	3	4	3.5
Expected	Negative	Negative	Positive	Positive	Positive	Positive	Positive
% positive	0%	0%	100%	100%	100%	100%	100%
% negative	100%	100%	0%	0%	0%	0%	0%

b. *Linearity/assay reportable range:*

Not applicable

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

There is no recognized standard or reference material for autoantibodies against phospholipase A2 receptor.

Stability:

Kit stability: The stability of EUROIMMUN Anti-PLA2R IFA was evaluated with both the real-time and accelerated/stress study.

The real-time stability was performed to evaluate the shelf life for original sealed and opened kit. Three production lots of reagents were stored original sealed at 2 – 8°C and opened at 2 – 8°C. The kits stored at -20°C were used as a reference. The kits were tested using both positive and negative samples at the point 0, 12, 24, and 36 months. Results were compared to the results of day 0 and did not exceed ±1. The data supports a stability claim of 18 months.

The accelerated stability was performed by storing three different kit lots (reagents and controls) at 37°C and -20°C for 14 days. The kits stored at -20°C were used as a reference. At the end of storage time, the kits were tested using 4 samples with fluorescence intensity at 0, 1, 2, and 4). The controls (positive and negative) stored at 37°C were also tested and compared to the results from those stored at -20°C. The results showed no deviation in fluorescence intensity level exceed ±1. For the establishment of the stability claim, a storage time of 14 days at 37°C equals 18 months at 2°C – 8°C.

The results support stability of the kits under the recommended storage of 2 – 8°C for

up to 18 months.

Controls: Positive control is manufactured from human serum samples containing antibodies to PLA2R which are diluted with buffered solution. Positive control is ready for use with a 3 – 4 fluorescence intensity. The quality control criteria for positive control are that the control serum shows expected fluorescence and the expected rated value titer of the control is equivalent to the reference. Negative control is manufactured with human autoantibody-free serum samples diluted with buffered solution. The negative control serum should show no specific fluorescence and rated value titer.

d. Detection limit:

Not applicable

e. Analytical specificity:

Interference: Study was done to evaluate the analytical specificity of EUROIMMUN anti-PLA2R IFA assay in the presence of endogenous interfering substances including hemoglobin, bilirubin and lipid. Three serum samples with fluorescence intensity at 0, 1, and 3 were tested by spiking with each interference substance in three different concentrations. For each test sample, the samples without spiking with interference substance were used as control. The recovery of spiked sample to the control sample was evaluated. Acceptance criterion was defined as deviation in fluorescence intensity level not to exceed ± 1. No interference was observed for concentrations up to 500 mg/dL for hemoglobin, 2000 mg/dL for triglyceride and 40 mg/dL for bilirubin.

Cross-reactivity: Cross reactivity was investigated using a panel of 60 clinically characterized sera positive for thyroiditis (n=5), systemic lupus erythematosus (SLE) (n=5), systemic sclerosis (SSc) (n=5), rheumatoid arthritis (RA) (n=5), cANCA (n=10), pANCA (n=10), Goodpasture Syndrome (GBM) (n=10) and hepatitis B surface antigen (HBsAg) (n=10). All 60 sera were negative, so no cross reactivity was found.

f. Assay cut-off:

The cut-off was established by testing serum samples from biopsy proven pMGN patients (n=35), non-membranous glomerulonephritis patients (n=182), and normal healthy blood donors (n=150 with 110 men and 40 women). At the serum dilution of 1:10, 23 out of 35 patient samples with pMGN (65.7%) were found positive with the EUROIMMUN Anti PLA2R IFA. All non-membranous glomerulonephritis and normal healthy subjects were negative. The 1:10 dilution was determined as the cut-off dilution.

2. <u>Comparison studies:</u>

a. *Method comparison with predicate device:*

Not applicable

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

A total of 560 clinically characterized samples (275 samples from patients diagnosed with pMGN and 500 samples from control groups) were evaluated to determine clinical sensitivity and specificity of the EUROIMMUN Anti-PLA2R IFA assay. pMGN diagnosis was based on renal biopsy and was considered to be idiopathic/primary when no secondary cause of MGN was suspected on the basis of clinical and laboratory criteria. The samples were drawn within 8 weeks after biopsy, before treatment. Patients who had been or were currently being treated with immunosuppressive drugs were excluded. In disease control groups, sMGN diagnosis was based on serological tests and clinical evaluation. sMGN samples were from patients with malignancy, SLE, cryoglobulinemia type II, RA, Still's Disease, IgG4-related disease, ANCA-positive crescentic glomerulonephritis and membranous nephritis, hepatitis B virus infection, hepatitis C virus infection, non-steroidal anti-inflammatory drug (NSAID), mercury, chronic inflammatory disease and others. The non-membranous glomerulonephritis samples were obtained from patients with focal segmental glomerulosclerosis (FSGS), IgA nephritis, minimal change disease, membranoproliferative glomerulonephritis, diabetes mellitus/diabetic nephropathy, and other non-glomerulonephritis diseases. The test was performed with the EUROIMMUN Anti PLA2R IFA using the cut-off dilution of 1:10 according to the package insert. The results are summarized in the following table:

