

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

A. 510(k) Number:

k091969

B. Purpose for Submission:

New assay

C. Measurand:

Anti-Desmoglein 1 and Anti-Desmoglein 3 IgG autoantibodies

D. Type of Test:

Semi-quantitative or qualitative immunoassay

E. Applicant:

Euroimmun US Inc.

F. Proprietary and Established Names:

EUROIMMUN Anti- Desmoglein 1 ELISA (IgG)

EUROIMMUN Anti-Desmoglein 3 ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5660, Multiple autoantibodies immunological test system

2. Classification:

Class II

3. Product code:

NBO, Autoantibodies, skin (Desmoglein 1 and Desmoglein 3)

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

See Indications for Use below

2. Indication(s) for use:

The EUROIMMUN Anti-Desmoglein 1 ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against desmoglein 1 in human serum and plasma. It is used as an aid in the diagnosis of pemphigus foliaceus (PF) in conjunction with other laboratory and clinical findings.

The EUROIMMUN Anti-Desmoglein 3 ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class autoantibodies against desmoglein 3 in human serum and plasma. It is used as an aid in the diagnosis of pemphigus vulgaris (PV), in conjunction with other laboratory and clinical findings.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Microplate reader capable of measuring OD at 450 nm and 620 to 650 nm.

I. Device Description:

The devices are packaged as kits containing: microplate strips and frame, three calibrators, positive and negative controls, HRP-conjugated rabbit anti-human IgG, ready-to-use sample buffer, 10x wash buffer concentrate, TMB substrate and sulfuric acid stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
RhiGene Mesacup DSG-1 and DSG-3 ELISA Test System
2. Predicate K number(s):
k000336
3. Comparison with predicate:

| Similarities | | |
|---------------------|---|---|
| Item | New Device | Predicate Device |
| | EUROIMMUN Anti-Desmoglein 1 ELISA (IgG) test kit EUROIMMUN Anti-Desmoglein 3 ELISA (IgG) test kit | RhiGene Mesacup DSG-1 and DSG-3 ELISA Test System |
| Intended use | Detection of IgG autoantibodies to desmoglein -1 as an aid in diagnosis of pemphigus foliaceus (PF). Detection of IgG autoantibodies to desmoglein -3 as an aid in diagnosis of pemphigus vulgaris (PV). | Detection of IgG autoantibodies to desmoglein-1 /-3 as an aid in diagnosis of certain pemphigus diseases. |
| Technology | ELISA | Same |
| Assay platform | 48-well microtiter plates (6 strips of 8 wells) | Same |
| Calibration | Relative arbitrary units | Same |
| Substrate | TMB | Same |
| Stop solution | 0.5 M sulphuric acid | Same |
| Cut Off level | 20 RU/mL | 20 U/mL |
| Differences | | |
| Item | New Device | Predicate Device |
| Assay format | Qualitative or semi-quantitative (using 1 calibrator only or standard curve based on the 3 calibrators) | Semi-quantitative |
| Antigen | Extracellular domain of desmoglein-1 or desmoglein-3 (5 subdomains). The protein based on human cDNA was produced recombinantly in mammalian cells. | Recombinant purified proteins of desmoglein-1 or desmoglein-3 |

| | | |
|----------------|---|---|
| Calibrators | 3 calibrators: 2, 20 and 200 RU/mL (for each test system) | 3 calibrators: 1 negative calibrator, 1 desmoglein-1 calibrator and 1 desmoglein 3 calibrator |
| Controls | 2 controls (for each test system) 1 positive, 1 negative | No controls (recommendation that user create own controls) |
| Conjugate | Rabbit anti-human IgG labeled with horseradish peroxidase | Mouse anti-human IgG labeled with horseradish peroxidase |
| Samples | Serum or plasma 1:101 dilution | 1:101 dilution Serum |
| Reported units | RU/mL or Ratio | U/mL |

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

None referenced.

