510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172582

B. Purpose for Submission:

New device

C. Measurand:

Human serum IgG autoantibodies to anti-neutrophil cytoplasmic antibodies (ANCA), serine proteinase 3 (PR3), myeloperoxidase (MPO), and glomerular basement membrane antigen (GBM)

D. Type of Test:

Qualitative and semi-quantitative indirect immunofluorescence (IFA) assay

E. Applicant:

EUROIMMUN US Inc.

F. Proprietary and Established Names:

EUROIMMUN IFA Granulocyte Mosaic EUROPattern
EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660, Multiple autoantibodies immunological test system
   21 CFR §866.4750, Automated indirect immunofluorescence microscope and software-assisted system for clinical use

2. Classification:

   Class II

3. Product code:

   MOB, antineutrophil cytoplasmic antibodies (ANCA)
   MVJ, devices, measure, antibodies to glomerular basement membrane (GBM)
   PIV, automated indirect immunofluorescence microscope and software-assisted system for clinical use

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use

   The EUROIMMUN IFA Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN IFA Granulocyte Mosaic EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel.

   The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) and the qualitative determination of IgG anti-PR3, anti-MPO, and, if included, anti-GBM antibodies in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel.

2. Indication for use:

   Same as intended use

3. Special conditions for use statements:

   - For prescription use only.
   - The device is for use by a trained operator in a clinical laboratory setting.
   - All software-aided results must be confirmed by the trained operator.

4. Special instrument requirements:

   For use only with EUROPattern Microscope, or with the EUROPattern Microscope and EUROPattern Software (K141827) in combination.

I. Device Description:

The device test system is a combination of BIOCHIPs arranged with: ethanol-fixed granulocytes (EOH); formaldehyde-fixed human granulocytes (HCHO); EUROPLUS purified human native antigens MPO, PR3, and recombinant GBM microdotted onto the chip; and the control BIOCHIP of HEp-2 cells spiked with ethanol-fixed granulocytes. Each device contains a slide with a mosaic of BIOCHIPs, fluorescein-labeled anti-human IgG (goat) conjugate with Evans Blue counterstain, positive controls, negative control, PBS, Tween-20, and mounting medium.

All reagents are ready for use, except for the wash buffer. The test procedure follows the TITERPLANE technique cleared previously (see K083850). While the core granulocyte BIOCHIPS of EOH and HCHO are always included in the device for sale, different combinations of BIOCHIPS and different number of slides in a kit can be purchased, e.g., with or without the purified antigen BIOCHIPS and Hep-2 cell substrate BIOCHIP.

J. Substantial Equivalence Information:

1. Predicate device names:
   EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic Test System,
   INOVA QUANTA Lite™ GBM ELISA

2. Predicate 510(k) numbers:
   K083850, K984336

3. Comparison with the predicate:

Similarities: **EUROIMMUN IFA Granulocyte Mosaic EUROPattern EUROPLUS Granulocyte Mosaic EUROPattern EOH, HCHO, PR3, and MPO BIOCHIPs**		
Item	EUROIMMUN IFA and EUROPLUS EUROPATTERN	EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic Test System (K083850)
Intended Use	The EUROIMMUN IFA Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies	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.

Similarities: **EUROIMMUN IFA Granulocyte Mosaic EUROPattern** **EUROPLUS Granulocyte Mosaic EUROPattern EOH, HCHO, PR3, and MPO BIOCHIPs**		
Item	EUROIMMUN IFA and EUROPLUS EUROPATTERN	EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic Test System (K083850)
	(ANCA) in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN IFA Granulocyte Mosaic EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel. The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) and the qualitative determination of IgG anti-PR3, anti-MPO, and, if included, anti-GBM antibodies in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel	This test is used as an aid in the diagnosis of Wegener's granulomatosis, microscopic arthritis, Churg-Strauss syndrome and classic polyarteritis nodosa diseases in conjunction with other laboratory and clinical findings. For in vitro diagnostics use.
Assay Format	Qualitative (PR3, MPO) and/or semi-quantitative (EOH, HCHO)	Same
Assay Technology	Indirect immunofluorescence assay (IFA), EUROIMMUN BIOCHIP TITERPLANE technology	Same

Similarities: **EUROIMMUN IFA Granulocyte Mosaic EUROPattern** **EUROPLUS Granulocyte Mosaic EUROPattern EOH, HCHO, PR3, and MPO BIOCHIPs**		
Item	EUROIMMUN IFA and EUROPLUS EUROPATTERN	EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic Test System (K083850)
Antigens	Human neutrophil granulocytes, ethanol-fixed; Human neutrophil granulocytes, formalin-fixed; Affinity-purified PR3 antigen dots; Affinity-purified MPO antigen dots	Same
Sample Matrix	Serum	Same
Controls	Two positive controls, one negative control	Same
Cut-Off	1:10	Same
Reported Results	Positive/negative with pattern (cANCA, pANCA, atypical) and titer; EUROPLUS antigen dots (PR3, MPO) positive/negative	Same

