

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

A. 510(k) Number:

k141827

B. Purpose for Submission:

Assay and instrument

C. Measurand:

Anti-nuclear antibodies (ANA)

D. Type of Test:

Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:

EUROIMMUN US, INC.

F. Proprietary and Established Names:

EUROIMMUN IFA 40: HEp-20-10 EUROPattern

EUROPattern Microscope and Software

G. Regulatory Information:

1. Regulation section:

21 §CFR 866.5100 - Antinuclear antibody immunological test system

2. Classification:

Class II

3. Product code:

DHN – Antinuclear Antibody, Indirect Immunofluorescent, Antigen, Control

PIV – Automated indirect immunofluorescence microscope and software-assisted system for clinical use

4. Panel:

Immunology (82)

H. Intended Use:

Instrument:

The EUROPattern Microscope and Software is an automated system consisting of fluorescent microscope and software that acquires, interprets, stores and displays digital images of stained indirect immunofluorescence slides. The EUROPattern Microscope and Software can only be used with cleared or approved EUROIMMUN *in vitro* diagnostic assays that are indicated for use on the device. All suggested results obtained with the EUROPattern Microscope and Software must be confirmed by trained personnel.

Assay:

The EUROIMMUN IFA 40: HEp-20-10 EUROPattern is an indirect immunofluorescence antibody test for the qualitative or semi-quantitative determination of IgG antibodies against anti-nuclear antibody (ANA) in human serum with the EUROPattern Microscope and software automated instrument. This *in vitro* diagnostic assay is used as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other laboratory and clinical findings. All suggested results obtained with the EUROPattern system must be confirmed by trained personnel.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

1. For prescription use only
2. This device is only for use with reagents that are indicated for use with the device.
3. The device is for use by a trained operator in a clinical laboratory setting.
4. All software-aided results must be confirmed by the trained operator.
5. Special instrument requirements: for use only with EUROPattern Microscope and Software

I. Device Description:

Assay kit components:

- Slides, each containing 10 BIOCHIPS coated with HEp-20-10 cells
- Fluorescein-labeled goat anti-human IgG with propidium iodide for EUROPattern Microscope and Software (EUROPattern), ready for use
- Positive control, autoantibodies against cell nuclei (ANA homogeneous), human, for EUROPattern, ready for use
- Negative control, autoantibody-negative, human, ready for use
- Salt for PBS, pH7.2

- Tween 20
- Embedding medium, ready for use
- Cover glasses (62 mm x 23 mm)
- Instruction booklet.

Reagent trays for the TITERPLANE technique are required but ordered separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):
EUROIMMUN IFA 40: HEp-20-10
2. Predicate 510(k) number(s):
k131791
3. Comparison with predicate:

Similarities		
Item	Device EUROIMMUN IFA 40: HEp-20-10 EUROPattern	Predicate EUROIMMUN IFA 40: HEp-20-10 (k131791)
Intended Use	Qualitative or semi-quantitative detection of antibodies against cell nuclei (ANA). The test system is used as an aid in the diagnosis of systemic rheumatic diseases, in conjunction with other laboratory and clinical findings.	Same
Sample Matrix	Serum	Same
Initial Dilution	1:40	Same
Slide Format/ Antigen Source	BIOCHIP TITERPLANE/ HEp-20-10 cells bound to the BIOCHIPS	Same
Methodology	Immunofluorescent assay (IFA)	Same
Procedure	Standard IFA technique: serum incubation with cells, followed by a wash step, incubation with IgG conjugate, wash step, embedding and reading fluorescence with a fluorescence microscope.	Same

Similarities		
Item	Device EUROIMMUN IFA 40: HEp-20-10 EUROPattern	Predicate EUROIMMUN IFA 40: HEp-20-10 (k131791)
Cut off level	1:40 dilution	Same
Results	Pattern and titer; Qualitative, Semi- quantitative titer	
Stability	18 months at +2°C and +8°C	Same
Controls	1 Positive control 1 Negative control	Same
Conjugate	Fluorescein-labelled goat anti human IgG	Same

Differences		
Item	Device EUROIMMUN IFA 40: HEp-20-10 EUROPattern	Predicate EUROIMMUN IFA 40: HEp-20-10 (k131791)
Counterstain (dye in conjugate)	Propidium Iodide (PI) as counter stain	None
Interpretation of results	EUROIMMUN EUROPattern Microscope and Software with trained operator verification	Manual fluorescence microscopy

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

Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510k) Submissions (January 22, 2009)

Guidance for the Content of Premarket submission for Software Contained in Medical Devices (May 11, 2005)

L. Test Principle:

Assay:

Patient samples are diluted 1:40 in PBS-Tween 20. Thirty microliters (30 µL) of diluted patient sample is added to a reaction field on the reagent tray. Reactions are started by fitting the BIOCHIP slides containing the sections from the substrate (HEp-20-10 cells) into the corresponding recesses of the reagent tray and incubating for 30 minutes at room temperature. Specific antibodies from the patient sample attach to antigens in the HEp-20-10 cells coating the BIOCHIP slide. After incubation, the BIOCHIP slides are washed with PBS-Tween to remove unbound antibodies. Twenty-five microliters (25 µL) of fluorescein-labelled anti-human IgG are added to each reaction field of a new reagent tray and the

BIOCHIP Slides are placed into the recesses of the tray. After 30 minutes of incubation at room temperature, the BIOCHIPS are again washed with PBS-Tween to remove any unbound fluorescein-labelled reagent. Ten microliters (10 μ L) of embedding medium are placed on a cover glass and the BIOCHIP Slides for each reaction field. The downward facing BIOCHIPS, are then placed onto the prepared cover glass. Fluorescence is read by EUROPattern Microscope and Software (EUROPattern).

