510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:
K172244

B. Purpose for Submission:
New Device on a previously cleared instrument

C. Measurand:
Anti-double stranded DNA (ds DNA) antibodies

D. Type of Test:
Qualitative or semi-quantitative

E. Applicant:
EUROIMMUN US Inc

F. Proprietary and Established Names:
EUROIMMUN IFA: Crithidia luciliae (anti-dsDNA) EUROPattern

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5100, Antinuclear antibody 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:
   KTL, anti-DNA indirect immunofluorescent solid phase
   PIV, automated indirect immunofluorescence microscope and software-assisted system for clinical use

4. Panel:
   82, Immunology

H. Intended Use:

1. Intended use(s):
   The EUROIMMUN IFA: Crithidia luciliae (anti-dsDNA) EUROPattern test kit is intended for the qualitative and semi-quantitative determination of human antibodies of immuno-globulin class IgG against anti-double stranded DNA (dsDNA) in human serum with the EUROPattern Microscope and Software automated instrument. It is used as an aid in the diagnosis of systemic lupus erythematosus

(SLE), 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 (cleared in K141827)

I. Device Description:

Materials provided. The kit contains enouth for 50 determinations:

- Slides, each containing 5 BIOCHIPs coated with a smear of Crithidia luciliae
- Conjugate, Fluorescein-labelled anti-human IgG (goat), ready for use with Evans Blue
- Positive control: autoantibodies against dsDNA, human, ready for use
- Negative control: autoantibody-negative, human, ready for use
- Salt for PBS pH 7.2
- Tween 20
- Mounting medium, glycerol, ready for use
- Cover glasses (62 mm x 23 mm)
- Instruction booklet

Needed and not provided:

- Reagent trays for slides containing up to 10 fields (order #ZZ 9999-0110)
- Fluorescence microscope (EUROPattern microscope & software): Equipped with a 450-490 nm excitation filter; 510 nm color separator & 515-565 nm bandpass filter with LED Bluelight
- Distilled or de-ionized water for wash buffer production
- Pipettes with a range of 10 µl to 200 µl
- Cuvettes or wash/staining dishes for PBS wash step
- Lint free towelling

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Quanta Lite® dsDNA ELISA

2. Predicate 510(k) number(s):
   K903898

3. Comparison with predicate:

Similarities		
Item	Device EUROIMMUN IFA: Crithidia luciliae (anti-dsDNA) EUROPattern	Predicate QUANTA Lite dsDNA ELISA (K903898)
Intended Use	For the qualitative or semi-quantitative determination of human antibodies of immuno-globulin class IgG against anti-double stranded DNA (dsDNA) in human serum with the EUROPattern Microscope and Software automated instrument. It is used as an aid in the diagnosis of systemic lupus erythematosus (SLE), in conjunction with other laboratory and clinical findings.	Enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of ds DNA autoantibodies in human serum. The presence of antibodies to ds DNA can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erithematosys (SLE)
Assay format	Qualitative or semi-qantitative	Semi-quantitative
Controls	1 Positive control 1 Negative control	Same
Sample matrix	Serum	Same
Procedure	Standard immunoassay: Serum incubation with antigen, followed by a wash step, incubation with labelled anti–human globulin, wash step, measurement/evaluation	Same

Differences		
Item	Device	Predicate
Technology	IFA	ELISA
Assay platform	BIOCHIP TITERPLANE technology	96-well microtiter plates
Antigen	Crithidia luciliae cells	Purified dsDNA antigen from calf thymus
Conjugate	Fluorescein labeled goat anti-human IgG	Horseraddish peroxidase, TMB chromogen reaction with 0.5 M sulphuric acid stopping

Differences		
Item	Device	Predicate
Calibrators	None	1 calibrator
Sample dilution	1:10	1:101
Measurement/ evaluation	Visual evaluation of fluorescence under microscope, IFA EUROPattern automatic evaluation with user verification	Photometric reading
Cutoff	Negative at <1:10 dilution Positive at 1:10 dilution	Sample OD / Calibrator OD 0-200 IU/ml: negative 201-300 IU/ml: equivocal 301-800 IU/ml: moderate positive ≥800 IU/ml: strong positive
Reported results	Qualitative, Titer	Qualitative, IU/ml

