510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083850

B. Purpose for Submission:
New Device

C. Measurand:
Human IgG Anti-Neutrophil Cytoplasmic Antibodies (ANCA)

D. Type of Test:
Qualitative and Semi-quantitative, Immunofluorescence Assay (IFA)

E. Applicant:
EUROIMMUN US Inc

F. Proprietary and Established Names:
EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic™ Test System

G. Regulatory Information:
1. Regulation section:
   21§CFR 866.5660 – Multiple autoantibodies immunological test system
2. Classification:
   Class II
3. Product code:
   MOB – Test system, anti-neutrophil cytoplasmic antibodies (ANCA)
4. Panel:
   Immunology (82)

H. Intended Use:
1. Intended use(s):
   The EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic™ Test System is intended for the qualitative or semi-quantitative determination of anti-neutrophil cytoplasmic antibodies (ANCA) in serum. This test is used as an aid in the diagnosis of Wegener's granulomatosis, microscopic arthritis, Churg-Strauses syndrome and classic polyarteritis nodosa diseases in conjunction with other laboratory and clinical findings. For *in vitro* diagnostics use.
2. Indication(s) for use:
   Same as intended use
3. Special conditions for use statement(s):
   The device is for prescription use only.
4. Special instrument requirements:
   Fluorescent microscope with a 488nm excitation filter, 510nm color separator, and 520nm blocking filter.

I. Device Description:
The device test system is a combination of BIOCHIPs with ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, and EUROPLUS™ MPO and PR3 and the controls BIOCHIPs of HEp-2 and monkey liver section. Each device contains a slide with a mosaic of BIOCHIPs, fluorescein-labeled anti-human IgG (goat), positive controls, negative control, PBS, Tween-20 and embedding medium. All reagents are ready for use except for the wash buffer. The test procedure follows

the TITERPLANE technique cleared previously in k051489, k061408 and k070763.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   EUROIMMUN ANCA IFA granulocyte BIOCHIP Mosaic^{TM} Test System
2. Predicate 510(k) number(s):
   k051489
3. Comparison with predicate:

Similarities		
Item	Predicate	New Device
Intended Use	Semi-quantitative detection of anti-neutrophil cytoplasmic antibodies (ANCA) in serum.	Same
Reagents	Fluorescein Human granulocytes native antigen	Same
Technology	IFA BIOCHIP TITERPLANE technology	Same
Substrate	Ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, and the controls BIOCHIPs of HEp-2 and monkey liver section	Same
Sample type and dilution	Serum 1:10 dilution	Same
Controls	1 positive control for cANCA/PR3 1 positive control for pANCA/MPO 1 negative control	Same
Cut off level	1:10 dilution	Same

Differences		
Item	Predicate	New Device
Substrate		Affinity purified PR3 and MPO antigen dots

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

L. Test Principle:
   The EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic^{TM} Test System is a combination of BIOCHIPs with ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, MPO- and PR3-specific microdots, and the control BIOCHIPs consisting of HEp-2 and monkey liver section. The BIOCHIP combinations of substrates are incubated with diluted patient samples. If the patient sample contains the antibody, the specific antibodies will bind to the antigens on the specific substrate slide sections. The specific reaction patterns are defined by fluorescein-labeled anti-human antibodies and then measured by fluorescence microscopy.

In the case of autoantibodies against the cytoplasm of granulocytes fixed with ethanol, two relevant fluorescence patterns can be differentiated: cANCA and pANCA. The cANCA pattern, in which the granular fluorescence is distributed evenly over the entire cytoplasm of the granulocytes, is caused by antibodies against proteinase 3 (PR3) localized in the azurophilic granules of the neutrophils.

The pANCA pattern, in which a smooth fluorescence wraps ribbon-like around the cell nuclei of the granulocytes, can be caused by antibodies of various specificities, such as myeloperoxidase (MPO), granulocyte-specific elastase, lactoferrin, lysozyme, beta-glucuronidase and cathepsin G. MPO can be further distinguished by granular fluorescence which is distributed evenly over the entire cytoplasm of the formaldehyde-fixed granulocytes.

Two additional antigens, MPO- and PR3- microdotted BIOCHIPS, are included for specific detection of autoantibodies against MPO and PR3. They help to facilitate the evaluation of problematic fluorescence patterns (e.g. unspecific fluorescence caused by other cytoplasm antibodies).

A control BIOCHIP substrate combination consisting of human epithelia cell (HEp-2) and /or primate liver is used to aid in interpreting the reading of co-existing antibodies such as the nuclear antigens (ANA).