L. Test Principle:

Calibrators, controls, or diluted patient samples are added to the wells and autoantibodies recognizing the antigen bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured human autoantibody. Excess unbound conjugate is removed by another wash step. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate. The intensity of color development is proportional to the concentration of autoantibody in the sample. Microtiter plates are read at 450 nm and a reference wavelength of 620 to 650 nm. The controls and patient results are interpreted by comparing them as a ratio of one of the calibrators (for a qualitative claim), or to a 3 point calibration curve (for the semi-quantitative claim).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of the tests were assessed by determining the intra- and inter-assay coefficients of variation (CV) of several sera with values across the claimed assay ranges. The intra-assay %CVs are based on 20 determinations and the inter-assay %CVs on 16 determinations (2 runs on 8 different days) or 24 determinations (4 runs on 6 different days).

Intra-assay precision:

| | Anti-Desmoglein 1 ELISA (IgG) | | | | | | | | |
|--------------------|-------------------------------|------|-----|-----|-----|-----|-----|-----|-----|
| Mean value (RU/mL) | 2 | 5 | 14 | 19 | 47 | 73 | 111 | 172 | 180 |
| SD (RU/mL) | 0.4 | 0.6 | 0.8 | 0.7 | 1.9 | 2.3 | 3.6 | 6.2 | 7.0 |
| % CV | 17.1 | 10.8 | 6.0 | 3.6 | 4.0 | 3.1 | 3.3 | 3.6 | 3.9 |

| | Anti-Desmoglein 3 ELISA (IgG) | | | | | | | | |
|--------------------|-------------------------------|-----|-----|-----|-----|-----|-----|------|--|
| Mean value (RU/mL) | 7 | 8 | 23 | 27 | 42 | 63 | 155 | 187 | |
| SD (RU/mL) | 0.7 | 0.6 | 1.7 | 1.5 | 1.8 | 1.6 | 9.2 | 5.63 | |
| % CV | 8.9 | 7.5 | 7.2 | 5.6 | 4.4 | 2.6 | 5.9 | 3.0 | |

Inter-assay precision:

| Anti-Desmoglein 1 ELISA (IgG) | | | | | | | | | | | |
|-------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Mean value (RU/mL) | 5 | 15 | 17 | 22 | 25 | 40 | 77 | 134 | 145 | 184 | 199 |
| SD (RU/mL) | 1.0 | 0.5 | 0.4 | 0.6 | 0.7 | 1.0 | 1.3 | 2.4 | 2.7 | 3.6 | 8.7 |
| % CV | 23.3 | 3.4 | 2.6 | 2.8 | 2.7 | 2.5 | 1.6 | 1.8 | 1.9 | 2.0 | 4.4 |
| n = | 24 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |

| Anti-Desmoglein 3 ELISA (IgG) | | | | | | | | | | | |
|-------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Mean value (RU/mL) | 7 | 14 | 16 | 18 | 22 | 35 | 60 | 69 | 154 | 180 | 195 |
| SD (RU/mL) | 1.0 | 1.0 | 1.0 | 0.5 | 1.7 | 1.2 | 2.3 | 3.6 | 4.2 | 4.7 | 8.8 |
| % CV | 13.9 | 6.8 | 6.5 | 2.7 | 7.5 | 3.3 | 3.8 | 5.3 | 2.8 | 2.6 | 4.5 |
| n = | 24 | 16 | 16 | 16 | 16 | 16 | 16 | 24 | 16 | 24 | 24 |

Reproducibility:

Samples near the assay cut-off of 20 U/mL from the inter-assay precision studies above were assessed using the qualitative method. Each sample was tested 16 times:

| Anti-Desmoglein 1 ELISA | | | Anti-Desmoglein 3 ELISA | | |
|-------------------------|-----------------|-------------------|-------------------------|-----------------|-------------------|
| Sample Mean (RU/mL) | Expected result | # Correct results | Sample Mean (RU/mL) | Expected result | # Correct results |
| 15 | Neg | 16/16 | 16 | Neg | 16/16 |
| 17 | Neg | 16/16 | 18 | Neg | 16/16 |
| 22 | Pos | 16/16 | 22 | Pos | 16/16 |
| 25 | Pos | 16/16 | | | |

b. Linearity/assay reportable range:

Serial dilutions of six positive patient sera (per assay) showed the assay was linear across the claimed measurement range of 2 – 200 RU/mL. Ordinary least squares linear regression of obtained values (y-axis) against expected values (x-axis) was performed for each sample and the coefficients of determination (R^2) calculated. Values above or below the measurement range were excluded from the calculation. Mean percent recovery with the Anti-Desmoglein 1 ELISA was 99% (range: 70 – 121%) and with the Anti-Desmoglein 3 ELISA 101% (range: 71 – 129%). In both assays, the samples that were outside of $\pm 10\%$ of the expected values were highly diluted samples (1:800 or higher).

| Anti-Desmoglein 1 ELISA (IgG) | | | |
|-------------------------------|----------------------------|---------------------|---------------------------------|
| Sample | Range of dilutions (RU/mL) | Regression equation | Coeff. of correlation (R^2) |
| 1 | 7 – 123 | $y = 1.03x + 0.58$ | 0.983 |
| 2 | 3 – 197 | $y = 1.05x - 4.94$ | 0.989 |
| 3 | 2 – 111 | $y = 1.05x - 3.02$ | 0.995 |

| Anti-Desmoglein 1 ELISA (IgG) | | | |
|-------------------------------|---------|--------------------|-------|
| 4 | 2 – 163 | $y = 1.03x - 0.40$ | 0.994 |
| 5 | 3 – 140 | $y = 1.03x - 3.52$ | 0.998 |
| 6 | 3 – 190 | $y = 1.03x - 0.96$ | 0.992 |

| Anti-Desmoglein 3 ELISA (IgG) | | | |
|-------------------------------|----------------------------|---------------------|---|
| Sample | Range of dilutions (RU/mL) | Regression equation | Coeff. of correlation (R ²) |
| 1 | 6 – 195 | $y = 1.04x + 2.75$ | 0.976 |
| 2 | 5 – 142 | $y = 1.03x - 1.53$ | 0.994 |
| 3 | 10 – 173 | $y = 1.04x + 1.13$ | 0.972 |
| 4 | 2 – 136 | $y = 1.03x + 0.14$ | 0.991 |
| 5 | 2 -162 | $y = 1.03x - 1.24$ | 0.994 |
| 6 | 14 – 130 | $y = 1.05x - 1.15$ | 0.957 |

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
 There are no international standards for desmoglein antibodies. The results are expressed as either relative units (RU/mL) using the three-point calibration, or as a ratio using the single calibrator 2.

Stability studies are conducted in accordance to the international standard DIN EN 13640:2002: Stability testing of in vitro diagnostics reagents. Three production lots of all kit reagents are tested. Real-time stability studies (+2°C to +8°C) are underway; current data supports at least a six month unopened stability claim. A preliminary stability claim of 10 months is supported by means of an accelerated stability test.

Opened reagents are stable until the expiration date if stored as directed in the instructions (+2°C to +8°C). Diluted (ready-to-use) wash buffer is stable for up to 28 days.

The sponsor recommends following the guidelines in CLSI H18-A3 for sample storage.

- d. *Detection limit:*
 Claimed assay range is 2 -200 RU/mL. The limit of detection (LoD) of the assays as determined by repeated measurement of the blank plus two times their standard deviations were well below 2 RU/mL.

- e. *Analytical specificity:*
 Cross-reactivity of the assays was tested with a panel of 20 bullous pemphigoid samples and 20 linear IgA dermatosis samples. All samples were negative by the anti-desmoglein 1 and the anti-desmoglein 3 assays. Cross-reactivity between anti-Desmoglein 1 and anti-Desmoglein 3 is shown in the clinical sensitivity and specificity section below.

To investigate the influence of potentially interfering substances, aliquots of three samples of differing concentrations were spiked with different concentrations hemoglobin, triglycerides and bilirubin. The spiked samples were measured using the Anti-Desmoglein 1 and Anti-Desmoglein 3 ELISA (IgG) according to the package insert and the results expressed in RU/ml. No significant interference was observed with hemolytic, lipemic or icteric samples 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:

The defined cut-off of 20 RU/mL was evaluated in the normal range study (see below). The average value plus 3 standard deviations was below 20 RU/mL in both assays.