Differences: **EUROIMMUN IFA Granulocyte Mosaic EUROPattern** **EUROPLUS Granulocyte Mosaic EUROPattern EOH, HCHO, PR3, and MPO BIOCHIPs**		
Item	EUROIMMUN IFA and EUROPLUS EUROPATTERN	EUROIMMUN ANCA IFA EUROPLUS Granulocyte Mosaic Test System (K083850)
Conjugate	Fluorescein-labelled anti-human IgG (goat) with additional dye (Evans Blue) for counterstaining	Fluorescein-labelled anti-human IgG (goat)
Measurement/Evaluation	Visual evaluation of fluorescence under microscope (manual), IFA EUROPattern automatic evaluation with user verification (partially automated)	Visual evaluation of fluorescence under microscope (manual)

Similarities: EUROPLUS Granulocyte Mosaic EUROPattern GBM BIOCHIP		
Item	EUROIMMUN EUROPLUS EUROPATTERN	INOVA QUANTA Lite GBM ELISA (K083850)
Sample Matrix	Serum	Same

Differences: EUROPLUS Granulocyte Mosaic EUROPattern GBM BIOCHIP		
Item	EUROIMMUN EUROPLUS EUROPATTERN	INOVA QUANTA Lite GBM ELISA (K083850)
Intended Use	The EUROIMMUN EUROPLUS Granulocyte Mosaic™ EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN EUROPLUS Granulocyte Mosaic™ EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel.The EUROIMMUN IFA Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN IFA Granulocyte Mosaic EUROPattern test kit is intended for use with the	QUANTA Lite GBM is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of glomerular basement membrane (GBM) IgG antibodies in human serum. The presence of GBM antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of autoimmune renal disorders such as Goodpasture's syndrome.

Differences: EUROPLUS Granulocyte Mosaic EUROPattern GBM BIOCHIP		
Item	EUROIMMUN EUROPLUS EUROPATTERN	INOVA QUANTA Lite GBM ELISA (K083850)
	EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel. The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern is intended as an indirect immunofluorescence test for the qualitative or semi-quantitative determination of immunoglobulin class IgG anti-neutrophil cytoplasmic antibodies (ANCA) and the qualitative determination of IgG anti-PR3, anti-MPO, and, if included, anti-GBM antibodies in human serum. It is used as an aid in the diagnosis of ANCA associated vasculitides, in conjunction with other laboratory and clinical findings. The EUROIMMUN EUROPLUS Granulocyte Mosaic EUROPattern test kit is intended for use with the EUROPattern microscope and software system. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel.	
Antigen	Recombinant GBM antigen	Purified GBM antigen
Assay Technology	Indirect immunofluorescence assay (IFA), EUROIMMUN BIOCHIP TITERPLANE technology	Enzyme-linked immunosorbent assay (ELISA), 12x8 microtiter plate
Assay Format	Qualitative	Qualitative or semi-quantitative
Calibrator	None	Low positive control used as calibrator to calculate

Differences: EUROPLUS Granulocyte Mosaic EUROPattern GBM BIOCHIP		
Item	EUROIMMUN EUROPLUS EUROPATTERN	INOVA QUANTA Lite GBM ELISA (K083850)
		Units
Controls	Positive controls sold separately, one negative control	Positive controls included in the kit, one negative control
Conjugate	Fluorescein-labelled anti-human IgG (goat) with additional dye (Evans Blue) for counterstaining	Horseradish peroxidase-labelled anti-human IgG (goat)
Measurement/Evaluation	Visual evaluation of fluorescence under microscope (manual), IFA EUROPattern automatic evaluation with user verification (partially automated)	Photometric
Reported Results	Positive/negative fluorescence	0-20 Units: negative 21-30 Units: weak positive >30 Units: moderate to strong positive
Sample Dilution	1:10	1:101
Cut-Off	1:10	21 Units

K. Standard/Guidance Document Referenced:

Not applicable

L. Test Principle:

Samples are diluted 1:10 in PBS and incubated with the antigen substrates fixed onto the BIOCHIPs. After incubation, unbound antibodies are washed off. The substrate is then incubated with anti-human IgG-FITC conjugate. The conjugate also contains Evans Blue that is used to detect the cells or microdots on the slide by the EUROPattern Microscope and Software. The blue dye is not visible by a traditional fluorescence microscope at the wavelength where FITC fluorescence is viewed. Unbound reagent is washed off the slides, and the cover slips are applied. Stained slides are read by manual fluorescence microscopy or scanned with the EUROPattern Microscope and Software. The resulting digital images are reviewed and interpreted from the computer monitor. ANCA positive samples exhibit an apple-green fluorescence corresponding to areas of the cell where autoantibody has bound. A titer of 1:10 or greater that results in a positive reaction is considered positive.