K. Standard/Guidance Document Referenced:

N/A

L. Test Principle:

For the detection of autoantibodies against native, double-stranded DNA (dsDNA, nDNA) by indirect immunofluorescence, EUROIMMUN uses as the assay substrate the Crithidia luciliae species. This protozoon possesses a giant mitochondrium containing double-stranded DNA (kinetoplast) which shows none of the remaining antigens contained in human cell nuclei. Antinuclear antibodies reacting with the kinetoplast are directed against dsDNA antigens.

Patient samples, controls and in separate steps conjugate and mounting medium are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. The fluids are confined to the recessed wells eliminating the need to use a conventional "humidity chamber".

Patient samples are diluted 1:10 in PBS-Tween, 30 µl of each diluted patient sample are added to each reaction field of the reagent tray. Reactions are started by fitting the BIOCHIP slides containing the sections from the substrate (Crithidia luciliae smears) into the corresponding recesses of the reagent tray and incubated for 30 minutes at room temperature. Specific antibodies attach to the Crithidia luciliae antigens. After incubation the BIOCHIP slides are washed with PBS-Tween to remove unbound antibodies. 25 µl of fluorescein–labelled anti–human globulin are added to each reaction field of a clean reagent tray and the BIOCHIP slides placed into the recesses of the tray. After a 30 minutes incubation at room temperature, the BIOCHIPs are again washed with PBS-Tween to remove any unbound fluorescein-labelled reagent. 10 µl of mounting medium are placed for each reaction field on a cover glass and the BIOCHIP slides, with the

BIOCHIPs facing downwards, placed onto the prepared cover glass. Fluorescence is read by EUROPattern fluorescence microscopy.

M. Performance Characteristics:

1. Analytical performance:

Modes of comparison:

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

Results of the analytical and clinical studies are given separately for the three modes (A, B and C) to demonstrate automated performance using the EUROPattern microscope and software compared to manual performance. 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 EUROPatter microscope and software.

Nomenclature:
CLIFT – Crithidia lucilae indirect immunofluorescence test
IIFT – Indirect immunofluorescence test
IF – Immunofluorescence
FI – Fluorescence Intensity

Titer:

Titer	Consideration
Negative	Less than 1:10
Low positive	1:10, 1:20, 1:40, 1:80
Medium positive	1:160, 1:320, 1:640
High positive	1:1280, 1:2560, 1:5120

Interpretation of results:
For a positive result, a distinct, homogeneous, in parts circular fluorescence of the kinetoplast can be identified, the same pattern is found as for the positive control. For negative samples, the kinetoplast shows no staining. 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.

Qualitative evaluation

Anti-dsDNA reactivity (IgG)	Evaluation
No reaction at 1:10	Negative. No antibodies against dsDNA detected in the patient sample.
Positive reaction at 1:10	Positive. Indication of antibodies to dsDNA in the patient sample.

Semi-quantitative evaluation: The endpoint titer is defined as the highest sample dilution factor for which specific fluorescence is identifiable. For a semi-quantitative evaluation all dsDNA positive samples should be reported with endpoint titers, depending on the user established titering protocol. It is stated in the labeling: "*For diagnosis, the clinical symptoms of the patient should always be taken into account*."

a. *Precision/Reproducibility:*
Repeatability/Reproducibility was investigated using a panel of serum samples representing the measurement range (range of patterns and titers) in a laboratory setting at multiple sites. Titer agreement and percent posititive agreement and percent negative agreements were evaluated and summarized. The following samples were tested:

Sample	Titer
Negative	<10
Low positive	1:40
Medium positive	1:160
High positive	1:2560

Repeatability:
Repeatability was determined by repeated measurements using four saples on five different days with two runs per day and two replicates per run (80 reads per mode). The IFA: Crithidia luciliae (anti-dsDNA) EUROPattern assays were processed according to the package insert. Each result was reported in three modes (A, B, C). Positive samples were not found negative and vice versa.

