

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

A. 510(k) Number:

k082130

B. Purpose for Submission:

Modified device

C. Measurand:

Anti-protease 3 Antibodies (PR3) IgG

D. Type of Test:

Qualitative and semi-quantitative

E. Applicant:

EUROIMMUN US INC

F. Proprietary and Established Names:

EUROIMMUN Anti-PR3-hn-hr ELISA (IgG) Kit

G. Regulatory Information:

1. Regulation section:
21 CFR § 866.5660, Multiple autoantibodies, immunological test systems
2. Classification:
Class II
3. Product codes:
MOB, Test System, antineutrophil antibodies (ANCA)
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The EUROIMMUN Anti-PR3-hn-hr ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against proteinase 3 (PR3) in human serum, EDTA plasma, lithium heparin plasma, and citrate plasma. It is used as an aid in the differential diagnosis of Wegener's granulomatosis 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 only.
4. Special instrument requirements:
Microplate reader capable of measuring OD at 450 and a reference wavelength of 620-650 nm. Microplate washer is optional.

I. Device Description:

Each device contains the following: microwell strips (12X8) coated with a mixture of recombinant and native proteinase 3, Calibrators 1-3 (2, 20, 200 RU /mL), rabbit anti-human IgG horse-radish peroxidase conjugate, TMB/H₂O₂ 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):
EUROIMMUN Anti-Proteinase 3 (PR3) ELISA IgG
EUROIMMUN Anti-Myeloperoxidase (MPO) ELISA IgG
2. Predicate K number(s):
k060700
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended 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	Qualitative and semi-quantitative	Same
Technology	ELISA	Same
Platform	96 well plates	Same
Calibrators	Three levels: 2, 20, 200 RU/mL	Same
Controls	Positive, Negative	Same
Enzyme-Conjugate	rabbit anti-human IgG HRP conjugate	Same
Substrate	TMB/H ₂ O ₂	Same
Wash buffer	10X Concentrate	Same
Stop solution	0.5M sulfuric acid	Same
Sample type	Serum and plasma	Same
Sample dilution	1:101	Same
Result reading	O.D. at 450 nm	
Calculation	Point to point Linear/linear	Same
Cut-off value	20 RU/mL	Same
Result Interpretation	Semi-quantitative: Negative: <20 RU/mL Positive: ≥20 RU/mL Qualitative OD (patient/OD _{Cal 2}) Negative: <1.0 Positive: ≥1.0	Same

Differences		
Item	Device	Predicate
Antigen	Mixture of recombinant and highly purified proteinase 3 isolated from human neutrophils	Highly purified proteinase 3 isolated from human neutrophils
Linear range	4 – 197 RU/mL	12 – 158 RU/mL
Detection limit	0.6 RU/mL	1 RU/mL

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

None provided.

L. Test Principle:

The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with a mixture of recombinant human and native human PR3. In the first reaction step, diluted patient samples, calibrators and controls are incubated in the wells. PR3 antigen specific auto-antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, goat anti-human IgG HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have Human IgG binding to the PR3 antigen. The wells are washed to remove any unbound HRP enzyme conjugate. 3,3',5,5'-tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for a stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

For the intra-assay study, seven samples for PR3, (weak, moderate and high titers) were analyzed 20 times in one run. For the inter-assay study, the same samples were analyzed 4 times in 6 different runs. The intra-assay CV ranges from, 1.8% to 5.2% . The inter-assay CV ranges from 3.9% to 11.2% (see table below).

Antigen	Sample #	Intra-assay		Inter-assay	
		Mean value	CV (%)	Mean value	CV (%)
PR3	1	11	5.2	12	6.2
	2	19	2.6	19	5.4
	3	21	2.8	20	5.9
	4	55	4.1	47	11.2
	5	89	2.6	85	4.3
	6	108	1.8	106	4.2
	7	152	2.8	159	3.9

Lot to lot reproducibility:

The inter-lot reproducibility was determined by testing 3 QC samples with different autoantibody concentrations for two runs on three lots. Specification was set at <12%. The lowest variability was 3.6% and highest variability was 7.7%. All three lots were within the 12% variability.

b. *Linearity/assay reportable range:*

Six samples with different autoantibody concentrations were serially diluted to cover the assay range (4 – 197 RU/mL). For each sample, there were four dilutions and the dilution factor for each was dependent on the autoantibody concentration. Results showed the assay was linear for the specified assay range.