M. Performance Characteristics (if/when applicable):
   1. Analytical performance:
      The analytical performance of the assay was assessed using a fluorescence intensity level scale which measures the intensity of the specific fluorescence expressed as a numeric value. These values can vary from "0" (no specific fluorescence) to "5" (extremely strong specific fluorescence). The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	Evaluation
0	no specific fluorescence
1	positive reaction, very weak specific reaction visible
2	positive reaction, weak specific reaction visible
3	positive reaction, specific reaction well visible
4	positive reaction, strong specific reaction visible
5	positive reaction, very strong specific reaction visible

   a. *Precision/Reproducibility:*

      Intra-assay reproducibility:
      Intra-assay reproducibility of the new antigen specific microdots was determined by repeated measurement of a normal human serum, three pANCA samples and three cANCA samples that contain different fluorescence intensities 10 times from 10 different slides. Each of the

pANCA and cANCA pattern samples was tested with two different dilutions (1:10 and 1:32) of known samples. The variation of fluorescence intensity was within ±1 intensity level unit.

Inter-assay reproducibility:
Intra-assay reproducibility of the new antigen specific microdots was performed by two different technicians for measurement of a normal human serum, three pANCA samples and three cANCA samples that contain different fluorescence intensities 5 times from 5 different slides. Each sample was tested with two different dilutions (1:10 and 1:32). The variation of fluorescence intensity was within of ±1 intensity level unit.

Inter-lot reproducibility:
The inter-lot reproducibility was determined by testing 7 different samples that contain different fluorescence intensities for anti-PR3 and anti-MPO with 3 lots of the kits. The results did not exceed of fluorescence intensity of ±1 intensity level unit.

The inter-lot reproducibility study is also demonstrated retrospectively by a summary of 177 quality control runs using 32 characterized sera (22 negative, 2 low positive, and 8 high positive) with 15 different slide lots. The results of all runs were within ±1 fluorescence intensity level unit from target value.

b. *Linearity/assay reportable range:*
The dilution starting point of the measurement system is 1:10. Samples can be further diluted by a factor of 10. There is no upper limit to the measurement range.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No recognized reference materials are available. Two positive controls (control serum with autoantibodies against cytoplasm of granulocytes, control serum with autoantibodies against myeloperoxidase) and one negative control (human autoantibody-negative serum) is included.

Stability:
- Kit stability:
  The stability of the kit was tested by storing kits at -20ºC for 7 days and at 37ºC for 7 days. The 7 days storage at 37ºC is equivalent to 1 year storage at +2ºC to +8ºC. The test was done using 3 characterized control samples (2 positive and 1 negative) with 3 different lots. No deviation was observed beyond ±1 fluorescence intensity level after storage at -20ºC and 37ºC for 7 days. The real-time stability data also showed no deviation beyond ±1 fluorescence intensity level for the kit stored at +2ºC to +8ºC for up to 18 months.

- Stability test of reconstituted PBS-Tween buffer:

The stability of the reconstituted PBS-Tween buffer has been established using the current device. The reconstituted reagent was measured at day 0, day 7 and day 28. The deviation of results did not exceed ±1 after the storage of 28 days for the reconstituted PBS-Tween buffer.

d. *Detection limit:*
    Not applicable.

e. *Analytical specificity:*
    Cross-Reactivity:
    To investigate if antibodies against MPO were recognized by the EUROPLUS Anti-PR3 BIOCHIP, 17 sera previously shown to contain anti-MPO antibodies were tested with the ANCA IFA using the EUROPLUS Anti-PR3 BIOCHIP. All 17 samples had a fluorescence intensity level of 0, indicating no significant cross reacitivty of anti-PR3 antibodies to sera containing anti MPO antigens. The anti-PR3 biochips were also tested with samples from patients with other autoimmune diseases such as 53 non-ANCA associated vasculitides, 100 systemic lupus erythematosus (SLE) patients, 196 Sjogren's syndrome and 229 rheumatoid arthritis. One positive was found in the non-ANCA associated vasculitides samples, 4 positives in 100 SLE samples, 5 positives in 196 Sjögren's syndrome samples, and 1 in rheumatoid arthritis sample.

    To investigate if antibodies against PR3 were recognized by the EUROPLUS Anti-MPO BIOCHIP, 40 sera, previously shown to contain anti-PR3 antibodies were tested with the ANCA IFA using the EURPLUS Anti-MPO BIOCHIP. All 40 samples had a fluorescence intensity level of 0, indicating no significant cross-reactivity of anti-MPO antibodies to the sera containing anti-PR3 antigens. The anti-MPO biochips were also tested in other autoimmune diseases such as 59 Wegener's granulomatosis, 53 non-ANCA associated vasculitides, 100 SLE, 196 Sjogren's syndrome and 229 rheumatoid arthritis. No cross reactivity were observed in Wegener's granulomatosis and Sjögren's syndrome, 1 positive was observed in 100 SLE, 2 in 53 non-ANCA associated vasculitides, and 6 in 229 rheumatoid arthritis.