Within mode agreement

Expected result	Mode A	Mode B	Mode C
	% titer agreement (within ±1)		
Negative	100	100	100
1:40	100	100	95
1:160	85	90	90
1:2500	100	100	100

Mode to mode agreement

N=80	Mode A/B	Mode A/C	Mode B/C
% positive agreement (95% CI)	100 (94.0–100.0)	100 (94.0–100.0)	100 (94.0–100.0)
% negative agreement (95% CI)	100 (83.2-100.0)	100 (83.2-100.0)	100 (83.2–100.0)
% overall agreement (95% CI)	100 (95.5–100.0)	100 (95.5–100.0)	100 (95.5–100.0)
% titer agreement (95% CI)	96.3 (89.4–99.2)	87.5 (78.2–93.8)	97.5 (91.3–99.7)

Reprodicibility:
Reproducibility was evaluated with the same four samples as repeatability and was determined by using four samples on five days with two runs per day and two replicates per run, performed at three different sites (60 replicates per sample and 240 reads per mode), at EUROIMMUN's laboratory and two external US clinical laboratories. The IFA: Crithidia luciliae (anti-dsDNA) EUROPattern assays were processed according to the package insert and evaluated by three different EUROPattern microscope and software systems. Each result was reported in three modes (A, B, C). Positive samples were not found negative and vice versa for mode B and C. The results are summarized in the tables below:

Agreement with expected results within mode

Sample N=60	Site	Mode A		Mode B		Mode C	
		% positive agreement	% titer agreement within±1	% positive agreement	% titer agreement within±1	% positive agreement	% titer agreement within±1
Negative	1	0	100	0	100	0	100
	2	5	95	0	100	0	100
	3	25	75	0	100	0	100
1:40	1	100	100	100	100	100	95
	2	100	100	100	100	100	100
	3	100	100	100	100	100	100
1:160	1	100	85	100	90	100	90
	2	95	85	100	100	100	100
	3	100	80	100	100	100	100
1:2560	1	100	100	100	100	100	100
	2	100	95	100	100	100	100
	3	100	100	100	100	100	100

Mode to mode agreement at each site
Mode to mode agreement is summarized in the tables below

Site 1 mode to mode agreement

Site 1, N=240	A/B	A/C	B/C
% positive agreement (95% CI)	100 (94.0–100)	100 (94.0–100)	100 (94.0–100)
% negative agreement (95% CI)	100 (83.2–100)	100 (83.2–100)	100 (83.2–100)
% overall agreement (95% CI)	100 (95.5–100)	100 (95.5–100)	100 (95.5–100)
% titer agreement (95% CI)	96.3 (89.4–99.2)	87.5 (78.2–93.8)	93.8 (86.0–97.9)

Site 2 mode-to-mode agreement

Site 2, N=240	A/B	A/C	B/C
% positive agreement (95% CI)	98.3 (91.1–100)	98.3 (91.1–100)	100 (94.0–100)
% negative agreement (95% CI)	95.0 (75.1–99.9)	95.0 (75.1–99.9)	100 (83.2–100)
% overall agreement (95% CI)	97.5 (91.3–99.7)	97.5 (91.3–99.7)	100 (95.5–100)
% titer agreement (95% CI)	93.8.0 (86.0–97.9)	92.5 (84.4–97.2)	100 (95.5–100)

Site 3 mode-to-mode agreement

Site 3, N=240	A/B	A/C	B/C
% positive agreement (95% CI)	100 (94.0–100)	100 (94.0–100)	100 (94.0–100)
% negative agreement (95% CI)	75.0 (50.9–91.3)	75.0 (50.9–91.3)	100 (83.2–100)
% overall agreement (95% CI)	93.8 (96.0–97.9)	93.8 (96.0–97.9)	100 (95.5-100)
% titer agreement (95% CI)	90.0 (81.2–95.6)	91.3 (82.8–96.4)	98.8 (93.2–100)