Interpretation of results:

A sample is considered positive for ANA antibodies if it has a discernable pattern at a sample dilution of 1:40 or greater. If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence, the results are not to be used and the test is to be repeated. The user manually prepares the dilutions and tests each on a separate BIOCHIP. The EUROPattern evaluates and suggests the titration endpoint based on the last dilution for which the cells exhibit fluorescence and a discernible pattern.

Qualitative evaluation:

A titer of 1:40 or greater that has results in a discernable pattern is considered positive.

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

The titers are classified as:

- 1:40 and 1:80 are considered low titers,
- 1:160 and 1:320 are considered medium titers, and
- 1:640 and greater are considered high titers.

The software-reported results include titers and staining pattern(s).

A large range of fluorescence reference images can be found on the EUROIMMUN website (www.euroimmun.com)

Software

IFA fluorescence microscopy digital images are taken by a camera and stored on the computer system. The EUROPattern software compares the unknown patterns of the stored image with known verified patterns from a pre-established image database of more than 5,000 images (115,000 cell references) and identifies the most probable IFA patterns using the principles of “k-nearest neighbor” (kNN) statistical techniques. The EUROPattern software operates with rule-based synthesizing of cell results to one result per sample and provides suggestion(s), as a confidence value, to the pattern(s) detected. The software is able to detect all patterns present in the image, whether they occur as a single pattern or in combination with other patterns.

The microscope information is in section N.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Nomenclature and acronyms used in studies:

The EUROPattern Microscope and Software (EUROPattern) system allows semi-automated imaging or manual imaging with the microscope. All studies were evaluated by comparing the three possible reading modes (A, B, and C) as shown in the table below; these modes are consistent throughout this document. Mode C (e.g. manual imaging and manual reading of the slides with a traditional fluorescence microscope) is considered the reference method to which all results are compared. All results generated by the EUROPattern software must be confirmed by a trained operator.

Modes:

Mode	Imaging	Reading
A	Automated	Automated (software interpretation)
B	Automated	Manual (read of digital image)
C	Manual	Manual (read of microscope fields)

The EUROPattern software identifies the following immunofluorescence (IF) patterns, abbreviated below:

IF patterns suggested	
ho	homogeneous
gr	granular
nc	nucleolar
ce	centromere
nm	nuclear membrane
nd	nuclear dots
cp	cytoplasmic

The following abbreviations are used for autoimmune diseases:

Panel	
Mixed connective tissue diseases	MCTD
Systemic lupus erythematosus	SLE
Drug-induced SLE (part of SLE panel)	DIL
Poly-dermatomyositis	PM/DM
Systemic sclerosis	SSc
Sjögren’s syndrome	SS
Primary Biliary Cirrhosis & Primary Sclerosing Cholangitis	PBC/PSC
Autoimmune hepatitis	AIH

Interpretation of manual test results:

The fluorescence intensity level is the intensity of the specific fluorescence expressed as a numeric value. These values can vary from “0” (no specific fluorescence) to “4+” (strong specific fluorescence). Fluorescence intensity was manually evaluated for the

purpose of this study and is not reported by the EUROPattern software with the final results. The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	Interpretation
0	Negative: no specific fluorescence
1+	Positive: Weak visible reaction; dim subdued fluorescence
2+	Positive: Moderate visible reaction; green fluorescence
3+	Positive: Strong visible reaction; brilliant green fluorescence
4+	High Positive: Very strong visible reaction; brilliant green fluorescence

A serum dilution is considered negative for ANA antibodies if the cells exhibit < 1+ fluorescence intensity and no discernible pattern. Cells appear reddish-orange due to the propidium iodide counterstain. Likewise, a serum dilution is considered positive for ANA antibodies if the cells exhibit \geq 1+ fluorescence intensity and a discernible pattern at a sample dilution of 1:40 or greater. Trained operators must confirm all titers and patterns identified by EUROPattern.

a. Precision/Reproducibility:

Repeatability and reproducibility of the assay was investigated using a panel of serum samples representing the range of patterns and titers identified by the device in 3 US laboratories. The manufacture's pre-defined acceptance criteria state that all results are to be within ± 1 titer level.

Repeatability was determined by repeated measurements on 3 sites, on 5 different days with 2 runs per day and 2 replicates per run for a total of 60 replicates per sample; each result was reported in all 3 modes (A, B and C). Nineteen (19) samples with a range of endpoint titers and representing all patterns except nm were tested. Assays were processed according to the package insert. Each result was determined for all 3 modes (A, B and C). Within-run, run-to-run, and day-to-day results were evaluated. Positive samples were not found negative and vice versa, and the observed patterns did not change. All results met the pre-determined acceptance criteria above.