<u>Interpretation of the Granulocyte Mosaic EUROPatterns Ethanol-fixed BIOCHIP:</u>

The two major patterns on ethanol-fixed (EOH) granulocyte substrate are cytoplasmic (cANCA), and perinuclear (pANCA). Atypical (aANCA) patterns, described in the table below, are less commonly found.

- cANCA presents as coarse granular speckled cytoplasmic fluorescence, often with accentuated staining between the nuclear lobes. This pattern is characteristic for antibodies reacting with proteinase 3 (PR3).
- pANCA presents as a smooth ribbon-like perinuclear staining, with or without nuclear extension. This pattern is usually characteristic for antibodies reacting with myeloperoxidase (MPO). Note that anti-nuclear antibody (ANA) positive samples (containing anti-DNA/histones) may react with the nuclei of ethanol-fixed neutrophils, causing nuclear staining, and may mask or mimic the P-ANCA pattern(s).

If the sera are negative, the granulocytes are dark. A granular cytoplasmic fluorescence of a small percentage of the granulocytes (eosinophils, basophils) is generally without significance. With ethanol-fixed granulocytes, some antibodies against nuclear antigens also react. However, this does not apply to antibodies against mitochondria. Granular fluorescence of the cell nuclei should not be confused for cANCA.

Interpretation of the Granulocyte Mosaic EUROPattern Formalin-fixed BIOCHIP:

On formalin-fixed (HCHO) granulocyte substrate, both MPO and PR3 antibodies appear as coarse cytoplasmic granular staining with interlobular accentuation. Formalin fixation also destroys and/or modifies most of the nuclear antigens, and ANA positive samples usually become negative, or show greatly reduced fluorescence.

The reactivity of the two granulocytes BIOCHIPs for the various patterns is summarized in the table below:

Pattern	**Ethanol-fixed granulocytes**	**Formalin-fixed granulocytes**
cANCA	cANCA pattern	positive
pANCA	pANCA pattern	positive
atypical	not negative (usually pANCA)	negative
atypical	not pANCA, not cANCA; not negative	positive
atypical	negative	positive
negative	negative	negative

The atypical pattern (aANCA), less commonly seen, comprises all fluorescences, other than cANCA or pANCA as described above. The most common aANCA (not negative, usually pANCA) and ANA patterns usually become negative on formalin-fixed substrate. All atypical results should be considered negative in the context of the intended use of this product, i.e., diagnosis of ANCA-associated vasculitides, but should be reported to aid in any recommendations for additional testing.

The Hep-2 BIOCHIP substrate is a control and is used to aid in interpreting the reading of co-existing antibodies that recognize the nuclear antigens (ANA).

Interpretation of the Granulocyte Mosaic EUROPattern PR3, MPO, and GBM BIOCHIPs:

The BIOCHIPs are coated with microscopically small droplets of highly purified or recombinant antigens. If a sample contains antibodies which are directed against these antigens, the respective antigen dots fluoresce as green circular areas on a dark background and are reported as positive. In a negative reaction, the entire BIOCHIP remains dark and the dot areas are faint or cannot be detected.

M. Performance Characteristics:

1. Analytical performance:

Modes of comparison:
The EUROPattern system allows both imaging and reading to be performed semi-automated or manually. Although any results created by the system must be manually verified, results of the analytical and clinical studies are given for the following processes separately to demonstrate automated performance using the EUROPattern microscope and software compared to manual performance.

Mode	Imaging	Reading
A	Automated	Automated – all chips
B	Automated	Manual, images on the computer monitor – all chips
C	Manual	Manual, on the microscope – all chips

Results of the analytical and clinical studies are given separately for three modes (A, B, and C) to demonstrate automated performance using the EUROPattern microscope and software compared to manual performance. Results for Mode A are included for information only. For Mode B and C, manufacturer's pre-determined acceptance criteria for performance characteristics were met. Trained operators must confirm all assessments made by the EUROPattern microscope and software.

Interpretation of results:
Qualitative: A fluorescence reaction in a sample titered at ≥1:10 is positive.