Summary, mode to mode overall agreement at each site

N=240	Overall agreement	A/B	A/C	B/C
Site 1	% overall agreement (95% CI)	100 (95.5–100)	100 (95.5–100)	100 (95.5–100)
	% titer agreement (95% CI)	96.3 (89.4–99.2)	87.5 (78.2–93.8)	93.8 (86.0–97.9)
Site 2	% overall agreement (95% CI)	97.5 (91.3–99.7)	97.5 (91.3–99.7)	100 (95.5–100)
	% titer agreement (95% CI)	93.8.0 (86.0–97.9)	92.5 (84.4–97.2)	100 (95.5–100)
Site 3	% overall agreement (95% CI)	93.8 (96.0–97.9)	93.8 (96.0–97.9)	100 (95.5–100)
	% titer agreement (95% CI)	90.0 (81.2–95.6)	91.3 (82.8–96.4)	98.8 (93.2–100)
Total	% overall agreement (95% CI)	97.1 (94.1–98.8)	97.1 (94.1–98.8)	100 (98.5–100)
	% titer agreement (95% CI)	93.3 (89.4–96.1)	90.4 (86.0–93.8)	98.8 (96.4–99.7)

Site to site agreement for each mode

Site to site agreement is summarized in the tables below for each mode. Study was evaluated using four samples on five days with two runs per day and two replicates per run, (80 reads per site).

Mode A, site to site comparison

Mode A, N=80	Site 1 vs. 2	Site 1 vs. 3	Site 2 vs. 3
% positive agreement (95% CI)	98.3 (91.1–100)	92.3 (83.0–97.5)	92.3 (83.0–97.5)
% negative agreement (95% CI)	95.0 (75.1–99.1)	100 (78.2–100)	100 (78.2–100)
% overall agreement (95% CI)	97.5 (91.3–99.7)	93.8 (86.0–97.9)	93.8 (86.0–97.9)
% titer agreement (95% CI)	90.0 (81.2–95.6)	85.0 (75.3–92.0)	90.0 (81.2–95.6)

Mode B, site to site comparison

Mode B, N=80	Site 1 vs. 2	Site 1 vs. 3	Site 2 vs. 3
% positive agreement (95% CI)	100 94.0–100	100 94.0–100	100 94.0–100
% negative agreement (95% CI)	100.0 83.2–100	100 83.2–100	100 83.2–100
% overall agreement (95% CI)	100 95.5–100	100 92.5–100	93.8 95.5–100
% titer agreement (95% CI)	95.0 87.7–98.6	92.5 84.4–97.2	97.5 91.3–99.7

Mode C, site to site comparison

Mode C, N=80	Site 1 vs. 2	Site 1 vs. 3	Site 2 vs. 3
% positive agreement (95% CI)	100 (94.0–100)	100 (94.0–100)	100 (94.0–100.0)
% negative agreement (95% CI)	100 (83.2–100)	100 (83.2–100)	100 (83.2–100.0)
% overall agreement (95% CI)	100 (95.5–100.0)	100 (95.5–100)	100 (95.5–100)
% titer agreement (95% CI)	95.0 (87.7–98.6)	91.3 (82.8–96.4)	93.8 (86.0–97.9)

Summary: site to site agreement for each mode

Mode N=80	% Agreement	Site 1 vs. 2	Site 1 vs. 3	Site 2 vs. 3
Mode A	% overall agreement (95% CI)	97.5 (91.3–99.7)	93.8 (86.0–97.9)	93.8 (86.0–97.9)
	% titer agreement (95% CI)	90.0 (81.2–95.6)	85.0 (75.3–92.0)	90.0 (81.2–95.6)
Mode B	% overall agreement (95% CI)	100 (95.5–100)	100 (92.5–100)	93.8 (95.5–100)
	% titer agreement (95% CI)	95.0 (87.7–98.6)	92.5 (84.4–97.2)	97.5 (91.3–99.7)
Mode C	% overall agreement (95% CI)	100 (95.5–100)	100 (95.5–100)	100 (95.5–100)
	% titer agreement (95% CI)	95.0 (87.7–98.6)	91.3 (82.8–96.4)	93.8 (86.0–97.9)