    Interferences:
    To investigate the influence of potentially interfering substances, hemolytic, lipemic and icteric samples that contain different fluorescence intensity level, were spiked with hemoglobin of 0, 2.5 mg/mL, 5 mg/mL, triglyceride of 0, 5 mg/mL, 20 mg/mL, and bilirubin of 0, 0.1 mg/mL, 0.4 mg/mL. The spiked samples were measured using the ANCA IFA. The variation of fluorescence intensity was within ±1 fluorescence intensity unit. No interference was observed with hemolytic, lipemic or icteric samples for concentrations of up to 500 mg/dL for hemoglobin, 2000 mg/dL for triglyceride and 40 mg/dL for bilirubin.

f. Assay cut-off:
The dilution starting point is 1:10. Samples can be further diluted by a factor of 10 so that the dilution series is 1:100, 1:1000 etc. There is no upper limit to the measurement range. The dilution starting point of 1:10 was pre-determined by the predicate device (EUROIMMUNE ANA IFA) which contains the same 4 Biochips.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   1049 samples were used for the comparison study between the predicate and the current device; they consisted of samples from 59 Wegener's granulomatosis, 47 pANCA positive, 53 cANCA positive, 100 SLE, 196 Sjögren's arthritis, 229 rheumatoid arthritis and 226 normal blood donors. The samples also included 65 biopsy proven AAV and 74 outpatient samples.

Comparison for pANCA formalin resistant:

n = 1049		EUROIMMUN ANCA IFA Granulocyte BIOCHIP Mosaic™ Test System pANCA formalin resistant	
		positive	negative
EUROIMMUN ANCA IFA EUROPLUS Granulocyte BIOCHIP Mosaic™ Test System EUROPLUS MPO BIOCHIP	positive	74	2
	negative	7	966

Negative agreement   966/968 = 99.8% 95% C.I.: 99.3%-100%
Positive agreement   74/81 = 91.4% 95% C.I.: 83.0%-96.5%
Overall agreement   1040/1049 = 99.1% 95% C.I.: 98.4%-99.6%

Comparison for cANCA formalin resistant:

n = 1049		EUROIMMUN ANCA IFA Granulocyte BIOCHIP Mosaic™ Test System cANCA formalin resistant	
		positive	negative
EUROIMMUN ANCA IFA EUROPLUS Granulocyte BIOCHIP Mosaic™ Test System EUROPLUS PR3 BIOCHIP	positive	130	1
	negative	14	904

Negative agreement   904/905 = 99.9% 95% C.I.: 99.4%-100.0%
Positive agreement   130/144 = 90.3% 95% C.I.: 84.2%/94.6%
Overall agreement   1034/1049 = 98.6% 95% C.I.: 97.7%-969.2%

b. *Matrix comparison:*
   Both devices use serum as a matrix.

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity:*
      Whole slide was read.
      Positive samples including clinically characterized autoimmune disease and Negative samples including the other autoimmune diseases were investigated for IgG antibodies against PR3 and MPO.

      For sensitivity and specificity study against PR3, 59 Wegener's granulomatosis, 53 biopsy proven cANCA positive AAV samples, 53 non-ANCA associated vasculitides, 100 Systemic lupus erythematosus, 196 Sjögren's syndrome, 229 Rheumatoid arthritis, and 226 healthy blood donors were tested.

		Diagnosis		
		Positives (Wegener's granulomatosis and biopsy proven cANCA positives)	Negative (Other autoimmune diseases and healthy controls)	Totals
EUROPLUS Anti-PR3	Positive	98	3	101
	Negative	14	801	815
	Total	112	804	916

Clinical Sensitivity: 87.5% (98/112) 95%CI 79.9 – 93%
Clinical Specificity: 99.6% (801/804) 95%CI 98.9 – 99.9%

For sensitivity and specificity study against MPO, 59 Wegener's granulomatosis, 47 biopsy proven pANCA positive AAV samples, 53 non-ANCA associated vasculitides, 100 Systemic lupus erythematosus, 196 Sjögren's syndrome, 229 Rheumatoid arthritis, and 226 healthy blood donors were tested.

		Diagnosis		
		Positives (biopsy proven pANCA positives)	Negative (Other autoimmune diseases and healthy controls)	Totals
EUROPLUS Anti-MPO	Positive	45	7	52
	Negative	2	856	858
	Total	47	863	910

Clinical Sensitivity: 95.7% (45/47) 95%CI 85.5 – 99.5%
Clinical Specificity: 99.2% (856/863) 95%CI 98.3 – 99.7%

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

Not applicable.

4. Clinical cut-off**:**
   See assay cut-off

5. Expected values/Reference range:
   A panel of 200 sera from normal healthy adult blood donors of mixed age and gender were tested to determine reference range with EUROPLUS anti-PR3 Biochip and EUROPLUS anti-MPO biochip. 1.5% prevalence of antibodies was found with anti-PR3 antibody and 1.0% prevalence of antibodies was found with Anti-MPO antibody.

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.