

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k051489

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 LLC

F. Proprietary and Established Names:

EUROIMMUN ANCA IFA Granulocyte BIOCHIP MOSAIC™ Test System
ANCA IFA for the detection of human IgG ANCA antibodies.

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):

This EUROIMMUN ANCA IFA Granulocyte Biochip Mosaic is designed for qualitative and semiquantitative determination of anti-Neutrophil cytoplasmic antibodies (ANCA) in serum. These antibodies are associated with Wegener's granulomatosis, microscopic arteritis, Churg-Strauss syndrome and classic polyarteritis nodosa. 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 488 nm excitation filter, 510 nm color separator, and 520 nm blocking filter.

I. Device Description:

The test system is a combination of BIOCHIPS with ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, and controls BIOCHIPS of HEp-2 and/or monkey liver sections. It includes slides with a mosaic of BIOCHIPS, Fluorescein-labeled goat anti-human IgG antibody (FITC), positive and negative controls, salt for PBS, Tween 20, embedding medium, cover glasses and an instruction booklet. Reagent trays for

the TITERPLANE technique are required but ordered separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Binding Site ANCA Combi Diagnostic Kit with IF-AIM Technology- ANCA Kit/Substrate Slides.
2. Predicate 510(k) number(s):
k001127
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended use	In vitro determination of anti-Neutrophil cytoplasmic antibodies (ANCA) in serum. The antibodies are associated with Wegener's granulomatosis, microscopic arteritis, Churg-Strauss syndrome and classic polyarteritis nodosa.	Same
Assay Format	Qualitative or Semi-quantitative	Same
Antigen Substrate	Human granulocyte ethanol/formalin fixed	Same
Sample Type	Serum	Same
Detection Method	Immunofluorescence	Same

Differences		
Item	Device	Predicate
Cut-off	Positive = $\geq 1:10$ Negative = $< 1:10$	Positive = $\geq 1:20$ Negative = $< 1:20$
Sample Dilution	1:10	1:20
Conjugate	FITC Goat anti-human IgG	FITC Sheep anti-human IgG
Incubation Time	Patient samples - 30 min Wash - 5 min Conjugate - 30min Wash - 5 min	Patient samples - 30 min Wash - 10 min Conjugate - 30min Wash - 10 min
Incubation Conditions	Humidity chamber not needed	Humidity chamber
Control Substrates	Monkey liver sections Hep2 cell culture	None
Slide Configuration	Combination of 4 substrates grouped together	Slides of either ethanol or formalin fixed
Patient Sample/ Reagent Application	Slides inverted over drops of diluted serum or reagent in the reagent tray	Diluted serum or reagent applied directly to substrate wells

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

None referenced.

L. Test Principle:

The EUROIMMUN ANCA IFA Granulocyte BIOCHIP MOSAIC™ Test System is a combination of BIOCHIPS with ethanol-fixed granulocytes, formaldehyde-fixed granulocytes, and control BIOCHIPS of HEP-2 and/or monkey liver sections.

The BIOCHIP combinations of substrates are incubated with diluted patient samples using TITERPLANE Technique reaction tray. If the reaction is positive, specific antibodies attach to the antigens on the specific substrate slide sections. Fluorescein-labeled goat anti-human IgG antibody reagent is added and if the specific antibodies are present, they are stained with the fluorescein reagent. The specific reaction patterns are then observed by fluorescence microscopy. The intensity of the specific fluorescence as a numeric value is called fluorescence intensity level. These values range from “0”(no specific fluorescence) to “5” (extremely strong specific fluorescence).

Ethanol-fixed human granulocytes (blood group O) are used as a standard substrate for the detection of antibodies against granulocyte cytoplasm (cANCA, pANCA) and nuclei of granulocytes (GS-ANA). It is often difficult to differentiate pANCA from antibodies against cell nuclei by means of indirect immunofluorescence. By using the primate liver control substrate, pANCA and ANA can be differentiated. The granulocytes in the sinusoids lie in immediate proximity to the nuclei of the hepatocytes and can thus be identified visually together as co-existing antibodies. If antibodies against myeloperoxidase (MPO) are present, the additional use of formaldehyde-fixed human granulocytes (blood group O) makes it possible to identify pANCA along with antinuclear antibodies detectable at the same time. Antibodies against c-ANCA / proteinase 3 can also be visualized on formaldehyde-fixed granulocytes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay reproducibility: Each of the two characterized samples, p-ANCA and c-ANCA (9 dilutions) and a normal serum were incubated in parallel on 10 slides on both ethanol and formalin fixed substrates. In semi-quantitative evaluation of the results there was no deviation in the intensity level. The normal serum resulted in negative or no fluorescence.