Operator to Operator Comparison

An additional study was performed to evaluate between operator precision by repeated measurments using three samples on five days with two runs per day and two replicates per run (60 replinates per operator). The assays were processed according to the package insert. Each result was reported in two modes (B and C). Each mode B and each mode C reading was performed by two different technicians independently. Acceptance criteria were met; positive samples were not found negative and vice versa. The samples used in the study are summarized in the table below:

Sample	Titer
Negative	<10
Low positive	1:40
Medium positive	1:320

Operator to operator agreement with expected results within mode

N=60	Mode B Two operators	Mode C Two operators
% positive agreement (95% CI)	100 (91.2–100)	100 (91.2–100)
% negative agreement (95% CI)	100 (83.2–100)	100 (83.2–100)
% overall agreement (95% CI)	100 (94.0–100)	100 (94.0–100)
% titer agreement (95% CI)	98.3 (91.1–100)	87.5 (94.0–100)

b. *Linearity/assay reportable range:*

To investigate linearity, three positive samples were serially diluted and assayed with The IFA: Crithidia luciliae (anti-dsDNA) 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 EUROPattern software creates one result based on all dilutions, it does not allow individual reading of single dilutions. The fluorescence intensities (FI) decreased with dilutions until negative. The results are shown in the table below.

Three sample were used in the study:

Sample No.	Sample	End titer results erstimate per sample		
		Mode A	Mode B	Mode C
1	High positive	1:2560	1:5120	1:5120
2	Medium positive	1:80	1:160	1:160
3	Low positive	1:20	1:20	1:20

The dilution fluorescenc intensity results (FI) 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	1:1280	1:2560	1:5120
1	5	5	5	5	5	5	5	3	2	1
2	5	5	4	2	1	0	0	0	0	0
3	2	1	0	0	0	0	0	0	0	0

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

Traceability:

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

Stability:

Stability studies were conducted in accordance with the international standard DIN EN 13640 / DIN EN ISO 23640: Stability testing of in vitro diagnostic reagents. Six positive and four negative samples were tested in an accelerated stability study. The data supports a stability claim of 18 months after the date of manufacturer if stored properly. The slides and reagents should be stored at +2°C to +8°C. The tests included three different kit lots and were performed according to the package insert with imaging and reading both being done manually (i.e. mode C). Kits must not be used beyond the expiration date noted on the kit label. After initial opeing, the reagents are stable until the expiry date when stored at +2°C to +8°C and protected from contamination,. Real time stability was tested on three lots at 0, 12 months, 24 months and 36 months. 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

Control:
Negative and positive controls are included in the kit, ready for use. The positive controls are ready to use human serum that contain anti-dsDNA antibodies that exhibit a distinct, homogeneous, in parts circular fluorescence of the kinetoplast. There is no target titer assigned to the positive controls. The negative control contains ready-to-use autoantibody-negative human sera and should exhibit a negative result. The controls are derived from native samples that are diluted with stabilizing buffer to reach the appropriate ready-to-use concentration. The reactivity of the positive and negative controls is assured by functional and stability testing. The manufacturer recommends using the positive and negative controls as stated within the labeling (Instructions for Use).

d. *Detection limit:*
Not applicable

e. *Analytical specificity:*

Cross reactivity:
The specificity of the IFA: Crithidia luciliae (anti-dsDNA) EUROPattern was verified using the ANA reference panel and ANCA (PR3, MPO) positive reference samples obtained from the CDC Centers for Disease Control and Prevention, Atlanta, USA (16 samples). The samples were assayed according to the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in three modes (A, B, C). The CDC sample specific for nDNA were positive by the assay; all other samples were negative (for mode B or C); RNP, SP and Ribosomal P-protein were positive for Mode A. The other samples represent other ANA-type antigens such as Scl-70, SS-A, SS-B, Jo-1, etc.