Reproducibility was determined by using same samples above, with the exception of one (n = 18). The EUROIMMUN IFA 40: HEp-20-10 EUROPattern assays were processed according to the package insert and evaluated with 3 EUROPattern systems (each at a different clinical site), 4 days, 3 lots, 2 operators, 1 replicate/run for 36 replicates, each read by two operators for 72 readings per mode per sample. Lot-to-lot, operator-to-operator, instrument-to-instrument and site-to-site results were evaluated. Each result was reported in all 3 modes (A, B, C). Positive samples were not found negative and vice versa, and the observed patterns did not change. All results met the pre-determined acceptance criteria outlined above.

b. Linearity/assay reportable range:

To investigate linearity, seven (7) patient samples with different patterns and titers were serially diluted and assayed with the EUROIMMUN IFA 40: HEp-20-10

EUROPattern test. Assays were processed according to the package insert and evaluated by the EUROPattern microscope and software. Patterns did not change when the samples were diluted; the same patterns were recovered in every dilution until negative. The results are summarized in the table below:

EUROIMMUN IFA 40:HEp-20-10 EUROPattern				
	Pattern	Mode A	Mode B	Mode C
1	ho	ho 640	ho 640	ho 640
2	gr	gr 2560	gr 2560	gr 2560
3	nc	nc 160	nc 320	nc 320
4	ce	ce 20480	ce 10240	ce 20480
5	nm	nm 1280	nm 1280	nm 1280
6	nd	nd 640	nd 640	nd 1280
7	cp	cp 10240	cp 10240	cp 10240

Measurement range:

The starting dilution for the EUROPattern is 1:40. Samples can be further diluted by a factor of 2 so that the dilution series is 1:80, 1:160, etc. There is no upper limit to the measurement range.

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

Traceability:

There is no recognized standard for anti-nuclear antibodies.

Controls:

Negative and positive controls are included in the kit. The positive control is a ready-to-use human serum base that contains anti-nuclear autoantibodies that show a homogenous staining pattern (ANA homogeneous). The negative control contains ready-to-use autoantibody-negative human sera and should exhibit a negative result. EUROIMMUN Inc. recommends using both the positive and negative controls as stated in the labeling.

Stability:

The EUROIMMUN IFA 40: HEp-20-10 kit is stable for a period of 18 months after the date of manufacture if properly stored at 2°C to 8°C. As the composition of the EUROIMMUN IFA 40: HEp-20-10 kit is very similar to the EUROIMMUN ANA IFA: Hep-20-10 kit, stability of most components has previously been demonstrated in k070763 and k131791. The sponsor stated that real-time stability tests of the new conjugate component, as well as the entire kit, are being conducted in accordance with the international standard DIN EN 13640:2002: Stability testing of in vitro diagnostics reagents. A preliminary stability claim for the new components (conjugate, positive control) of 18 months at +2°C to +8°C is supported by means of accelerated stability tests. Real time stability currently supports a claim of 12 months and is ongoing.

d. *Detection limit:*
Not applicable

e. *Analytical specificity:*

Cross-reactivity:

Analytical cross-reactivity was determined by using the ANA reference panel of the Centers for Disease Control and Prevention (CDC), Atlanta, USA. The results are in line with the characterization by the CDC and literature and did not indicate cross-reactivity to ANA. In addition to the CDC panel, clinical samples positive for ANCA-associated vasculitis, Crohn's disease, ulcerative colitis, celiac disease and infectious diseases (Chlamydia pneumonia and Epstein-Barr virus) (each 10 samples) were tested. All samples were negative in each of the 3 modes. The results therefore do not indicate any cross reactivity.

Interfering substances:

The effect of interfering endogenous substances on assay results were tested by spiking clinical samples with hemoglobin (0, 250 and 500 mg/dl), bilirubin (0, 10 and 40 mg/dl) and triglycerides (0, 150, 500 and 2000 mg/dl). The samples consisted of negative (< 1:40), weak positive (fluorescent intensity +1) and strong positives (fluorescent intensity 3+ and 4+) samples with varying pattern specificities. Interference by rheumatoid factor (RF) was tested by diluting these samples 1:1 with a high positive RF serum respectively. The spiked samples were incubated with the EUROIMMUN IFA 40: HEp-20-10 EUROPattern according to the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in all 3 modes (A, B, C). The results are summarized in the table below:

Interferants tested	Patterns tested	No interference up to concentrations below
Hemoglobin	ho, gr, nc, ce, nm, nd, cp, neg	500 mg/dL
Billirubin	ho, gr, nc, ce, nm, nd, cp, neg	40 mg/dL
triglycerides	ho, gr, nc, ce, nm, nd, cp, neg	2000 mg/dL
RF	ho, gr, nc, ce, nm, nd, cp, neg	55 IU/ml

f. *Assay cut-off:*

The cut-off is the same as in the predicate k131791. The recommended starting dilution, above which the result is reported as positive and below which the result is reported as negative, is 1:40. The manufacturer suggests performing two-fold dilutions but recommends that each laboratory establish its own titering protocol.

2. Comparison studies:

a. *Method comparison with predicate device:*

Comparison to predicate device:

To evaluate whether the addition of the PI counterstain affects the performance of the assay relative to the predicate, 70 clinical samples were tested on the proposed device and the predicate device according to the manufacturer's instructions; sixty of the samples tested in this study were positive by the predicate. Each sample was read manually (Mode C) at two sites by two technicians. The agreement results are summarized below:

Agreement (positive/negative results) between predicate and new assay with manual method at different sites and with different technicians			
N=70	Positive Sample Agreement (95%CI)	Negative Sample Agreement (95%CI)	Total Sample Agreement (95%CI)
Site 1 Tech 1	59/60 = 98.3% (91.1% - 100%)	8/10 = 80% (44.4% - 97.5%)	67/70 = 95.7% (88.0% - 99.1%)
Site 1 Tech 2	59/60 = 98.3% (91.1% - 100%)	8/10 = 80% (44.4% - 97.5%)	67/70 = 95.7% (88.0% - 99.1%)
Site 2 Tech 1	59/60 = 98.3% (91.1% - 100%)	9/10 = 90.0% (55.5% - 99.7%)	68/70 = 97.1% (90.1% - 99.7%)
Site 2 Tech 2	59/60 = 98.3% (91.1% - 100%)	9/10 = 90.0% (55.5% - 99.7%)	68/70 = 97.1% (90.1% - 99.7%)

Comparison of 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 studies. Mode C (manual imaging and manual reading) is considered the reference method.

