510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k060700

B. Purpose for Submission:
New Devices

C. Measurand:
Anti-proteinase 3 IgG and anti-myeloperoxidase IgG

D. Type of Test:
Qualitative and semi-quantitative, enzyme-linked immunosorbent assay (ELISA)

E. Applicant:
EUROIMMUN US, LLC

F. Proprietary and Established Names:
EUROIMMUN Anti-Proteinase 3 (PR3) ELISA IgG
EUROIMMUN Anti-Myeloperoxidase (MPO) ELISA IgG

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5660, Multiple Autoantibodies Immunological Test System
2. Classification:
   II
3. Product code:
   MOB, Test System, Antineutrophil Cytoplasmic Antibodies (ANCA)
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The EUROIMMUN Anti-Proteinase 3 (PR2) ELISA IgG test kit is designed for the determination of anti-proteinase 3 antibodies (PR3) in human serum and plasma. This test is used as an aid in the differential diagnosis Wegener's granulomatosis and other autoimmune vasculitides, in conjunction with other laboratory and clinical findings.

   The EUROIMMUN Anti-Myeloperoxidase (MPO) ELISA IgG test kit is designed for the determination of anti-myeloperoxidase antibodies (MPO) in human serum and plasma. This test is used as an aid in the differential diagnosis of microscopic polyangitis, Churg-Strauss syndrome and other autoimmune vasculitides, 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:
   Microplate reader capable of measuring OD at 450 and a reference wavelength of 620-650 nm. Microplate washer optional.

I. Device Description:

Each device contains the following: microwell strips (12x8) coated with PR3 or MPO, Calibrators 1-3 (2, 20 and 200 RU/mL), rabbit anti-human IgG horse-radish peroxidase conjugate, TMB/H_2O_2 substrate, positive control, negative control, sample buffer, wash buffer (10x concentrate) and 0.5M sulfuric acid stop solution. All reagents are ready for use except for the wash buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Bindazyme Anti-PR3 ELISA Kit
   Bindazyme Anti-MPO ELISA Kit
2. Predicate 510(k) number:
   k981029 (Anti-PR3)
   k981030 (Anti-MPO)
3. Comparison with predicate:

Anti-PR3

Similarities		
Item	New Device	Predicate Device
Intended Use/Indications for Use	Detection of IgG antibodies to PR3 as an aid in the diagnosis of Wegener's granulomatosis and other autoimmune vasculitis , in conjunction with other laboratory and clinical findings	Same
Assay format	Semi-quantitative	Same
Technology	ELISA	Same
Antigen	Purified	Same
HRP anti-human IgG conjugate	Rabbit	Same
Controls	Positive and negative	Same
Result reading	O.D. at 450 nm	Same
Differences		
Item	New Device	Predicate
Sample	Serum or plasma 1:101 dilution	Serum 1:100 dilution
Calibrators	Three levels: 2, 20, 200 RU/mL	5 levels: 1.23, 3.7, 11.1, 33.3 and 100 U/mL
Controls	Human IgG	Diluted human serum
Wash Buffer	10X concentrate	20X concentrate
Stop solution	0.5M Sulfuric acid	3M Phosphoric Acid
Calculation	Point to point Linear/linear	Not point to point Log/linear
Cut-off value	20 RU/mL	3.5 U/mL
Results interpretation	Semi quantitative: Negative: <20 RU/mL Positive: ≥20 RU/mL Qualitative (OD $_{(patient)}$/OD$_{Cal\ 2}$) Negative: <1.0 Positive: ≥1.0	Semi-quantitative: Negative: ≤3.5 U/mL Positive: >3.5 U/mL

Anti-MPO

Similarities		
Item	New Device	Predicate Device
Intended Use	Detection of IgG antibodies to MPO	Same
Assay format	Semi-quantitative	Same
Technology	ELISA	Same
Antigen	Purified	Same
HRP anti-human IgG conjugate	Rabbit	Same
Controls	Positive and negative	Same
Result reading	O.D. at 450 nm	Same
Differences		
Item	New Device	Predicate
Indications for Use	As an aid in the differential diagnosis of microscopic polyangitis, Churg-Strauss syndrome and other autoimmune vasculitides, in conjunction with other laboratory and clinical findings	As an aid to the diagnosis of certain types of autoimmune vasculitis including crescentric glomerulonephritis and microscopic polyarteritis, in conjunction with other clinical findings.
Sample	Serum or plasma 1:101 dilution	Serum 1:100 dilution
Calibrators	Three levels: 2, 20, 200 RU/mL	5 levels: 1.23, 3.7, 11.1, 33.3 and 100 U/mL
Controls	Human IgG	Diluted human serum
Wash Buffer	10X concentrate	20X concentrate
Stop solution	0.5M Sulfuric acid	3M Phosphoric Acid
Calculation	Point to point Linear/linear	Not point to point Log/linear
Cut-off value	20 RU/mL	3.5 U/mL
Results interpretation	Semi quantitative: Negative: <20 RU/mL Positive: ≥20 RU/mL Qualitative (OD $_{(patient)}$/OD$_{Cal\,2}$) Negative: <1.0 Positive: ≥1.0	Semi-quantitative: Negative: ≤3.5 U/mL Positive: >3.5 U/mL