2. Comparison studies:

a. Method comparison with predicate device:

A study was performed in cooperation with the dermatological reference center of the university hospital Lübeck, Germany. In total, 190 samples (50 from PF patients, 71 from PV patients, 48 from BP patients and 21 from LAD patients) were tested with the EUROIMMUN Anti-Desmoglein 1 and Anti-Desmoglein 3 ELISA (IgG) and the predicate assays using the cut-offs recommended by the respective test instructions. The results are shown in the tables below. 95% C.I. are calculated by the exact method. Samples exceeding the assay range of 2 – 200 RU/mL were excluded from the calculations: 66 anti-Desmoglein 1 samples remained and 54 anti-Desmoglein 3 samples remained:

| n = 66 | | Predicate Assay | | | |
|--------------------------------|----------|-----------------|---------------|----------|-------|
| | | positive | indeterminate | negative | Total |
| EUROIMMUN Anti-Desmoglein 1 | positive | 37 | 4 | 2 | 43 |
| | negative | 2 | 2 | 19 | 23 |
| | Total | 39 | 6 | 21 | 66 |

Indeterminate samples considered as positive:

| | | |
|--------------------|-------------|----------------------|
| Positive agreement | 41/45=91.1% | 95% C.I.:78.8%-97.5% |
| Negative agreement | 19/21=90.5% | 95% C.I.:69.6%-98.8% |
| Overall agreement | 60/66=84.8% | 95% C.I.:73.9%-92.5% |

Indeterminate samples considered as negative:

| | | |
|--------------------|-------------|----------------------|
| Positive agreement | 37/39=94.9% | 95% C.I.:82.7%-99.4% |
| Negative agreement | 21/27=77.8% | 95% C.I.:57.7%-91.4% |
| Overall agreement | 58/66=87.9% | 95% C.I.:77.5%-94.6% |

| n = 54 | | Predicate | | | |
|---|----------|-----------|---------------|----------|-------|
| | | positive | indeterminate | negative | Total |
| EUROIMMUN Anti-Desmoglein 3 ELISA (IgG) | positive | 37 | 1 | 1 | 39 |
| | negative | 1 | 0 | 14 | 15 |
| | Total | 38 | 1 | 15 | 54 |

Indeterminate samples considered as positive:

| | | |
|--------------------|-------------|----------------------|
| Positive agreement | 38/39=97.4% | 95% C.I.:86.5%-99.9% |
| Negative agreement | 14/15=93.3% | 95% C.I.:68.1%-99.8% |
| Overall agreement | 52/54=96.3% | 95% C.I.:87.3%-99.5% |

Indeterminate samples considered as negative:

| | | |
|--------------------|-------------|----------------------|
| Positive agreement | 37/38=97.4% | 95% C.I.:86.2%-99.9% |
| Negative agreement | 14/16=87.5% | 95% C.I.:61.7%-98.4% |
| Overall agreement | 51/54=94.4% | 95% C.I.:84.6%-98.8% |

b. Matrix comparison:

The purpose of this study was to demonstrate that recovery of analyte from lithium heparin plasma, citrate plasma and EDTA plasma collection tubes was equivalent to the analyte recovered from serum collection tubes. Samples from the same patient were collected in all four collection tubes and tested according to the directions for use. Samples spanned the diagnostically important range, i.e. the lower half of the calibration curve and the cut-off area measuring range of both assays; anti-Desmoglein 1 samples ranged from 7 – 107 RU/mL and anti-Desmoglein 3 samples ranged from 7 – 131 RU/mL. 12 samples were tested for both assays. Regression analysis was performed using serum as the comparator; results are shown in the table below:

| Matrix | anti-Desmoglein 1 | anti-Desmoglein 3 |
|----------------|----------------------------------|----------------------------------|
| heparin plasma | $y = 0.96x + 0.57$ R2 = 0.994 | $y = 0.96x + 1.34$ R2 = 0.994 |
| citrate plasma | $y = 0.97x - 0.30$ R2 = 0.992 | $y = 0.98x + 1.43$ R2 = 0.991 |
| EDTA plasma | $y = 0.98x - 0.21$ R2 = 0.988 | $y = 0.92x + 0.28$ R2 = 0.983 |

