

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k071961

B. Purpose for Submission:

New devices.

C. Measurand:

Autoantibodies to BP180 and BP230

D. Type of Test:

Semi-quantitative, ELISA

E. Applicant:

MBL International Corporation

F. Proprietary and Established Names:

MESACUP BP180 ELISA Kit and MESACUP BP230 ELISA Kit

G. Regulatory Information:

1. Regulation section:
21 CFR § 866.5660 Multiple Autoantibodies, Immunological Test System
2. Classification:
Class II
3. Product codes:
OEG Autoantibodies, Skin (Bullous Pemphigoid 180 and Bullous Pemphigoid 230)
4. Panel:
Immunology, 82

H. Intended Use:

1. Intended use(s):
The MESACUP BP180 Test is a semi-quantitative, enzyme-linked immunosorbent assay (ELISA) for the detection of anti BP180 antibodies in human serum. The MESACUP BP180 Test is intended for in-vitro diagnostic use as an aid in the diagnosis of bullous pemphigoid in conjunction with other laboratory and clinical findings. Patients with bullous pemphigoid are known to have either BP180 or BP230 or both types of antibodies in serums. It is recommended that each patient be tested for both BP180 and BP230 antibodies.

The MESACUP BP230 Test is a semi-quantitative, enzyme-linked immunosorbent assay (ELISA) for the detection of anti BP230 antibodies in human serum. The MESACUP BP230 Test is intended for in-vitro diagnostic use as an aid in the diagnosis of bullous pemphigoid in conjunction with other laboratory and clinical findings. Patients with bullous pemphigoid are known to have either BP180 or BP230 or both types of antibodies in serums. It is recommended that each patient be tested for both BP180 and BP230 antibodies.

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 (wavelength: 450nm, 620nm/reference)

Automatic washer

I. Device Description:

The device consists of the following:

BP180 MICROWELL STRIPS - 48 wells microwell strips (6 x 8 wells) coated with recombinant purified BP 180NC16a antigen, the breakaway strips packed in a strip holder and sealed in a foil envelope with desiccant, are stable at 2-8°C until labeled expiration date.

BP230 MICROWELL STRIPS - 48 wells microwell strips (6 x 8 wells) coated with recombinant purified BP230-N and BP230C antigen, the breakaway strips packed in a strip holder and sealed in a foil envelope with desiccant, are stable at 2-8°C until labeled expiration date.

Calibrator 1 (0U/ml) - One vial containing 1.5ml of Assay Diluent including 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

Calibrator 2 (100U/ml) - One vial containing 1.5ml of anti BP180 or BP 230 antibody positive human serum with Assay Diluent including 0.09 sodium azide. Stable at 2-8°C until labeled expiration date.

Conjugate Reagent - One vial containing 8ml of horseradish peroxidase (HRP) conjugated goat anti human IgG. Stable at 2-8°C until labeled expiration date.

Assay Diluent - One vial containing 50ml of PBS, Tween 20 and 0.09% sodium azide. Stable at 2-8°C until labeled expiration date.

Wash Concentrate (10x) - One vial containing 100ml of PBS and Tween 20 as a 10x concentrate. Stable at 2-8°C until labeled expiration date.

Substrate- One vial containing 20ml of 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H₂O₂). Stable at 2-8°C until labeled expiration date.

Positive and negative controls are not supplied with device.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Scimedx Anti-Skin Antibody Test Kit

2. Predicate K number(s):

k902237

3. Comparison with predicate:

| Similarities | | |
|---------------------|---|---|
| Item | Device | Predicate |
| Devices | MESACUP BP180/ MESACUP BP230 ELISA Kits | Scimedx Anti-Skin Antibody Test System |
| Indications for Use | Diagnosis of bullous pemphigoid | Diagnosis of various skin disorders and bullous pemphigoid autoimmune diseases |
| Sample type | Serum | Serum |

| Similarities | | |
|--------------|-------------------|--------------------------------|
| Item | Device | Predicate |
| Type of test | Semi-quantitative | Qualitative, semi-quantitative |