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
Diluted calibrators, controls, and diluted patient samples are added to microwell coated with either PR3 or MPO antigen. Anti-PR3 or anti-MPO autoantibodies will bind to the respective antigen in the microtiter wells. After washing to remove any unbound serum proteins, horseradish peroxidase conjugated rabbit anti human IgG, is added and incubated. Following another washing step, the peroxidase substrate (TMB) is added and incubated for an additional period of time. If the HRP enzyme conjugate is present in the well, it will react with the substrate and produce a blue color. A stop solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The optical density of the color reaction is measured by spectrophotometry. The amount of antigen specific bound antibody is

proportional to the color intensity.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance</u>:
   a. *Precision/Reproducibility*:
   For each of the device, eight samples with different autoantibody concentrations were used in the reproducibility studies. Samples were assayed 20 times in one run for the intra-assay reproducibility study and 4 times on 4 different runs for the inter-assay reproducibility study. The assay specification was <12% CV. Results are summarized below.

	Anti-PR3				Anti-MPO			
	Intra-Assay		Inter-Assay		Intra-Assay		Inter-Assay	
Serum	Mean RU/mL	CV (%)	Mean RU/mL	CV (%)	Mean RU/mL	CV (%)	Mean RU/mL	CV (%)
1	12	11.1	10	5.1	15	7.8	19	9.9
2	21	7.6	16	9.8	21	11.3	20	4.9
3	22	10.6	16	7.6	30	6.9	27	6.9
4	33	9.9	33	9.6	31	6.5	37	5.4
5	40	3.8	42	8.0	58	4.4	59	5.1
6	85	3.4	94	3.2	67	2.3	71	4.6
7	108	3.4	125	3.5	125	2.1	125	3.5
8	152	4.0	139	3.0	159	1.7	164	3.6

The inter-lot reproducibility for each device was determined by testing 6 QC samples with different autoantibody concentrations for two runs on three lots of kits. Speciation was set at <12% CV. Results are summarized in the following table.

		Anti-PR3						Anti-MPO					
Sample		1	2	3	4	5	6	1	2	3	4	5	6
Lot	Run	Concentrations (RU/mL)											
1	1	116	72	46	20	33	20	85	72	187	20	17	25
1	2	115	72	43	19	37	21	77	74	163	20	18	26
2	1	124	82	47	23	30	22	83	81	170	20	18	26
2	2	129	80	49	22	37	22	83	76	181	20	17	27
3	1	118	79	47	18	29	20	91	70	165	18	16	23
3	2	127	80	47	18	34	20	92	71	165	19	16	23
Mean value		122	78	46	20	33	21	85	74	172	19	17	25
SD		6.0	4.4	1.8	2.1	3.4	1.1	5.5	4.0	9.9	0.8	1.0	1.6
CV%		4.9	5.7	3.8	10.1	10.1	5.2	6.5	5.4	5.8	4.1	6.2	6.5

   b. *Linearity/assay reportable range:*
   For each device, six samples with different autoantibody concentrations were serially diluted to cover the assay range. For each sample, there were four dilutions and the dilution factor for each was dependent on the autoantibody concentration. Results showed the assays were linear for the specified assay range.
   The assay ranges for anti-PR3 and anti-MPO are 12-158 RU/ML and 7-181 RU/mL respectively.
   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

There is no reference standard for anti-PR3 and anti-MPO. The calibrators, positive and negative controls are prepared in-house and arbitrary units are assigned during development process.

d. *Detection limit:*
The detection limit is determined by assaying the sample buffer 20 times in one run. The mean and standard deviation (SD) were calculated. The detection limit is defined as 3SD above the mean value of the sample buffer and is approximately 1 RU/mL.

e. *Analytical specificity:*
Interference by endogenous substances: Aliquots of three samples were spiked with three different concentrations of endogenous substances, namely hemoglobin, bilirubin and triglyceride were tested on both anti-PR3 and anti-MPO devices. The acceptance criterion for the mean % recovery ranged from 89% to 115%. Both devices did not appear to be affected by hemoglobin (up to 1000 mg/dL), bilirubin (up to 40 mg/dL) and triglyceride (up to 2000 mg/dL).