Sample	Concentration range of dilutions (RU/ml)	Regression equation	Coeff. of determination (R ²)
1	4 – 57	$y = 97789x - 5.06$	0.9956
2	28 – 147	$y = 106630x + 16.52$	0.9909
3	47 – 197	$y = 16591x + 37.48$	0.9712
4	36 – 164	$y = 113252x + 26.39$	0.9825
5	35 – 171	$y = 60633x + 24.70$	0.9772
6	45 – 172	$y = 224835x + 36.13$	0.9776

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

There are no reference standards for anti-PR3. The calibrators and controls (positive and negative) were prepared in-house and assigned arbitrary units (RU/mL) during the 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 0.6 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 anti-PR3-hn-hr Elisa (IgG) device. The acceptance criterion for the mean % recovery ranged from 85%-115%. The device 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: Sixty seven samples with other autoimmune diseases (20 SLE, 10 Ulcerative Colitis, 10 Crohn’s disease, 15 Primary Biliary Cirrhosis and 12 samples with lactoferrin, elastase, and MPO) were tested. 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 429 normal samples and 725 patient sera [47 Wegener’s’ granulomatosis (WG), 93 ANCA associated vasculitides (AAV), 585 other autoimmune diseases (100 SLE, 200 SS, 230 RA, 55 non-AAV)]. All normal sera were negative with 20 RU/mL cut-off except one with 37.6 RU/mL result.

2. Comparison studies:

a. *Method comparison with predicate device:*

Testing was performed on 1255 samples which included 429 samples from asymptomatic blood donors and 826 patient sera [47 Wegener’s’

granulomatosis (WG); 140 ANCA associated vasculitides (AAV including relapses); 585 other autoimmune diseases (100 SLE, 200 SS, 230 RA, 55 non-AAV); and 78 prospective ANCA positive samples by IFA] on the new device and the predicate device. Results are summarized in table below.

N = 1255		EUROIMMUN Anti-PR3 (IgG)		
		Positive	Negative	Total
EUROIMMUN Anti-PR3-hn- hr (IgG)	Positive	162	36*	198
	Negative	1	1056	1057
	Total	163	1092	1255

*Of the 36 samples found to be anti-PR3-hn-hr (IgG) positive, yet negative with predicate device, 13 were from WG, 14 from AAV, 7 from suspected vasculitis, 1 from SS and 1 from healthy blood donor.

Positive percent agreement: 99.4% (162/163)
 Negative percent agreement: 96.7 % (1056/1092)
 Overall percent Agreement: 97.1% (1218/1255)

b. Matrix comparison:

EDTA, lithium heparin and citrate plasmas are claimed as additional sample types to serum. Comparison studies were performed by testing 14 matched serum/ plasma pairs for each anticoagulant. The concentration of the samples ranged from 6-193 RU/mL. The samples were also serially diluted and linear regression analyses were performed. The results of the regression analysis were as follows:

	EDTA-Plasma	Heparin-Plasma	Citrate-Plasma
Regression equation	$y = 0.078 + 1.044 x$	$y = 0.207 + 1.005 x$	$y = -1.362 + 0.997 x$
95% C.I. of intercept	-5.146 to 1.707	-2.809 to 3.955	-4.634 to 2.107
95% C.I. of slope	0.995 to 1.137	0.956 to 1.053	0.964 to 1.044

y = concentration in serum (RU/mL), x = concentration in plasma (RU/mL)

3. Clinical studies:

a. Clinical Sensitivity and specificity:

The clinical sensitivity and specificity study were evaluated on 1177 clinically defined samples from patients with the following diagnosis: 47 WG, 58 Biopsy-proven AAV, 35 AAV outpatients, 23 AAV relapses, 55 non-ANCA associated vasculitis, 230 RA, 100, SLE, 200 SS and 429 asymptomatic blood donors. The EUROIMMUN anti-PR3-hn-hr Elisa (IgG) device assay sensitivity and specificity were 93.8% (152/162) and 99.4% (1009/1015) respectively (refer to table below).

		Diagnosis		
		Positives	Negative	Totals
EUROIMMUN Anti-PR3-hn-hr ELISA (IgG)	Positive	152	6	158
	Negative	10	1009	1019
	Total	162	1015	1177

c. Other clinical supportive data (when a. and b. are 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-hn-hr ELISA (IgG), the incidence of anti-PR3 in a German cohort of Wegener's granulomatosis was 91.5%.

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.