| Differences | | |
|------------------|--|--|
| Item | Device | Predicate |
| Technology | ELISA | Indirect Immunofluorescence |
| Intended Use | Detection of autoantibodies against BP180 and BP 230 antibodies | Detection of both anti-basement membrane and anti-intercellular antibodies. |
| Antigen | BP 180 Antigen: recombinant purified BP 180NC16a BP230 antigen: recombinant purified BP 230-N and BP 230C | Monkey esophagus |
| Calibrators | Two levels: 0 U/mL and 100 U/mL | Not applicable |
| Controls | No positive and negative controls supplied. Recommended to use. | Positive and negative controls, ready to use |
| Assay Diluent | PBS with Tween 20 | PBS |
| Wash concentrate | PBS and Tween 20: 10X concentrate | PBS |
| Conjugate | HRP conjugated anti-human IgG | Fluorescein isothiocyanate (FITC) conjugated anti-human IgG |
| Substrate | TMB/H ₂ O ₂ | Not applicable |
| Stop solution | 1.0N. sulfuric acid | Not applicable |
| Incubation time | 60-60-30 minutes | 30-30 minutes |
| Result reading | O.D. at 450/620 nm | Green fluorescence at the sites of autoantibodies binding to the substrate |
| Cut-off | <9 U/mL: Negative ≥9 U/mL: Positive | Negative: No fluorescence Positive: Fluorescence at 1:10 dilution or more |

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

None

L. Test Principle:

The MESACUP BP180 Test measures anti-BP180 antibodies present in the serum by ELISA. Calibrators and patient sera are added to microwell coated with BP180 antigen, allowing anti-BP180 antibodies to react with the immobilized antigen (Sample incubation). After washing to remove any unbound serum proteins,

horseradish peroxidase conjugated anti-human IgG antibody is added and incubated (Conjugate incubation). Following another washing step, the peroxidase substrate is added and incubated for an additional period of time (Substrate incubation). Acid solution is then added to each well to terminate the enzyme reaction and to stabilize the color development. The assay can be quantified by measuring the reaction photometrically.

The MESACUP BP230 TEST measures anti-BP230 antibodies present in the serum by ELISA. Same as for the MESACUP BP180 Test.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Intra-assay Precision

Variability within a plate (intra-assay precision) was determined by testing each of three samples 8 times on three separate assay runs using two different lots. The three separate plates employed were randomly selected from each plate coating run (kit lot). An additional study covering the low end of the measuring range was requested and data provided. Resulting intra-assay coefficients of variation (%CVs) were determined from values obtained and summarized in the tables below.

Anti-BP180 Intra-assay:

| Lot | Sample | Mean value (U/mL) | CV (%) | Sample | Mean value (U/mL) | CV (%) |
|-----|--------|-------------------|--------|--------|-------------------|--------|
| A | 1 | 50.7 | 1.1 | 4 | 5.4 | 5.8 |
| A | 2 | 116.6 | 0.6 | 5 | 9.4 | 2.9 |
| A | 3 | 201.5 | 1.2 | 6 | 17.8 | 3.1 |
| B | 1 | 52.2 | 1.6 | 4 | 5.3 | 1.3 |
| B | 2 | 117.0 | 1.3 | 5 | 10.0 | 1.6 |
| B | 3 | 191.8 | 2.2 | 6 | 18.0 | 3.2 |

Anti-BP230 Intra-assay:

| Lot | Sample | Mean value (U/mL) | CV (%) | Sample | Mean value (U/mL) | CV (%) |
|-----|--------|-------------------|--------|--------|-------------------|--------|
| A | 1 | 20.6 | 1.9 | 4 | 5.9 | 2.2 |
| A | 2 | 66.0 | 3.2 | 5 | 11.3 | 2.1 |
| A | 3 | 139.6 | 1.1 | 6 | 16.7 | 3.4 |
| B | 1 | 23.5 | 3.9 | 4 | 5.2 | 4.5 |
| B | 2 | 70.3 | 1.5 | 5 | 10.6 | 3.2 |
| B | 3 | 130.9 | 3.4 | 6 | 17.0 | 2.6 |

Inter-assay Precision

Day to day variability within plates of the same lot was determined by testing three samples in 8 repetitive assays on five consecutive days. Resulting inter-assay %CVs were determined and summarized in the table below.

An additional study covering the low end of the measuring range was requested and data provided. Resulting inter-assay coefficients of variation (%CVs) were determined from values obtained and summarized in the tables below.

