

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

**A. 510(k) Number:**

k083615

**B. Purpose for Submission:**

New device

**C. Measurand:**

Anti-BP180 (IgG) autoantibodies

**D. Type of Test:**

Qualitative or Semi- Quantitative Enzyme immunoassay

**E. Applicant:**

EUROIMMUN US Inc.

**F. Proprietary and Established Names:**

Proprietary name - EUROIMMUN Anti-BP180-4X ELISA (IgG)

**G. Regulatory Information:**

1. Regulation  
21 CFR 866.5660 Multiple autoantibodies immunological test system
2. Classification  
Class II
3. Product Code  
OEG Autoantibodies, skin (bullous pemphigoid 180 and bullous pemphigoid 230
4. Panel  
Immunology 82

**H. Intended Use:**

1. Intended use(s):  
The EUROIMMUN Anti-BP180-4X ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against BP180 in human serum and plasma. It is used as an aid in the diagnosis of bullous pemphigoid (PB), 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:  
A microtiter plate reader capable of measuring optical densities at 450 nm and a reference wavelength of between 620 nm and 650 nm

**I. Device Description:**

The Anti-BP180-4X ELISA (IgG) test kit is composed of ready-to-use 6 microplate strips containing 8 individual break-off wells in a frame, three levels of calibrators (2 mL each, at 2, 20, and 200 RU/mL human IgG), positive and negative controls (2.0 mL each), 1x12 mL bottle peroxidase-labelled rabbit anti-human IgG, 1 x 100 mL bottle sample buffer, 1x12 mL bottle TMB/H<sub>2</sub>O<sub>2</sub> chromogen/substrate, 1x12 mL bottle stop solution, and a 1x100 mL bottle of 10X concentrated wash solution.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

MESACUP BP180 ELISA Kit manufactured by MBL International Corp.

2. Predicate K number(s):

K071961

3. Comparison with predicate:

	Similarities	
Item	New Device	Predicate Device
Intended use	Detection of IgG antibodies to BP180 as an aid in diagnosis of bullous pemphigoid (PB).	Same
Technology	ELISA	Same
Assay platform	48-well microtiter plates (6 strips x 8 wells)	Same
Calibration	Relative arbitrary units	Same

	Differences	
Item	New Device	Predicate Device
Assay format	Qualitative or semi-quantitative (using either the 3 calibrators or 1 calibrator)	Semi-quantitative
Antigen	Recombinant tetramer protein of the NC16A domain (based on human cDNA), produced in E.coli.	Recombinant purified BP 180 NC16a.
Calibrators	3 calibrators 2, 20, and 200 RU/mL	2 calibrators 0 and 100 U/mL
Controls	2 controls 1 positive, 1 negative	No controls (recommendation that user create own controls)
Samples	Serum or plasma 1:101 dilution	Serum 1:101 dilution
Reported units	RU/mL or Ratio	U/mL
Cut Off level	20 RU/mL	9 U/mL

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

None Listed

**L. Test Principle:**

The device utilizes direct binding enzyme immunoassay technology. 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 (where 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 4 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. Neither inter-assay variation nor intra-assay variation should result in a CV% of over 12%. The following results were obtained:

Intra-assay reproducibility

n = 20	Sample 1	Sample 2	Sample 3	Sample 4
	Concentration (RU/mL):			
Mean value (x):	177	61	119	27
Standard deviation (SD):	3.6	0.7	1.9	0.8
Coefficient of variation (CV, %):	2.1	1.2	1.6	3.0
Mean CV (%):	2.0			

Inter-assay reproducibility

n = 4 tests / 6 days = 24	Sample 1	Sample 2	Sample 3	Sample 4
	Concentration (RU/mL):			
Mean value (x):	174	61	119	26
Standard deviation (SD):	4.6	1.9	5.0	1.3
Coefficient of variation (CV, %):	2.6	3.1	4.2	4.8
Mean CV (%):	3.7			

The Lot to Lot reproducibility is checked during the validation of the kit. 3 different lots are incubated with 3 different QC samples each. For each sample the coefficient of variation (CV) is calculated. Inter-lot variation should show results below CV = 12%. The following results were obtained:

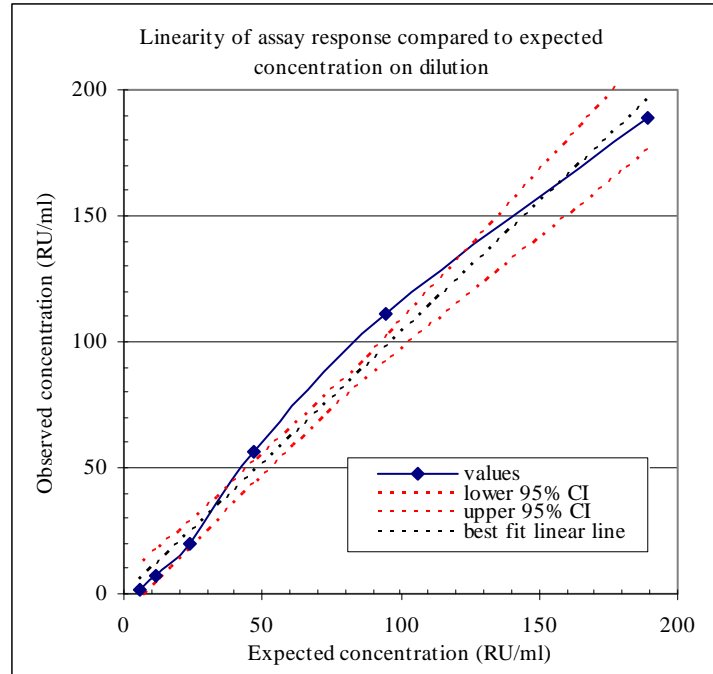
Lot to Lot reproducibility

n = 3 lots x 2 runs = 6	Sample 1	Sample 2	Sample 3
	Concentration (RU/mL):		
Mean value (x):	145	48	99
Standard deviation (SD):	5.5	2.1	2.3
Coefficient of variation (CV, %):	3.8	4.4	2.4
Mean CV (%):	3.5		

b. *Linearity/assay reportable range:*

The linearity of the ELISA was determined by assaying two-fold serial dilutions of 8 serum samples (6 dilutions, covering the concentration range of 2 – 200 RU/mL). Four of the 8 samples were not linear in this range when evaluated by

CLSI EP-6-A (Evaluation of linearity of quantitative measurement procedures). The deviation of 1 or more (up to 3 points in the supplied data) of the 6 points in the dilution series for the 4 samples was outside the 95% confidence interval for the linear line. A typical sample is shown as follows:



Since no reference standard material exists and units for the assay are arbitrary, semi-quantitative linearity may be of lesser importance than a qualitative result (positive or negative) during initial diagnosis.

The reportable range of the assay is 2 RU/mL (the lowest calibrator) to 200 RU/mL (the highest calibrator).

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
 No reference standard material exists for antibody to NC16A domain of BP180. Units for the assay are arbitrary and linked to value assignment using a recombinant protein produced in bacteria (*E. coli*). Value assignment of calibrators is made for a new lot against a current lot using a panel of reference sera. Target values are adjusted by dilution or spiking. The single value positive and single value negative controls used in the assay are derived from human blood donor material. The positive control is assigned an acceptable range using the same criteria ( $\pm$  a stated percentage) regardless of actual concentration.

Stability studies are conducted in accordance the international standard DIN EN 13640:2002: Stability testing of in vitro diagnostics reagents. Three production lots of all kit reagents are tested. For sealed original reagents, real-time stability testing currently supports 12 months duration at 2-8°C. Once opened, real-time stability currently supports 12 months duration at 2-8°C, however upon reconstitution, stability of the wash buffer is currently only 28 days when stored at 2-8°C.

d. *Detection limit:*

The limit of blank is the lowest arbitrary concentration from a replicate series of samples without analyte. It is defined for this assay as a value of three times the standard deviation of the sample buffer. The detection limit of the Anti-BP180-4X ELISA (IgG) is 0.6 RU/mL determined from 20 replicate determinations of sample buffer only. Due to the high dilution factor of the samples, no influence of the sample material (serum or plasma) is expected. The lowest quantified concentration is the lowest calibrator used in the assay (2 RU/mL).

e. *Analytical specificity:*

Cross reactivity with serum from related autoimmune diseases was investigated using a panel of 27 sera from different sources positive for Pemphigus vulgaris (n = 8), Pemphigus foliaceus (n = 2) and Goodpasture syndrome (n = 17). All 27 sera were negative in the Anti-BP180-4X ELISA (IgG).

To investigate interference from hemoglobin, triglycerides, and bilirubin, 4 different specimens at 4 different anti-BP180 concentrations (29, 73, 123, and 186 RU/mL) were spiked with potential interfering substances and were incubated with the test system. The recovery in relation to the unspiked sample without interferent was calculated. The individual value of the recovery was within the acceptable range of 75-125%. No interference was observed for concentrations of up to 1000 mg/dl for hemoglobin, 2000 mg/dl for triglyceride and 40 mg/dl for bilirubin.

f. *Assay cut-off:*

Receiver operator characteristics curve analysis was utilized to determine a cutoff. Disease subject samples were obtained from 118 patients with Bullous pemphigoid or 20 patients with Pemphigoid gestationis from 2 sites not in the United States. Non-diseased subject samples were obtained from the following patients groups from various sites not in the United States:

Panel	N (men, women)	Mean age (age range)
Rheumatoid arthritis	107 (21, 50) (36 unknown)	56 y (30 – 76) (36 unknown)
Progressive systemic sclerosis	50	55 y (22 – 82) (1 unknown)
Systemic lupus erythematosus	72 (1, 29) (42 unknown)	54 y (26 – 87 y) (40 unknown)
Asymptomatic blood donors	494 (309, 185)	39 y (18 – 67 y)
Total	723	

At a cutoff of 20 RU/mL, 98% of non-disease subjects were negative in the assay. Qualitative results are evaluated by calculating a ratio of the Optical Density value of the control or patient sample over the Optical Density value of the 20 RU/mL calibrator. A ratio greater than or equal to 1.0 is considered positive.