In addition, the World Health Organization (WHO) Reference Reagent Wo/80 for anti-dsDNA was evaluated by The IFA: Crithidia luciliae (anti-dsDNA) EUROPattern. The WHO sample specific for dsDNA was positive by the assay.

Additional information on cross-reactivity can be seen in the clinical study differential diagnosis samples that were evaluated for specificity, please see below table in Sensitivity/specificity section, *Summary: Sensitivity/Specificity, Correlation with Clinical Diagnosis*

Interference:
The effect of interfering substances on assay results were tested by spiking normal and positive serum samples with endogenous serum components and drugs. The samples consisted of negative (< 1:10), weak positive, medium positive, and strong positives samples. The spiked samples were processed according to the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in three modes (A, B, C). For Modes B and C the deviation in titer level did not exceed +/- 1 titer within modes and positive samples were not found negative and vice versa. In the triglycerides study the results for one sample were in a range of four titer levels between mode A (at 1:160) and modes B and C (at 1:20). The summary results are shown in the

tables below. Rheumatoid Factor (RF) was tested and interfering effect was obtained with RF IgG (at 61 U/ml) and with RF IgM (at 1200 U/mL), so interference occurs with RF and with RF IgM. No significant interference was observed up to the levels indicated in the table below:

	Substance	No interference up to
Endogenous	Hemoglobin	500 mg/dL
	Bilirubin	40 mg/dL
	Triglyceride	2000 mg/dL
	Cyclic Citrullinated Peptide (CCP)	Ratio 8.7 (sample over calibrator)
Drugs	Cyclophosphamide monohydrate	14.4 nmol/L
	Methotrexate hydrate	9.1 mg/mL
	Azathioprine	29.0 mg/L
	Mycophenolate mofetil	350 mg/L
	Prednisone	3.0 mg/L
	Hydroxychloroquine sulfate	33.0 µg/mL
	Ibuprofen	5.0 mg/mL
	Naproxen sodium	21.7 mmol/L
	Rituximab	1.1 mg/mL
	Belimumab	1.1 mg/mL

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 also recommends that each laboratory establish its own titering protocol. Assay cut-off of 1:10 was determined from Renato Tozzoli, MD, Nicola Bizzaro, MD, Elio Tonutti, MD, Danilo Villalta, MD, Danila Bassetti, MD, Fabio Manoni, MD, Anna Piazza, MD, Marco Pradella, MD, and Paolo Rizzotti, MD. Guidelines for the Laboratory Use of Autoantibody Tests in the Diagnosis and Monitoring of Autoimmune Rheumatic Diseases. Am J Clin Pathol 2002;117:316 – 324.

2. Comparison studies:
Method comparison and clinical evaluation of sensitivity and specificity was performed with 364 samples, the samples are detailed in the table below:

Panel/Disease	Acronym	N (men,women, unknown)	Mean age (range)
Systemic lupus erythematosus	SLE	98 (20, 78)	49 (18-85)
ANCA Associated Vasculitis	AAV	15 (9, 6)	67 (31-92)
Poly-dermatomyositis	PM/DM	10 (4, 6)	56 (42-86)
Systemic sclerosis	SSc	12 (6, 6)	52 (39-79)
Sjögren's syndrome	SS	17 (0, 17)	53 (34-88)
Mixed connective tissue disease	MCTD	18 (8, 10)	48 (19-75)
Rheumatoid artritis	RA	49 (11, 38)	56 (22-87)
Anti-Phospholipid Syndrome	APS	30 (20, 10)	53 (30, 84)
Drug-Induced Lupus	DIL	30 (2, 28)	52 (25, 84)
Autoimmune hepatitis	AIH	20 (6, 6, 8)	56 (33-77)
Autoimmune thyroid disease	AITD	16 (6, 10)	45 (23-63)
Hepatitic C virus	HCV	29 (14, 15)	48 (19-70)
Clamydia pneumoniae	CP	10 (9, 1)	54 (32-73)
Epstein-Barr virus	EBV	10 (8, 2)	44 (27-72)
Total		364	