Cross-reactivity: One hundred and sixteen samples from patients with other autoimmune diseases (34 SLE, 5 Ulcerative colitis (UC), 4 Crohns, 23 PBC, 30 glomerular basement membrane (GBM) and 20 RA) were tested by both devices. No cross-reactivity was observed for the samples tested.

f. *Assay cut-off:*
The cut-off value of 20 RU/mL was based on testing 148 normal serum and 155 patient sera [113 Wegener's granulomatosis (WG), 16 Churg-Strauss syndrome (CSS), 13 Polyarteritis nodosa (PN), 11 Microscopic arteritis (MA)]. All normal sera were negative with the 20 RU/mL cut-off.

2. Comparison studies:
    a. *Method comparison with predicate device:*
        Anti-PR3
        Testing was performed on 270 samples which included 148 samples from healthy adults, 37 WG, 5 CSS, 9 PN, 5 MA, 34 SLE, 5 UC, 4 Crohns and 23 PBC on the new device and the predicate device, Bindazyme Anti-PR3 ELISA. Two samples (one SLE and one PBC) were positive with the predicate device and negative with the new device. Results are summarized in table below.

		Bindazyme Anti-PR3 ELISA		
		Positive	Negative	Total
EUROIMMUN Anti-PR3 ELISA	Positive	37	0	37
	Negative	2	231	233
	Total	39	231	270

Positive percent Agreement: 94.9% (37/39)
Negative percent agreement: 100% (231/231)
Overall percent agreement: 99.3% (268/270)

Anti-MPO

The same 270 samples were also tested on the EUROIMMUN Anti-MPO ELISA and the predicate device, Bindazyme Anti-MPO ELISA. Three samples (two SLE and one PBC) were positive with the predicate device and negative with the new device. Results are summarized in table below.

		Bindazyme Anti-MPO ELISA		
		Positive	Negative	Total
EUROIMMUN Anti-MPO ELISA	Positive	24	0	24
	Negative	3	243	246
	Total	27	243	270

Positive percent Agreement: 88.9% (24/27)
Negative percent agreement: 100% (243/243)
Overall percent agreement: 98.9% (267/270)

Other studies to support performance:
Study 1: 342 clinically defined samples from patients with the following diagnosis: 113 WG, 16 CSS, 13 PN, 11 MA and 208 healthy subjects were evaluated internally. Sex and age distribution of the subjects were provided. The Anti-PR3 device was positive only for the WG patient samples and the Anti-MPO device for the CSS, PN and MA samples. None of the samples from the healthy controls reacted in either device.

Study 2: This study used the same samples for the k051489 which was for the EUROIMMUN ANCA IFA Granulocyte BIOCHIP MOSAIC Test to determine the sensitivity and specificity of the Anti-MPO device. Sera samples used consisted of 30 microscopic polyangitis (MPA), 327 sera from patients with other autoimmune diseases (WG, RA, SLE, MCTD, Sjogren's syndrome and scleroderma) and 206 normal blood donors. Of the MPA samples, 53% (16/30) were positive as compared to 0.6% (3/533) in the control group (other autoimmune diseases and blood donors combined). One of the false positive samples was from a patient with WG. When compared to EUROIMMUN ANCA IFA, the % positive agreement for the MPA group was 93.3% (14/15), % negative agreement was 86.7% (13/15) and total agreement was 90% (27/30). As for the control group, the % total agreement was 99.2% (529/533).

b. *Matrix comparison:*
   EDTA, heparin and citrate plasmas are claimed as additional sample types to serum. Comparison studies were performed by testing 15 matched serum/plasma pairs for each anti-coagulant. The concentration of the samples ranged from 14-187 RU/mL for anti-PR3 and 10-181 RU/mL for anti-MPO. The samples were also serially diluted and linear regression analyses were performed in addition to mean % recovery for each serum/plasma pair. Results are summarized below:

	Anti-PR3			Anti-MPO		
	EDTA	Heparin	Citrate	EDTA	Heparin	Citrate
% recovery	101	99	101	106	98	96
Correlation coefficient	0.990	0.985	0.978	0.997	0.996	0.997

3. Clinical studies:
   a. *Clinical Sensitivity and specificity:*
      Not provided.
   b. *Other clinical supportive data (when a is not applicable):*
      Not applicable.
4. Clinical cut-off:
   Same as assay cut-off.
5. Expected values/Reference range:
   Expected values in the normal population should be negative. Using the
   EUROIMMUN Anti-PR3 IgG ELISA, the incidence of anti-PR3 IgG in a German
   cohort of Wegener's granulomatosis was 85%. Using the EUROIMMUN anti-
   MPO IgG ELISA, the incidence of anti-MPO IgG in a German cohort of
   Microscopic polyangitis was 53%.

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.