2. Comparison studies:

a. *Method comparison with predicate device:*

An external study was performed in collaboration with several hospitals outside the United States on 347 samples tested with the proposed assay and with the predicate device, using the cut-offs recommended by the respective test instructions. The panel consisted of 61 men and 158 women (128 unknown gender). Age ranged from 22 to 99 years with an average age of 65 years (with 80 unknown). The results were categorized as positive or negative and are shown in the table below.

EUROIMMUN	Predicate		
	> 9 U/mL	< 9 U/mL	
> 20 RU/mL	107	5	112
< 20 RU/mL	9	226	235
	116	231	347

	estimate	standard error	lower 95% CI	upper 95% CI
positive agreement	92.2% (107/116)	± 2.5%	85.8%	96.4%
negative agreement	97.8% (226/231)	± 1.0%	95.0%	99.3%
total agreement	96.0% (333/347)	± 2.5%	93.3%	97.8%

b. *Matrix comparison:*

Sixteen samples of serum and the corresponding EDTA, heparin and citrate plasma were tested in the Anti-BP180-4X ELISA. The samples cover concentrations in the diagnostically important range. Passing-Bablok regression was calculated for the comparison of serum to each type of plasma. The slopes and 95% confidence intervals for each comparison are as follows:

Parameter	Serum vs. EDTA-Plasma	
Intercept	1.00	95% C.I.: -2.18 to 3.54
Slope	1.00	95% C.I.: 0.96 to 1.04
	Serum vs. Heparin-Plasma	
Intercept	-3.24	95% C.I.: -5.71 to 0.32
Slope	1.02	95% C.I.: 0.98 to 1.05
	Serum vs. Citrate-Plasma	
Intercept	2.03	95% C.I.: -2.94 to 5.84
Slope	0.98	95% C.I.: 0.91 to 1.06

A comparison in which the 95% confidence interval of the slope contains 1.0 and the 95% confidence interval of the intercept contains 0 indicates equivalence of concentration between serum and the corresponding plasma matrices. The comparisons above satisfy this condition.

3. Clinical studies:

In a clinical study performed in cooperation with several hospitals outside the United States, a total of 841 clinically characterized samples were investigated for anti-

BP180 antibodies using the proposed assay. The EUROIMMUN Anti-BP180-4X ELISA (IgG) showed a sensitivity of 89.8% for Bullous pemphigoid and a specificity of 97.9%. The results are shown in the tables below. 95% C.I. are calculated by the exact method.

*a. Clinical Sensitivity:*

Panel	n (men, women)	Mean age (age range)	Anti-BP180-4X ELISA (IgG)		
			positive	%	95% C.I.
Bullous pemphigoid	118 (39, 79)	76 y (25 – 99 y) (1 unknown)	106	89.8%	82.9 – 94.6%
Pemphigoid gestationis	20 (0, 18) (2 unknown)	32 y (19 – 40) (9 unknown)	20	100.0%	83.2 – 100.0%

*b. Clinical specificity:*

Panel	n (men, women)	Mean age (age range)	Anti-BP180-4X ELISA (IgG)		
			negative	%	95% C.I.
Rheumatoid arthritis	107 (21, 50) (36 unknown)	56 y (30 – 76) (36 unknown)	105	98.1%	93.4 – 99.8%
Progressive systemic sclerosis	50	55 y (22 – 82) (1 unknown)	48	96.0%	86.3 – 99.5%
Systemic lupus erythematosus	72 (1, 29) (42 unknown)	54 y (26 – 87 y) (40 unknown)	71	98.6%	92.5 – 100.0%
Asymptomatic blood donors	494 (309, 185)	39 y (18 – 67 y)	484	98.0%	96.3 – 99.0%
Total	723		708	97.9%	96.6 – 98.8%

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

4. Clinical cut-off:

No cutoff has been established.

5. Expected values/Reference range:

The levels of anti-BP180 antibodies were analyzed with the EUROIMMUN Anti-BP180-4X ELISA (IgG) in a panel of 494 apparently healthy blood donors. The subjects consisting of 309 men and 185 women with an age range of 18-67 years (average age: 39 years). The 98th percentile of the healthy blood donors was 27.4 RU/mL.

Positives	10
Negatives	484
Lowest value	0.01 RU/mL
Highest value	232.16 RU/mL
Mean value	4.73 RU/mL
Std dev. (SD)	15.87 RU/mL

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.