Anti-BP180 Inter-assay:

| Lot | Sample | Mean value (U/mL) | CV (%) |
|-----|--------|-------------------|--------|
| A | 1 | 23.0 | 2.4 |
| A | 2 | 46.1 | 1.5 |
| A | 3 | 89.5 | 1.7 |
| B | 1 | 23.2 | 2.8 |
| B | 2 | 46.7 | 1.9 |
| B | 3 | 90.3 | 0.7 |
| A | 4 | 8.1 | 3.4 |
| A | 5 | 11.9 | 4.1 |
| B | 4 | 7.7 | 4.1 |
| B | 5 | 12.1 | 4.7 |

Anti-BP230 Inter-assay:

| Lot | Sample | Mean value (U/mL) | CV (%) |
|-----|--------|-------------------|--------|
| A | 1 | 19.1 | 9.7 |
| A | 2 | 71.3 | 7.8 |
| A | 3 | 141.0 | 4.9 |
| B | 1 | 22.4 | 9.1 |
| B | 2 | 74.2 | 6.7 |
| B | 3 | 130.0 | 4.2 |
| A | 4 | 8.2 | 5.0 |
| A | 5 | 17.2 | 1.6 |
| B | 4 | 8.7 | 6.9 |
| B | 5 | 17.5 | 2.1 |

Inter-lot Precision

To determine the amount of variability between plates of the different lots, three samples were tested eight times in microwells from different plate lots. This was performed on three separate lots. Results of inter-lot CVs were determined and summarized below. An additional study covering the low end of the measuring range was requested and data provided.

Anti-BP180 Inter-lot study:

| Sample ID | Mean value (U/mL) | CV (%) |
|-----------|-------------------|--------|
| 1 | 52.5 | 3.1 |
| 2 | 115.4 | 2.1 |
| 3 | 197.9 | 4.1 |
| 4 | 5.4 | 0.86 |
| 5 | 9.6 | 3.13 |
| 6 | 19.3 | 3.44 |

Anti-BP230 Inter-lot study:

| Sample ID | Mean value (U/mL) | CV (%) |
|-----------|-------------------|--------|
| 1 | 21.5 | 7.8 |
| 2 | 68.9 | 3.6 |
| 3 | 133.3 | 4.1 |
| 4 | 5.5 | 7.06 |
| 5 | 8.5 | 3.94 |
| 6 | 18.0 | 4.10 |

- b. *Linearity/assay reportable range:*
There is no claim for linearity for this assay.
The assay reportable range is from 5-150 U/mL.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no reference standard for Anti-BP180 and BP230. The calibrators are prepared in-house and arbitrary units are assigned during development process. Positive and negative controls are not supplied but are recommended.

Specimens that were positive by both ELISA and IFA were evaluated to determine the assay's upper optical density detection limit. Fifty percent of the upper detection limit was arbitrarily assigned a value of 100 U/mL. Subsequent calibrators are prepared and compared to initial testing. Kit recovery is correlated with previous lots by testing a serum panel and comparing historical values.

Three lots each of BP180 kit and BP230 kit were put into stability program to assure consistent and reliable results over time. All kit components were stored appropriately at 2-8°C and tested at nine month intervals. The assays were performed using BP180 and BP230 reactive samples and healthy blood donor samples. All the sample results were within expected value in the study. The shelf life of the BP180 and BP230 kits was determined to be 13 months from date of production.

- d. *Detection limit:*
Not applicable.
- e. *Analytical specificity:*
Interfering Substances
Potentially interfering substances (bilirubin C = 39.0 mg/dL, bilirubin F = 37.2 mg/dL, hemoglobin = 440.0 mg/dL, chyle = 2350 turbidity units, and RF = 1000 IU/mL) were spiked into three patient specimens (negative, low and high positive). No significant effect on assay results was noted by addition of these substances.

Cross Reactivity

Two cross-reactivity studies were performed, one using samples from Japan and the other from US for both BP180 and BP230 ELISA tests. Results of the two studies are shown below.