Semi-quantitative: The endpoint titer is defined as the highest sample dilution factor for which specific fluorescence remains identifiable. For a semi-quantitative evaluation, all ANCA positive samples should be reported with patterns and endpoint titers.

a. Precision/Reproducibility:

The repeatability and reproducibility of the Granulocyte (EOH and HCHO), MPO, and PR3 BIOCHIPs were investigated using 13 serum samples representing a range of patterns and titers (<10, 1:10, 1:20, 1:40, 1:160, and 1:320) at multiple sites in U.S. laboratories; the samples were five cANCA/PR3 samples, five pANCA/MPO, two positive GBM samples, and one negative sample. The repeatability and

reproducibility of the GBM BIOCHIP was investigated with three serum samples: a positive, a low positive, and a negative.

Repeatability of all five BIOCHIPs was evaluated by qualitative agreement (positive or negative), while the Granulocytes BIOCHIPS were also assessed for pattern agreement and titer agreement. The samples were tested on four different days with two runs per day and two replicates per run (n = 16 replicates per sample). Each result was determined for all 3 modes (A, B and C). Overall qualitative agreement of the results of the human reading or confirmation modes (B or C) was 99.7%; pattern agreement was 99.5%; and titer level agreement was 98.6% (± 1 dilution level) for the Granulocyte BIOCHIPs. Positive qualitative agreement of the PR3, MPO and GBM BIOCHIP results was 100.0%.

Reproducibility was determined by repeated measurements on four different days with two runs per day and two replicates per run, tested at three different sites, one was the manufacturer's site, and two external laboratories. The EUROPLUS Granulocyte Mosaic EUROPattern test kits were evaluated by three different EUROPattern microscope and software systems. Results for each BIOCHIP were reported in three modes (A, B, C). Overall qualitative agreement of the results of the human reading or confirmation modes (B or C) was 99.8%; pattern agreement was 99.7%; and titer level agreement was 98.9% (± 1 titer level) for the Granulocyte BIOCHIPs. Positive agreement of the PR3, MPO, and GBM BIOCHIP results was 100.0%.

Reproducibility between the three modes**,** i.e., A to B; A to C; B to C, was evaluated by combining the results for all 13 samples (n = 208 measurements) for the Granulocyte, MPO, and PR3 BIOCHIPs and the three GBM samples (n = 48 measurements) at each site:

Mode-to-Mode agreement: Granulocyte BIOCHIPs			
Site 1, n = 208	**A/B**	**A/C**	**B/C**
% Positive agreement (95% CI)	99 (97–100)	99 (97–100)	100 (98–100)
% Negative agreement (95% CI)	98 (89–100)	98 (89–100)	100 (93–100)
% Pattern agreement (95% CI)	99 (97–100)	99 (97–100)	100 (95.5–100)
% Titer agreement (95% CI)	99 (96–100)	98 (95–100)	99 (96–100)
Site 2, n = 208	**A/B**	**A/C**	**B/C**
% Positive agreement (95% CI)	96 (92–99)	96 (91–98)	99 (96–100)
% Negative agreement (95% CI)	98 (89–100)	100 (93–100)	100 (93–100)
% Pattern agreement (95% CI)	96 (93–98)	97 (93–99)	99 (97–100)
% Titer agreement (95% CI)	99 (96–100)	99 (96–100)	100 (97–100)
Site 3, n = 208	**A/B**	**A/C**	**B/C**

% Positive agreement (95% CI)	93 (88–97)	93 (88–97)	100 (98–100)
% Negative agreement (95% CI)	100 (93–100)	100 (93–100)	100 (93–100)
% Pattern agreement (95% CI)	95 (91–97)	95 (91–97)	100 (98-100)
% Titer agreement (95% CI)	97 (93–99)	97 (93–99)	99 (97–100)

Mode-to-Mode agreement: Purified BIOCHIPs			
Site 1	**A/B**	**A/C**	**B/C**
PR3, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	98 (93–100)	98 (93–100)	100 (97–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			
% Positive agreement (95% CI)	100 (89–100)	100 (89–100)	100 (89–100)
% Negative agreement (95% CI)	94 (70–100)	94 (70–100)	100 (79–100)
Site 2	**A/B**	**A/C**	**B/C**
PR3, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	99 (96–100)	99 (96–100)	100 (97–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			
% Positive agreement (95% CI)	100 (89–100)	100 (89–100)	100 (89–100)
% Negative agreement (95% CI)	100 (79–100)	100 (79–100)	100 (79–100)
Site 3	**A/B**	**A/C**	**B/C**
PR3, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	98 (94–100)	98 (94–100)	100 (97–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			

% Positive agreement (95% CI)	97 (84–100)	97 (84–100)	100 (89–100)
% Negative agreement (95% CI)	100 (79–100)	100 (79–100)	100 (79–100)

Reproducibility between the three sites was evaluated by combining the results for all 13 samples (n = 208 measurements) for the Granulocyte, MPO, and PR3 BIOCHIPs and the three GBM samples (n = 48 measurements) for each mode:

Site-to-Site agreement: Granulocyte BIOCHIPs			
Mode A, n = 208	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
% Positive agreement (95% CI)	96 (93–98)	95 (91–95)	94 (90–97)
% Negative agreement (95% CI)	87 (76–95)	83 (71–92)	86 (75–94)
% Pattern agreement (95% CI)	96 (93–98)	95 (91–97)	93 (88–96)
% Titer agreement (95% CI)	98 (95–99)	96 (92–98)	96 (93–99)
Mode B, n = 208	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
% Positive agreement (95% CI)	99 (97–100)	99 (97–100)	99 (96–100)
% Negative agreement (95% CI)	96 (86–100)	100 (93–100)	100 (93–100)
% Pattern agreement (95% CI)	99 (96–100)	100 (97–100)	99 (97–100)
% Titer agreement (95% CI)	99 (96–100)	98 (95–99)	99 (96–100)
Mode C, n = 208	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
% Positive agreement (95% CI)	99 (97–100)	99 (97–100)	100 (98–100)
% Negative agreement (95% CI)	100 (93–100)	100 (93–100)	100 (93–100)
% Pattern agreement (95% CI)	100 (97–100)	100 (97–100)	100 (98-100)
% Titer agreement (95% CI)	99 (97–100)	98 (95–99)	99 (96–100)

Site to Site agreement: Purified BIOCHIPs			
Mode A,	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
PR3, n = 208			
% Positive agreement (95% CI)	99 (93–100)	98 (91–100)	98 (91–100)
% Negative agreement (95% CI)	98 (93–100)	98 (93–100)	99 (96–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			
% Positive agreement	100	100	100

(95% CI)	(89–100)	(89–100)	(89–100)
% Negative agreement (95% CI)	94 (70–100)	88 (64–99)	94 (71–100)
Mode B,	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
PR3, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			
% Positive agreement (95% CI)	100 (89–100)	100 (89–100)	100 (89–100)
% Negative agreement (95% CI)	100 (79–100)	100 (79–100)	100 (79–100)
Mode C,	**Site 1/Site 2**	**Site 1/Site 3**	**Site 2/Site 3**
PR3, n= 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
MPO, n = 208			
% Positive agreement (95% CI)	100 (95–100)	100 (95–100)	100 (95–100)
% Negative agreement (95% CI)	100 (97–100)	100 (97–100)	100 (97–100)
GBM, n = 48			
% Positive agreement (95% CI)	100 (89–100)	100 (89–100)	100 (89–100)
% Negative agreement (95% CI)	100 (79–100)	100 (79–100)	100 (79–100)

Operator-to-operator imprecision was evaluated using 205 samples representing the range of patterns and titer intensities. The samples were assayed with the EUROPLUS Granulocyte Mosaic EUROPattern according to the package insert in single determinations with modes B and C. Each mode was read and recorded by 2 different technicians independently. Overall qualitative agreement of the results was 98.6%, pattern agreement was 98.3% and titer level agreement was 97.6% (±1 titer level) and 99.3% (±2 titer levels). Agreement for each mode is shown below:

	Mode B	**Mode C**
Positive/Negative Correlation		
% Positive Agreement:	98 (93–100)	96 (91–99)
% Negative Agreement:	100 (96–100)	100 (96–100)
% Pattern Correlation	99	98

	Mode B	**Mode C**
	(96–100)	(95–99)
% Titer Correlation (±1 titer)	97	98

The **lot-to-lot imprecision** of the GBM BIOCHIP was evaluated using three different lots of BIOCHIPs that were assayed manually (mode C) with two positive samples with florescence intensities 2 and 4 and five negative samples according to the package insert. No deviation was obtained between the results of the three lots. The lot-to-lot reproducibility of the Granulocyte, PR3, and MPO BIOCHIPs were evaluated in K083850.

b. *Linearity/assay reportable range:*
To investigate linearity, four positive samples were serially diluted and assayed with the EUROPLUS Granulocyte Mosaic EUROPattern system. Assays were processed according to the package insert and evaluated by the EUROPattern microscope and software. Individual results per dilution were read visually and reported using a fluorescence intensity scale from "0" (negative) to "5" (high positive).