Clinical samples (n=129) that were being evaluated for systemic autoimmune disease were obtained from two US sites and read by two technicians. All samples were tested with IFA 40: HEp-20-10 EUROPattern assay at both sites and interpreted in each mode (A/B/C) for positive/negative agreement. The correlation between the modes was evaluated. The results are summarized in the table below:

Agreement between modes:

	Percent sample agreement (95% CI)		
	A/B	A/C	B/C
	Site 1		
Positive Sample Agreement	64/68=94.1% (85.6-98.4%)	64/69=92.8% (83.9-97.6%)	68/68=100.0% (94.7-100.0%)
Negative Sample Agreement	61/61=100.0% (94.1-100.0%)	60/60=100.0% (94.0-100.0%)	61/61=100.0% (94.1-100.0%)
Overall Agreement	125/129 =96.0% (92.3-99.1%)	124/129 = 96.1% (91.2-98.7%)	129/129=100.0% (97.2-100.0%)

	Percent sample agreement (95% CI)		
	A/B	A/C	B/C
	Site 2		
Positive Sample Agreement	68/68=100.0% (94.7-100.0%)	68/68=100.0% (94.7-100.0%)	68/68=100% (94.7-100.0%)
Negative Sample Agreement	59/61=96.7% (88.7-99.6%)	59/61=96.7% (88.7-99.6%)	61/61=100% (94.1-100.0%)
Overall Agreement	127/129 = 95.7% (94.5-99.8%)	127/129 = 95.7% (94.5-99.8%)	129/129=100.0% (97.2-100.0%)

Titer estimate agreement between modes:

Mode	Titer Agreement within ± 1 dilution	Titers > 1 Dilution from Other Mode
Site 1		
A/B	132/149 (88.6%)	17/149 (11.4%)
A/C	130/150 (86.7%)	20/150 (13.3%)
B/C	139/141 (98.6%)	2/141 (1.4%)
Site 2		
A/B	136/159 (85.5%)	23/159 (14.5%)
A/C	136/158 (86.1%)	21/158 (13.9%)
B/C	142/142 (100%)	0/142 (0%)

In another study performed in Germany, 442 clinical samples were tested with the IFA 40: HEP-20-10 EUROPattern assay; 212 samples were positive and 230 samples were negative. A study was done to compare results between the modes (A/B/C). The correlation between the modes was evaluated. The results are summarized in the tables below:

Agreement between modes:

	Percent sample agreement (95% CI)		
	A/B	A/C	B/C
Positive Sample Agreement	208/212 = 98.1% (95.2– 99.5%)	208/212 = 98.1% (95.2 – 99.5%)	212/212 = 100% (98.3 – 100%)
Negative Sample Agreement	175 / 230 = 76.1% (70.0 – 81.4%)	175 / 230 = 76.1% (70.0 – 1.4%)	219 / 230 = 95.2% (91.6 – 97.6%)
Overall Agreement	383 / 442 = 86.6% (83.1 – 89.7%)	383 / 442 = 86.6% (83.1 – 89.7%)	431 / 442 = 97.5% (83.1 – 89.7%)

Titer estimate agreement between modes:

MODE	Titer Agreement within ± 1 dilution	Titers > 1 Dilution from Other Mode
A/B	452/506 (89.3%)	54/506 (14.0%)
A/C	443/497 (89.1%)	54/497 (10.9%)
B/C	566/586 (96.6%)	20/586 (3.4%)

A final study was performed using 226 clinical samples with a high likelihood of systemic autoimmune disease. The samples were tested with the IFA 40: HEp-20-10 EUROPattern assay at three US sites, read by two technicians and interpreted by all 3 modes (A/B/C). The correlation between the modes was evaluated and the results are summarized below:

Agreement between modes:

	Percent sample agreement (95% CI)		
	A/B	A/C	B/C
Site 1			
Positive Sample Agreement	140/141= 99.3% (96.1-100%)	139/140 = 99.3% (96.1-100%)	140/140 = 100% (97.4-100%)
Negative Sample Agreement	76/85 = 89.4% (80.8-95.0%)	76/86=88.4% (79.7-94.3%)	85/86 = 98.8% (93.7-100%)
Overall Agreement	216/226 = 95.6% (92.0 – 97.9%)	215/226 = 95.1% (91.5 – 97.5%)	225/226 = 99.6% (97.6 – 100%)
Site 2			
Positive Sample Agreement	137/141= 96.5% (92.9 - 99.2%)	140/143= 97.9% (94.0 - 99.6%)	140/140 =100% (97.4 - 100%)
Negative Sample Agreement	78/85= 91.8% (83.8 - 96.6%)	76/83= 91.6% (83.4 - 96.5%)	85/86 = 98.8% (93.7 -100%)
Overall Agreement	215/226 = 95.1% (91.5 – 97.5%)	216/226 = 95.6% (92.0 – 97.9%)	225/226 = 99.6% (97.6 – 100%)
Site 3			
Positive Sample Agreement	138/140 = 98.6% (94.9 - 99.8%)	138/140 = 98.6% (94.9 - 99.8%)	140/140 =100% (97.6 - 100%)
Negative Sample Agreement	81/86 = 94.2% (87.0 - 98.1%)	81/86 = 94.2% (87.0-98.1%)	86/86 = 98.8% (95.8 - 100%)
Overall Agreement	219/226 = 96.9% (93.7 – 98.7%)	219/226 = 96.9% (93.7 – 98.7%)	226/226 = 100% (98.4 – 100%)