a. *Method comparison with predicate device:*
Samples were tested with the IFA: Crithidia luciliae (anti-dsDNA) EUROPattern assay and the predicate QUANTA Lite ds DNA ELISA assay. The QUANTA Lite ds DNA assay has posiive, negative and equivocal assay range. The QuantaLite equivocal results were evaluated as positive or as negative. The results are summarized in the tables below:

Correlation between new device and predicate

N=364		Quanta Lite dsDNA ELISA			
		Positive	Equivocal	Negative	Total
IFA: Critedia lucilae (anti-dsDNA) EUROPattern Mode C	Positive	31	3	6	40
	Negative	20	12	292	324
	Total	51	15	298	364

Equivocal as positive:

% positive agreement	34/66 = 51.5%	95% CI: 38.9–64.0
% negative agreement	292/298 = 98.0%	95% CI: 95.7–99.3
% overall agreement	326/364 = 89.6%	95% CI: 86.0–92.5

Equivocal as negative:

% positive agreement	31/51 = 60.8%	95% CI: 46.1–74.2
% negative agreement	304/313 = 97.1%	95% CI: 94.6–98.7
% overall agreement	335/364 = 92.0%	95% CI: 88.8–94.6

Comparison between modes:

Mode	A/B	A/C	B/C
% positive agreement (95% CI)	44/45=97.8% (88.2–99.9)	40/40=100% (91.2–100)	40/40=100% (91.2–100)
% negative agreement (95% CI)	286/319=89.7% (85.8–92.8)	287/324=88.6% (84.6–91.8)	319/324=98.5% (96.4–99.5
% overall agreement (95% CI)	330/364=90.7% (87.2–93.4)	327/364=89.8% (86.3–92.7)	359/364=98.6% (96.8–99.6)
%titer agreement ±1 titer (95% CI)	312/364=85.7% (81.7–89.1)	315/364=86.5% (82.6–89.0)	356/364=97.8% (95.7–99.0)

b. Matrix comparison:
Not applicable, ony serum is used in the assay.

3. Clinical studies:
Clinical studies were performed at three sites (two external US clinical laboratories and EUROIMMUN's laboratory). In total 364 clinically characterized samples, diagnosed according to the American College of Rheumatology (ACR) or other appropriate classification criteria, were investigated for anti-dsDNA antibodies. Samples were assayed with the IFA: Crithidia luciliae (anti-dsDNA) EUROPattern according to the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in three modes (A, B, C). The overall sensitivity and specificity of the system was evaluated, 95% CI are calculated by the exact method.

a. Clinical Sensitivity/Specificity:
Sensitivity and specificity was evaluated for each mode at each site and at the three sites combined using 364 clinical samples (364 x 3 sites=1092 total samples; 98 positive x 3 sites=294; 266 negative X 3 Sites=798)

Correlation to clinical diagnosis for each mode at the three sites

N=1092		Clinical diagnosis	
		Positive/per total SLE	Negative/per total non SLE
IFA: Critedia lucilae(anti-dsDNA) EUROPattern	Mode A	114/294	731/798
	Mode B	105/294	765/798
	Mode C	97/294	774/798

Summary: clinical overall agreement for all three sites for each mode

Overall sensitivity/specificity (95% CI)	Mode A	Mode B	Mode C
Sensitivity N=294	114/294=38.8% (33.2–44.6)	105/294=35.7% (30.2–41.5)	97/294=33.0% (27.6–38.7)
Specificity N=798	731/798=91.6% (89.5–93.4)	765/798=95.9% (94.2–97.1)	774/798=97.0% (95.6–98.1)