Japanese population:

BP180 ELISA and BP230 ELISA: Cross Reactivity Study:

| Disease Group | Number Tested | Number Positive | % Positive |
|--------------------------|---------------|-----------------|------------|
| Pemphigus | 62 | 0 | 0 |
| Epstein barr virus (EBV) | 7 | 0 | 0 |
| Treponema pallidum | 9 | 0 | 0 |
| SLE | 7 | 0 | 0 |
| SjS | 6 | 0 | 0 |
| RA | 8 | 0 | 0 |
| MCTD | 7 | 0 | 0 |

US population:

BP180 ELISA: Cross Reactivity Study:

| Disease Group | Number Tested | Number Positive | % Positive |
|---|---------------|-----------------|------------|
| Pemphigus | 67 | 9 | 13.4 |
| Linear IgA bullous erythematosus (LABD) | 7 | 0 | 0 |
| Epidermolysis bullous acquisita (EBA) | 3 | 1 | 33.3 |
| Bullous systemic lupus erythematosus (BSLE) | 1 | 0 | 0 |

BP230 ELISA: Cross Reactivity Study:

| Disease Group | Number Tested | Number Positive | % Positive |
|---------------|---------------|-----------------|------------|
| Pemphigus | 67 | 5 | 7.5 |
| LABD | 7 | 0 | 0 |
| EBA | 3 | 0 | 0 |
| BSLE | 1 | 0 | 0 |

Combined US and Japanese populations:

BP180 ELISA: Cross Reactivity Study:

| Disease Group | Number Tested | Number Positive | % Positive |
|--------------------|---------------|-----------------|------------|
| Pemphigus | 129 | 9 | 7 |
| LABD | 7 | 0 | 0 |
| EBA | 3 | 1 | 33.3 |
| BSLE | 1 | 0 | 0 |
| RA | 8 | 0 | 0 |
| MCTD | 7 | 0 | 0 |
| EBV | 7 | 0 | 0 |
| Treponema pallidum | 9 | 0 | 0 |
| SLE | 7 | 0 | 0 |
| SjS | 6 | 0 | 0 |

BP230 ELISA: Cross Reactivity Study:

| Disease Group | Number Tested | Number Positive | % Positive |
|--------------------|---------------|-----------------|------------|
| Pemphigus | 129 | 5 | 3.9 |
| LABD | 7 | 0 | 0 |
| EBA | 3 | 0 | 0 |
| BSLE | 1 | 0 | 0 |
| RA | 8 | 0 | 0 |
| MCTD | 7 | 0 | 0 |
| EBV | 7 | 0 | 0 |
| Treponema pallidum | 9 | 0 | 0 |
| SLE | 7 | 0 | 0 |
| SjS | 6 | 0 | 0 |

RA: Rheumatoid Arthritis MCTD: Mixed Connective Tissue Disease

SLE: Systemic Lupus Erythematosus SjS: Sjögren's Syndrome

f. Assay cut-off:

See expected section.

2. Comparison studies:*a. Method comparison with predicate device:*

Testing was performed on 136 samples which included 68 healthy blood donors and 68 bullous pemphigoid patient samples.

The MBL Anti-BP180 ELISA positive percent agreement was 70.8% (46/65); the negative percent agreement was 93.0% (66/71); and the overall agreement was 82.4% (112/136). Results are summarized below.

| | | Scimedx Anti-Skin Antibody IIF Test | | |
|----------------------|----------|-------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| MBL Anti-BP180 ELISA | Positive | 46 | 5 | 51 |
| | Negative | 19 | 66 | 85 |
| | Total | 65 | 71 | 136 |

Positive percent Agreement: 70.8% (46/65)

Negative percent agreement: 93.0% (66/71)

Overall percent agreement: 82.4% (112/136)

The MBL Anti-BP230 ELISA positive percent agreement was 73.0% (46/63); the negative percent agreement was 98.6% (72/73); and the overall agreement was 86.8% (118/136). Results are summarized below.

| | | Scimedx Anti-Skin Antibody IIF Test | | |
|----------------------|----------|-------------------------------------|----------|-------|
| | | Positive | Negative | Total |
| MBL Anti-BP230 ELISA | Positive | 46 | 1 | 47 |
| | Negative | 17 | 72 | 89 |
| | Total | 63 | 73 | 136 |

Positive percent Agreement: 73.0% (46/63)
Negative percent agreement: 98.6% (72/73)
Overall percent agreement: 86.8% (118/136)