Titer estimate agreement between modes:

Mode	Titer Agreement within ± 1 dilution	Titers > 1 Dilution from Other Mode
Site 1		
A/B	300/342 (87.7%)	41/342 (11.9%)
A/C	298/341 (87.4%)	43/341 (12.6%)
B/C	334/334 (100%)	0/334 (0%)
Site 2		
A/B	302/344 (87.8%)	42/344 (12.3%)
A/C	302/344 (87.8%)	42/344 (12.2%)
B/C	332/333 (99.7%)	1/333(0.3%)

Mode	Titer Agreement within ± 1 dilution	Titers > 1 Dilution from Other Mode
Site 3		
A/B	298/341 (87.4%)	43/341(12.6%)
A/C	299/340 (87.9%)	41/340 (12.1%)
B/C	331/331 (100%)	0/331 (0%)

Agreement summaries for all sites:

Positive/negative agreement between modes for all sites:

	Overall % agreement positive/negative results for all sites					
	US 2		US 3 Sites			EU
	Site 1	Site 2	Site 1	Site 2	Site 3	EU
A/B	96%	95.7%	95.6%	95.1%	96.9%	86.6%
A/C	96.1%	95.7%	95.1%	95.6%	96.9%	86.6%
B/C	100%	100%	99.6%	99.6%	100%	97.5%

Titer estimate agreement between modes for all sites:

Site	Titer Agreement within ± 1 dilution			Titers > 1 Dilution from Other Mode		
	A/B	A/C	B/C	A/B	A/C	B/C
2 Site US study						
US1/2	88.6%	86.7%	98.6%	11.4%	13.3%	1.4%
US2/2	85.5%	86.1%	100%	14.5%	13.9%	0%
3 Site US study						
US 1/3	87.7%	87.4%	100%	11.9%	12.6%	0.0%
US 2/3	87.8%	87.8%	99.7%	12.2%	12.2%	0.3%
US 3/3	87.4%	87.9%	100%	12.6%	12.1%	0%
EU Study						
EU	89.3%	89.1%	96.6%	14.0%	10.9%	3.4%

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Clinical samples (n = 226) with known diagnoses were assayed with the EUROIMMUN IFA 40: HEP-20-10 EUROPattern assay according to the package insert and evaluated by the EUROPattern Microscope and Software at 3 United States (US) sites. Studies were performed in the manufacturer's internal laboratory and two regional clinical laboratories. The results were reported in all modes (A, B and C).

The clinical diagnostic criteria used were consistent with the diagnostic criteria used in the US as published by and/or in collaboration with the American College of Rheumatology. The results are summarized in the tables below:

The table below details the samples used in the study:

Sample type		n
<i>Systemic rheumatic diseases and autoimmune liver diseases</i>		
MCTD		8
SLE		15
PM/DM		18
SSc		11
SS		14
PBC & PSC		32
AIH		20
Total		118
<i>Other diseases:</i>		
		n
Infections	HBV HCV	13
Neurological	Multiple Sclerosis	3
Thyroid /Hormonal	Hashimoto's Graves' Disease Thyroid Disease Diabetes Mellitus	17
Blood/Circulatory	Anemia Hypertension Raynaud's syndrome	27
Skin	Psoriasis alopecia dermatitis	6
Renal	Acute Renal Failure	13
Joint	Rheumatoid Arthritis Gout Osteoarthritis/degenerative joint disease	26
Miscellaneous	Chronic Fatigue Syndrome GERD	3
Total		108

The frequency of patterns and samples with mixed patterns (no disease association) in the data set was tabulated for all modes and at the three sites. Many samples showed more than one pattern; thus, the total number of patterns reported is more than the number of samples tested.

Frequency of patterns and number of samples with mixed patterns: systemic rheumatic and autoimmune liver diseases:

	Mode A			Mode B			Mode C		
Site	1	2	3	1	2	3	1	2	3
Pattern	n	n	n	n	n	n	n	n	n
ho	44	47	43	41	42	40	40	40	40
gr	67	67	68	72	72	72	72	72	72
cp	63	61	60	64	64	64	64	64	64
ce	8	8	8	8	8	8	8	8	8
nc	3	4	4	6	6	6	6	6	6
nd	2	3	2	7	7	7	7	7	7
nm	6	6	6	8	8	8	8	8	8
Total	193	196	191	206	207	205	205	205	205
Mixed Patterns	59	64	58	69	69	69	69	69	69

Frequency of patterns and number of samples with mixed patterns: other diseases

Mode	Mode A			Mode B			Mode C		
Site	1	2	3	1	2	3	1	2	3
Pattern	n	n	n	n	n	n	n	n	n
ho	19	19	18	17	17	17	17	17	16
gr	19	18	17	16	16	16	16	16	17
cp	4	4	4	4	4	4	4	4	4
ce	1	1	1	1	1	1	1	1	1
nc	0	0	0	0	0	0	0	0	0
nd	3	3	3	3	3	3	3	3	3
nm	6	6	6	8	8	8	8	8	8
Total	46	45	43	41	41	41	41	41	41
Mixed Patterns	12	12	12	12	12	12	12	12	12