The fluorescence intensities (FI) decreased with dilutions until negative. Patterns did not change when the samples were diluted. The same patterns were recovered in every dilution until negative. The samples and end titer results by mode are shown in the table below:

Sample No.	ANCA Pattern	End titer results estimate per sample		
		Mode A	Mode B	Mode C
1	cANCA	1:80	1:160	1:160
2	cANCA	1:320	1:320	1:320
3	pANCA	1:80	1:160	1:160
4	pANCA	1:320	1:640	1:640

The dilution FI results are summarized in the table below:

Sample No.	Result per dilution (FI level) Mode C						
	1:10	1:20	1:40	1:80	1:160	1:320	1:640
1	5	4	3	2	1	0	0
2	5	4	3	2	2	1	0
3	5	5	4	3	2	0	0
4	5	5	4	3	3	2	1

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

A recognized standard or reference material for anti-dsDNA antibodies is not available.

Stability:
As the composition of the EUROPLUS Granulocyte Mosaic EUROPattern kits are very similar to the manual kit versions (see above in Section J.3), the stability of many components was demonstrated in the predicate, K083850. To evaluate the changed components, i.e., the anti-GBM biochip and the addition of Evans Blue to the conjugate, stability studies were conducted in accordance with the international standard DIN EN 13640/DIN EN ISO 23640*: Stability testing of in vitro diagnostic reagents*. Three cANCA positive, three pANCA positive, a positive GBM sample, and a negative sample were tested using three lots of reagents in a real-time stability study. The tests were performed according to the package insert directions with imaging and reading done manually (mode C). The data supports a stability claim of 18 months after the date of manufacturer if stored properly.

In a separate study, three different lots of the reagents were stored refrigerated (4°C) or frozen (-20°C) and at 37°C for 14 days. After the end of the storage time, assays were run using a set of positive and negative samples. The tests were performed according to the package insert directions. The deviation in fluorescence intensity level (0-5) did not exceed ±1. The stressing of the reagents at 37°C for 14 days suggests that the device may withstand temperature deviations outside of the stated storage (2°C to 8°C) within the range of -20°C to 37°C for a period of ≤ 14 days, e.g., shipping conditions, without any significant impact on the expected results.

The manufacturer states the slides and reagents should be stored at 2°C to 8°C. Kits must not be used beyond the expiration date noted on the kit label. After initial opening, the reagents are stable until the expiry date when stored at 2°C to 8°C and protected from contamination. The stability claims are summarized in the table below:

	Temperature	Stability claim
Shelf life	2°C to 8°C	18 months
Open	2°C to 8°C	18 months
Shipping	37°C	≤14 days

Controls:
Positive controls for cANCA/anti-PR3, pANCA/anti-MPO, and anti-GBM and negative controls are included in the kit or available separately.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Cross reactivity:

The analytical specificity of the assay was verified using the ANA reference panel obtained from the Centers for Disease Control and Prevention, Atlanta, GA, USA and included 14 samples: CDC-1 through CDC-12, CDC-15, and CDC-16. The samples were assayed per the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in 3 modes: A, B, and C). All samples CDC-1 through CDC-12 samples had various titers of the atypical pattern in all three modes except for CDC-5 (Sm) sample in mode A. This sample had a mixed (cytoplasmic and atypical) pattern in mode A and an atypical pattern in modes B and C. All the CDC samples were negative with the microdot chips (PR3, MPO, and GBM) except for CDC-5 (Sm) sample on the PR3 microdot in Mode A, which was scored as positive. Based on the above results, the following limitation is contained in the Package Insert: "*In addition to ANCA, certain other autoantibodies (e.g. ANA) may also react with the substrates. Occasionally samples can be seen with more than one autoantibody. For example, cANCA and pANCA or ANCA plus ANA. Only specific ANCA fluorescence patterns i.e. cANCA, pANCA, should be evaluated.*"

Samples CDC-15, characterized as MPO-ANCA, and CDC-16, characterized as PR3-ANCA was evaluated with all modes and performed as expected.

Interference:

The effect of potential interfering substances (endogenous serum components, and drugs commonly used to treat vasculitis) on assay results were tested by spiking clinical samples with the potential interferents. For modes B and C, the deviation in titer level did not exceed ±1 dilution in the granulocyte BIOCHIPs; positive samples were not found negative and vice versa with the following interferents: hemoglobin (up to 500 mg/dL); bilirubin (up to 40 mg/dL); triglycerides (up to 2000 mg/dL); methylprednisolone (at 1.4 mg/L); cyclophosphamide monohydrate (at 14.4 mmol/L); rituximab (at 9.0 mg/mL); methotrexate hydrate (at 9.1 mg/mL); and azathioprine (at 29 mg/L). High concentrations of RF IgG/IgM (61/1200 U/mL) changed the result of the PR3 and MPO low and medium titer samples from positive to negative. The manufacturer has added a Limitation in the Package Insert to alert users stating: *"High concentrations of RF may change the MPO and PR3 BIOCHIP test results of low titer samples*."

f. Assay cut-off:

The recommended starting dilution, above which the result is reported as positive and below which the result is reported as negative, is 1:10. The manufacturer suggests performing two-fold dilutions and recommends that each laboratory establish its own titering protocol. The recommended starting dilution is the same as that of the predicate (K083850).