Inter-assay reproducibility: Five double determinations for each of two characterized samples, p-ANCA and c-ANCA (9 dilutions) and a normal serum were incubated in parallel on both ethanol and formalin fixed substrates on at least two different days. In semi-quantitative evaluation of the results there was no deviation in the intensity level and the normal serum resulted in negative or no fluorescence.

Inter-lot reproducibility: Thirty-three characterized sera were tested with different slide lots within a specific time period to estimate any deviations

between individual lots. In semi-quantitative evaluation of results, the deviation amounted to no more than ± 1 fluorescence intensity level.

b. Linearity/assay reportable range:

The dilution starting point for 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 claim was made for traceability to a reference standard.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Cross-reactivity: The cross reactivity for other autoimmune diseases was determined by evaluation of the AF-CDC ANA Reference serum panel. The manufacturer states there was no detectable cross-reactivity noted for any of the 11 CDC panel members. In addition, a total of 66 potential cross reactors [34 systemic lupus erythematosus (SLE) patients, 5 ulcerative colitis (UC) patients, 4 Crohn's disease patients and 23 primary biliary cirrhosis (PBC) patients], were also run and found to yield no false positive reaction.

Interference: Normal and positive sera samples were spiked with fixed concentrations of hemoglobin, triglycerides and bilirubin. None of the serum components were found to show interference.

f. Assay cut-off:

Qualitative evaluation: titer of 1:10 or greater that results in a positive reaction is considered positive. This cut off was determined based on the evaluation of clinically defined donor samples yielding the appropriate clinical sensitivity and specificity meeting the input criteria. One hundred fifty three patient samples and 526 normal health adult sera were used for validation. Of the 526 normal samples 517 (98.3%) were negative at 1:10 dilution on the ethanol fixed slides and 524 (99.6%) on the formalin fixed slides.

Semi-quantitative evaluation: the endpoint titer is defined as the highest sample dilution factor for which specific fluorescence is identifiable.

2. Comparison studies:

a. Method comparison with predicate device:

Comparison studies between the EUROIMMUN device and the predicate device were performed in 270 clinically diagnosed patient sera in both substrates. The patient population included: 37 Wegener's granulomatosis patients, 5 Churg-Strauss syndrome patients, 9 Polyarteritis nodosa and 5 microscopic arteritis patients. In addition, sera from 34 SLE patients, 5 UC patients, 4 Crohn's disease patients, 23 PBC patients and 148 normal healthy individuals were included. Determinations were made in both substrates and identical results were found. Overall agreement between the new device and the predicate was 99.6%. The results are summarized in the comparison table below:

Formalin/Ethanol Fixed Granulocyte Biochip

		Predicate Device		
		(+)	(-)	Total
EUROIMMUN	(+)	55	1	56
	(-)	0	214	214
	Total	55	215	270

Positive percent agreement: 100% (55/55)

Negative percent agreement: 99.5% (214/215)

Overall agreement: 99.6% (269/270)

b. Matrix comparison:

Both assays use human serum.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The clinical sensitivity and specificity were evaluated on 153 ANCA related disease characterized samples and 189 normal donor samples. Clinical sensitivity of the new device was 98.0%, with a clinical specificity of 98.4% and an overall agreement of 98.2% for both substrates. The results are summarized in the table below:

		Clinically Diagnosed Patient Samples		
		(+)	(-)	Total
EUROIMMUN	(+)	150	3	153
	(-)	3	186	189
	Total	153	189	342

Clinical Sensitivity: 98.0% (150/153)

Clinical Specificity: 98.4% (186/189)

Agreement: 98.2% (336/342)

b. 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:

The expected value in the normal population is negative at a 1:10 dilution.

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 substantial equivalence decision.