The resulting sensitivity and specificity are summarized in the tables below:

	Sensitivity (95% CI)		
	Mode A	Mode B	Mode C
Site 1	95/118 = 80.5% (72.2 - 87.2)	99/118 = 83.9% (76.0-90.0)	98/118 = 83.1% (75.0-89.3)
Site 2	96/118 = 81.4% (73.1 - 87.9)	99/118 = 83.9% (76.0-90.0)	98/118 = 83.1% (75.0-89.3)
Site 3	97/118 = 82.2% (74.1 - 88.6)	98/118 = 83.1% (75.0-89.3)	98/118 = 83.1% (75.0-89.3)

	Specificity (95% CI)		
	Mode A	Mode B	Mode C
Site 1	79/108 = 73.1% (63.8 - 81.2)	83/108 = 76.9% (67.8 - 84.4)	83/108 = 76.9% (67.8 - 84.4)
Site 2	80/108 = 74.1% (64.8 - 82.0)	83/108 = 76.9% (67.8 - 84.4)	83/108 = 76.9% (67.8 - 84.4)
Site 3	82/108 = 75.9% (66.7 - 83.6)	83/108 = 76.9% (67.8 - 84.4)	83/108 = 76.9% (67.8 - 84.4)

Systemic rheumatic diseases and autoimmune liver diseases: agreement with clinical diagnosis:

Panel	Percent Positive Samples								
	Site 1			Site 2			Site 3		
	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C
MCTD	100%	100%	100%	100%	100%	100%	100%	100%	100%
SLE	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%	93.3%
PM/DM	88.9%	88.9%	88.9%	94.4%	88.9%	88.9%	94.4%	88.9%	88.9%
SSc	54.5%	63.6%	63.6%	63.6%	63.6%	63.6%	63.6%	63.6%	63.6%
SS	78.6%	85.7%	85.7%	78.6%	85.7%	85.7%	92.9%	85.7%	85.7%
PBC& PSC	78.1%	84.4%	84.4%	78.1%	84.4%	84.4%	78.1%	84.4%	84.4%
AIH	75.0%	75.0%	70.0%	70.0%	75.0%	70.0%	70.0%	70.0%	70.0%
Total	80.5%	83.9%	83.1%	81.4%	83.9%	83.1%	82.2%	83.1%	83.1%

Other diseases: agreement of with clinical diagnosis:

Panel	Percent Negative Samples								
	Site 1			Site 2			Site 3		
	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C
Infectious	76.9%	76.9%	76.9%	76.9%	76.9%	76.9%	76.9%	76.9%	76.9%
Neurological	100%	100%	100%	100%	100%	100%	100%	100%	100%
Thyroid/ Hormonal	58.8%	58.8%	58.8%	58.8%	58.8%	58.8%	58.8%	58.8%	58.8%
Blood/ Circulatory	59.3%	66.7%	66.7%	62.9%	66.7%	66.7%	66.7%	66.7%	66.7%
Skin	83.3%	83.3%	83.3%	83.3%	83.3%	83.3%	83.3%	83.3%	83.3%
Renal	92.3%	92.3%	92.3%	92.3%	92.3%	92.3%	92.3%	92.3%	92.3%
Joint	80.8%	84.6%	84.6%	80.8%	84.6%	84.6%	84.6%	84.6%	84.6%
Misc	66.7%	100%	100%	66.7%	100%	100%	66.7%	100%	100%
Total	73.1%	76.9%	76.9%	74.1%	76.9%	76.9%	75.9%	76.9%	76.9%

An additional clinical study was performed in cooperation with two different US sites; in the manufacturer's internal laboratory and a clinical laboratory in a mid-size regional hospital. In total, 129 clinically characterized samples (25 males; 104 females ranging in age from 17 to 91 years of age, averaging 51.5 years) were investigated for anti-nuclear antibodies (IgG). Samples were prospectively collected

from patients being evaluated for systemic autoimmune disease and submitted for ANA testing; samples were tested with the EUROIMMUN IFA 40: HEp-20-10 EUROPattern assay according to the package insert and evaluated in all modes. The results are summarized below:

Samples used in the study:

<i>Systemic rheumatic diseases and autoimmune liver diseases</i>		n
SLE		13
SS		10
SSc		5
MCTD		7
PM/DM		7
Total		42
<i>Other diseases:</i>		
Infections	Hepatitis C Herpes simplex Herpes zoster (shingles) Klebsiella pneumonia (K. pneumoniae) Bornholm disease (epidemic pleurodynia) Helicobacter pylori (H. pylori) Meningitis	12
Neurological	Fibromyalgia Parkinson's disease Multiple Sclerosis Alzheimer's disease Idiopathic peripheral neuropathy Bell's Palsy Seizure Disorder (Epilepsy) Essential Tremor (ET) Chronic pain syndrome	23
Lung	Chronic obstructive pulmonary disease Pulmonary hypertension ILD (interstitial lung disease)	10
Gastrointestinal	Ulcerative/Ischemic Colitis Celiac Disease (Gluten enteropathy) Irritable Bowel Syndrome (IBS) Gastroesophageal reflux (GERD)	13
Thyroid/Hormonal	Diabetes mellitus (I) Graves' disease Hypothyroidism/hyperparathyroidism	24
Blood/Circulatory	Sickle cell-beta-thalassemia Thrombocytopenia Behcet's disease APL (Antiphospholipid Syndrome) Raynaud's phenomenon PVD (peripheral vascular disease) Essential/Idiopathic hypertension Cerebellar stroke syndrome PAH (pulmonary artery hypertension)	23