2. Comparison studies:

a. *Method comparison with predicate device:*

To compare the performance of the candidate device and the predicate, 204 serologically characterized samples were tested with both kits using Mode C. The samples included all patterns and a representative spectrum of endpoint titers. For the EOH and HCHO chips, the results reported the pattern and final titer. The negative samples included atypical patterns, tabulated as negative.

		Predicate		
		Positive	Negative	Total
EUROPattern Granulocyte BIOCHIPs	Positive	106	3	109
	Negative	0	95	95
	Total	106	98	204

% Positive agreement	106/106=100.0%	95% CI: 96.5–100.0
% Negative agreement	95/98=96.9%	95% CI: 91.4–99.0

The final titer determination showed 96.0% of the samples were within ±1 dilution level and 99.0% of the samples were within ±2 dilution levels. A comparison of the patterns (n = 206 because some samples had more than one pattern) showed 98.1% agreement: 202/206, 95% CI: 95.1–99.2).

Using the same samples above, the qualitative PR3 and MPO BIOCHIPs were compared to the predicate BIOCHIPs:

PR3 and MPO Mosaic BIOCHIPs, Mode C, n = 204		Predicate PR3 Chip		Predicate MPO Chip	
		Positive	Negative	Positive	Negative
PR3 EUROPattern	Positive	52	0		
	Negative	8	144		
MPO EUROPattern	Positive			46	4
	Negative			5	149

PR3 BIOCHIP:

% Positive agreement	52/60=86.7%	95% CI: 75.8–93.1
% Negative agreement	144/144=100.0%	95% CI: 97.4–100.0

MPO BIOCHIP:

% Positive agreement	46/51=90.2%	95% CI: 79.0–95.7
% Negative agreement	149/153=97.4%	95% CI: 93.5–99.0

The qualitative GBM BIOCHIP was compared to the predicate ELISA in all three modes using 213 samples from the clinical study (see below: 16 GBM positive samples and 197 differential samples):

			Predicate ELISA	
			Positive	Negative
EUROPLUS Granulocyte Mosaic EUROPattern GBM BIOCHIP	Mode A	Positive	16	5
		Negative	0	192
	Mode B	Positive	16	1
		Negative	0	196
	Mode C	Positive	16	1
		Negative	0	196

Mode A:
% Positive agreement 16/16=100.0% 95% CI: 80.6–100.0
% Negative agreement 192/197=97.5% 95% CI: 94.2–98.9

Mode B:
% Positive agreement 16/16=100.0% 95% CI: 80.6–100.0
% Negative agreement 196/197=99.5% 95% CI: 97.2–99.9

Mode C:
% Positive agreement 16/16=100.0% 95%CI: 80.6–100.0
% Negative agreement 196/197=99.5% 95%CI: 97.2–99.9

Comparison of clinical samples by modes:
Mode C (manual imaging and manual reading) is compared with Mode A (automated imaging and automated reading) and Mode B (automated imaging and manual reading with verification of results) in the following study. Mode C is considered the reference method.

The clinical samples (see below, n = 516) were tested with the EUROPLUS Granulocyte Mosaic EUROPattern assay and interpreted in each mode for positive/negative agreement. The correlation between the modes and their titer agreement was evaluated. The results are summarized in the table below:

	Percent sample agreement (95% CI)		
	A/B	A/C	B/C
Positive Sample Agreement	109/112=97.3% (92.4–99.1%)	109/111=98.2% (94–100%)	111/111=100% (99–100%)
Negative Sample Agreement	398/404=98.5% (96.8– 99.3%)	399/405=98.5% (96.8–99.3%)	404/405=99.8% (98.6–100.0%)

Titer estimate agreement between modes with positive samples:

MODE	Titer Agreement within ±1 dilution	Titers > 1 Dilution from Other Mode
A/B	194/228 (85.1%)	34/228 (14.9%)
A/C	199/229 (86.9%)	30/229 (13.1%)
B/C	243/244 (99.6%)	1/244 (0.4%)

b. Matrix comparison:

Not applicable; this assay is only indicated for serum.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A clinical study was performed using 516 clinically characterized samples diagnosed according to the American College of Rheumatology (ACR) or other appropriate classification criteria. The samples were assayed with the EUROPLUS Granulocyte Mosaic EUROPattern using the package insert directions and evaluated by the EUROPattern microscope and software. The samples are described below, with the sensitivity and specificity findings are summarized for all three modes: A, B, and C.