Clinical Sensitivity				
Panel	N	Positive	% Positive	95% CI
pMGN	275	212	77.1%	71.7 – 81.9%
Clinical Specificity				
Panel	N	Negative	% Negative	95% CI
sMGN	68	68	100.0%	94.8 – 100.0%
Non-membranous glomerulonephritis	63	63	100.0%	94.3 – 100.0%
SLE	30	30	100.0%	88.4 – 100.0%
Systemic sclerosis (SSc)	30	30	100.0%	88.4 – 100.0%
Psoriasis arthritis (PSA)	30	30	100.0%	88.4 – 100.0%
Rheumatoid arthritis (RA)	14	14	100.0%	76.8 – 100.0%

Thyroiditis	50	50	100.0%	92.9 – 100.0%
Total	285	285	100.0%	98.7– 100.0%

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 reference range of anti-PLA2R in the normal population is negative at a 1/10 dilution of serum. This reference range was analyzed with EUROIMMUN anti-PLA2R IFA using a panel of 128 samples from normal healthy European population (103 men and 25 women with age ranging of 18 – 68 years) and a panel of 189 samples from normal healthy US population (106 men and 83 women with age ranging of 20 - 50 years). At the initial dilution of 1:10, all samples were found negative.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10, 21 CFR 801.109, and the special controls for this type of device.

O. Identification Potential Risks and Required Mitigation Measures:

Identified Potential Risks	**Required Mitigation Measures**
Inaccurate test results that provide false positive and false negative results	Special controls (1), (2), and (3)
Failure to correctly interpret test results can lead to false positive and false negative results	Special controls (1) (iii), (2), and (3)

O. Benefit/Risk Analysis:

Summary of the Benefit(s)	The test can help differentiate pMGN from sMGN in the vast majority of patients. The test is straightforward and uses a standard (IFA) technique. This device is a non-invasive methodology that aids in the diagnosis of pMGN. The rationale behind the test is credible and respected scientific data showing that in some patients (generally those with pMGN) there are high levels of circulating serum anti-PLA2R antibodies of immunoglobulin class IgG. In the clinical study, the assay demonstrates a specificity of over 99% and a sensitivity of 77.1% for pMGN depending on the cohort individuals. The test result may aid the clinician to provide more specified therapy. Finally, a more directed evaluation of secondary causes of MGN may be feasible based, in part, on anti-PLA2R test results.
Summary of the Risk(s)	The risks to health associated with this test are inaccurate test results that provide false positive and false negative results, or failure to correctly interpret test results. Patients may be subjected to additional blood specimen collection based on a negative test result, but that is very unlikely given the frequency of times patients with MGN require blood analysis. Therefore, this test can be done using blood obtained during routine (scheduled) phlebotomy. Since IFA requires only 50-100 µL of serum, the amount of blood sample required will be negligible. The main risk of the test is a false negative result. Indeed, in the clinical study, 22.9% of patients with the clinical diagnosis of pMGN had a negative anti-PLA2R antibody result. A false negative result may result in delay of complete evaluation and appropriate therapy. This would be mitigated by the fact that the test is designed to be an adjunct, not a stand-alone test. A false positive result may result in the delay of therapy for the underlying cause of sMGN, or proper therapy for the renal disease. Similar to a false negative result, this may cause unnecessary testing or treatment. As with the false negative result, this would be mitigated by the fact that the test is designed to be an adjunct, not stand-alone test.
Summary of Other Factors	None

Conclusions Do the probable benefits outweigh the probable risks?	MGN is a renal disease that is often slowly progressive. Current therapy requires accurate diagnosis. In some patients, it can be difficult to differentiate between pMGN and sMGN. This is crucial since the choice of optimal therapy and favorable prognosis requires accurate identification of disease sub-type. Acutely, the disease may cause breathing problems or infection. Through various mechanisms, kidney function may slowly decline, resulting in high blood pressure, inability to properly balance body chemicals, and bone and heart disease. Therapy for MGN may include medications that have significant side effects. While some patients may experience a rapid decline in kidney function, others may take decades to reveal a significant progression. The response to therapy is dependent, in part, on the accurate diagnosis and differentiation of sub-type. The benefits of the test outweigh the risks. Basically, this test can help differentiate pMGN from sMGN. The accuracy of the test, most prominently for a negative result, is very good, especially for renal disease in which biomarkers are scarce. The test is non-invasive and based on standard technology (IFA). Moreover, the test is based on solid scientific data. The risks are basically related to the potential (albeit low) for a false negative or positive result. Yet, these risks would be mitigated by the fact that the test is designed to be an adjunct, not stand-alone test, and given the combination of general and special controls.

P. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.5780 with special controls. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code:	PGV
Device Type:	Anti-phospholipase A2 receptor immunological test system
Class:	II (special controls)
Regulation:	21 CFR 866.5780

(a) *Identification*. An anti-phospholipase A2 receptor immunological test system is a device that consists of the reagents used to measure by immunochemical techniques the autoantibodies in human blood samples that react with phospholipase A2 receptor. The measurements aid in the diagnosis of primary membranous glomerulonephritis (pMGN), in conjunction with other laboratory and clinical findings.

(b) *Classification.* Class II (special controls). The special controls for this device are:

(1) Design verification and validation must include:
  (i) A detailed description of the device that includes:
    (A) A detailed description of all parts of the test system, including a description of the assay parts in the kit and all required ancillary reagents;
    (B) A detailed description of instrumentation and equipment, and illustrations or photographs of non-standard equipment or methods if applicable;
    (C) Detailed documentation of the device software, including standalone software applications and hardware-based devices that incorporate software where applicable;
    (D) A detailed description of appropriate internal and external quality controls that are recommended or provided. The description must identify those control elements that are incorporated into the recommended testing procedures;
    (E) Detailed specifications for sample collection, processing and storage;
    (F) A detailed description of methodology and assay procedure; and,
    (G) Detailed specification of the criteria for test results interpretation and reporting.
  (ii) Information that demonstrates the performance characteristics of the device, including:
    (A) Device precision/reproducibility data generated from within-run, between-run, between-day, between-lot, between-operator, between-instruments, between-site, and total precision for multiple nonconsecutive days as applicable. A well characterized panel of patient samples or pools from the intended use population that covers the device measuring range must be used;
    (B) Device linearity data generated from patient samples covering the assay measuring range if applicable;
    (C) Information on traceability to a reference material and description of value assignment of calibrators and controls if applicable;
    (D) Device analytical sensitivity data, including limit of blank, limit of detection and limit of quantitation if applicable;
    (E) Device analytical specificity data, including interference by endogenous and exogenous substances, as well as cross-reactivity with samples derived from patients with other autoimmune diseases or conditions;
    (F) Device instrument carryover data when applicable;
    (G) Device stability data including real-time stability under various storage times and temperatures;
    (H) Specimen stability data including stability under various storage times, temperatures, freeze-thaw and transport conditions where appropriate;
    (I) Method comparison data generated by comparison of the results obtained with the device to those obtained with a legally marketed predicate device with similar indication of use. Patient samples from

the intended use population covering the device measuring range must be used;

(J) Specimen matrix comparison data if more than one specimen type or anticoagulant can be tested with the device. Samples used for comparison must be from patient samples covering the device measuring range;

(K) A description of how the assay cut-off (the medical decision point between positive and negative) was established and validated as well as supporting data;

(L) Clinical performance must be established by comparing data generated by testing samples from the intended use population and differential diagnosis groups with the device to the clinical diagnostic standard. Diagnosis of primary membranous glomerulonephritis must be based primarily on clinical history, physical examination, laboratory tests, including urinalysis, and renal biopsy. Membranous glomerulonephritis is considered to be idiopathic/primary when no secondary cause can be elucidated on the basis of clinical and laboratory criteria. The differential diagnosis groups must include secondary membranous glomerulonephritis, membranoproliferative glomerulonephritis, lupus nephritis, focal segmental glomerulosclerosis, IgA nephritis, diabetic nephropathy, systemic lupus erythematosus, systemic sclerosis, and Goodpasture syndrome. Diagnosis of autoimmune and immune-mediated diseases that are associated with membranous glomerulonephritis must be based on established diagnostic criteria and clinical evaluation. For all samples, clinical criteria, including demographic information must be considered in the differentiation between primary membranous glomerulonephritis and secondary membranous glomerulonephritis. The clinical validation results must demonstrate correlation clinical sensitivity and clinical specificity between the test values and the presence or absence of primary membranous glomerulonephritis. The data must be summarized in tabular format comparing the interpretation of results to the disease status; and

(M) Expected/reference values generated by testing an adequate number of samples from apparently healthy normal individuals.

(iii) Identification of risk mitigation elements used by the device, including a description of all additional procedures, methods, and practices incorporated into the directions for use that mitigate risks associated with testing.

(2) The label required under § 809.10(a) and labeling required under § 809.10(b) of this chapter must include warnings relevant to the assay including:

(i) A warning statement that explains: The device is for use by laboratory professionals in a clinical laboratory setting;

(ii) A warning statement that explains: The test is not a stand-alone test but an adjunct to other clinical information. A diagnosis of pMGN or secondary

MGN should not be made on a single test result. The clinical symptoms, results on physical examination, and laboratory tests (e.g., serological tests), when appropriate, should always be taken into account when considering the diagnosis of primary versus secondary MGN;

(iii) A warning statement that explains: Absence of circulating PLA2R autoantibody does not rule out a diagnosis of pMGN; and

(iv) A warning statement that explains: The assay has not been demonstrated to be effective for monitoring the stage of disease or its response to treatment.

(3) The labeling required under § 809.10(b) of this chapter must include a description of the protocol and performance studies performed in accordance with paragraph (b)(1)(ii) of this section and a summary of the results.