Cancer	Malignant Tumor (Prostate) Pericardial Tumor Malignant Tumor (Breast) Intraepithelial neoplasia/carcinoma Leiomyosarcoma/Osteosarcoma Hepatic hemangioma	6
Skin	Eczema/Dermatitis/Cellulitis/Folliculitis Psoriasis Alopecia Rosacea	13
Renal	Chronic kidney disease Acute renal Failure Nephrosclerosis	6
Heart	CHF (Chronic Heart Failure) Chronic Pulmonary Heart Disease Hypertensive heart disease Multiple vessel coronary artery disease Aortic valve endocarditis	8
Joint	Rheumatoid Arthritis (RA) Osteoarthritis Polymyalgia rheumatica Acute Gout	16
Miscellaneous	Chronic Fatigue Syndrome (CFS) Fatty Liver Disease (FLD) Viral syndrome	4
Total negative		158

The frequency of patterns and samples with mixed patterns (no disease association) in the data set was tabulated for all modes and at the two sites. Many samples showed more than one pattern; thus, the total number of patterns reported is more than the number of samples tested.

Frequency of patterns and number of samples with mixed patterns: systemic rheumatic diseases and autoimmune liver diseases:

Mode	Mode A		Mode B		Mode C	
Site	1	2	1	2	1	2
Pattern	n	n	n	n	n	n
ho	32	24	27	29	27	28
gr	35	30	35	33	35	34
cp	6	9	9	6	8	6
ce	6	16	3	4	4	4
nc	3	3	3	3	3	3
nd	0	1	0	0	0	0
nm	0	0	0	0	0	0
Total	82	83	77	75	77	75
Mixed pattern	32	26	30	28	31	29

Frequency of patterns and number of samples with mixed patterns: other diseases

Mode	Mode A		Mode B		Mode C	
	1	2	1	2	1	2
Pattern	n	n	n	n	n	n
ho	21	21	19	19	19	19
gr	20	21	18	18	18	17
cp	1	3	3	3	3	3
ce	1	8	1	1	1	1
nc	2	2	2	2	2	2
nd	0	1	2	2	2	2
nm	0	0	0	0	0	0
Total	45	56	45	45	45	44
Mixed pattern	19	20	18	18	18	17

The resulting sensitivity and specificity is summarized in the tables below:

	Sensitivity (95% CI)		
	Mode A	Mode B	Mode C
Site 1	38/42 = 90.5% (77.4 - 97.3)	40/42 = 95.2% (83.8 - 99.4)	41/42 = 97.6% (87.4 - 99.9)
Site 2	40/42 = 95.2% (83.8 - 99.4)	41/42 = 97.6% (87.4 - 99.9)	41/42 = 97.6% (87.4 - 99.9)

	Specificity (95% CI)		
	Mode A	Mode B	Mode C
Site 1	111/158 = 70.3% (62.5 - 77.3)	106/158 = 67.1% (59.2 - 74.3)	106/158 = 67.1% (59.2 - 74.3)
Site 2	104/158 = 65.8% (57.9 - 73.2)	106/158 = 67.1% (59.2 - 74.3)	106/158 = 67.1% (59.2 - 74.3)

Systemic rheumatic diseases and autoimmune liver diseases :agreement with clinical diagnosis:

Panel	Percent Positive Samples					
	Site 1			Site 2		
	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C
MCTD	100%	100%	100%	85.7%	100%	100%
SLE	100%	100%	100%	100%	100%	100%
PM/DM	100%	100%	100%	100%	100%	100%
SSc	60.0%	80.0%	100%	100%	100%	100%
SS	88.9%	90.0%	90.0%	90.0%	90.0%	90.0%
Overall	90.5%	95.2%	97.6%	95.2%	97.6%	97.6%

Other diseases: agreement of with clinical diagnosis:

Panel	Percent Negative Samples					
	Site 1			Site 2		
	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C
Infections	75.0%	75.0%	75.0%	66.7%	75.0%	75.0%
neurological	65.2%	65.2%	65.2%	65.2%	65.2%	65.2%
Lung	70.0%	70.0%	70.0%	70.0%	70.0%	70.0%
Gastrointestinal	69.2%	53.8%	53.8%	53.8%	53.8%	53.8%
Thyroid/ hormonal	70.8%	70.8%	70.8%	66.7%	70.8%	70.8%
Blood/circulatory	78.3%	78.3%	78.3%	78.3%	78.3%	78.3%
Cancer	83.3%	83.3%	83.3%	83.3%	83.3%	83.3%
Skin	46.2%	46.2%	46.2%	46.2%	46.2%	46.2%
Renal	83.3%	66.7%	66.7%	66.7%	66.7%	66.7%
Heart	62.5%	62.5%	62.5%	62.5%	62.5%	62.5%
Joint	68.8%	56.3%	56.3%	56.3%	56.3%	56.3%
miscellaneous	100%	100%	100%	100%	100%	100%
Overall	70.3%	67.1%	67.1%	65.8%	67.1%	67.1%

Summary of sensitivity and specificity for both studies:

Site		Mode A	Mode B	Mode C
US 3 site study (N=226)				
US 1/3	Sensitivity	80.5%	83.9%	83.1%
	Specificity	73.1%	76.9%	76.9%
US 2/3	Sensitivity	81.4%	83.9%	83.1%
	Specificity	74.1%	76.9%	76.9%
US 3/3	Sensitivity	82.2%	83.1%	83.1%
	Specificity	75.9%	76.9%	76.9%
US 2 site study (N=129)				
US 1/2	Sensitivity	90.5%	95.2%	97.6%
	Specificity	70.3%	67.1%	67.1%
US 2/2	Sensitivity	95.2%	97.6%	97.6%
	Specificity	65.8%	67.1%	67.1%

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Reference range: The reference range of ANA was analyzed for the EUROIMMUN IFA 40: HEp-20-10 EUROPattern assay using a panel of 197 sera from normal U.S. healthy adult blood donors of mixed age and gender (76 men, 121 women, mean age 35 years, range 17 to 50 years). Samples were evaluated by the EUROPattern. The results showed a prevalence of 33.0% (at a cut-off of 1:40) in all three modes (A, B, and C). This finding is in concordance with data reported in literature.^{1,2}

It is recommended that each laboratory determine its own normal range based on the local population.

N. Instrument Name:

EUROIMMUN EUROPattern Microscope and Software

O. System Descriptions:

The EUROIMMUN EUROPattern Microscope and Software (EUROPattern) is an automated system including microscope and software that acquires, interprets, stores and displays digital images of stained indirect immunofluorescence slides. The EUROPattern software is designed to support input of results from the EUROPattern into electronic laboratory data management systems. The EUROPattern should only be used with EUROIMMUN assays that are cleared or approved for use on the instrument. All suggested results obtained with the EUROPattern software must be confirmed by trained personnel.

EUROPattern is intended to support trained laboratory professional's evaluation of fluorescence images obtained from EUROIMMUN IFA slides. The EUROPattern software generates a result proposal including positive/negative classification, patterns, and estimates of titer based on the last dilution with immunofluorescence and discernable pattern(s). Any proposal from the software needs to be verified by trained laboratory professionals. Results should always be interpreted in conjunction with other clinical findings.

For the EUROIMMUN IFA 40: HEp-20-10 EUROPattern assay the following patterns can be discriminated by the software: homogeneous, granular, nuclear dots, nucleolar, centromere, nuclear membrane, cytoplasmic and negative. Also pattern combinations can be identified. Based on immunofluorescence pictures from dilution series the brightness is

¹ Satoh, M., et al., Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis & Rheumatism*, 64: 2319–2327, 2012.

² Tan, E. M. (1997), Range of antinuclear antibodies in “healthy” individuals. *Arthritis & Rheumatism*, 40: 1601–1611, 1997.

analyzed and a titer estimated for each pattern. The results of the automatic evaluation are presented to the user as a proposal that needs to be verified. The pictures, the proposal, the evaluation by the user and the final result are stored and tracked by the software. Results can be exported into laboratory information management systems after verification by trained operator. A detailed description of all functions is included in the user manual.

General recommendation for EUROPattern microscope:

- Read the fluorescence: objective 20x (slides, each containing 10 BIOCHIPs coated with HEp-20-10 cells)
- Excitation filter: 450-490 nm; color separator: 510 nm, long-pass filter: 515-565 nm.
- Visual examination: excitation filter: 450-490 nm; color separator: 510 nm, locking filter: 515 nm.
- Light source: EUROIMMUN LED, EUROStar Bluelight

1. Modes of Operation:

Semi-automated (automated imaging with manual reading) and manual modes (manual imaging with manual reading)

2. Software:

1. General

EUROPattern is an integrated digital imaging system constructed of a microscope, motorized multi-slide stage, camera, and a workstation. The processed IFA slides are placed on the multi-slide stage of the EUROPattern Microscope. The EUROPattern Microscope automatically focuses on the areas of biological material on the IFA slides. Pictures are stored and evaluated by the EUROPattern software. The software performs a positive/negative and pattern classification of the cells by comparison of the FITC fluorescence picture with known verified patterns from a reference database. The most probable IFA patterns are identified by principles of k-nearest neighbor (kNN) statistical techniques. The software suggests the result as a qualitative result (positive, negative, not evaluable) and, for positive samples, suggests a pattern and titer. The trained operator evaluates the digital image on the screen and confirms or changes the software-generated result before the result is finalized.

2. Device and Software Description

Software: Version 1

Level of Concern: Level of concern was determined according to the “Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices” dated May 11, 2005. Following the recommendations of this standard, the EUROPattern software is assigned moderate level of concern.

FDA has reviewed applicant’s Hazard Analysis and software development processes for this line of product types:

Yes ___X___ No _____

3. Specimen Identification:

Manual sample identification and/or Barcode

4. Specimen Sampling and Handling:

Not applicable

5. Calibration:

There is no calibration of the instrument by the user, except for the monitor calibration. The monitor must be calibrated before the camera settings can be configured. This applies to all other monitors that are used to view images on other workstations. For monitor calibration EUROIMMUN recommends using the “Monitor Calibration Wizard”.

The cLED source has an integrated regulator to ensure that the light intensity for fluorescence excitation remains at a constant level. cLED calibration & recalibration is performed by EUROIMMUN customer service before delivery of the EUROPattern Microscope. Recalibration is then required every 12 months to ensure the proper functioning of the EUROPattern Microscope done by EUROIMMUN technical service only.

6. Quality Control:

Positive and negative controls are supplied with the assay reagents (see assay description above).

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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