	Panel	
Diseases and conditions (n)	**ANCA, PR3 and MPO**	**GBM**
ANCA-associated vasculitis (AAV, 92), necrotizing and crescentic glomerulonephritis (5)	Sensitivity	Specificity
Anti-GBM disease (16)	Specificity	Sensitivity
Non-AAV (33), systemic lupus erythematosus (SLE, 35); Sjögren's syndrome (SjS, 20); primary biliary cirrhosis (PBC, 7); rheumatoid arthritis (RA, 48); autoimmune hepatitis (AIH, 33); progressive systemic sclerosis (PSS, 32); hepatitis B/C virus (HCB/HCV, 43); Crohn's disease (20); ulcerative colitis (11); celiac disease (30); asthma (10); allergy (10); sinusitis (10); acute renal failure (ARF, 8); nephrotic syndrome (NeS, 27); leukemia (9); lymphoma (10); and lung cancer (7)	Specificity	Specificity

Sensitivity	**Mode A**	**Mode B**	**Mode C**
ANCA (cANCA and/or pANCA)	80/97 = 82.5% (73.7–88.8%)	82/97 = 84.5% (76.0–90.4%)	81/97 = 83.5% (74.9–89.6%)
PR3	57/97 = 58.8% (48.8–68.0%)	56/97 = 57.7% (47.8–67.1%)	56/97 = 57.7% (47.8–67.1%)
MPO	18/97 = 18.6% (12.1–27.4%)	20/97 = 20.6% (13.8–29.7%)	20/97 = 20.6% (13.8–29.7%)
GBM	16/16 = 100% (80.6–100%)	16/16 = 100% (80.6–100%)	16/16 = 100% (80.6–100%)

Specificity	**Mode A**	**Mode B**	**Mode C**
ANCA (cANCA and/or pANCA)	384/419 = 91.6% (88.6–94.0%)	389/419 = 92.8% (90.0–94.9%)	389*/419 = 92.8% (90.0–94.9%)

Specificity	Mode A	Mode B	Mode C
PR3	401/419 = 95.7% (93.3–97.3%)	416/419 = 99.3% (97.9–99.8%)	416/419 = 99.3% (97.9–99.8%)
MPO	407/419 = 97.1% (95–99%)	416/419 = 99.3% (97.9–99.8%)	416/419 = 99% (97.9–99.8%)
GBM	490/500 = 98% (96.1–98.4%)	498/500 = 99.6% (98.6–99.9%)	498/500 = 99.6% (98.6–99.9%)

*****The 30 discrepant samples (false positives) were: GBM samples (5), Non-AAV (2); SLE (6); SjS (2); RA (2); AIH (7); HCV (1); Crohn's (1); allergy (1); ARF (2); NeS (1)

The following tables show Mode C's sensitivity results for the patterns and purified BIOCHIPs:

Panel Results (Mode C)	**n**	**cANCA**	**PR3**	**pANCA**	**MPO**
ANCA-associated vasculitis (AAV)	92	55 (60%)	56 (61%)	22 (24%)	19 (21%)
Granulomatosis with polyangiitis (Wegener's granulomatosis)	*64*	*49 (77%)*	*50 (78%)*	*8 (13%)*	*6 (9%)*
Eosinophilic GPA (Churg-Strauss syndrome)	*8*	*0*	*0*	*3 (38%)*	*2 (25%)*
Microscopic polyangiitis	*4*	*1 (25%)*	*0*	*2 (50%)*	*3 (75%)*
ANCA-associated vasculitis, not further differentiated between GPA, EGPA, MPA, etc.	*16*	*5 (31%)*	*6 (38%)*	*9 (56%)*	*8 (50%)*
Necrotizing and crescentic glomerulonephritis	5	1 (20%)	0	3 (60%)	1 (20%)
Total	97	56	56	2	20
	n	**EUROPLUS GBM**			
Anti-GBM disease	16	16 (100%)			

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:
   Positive ANCA results detected by IFA are not commonly seen in normal populations and the expected value in the normal population is "negative" at a 1:10 starting dilution. 127 sera from normal U.S. healthy adult blood donors of mixed age and gender (50 men, 77 women, mean age 37 years, range 19-50 years) were analyzed with the EUROPLUS Granulocyte Mosaic EUROPattern system. The samples were assayed according to the package insert directions and evaluated by the EUROPattern microscope and software.

Results of the reference range study are as follows:

	Pattern		Purified BIOCHIPs		
	cANCA	pANCA	PR3	MPO	GBM
Mode A	0.8% (n=1)	0	7.1% (n=9)	3.9% (n=5)	5.5% (n=7)
Mode B	1.6% (n=2)	0	0	0.8% (n=1)	0.8% (n=1)
Mode C	0.8% (n=1)	0	0	0.8% (n=1)	0

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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