3. Clinical studies:

a. Clinical sensitivity and specificity:

An external clinical laboratory study was performed in cooperation with the dermatological reference center of the university hospital in Lübeck, Germany. In total, 190 samples [50 from PF patients, 71 from PV patients, 48 from Bullous Pemphigoid (BP) patients and 21 from Linear IgA-Dermatosis (LAD) patients] were tested with the EUROIMMUN Anti-Desmoglein 1 and Anti-Desmoglein 3 ELISA (IgG). Results of both assays were compared to the clinical diagnosis:

Anti-Desmoglein 1 Study:

| All samples | | Pemphigus foliaceus | | |
|-------------------|--------------|---------------------|----------|------------|
| | | Positive | Negative | Total |
| Anti-Desmoglein 1 | Positive | 48 | 34 | 82 |
| | Negative | 2 | 106 | 108 |
| | Total | 50 | 140 | 190 |

Sensitivity: 96.0% 95% CI: 86.5 – 98.9 %
 Specificity: 75.7% 95% CI: 68.0 – 82.1%

Of the 71 PV samples tested, 33 were positive for anti-Desmoglein 1 and anti-Desmoglein 3. In the mucosal variant of pemphigus vulgaris, autoantibodies exclusively react with desmoglein 3 whereas patients with the mucocutaneous subtype raise antibodies against both desmoglein 3 and 1. Clinical information was not available to determine which variant of PV the patients that provided the samples suffered from.

In the absence of this information, we analyzed the data without including the 71 PV samples:

| Without PV Samples | | Pemphigus foliaceus | | |
|--------------------|--------------|---------------------|----------|------------|
| | | Positive | Negative | Total |
| Anti-Desmoglein 1 | Positive | 48 | 1 | 49 |
| | Negative | 2 | 68 | 70 |
| | Total | 50 | 69 | 119 |

Sensitivity: 96.0% 95% CI: 86.5 – 98.9 %
 Specificity: 98.6% 95% CI: 92.2 – 99.7%

Anti-Desmoglein 3 Study:

| All Samples | | Pemphigus vulgaris | | |
|-------------------|--------------|--------------------|----------|------------|
| | | Positive | Negative | Total |
| Anti-Desmoglein 3 | Positive | 71 | 1 | 72 |
| | Negative | 0 | 118 | 118 |
| | Total | 71 | 119 | 190 |

Sensitivity: 100.0% 95% CI: 94.9 – 100.0 %
 Specificity: 99.2% 95% CI: 95.4 – 99.8%

b. Other clinical supportive data (when a. is not applicable):
 Not applicable

4. Clinical cut-off:
 Not applicable.

5. Expected values/Reference range:

The frequency distribution for anti-desmoglein 1 antibodies was analyzed with the EUROIMMUN Anti-Desmoglein 1 in a panel of 251 apparently healthy blood donors. The panel for anti-desmoglein 1 consisted of 123 men and 71 women with an age range of 18 – 66 years (average age: 39 years).

The frequency distribution for anti-desmoglein 1 antibodies was analyzed with the EUROIMMUN Anti-Desmoglein 3 in a panel of 251 apparently healthy blood donors. The panel for anti-desmoglein 3 consisted of 153 men and 98 women with an age range of 18 – 68 years (average age: 38 years).

| | Anti-Desmoglein 1 ELISA | Anti-Desmoglein 3 ELISA |
|---------------|----------------------------|----------------------------|
| n | 251 | 251 |
| Positives | 2 | 0 |
| Negatives | 249 | 251 |
| Lowest value | 0.4 RU/mL | 0.4 RU/mL |
| Highest value | 39.0 RU/mL | 12.6 RU/mL |
| Mean value | 1.9 RU/mL | 1.9 RU/mL |
| Std dev. (SD) | 3.6 RU/mL | 2.1 RU/mL |
| Prevalence | 0.8% | 0.0% |

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.