- b. Matrix comparison:*
Not applicable.
3. Clinical studies:
- a. Clinical sensitivity and specificity:*
- US population:** Serum samples from sixty nine (69) US patients diagnosed with bullous pemphigoid and 82 healthy blood donors were tested by BP180 ELISA and BP230 ELISA to determine clinical sensitivity and specificity.
Sensitivity: BP180 ELISA kit: 73.9% (51/69)
BP230 ELISA kit: 69.6% (48/69)
- Specificity: BP180 ELISA kit: 98.8% (81/82)
BP230 ELISA kit: 100%
- Japanese population:** Serum samples from two hundred thirty nine (239) Japanese patients diagnosed with bullous pemphigoid and 109 healthy blood donors were tested by BP180 ELISA and BP230 ELISA to determine clinical sensitivity and specificity.
Sensitivity: BP180 ELISA kit: 69.9% (167/239)
BP230 ELISA kit: 72.8% (174/239)
- Specificity: BP180 ELISA kit: 100%
BP230 ELISA kit: 99.1% (108/109)
- Combined US and Japanese Population**
Sensitivity: BP180 ELISA kit: 70.8% (218/308)
BP230 ELISA kit: 72.1% (222/308)
- Specificity: BP180 ELISA kit: 99.5% (190/191)
BP230 ELISA kit: 99.5% (190/191)
- Clinical sensitivities of 70.8% for BP180 and 72.1% for BP230 were supported by literature with values ranging from 66.3% to 95.3%.
- b. Other clinical supportive data (when a. is not applicable):*
None provided.
4. Clinical cut-off:
Same as assay cut-off.
5. Expected values/Reference range:
Expected values in the normal population should be negative.

A ROC analysis using 72 BP patient samples, 336 healthy blood donor samples, and 111 related skin disease patient samples demonstrated the best performance

data of 9.1 U/mL, where the sensitivity was 73.6% and the specificity was 98.9%. Based on this analysis, cut-off value for MESACUP BP180 ELISA and MESACUP BP230 ELISA was determined at 9 U/mL.

| | |
|--------------------------------|-----------|
| Negative for anti-BP180 Ab and | |
| < 9 U/mL | BP230 Ab |
| Positive for anti-BP180 Ab and | |
| ≥ 9 U/mL | BP 230 Ab |

The incidence of BP180 and BP230 were examined in various populations, with the following results.

BP180 ELISA: Incidence of BP180 in Various Populations (Internal studies):

| Population | No. Tested | No. Positive | % Positive |
|--|------------|--------------|------------|
| Healthy blood donors (US) | 82 | 1 | 1.2% |
| Healthy blood donors (Japan) | 109 | 0 | 0.0% |
| Bullous pemphigoid (US) | 69 | 51 | 73.9% |
| Bullous pemphigoid (Japan) | 239 | 167 | 69.9% |
| Other autoimmune skin diseases (US) (1) | 88 | 11 | 12.5% |
| Other autoimmune skin diseases (Japan) (2) | 62 | 0 | 0.0% |
| Infectious diseases (Japan) (3) | 16 | 0 | 0.0% |
| Other autoimmune diseases (Japan) (4) | 28 | 0 | 0.0% |

BP230 ELISA: Incidence of BP230 in Various Populations (Internal studies):

| Population | No. Tested | No. Positive | % Positive |
|--|------------|--------------|------------|
| Healthy blood donors (US) | 82 | 0 | 0.0% |
| Healthy blood donors (Japan) | 109 | 0 | 0.0% |
| Bullous pemphigoid (US) | 69 | 48 | 69.6% |
| Bullous pemphigoid (Japan) | 239 | 167 | 72.8% |
| Other autoimmune skin diseases (US) (1) | 88 | 6 | 6.8% |
| Other autoimmune skin diseases (Japan) (2) | 62 | 0 | 0.0% |
| Infectious diseases (Japan) (3) | 16 | 0 | 0.0% |
| Other autoimmune diseases (Japan) (4) | 28 | 0 | 0.0% |

(1): pemphigus, linear IgA bullous dermatosis, epidermolysis bullous acquisita, bullous SLE

(2): pemphigus

(3): epstein barr virus, treponema pallidum

(4): SLE, SjS, RA, MCTD

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.