Correlation with SLE clinical diagnosis in each mode at each site:

Sensitivity (95% CI) N=98	Mode A	Mode B	Mode C
Site 1	46/98=46.9% (36.8–57.3)	35/98=35.7% (26.3–46.0)	32/98=32.7% (23.5–42.9)
Site 2	32/98=32.7% (23.5–42.9)	33/98=33.7% (24.4–43.9)	29/98=29.6% (20.8–39.7)
Site 3	36/98=36.7% (27.2–47.10)	37/98=37.8% (28.2–48.1)	36/98=36.7% (27.2–47.1)

Correlation with differential diagnosis in each mode at each site:

Specificity (95% CI) N=266	Mode A	Mode B	Mode C
Site 1	235/266=88.3% (83.3–91.90)	256/266=96.2% (93.2–98.2)	258/256=97.0% (94.2–98.7)
Site 2	243/266=91.4% (87.3–94.4)	252/266=94.7% (91.3–97.1)	258/266=97.0% (94.2–98.7)
Site 3	253/266=95.1% (91.8–97.4)	257/266=96.6% (93.7–98.4)	258/266=97.0% (94.2–98.7)

Summary: sensitivity/specificity, correlation with clinical diagnosis by disease

Disease	N	Site 1			Site 2			Site 3		
		Mode A	Mode B	Mode C	Mode A	Mode B	Mode C	Mode A	Mode B	Mode C
		Sensitivity SLE:								
SLE	98	32.7	35.7	32.7	32.7	33.7	29.6	36.7	37.8	36.7
		Specificity Non-SLE:								
AAV	15	80.0	93.3	100	66.7	86.7	100	100	100	100
PM/DM	10	100	100	100	70.0	90.0	100	100	100	100
SSc	12	91.7	100	100	100	100	100	83.3	100	100
SS	17	94.1	100	100	76.5	94.1	100	94.1	94.1	100
MCTD	18	94.4	100	100	100	100	100	100	100	100
RA	49	87.8	98.0	100	98.0	100	100	95.9	100	100
APS	30	100	100	100	100	100	100	100	100	100
DIL	30	73.3	86.7	86.7	86.7	86.7	86.7	93.3	86.7	86.7
AIH	20	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
AITD	16	87.5	93.8	93.8	93.8	93.8	93.8	93.8	93.8	93.8
HCV	29	89.7	96.6	96.6	96.6	96.6	96.6	96.6	96.6	96.6
CP	10	90.0	100	100	100	100	100	100	100	100
EBV	10	70.0	100	100	80	80	100	80.0	90.0	100
Total	266	88.3	96.2	97.0	91.4	94.7	97.0	95.1	96.6	97.0

b. Clinical specificity:
See Clinical Sensitivity

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

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

5. Expected values/Reference range:
Positive dsDNA 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. Anti-dsDNA antibodies were analyzed with the IFA: Crithidia luciliae (anti-dsDNA) EUROPattern system using a panel of 124 sera from normal U.S. healthy adult blood donors of mixed age and gender (47 men, 77 women, mean age 37 years, range 20-50 years). The samples were assayed according to the package insert and evaluated by the EUROPattern microscope and software. Each result was reported in three modes (A, B, C). At the cut-off dilution of 1:10, the prevalence was found to be 0% for mode B or C and 9.7% for mode A. For mode B and C samples were 100% negative.

The manufacturer recommends that each laboratory establish its own normal range based on the population and equipement used.

N. Instrument Name:
EUROPattern: The EUROPATTERN Microscope and Software were cleared in K141827

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes _X_____ or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No __X_____

2. Software:

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

Yes _X_____ or No _____

3. Specimen Identification:
   Human serum

4. Specimen Sampling and Handling:
   Starting dilution is 1:10

5. Calibration:
   Samples are evaluated on the microscop, there is no calibrator in the assay.

6. Quality Control:
   Positive and negative control are part of the assay kit

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

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

R. Conclusion:

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