

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

**A. 510(k) Number:**

k140224

**B. Purpose for Submission:**

New device

**C. Measurand:**

IgG antibodies specific for nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, and Ribosomal P-proteins

**D. Type of Test:**

Manual and automated qualitative immunoblot assay

**E. Applicant:**

EUROIMMUN US Inc.

**F. Proprietary and Established Names:**

EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG)

**G. Regulatory Information:**

1. Regulation section:

21CFR§866.5100 – Antinuclear Antibody Immunological Test System

2. Classification:

Class II

3. Product code:

<b>EUROIMMUN EUROLINE ENA Profile Autoantibodies</b>	<b>Product Code</b>
Anti-nRNP/Sm	<b>LKO</b> , Anti-RNP Antibody, Antigen and Control
Anti-Sm	<b>LKP</b> , Anti-Sm Antibody, Antigen and Control

Anti-SS-A	<b>LLL</b> , Extractable Antinuclear antibody, Antigen and Control
Anti-Ro-52	
Anti-SS-B	
Anti-Scl-70	
Anti-Jo-1	
Anti-CENP B	<b>LJM</b> , Antinuclear Antibody (Enzyme-labeled), Antigen, Controls
Anti-Ribosomal P-proteins	<b>MQA</b> , Anti-Ribosomal P Antibodies

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

The EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG) kit is an immune lineblot strip test intended for the qualitative detection of IgG class antibodies against nRNP/Sm, Sm, SS-A Ro-52, SS-B, Scl-70, Jo-1, CENP B and ribosomal P-proteins in human serum. Detection of these antibodies is used as an aid in the diagnosis of systemic lupus erythematosus, systemic sclerosis, poly-/dermatomyositis, mixed connective tissue disease and Sjögren's syndrome, in conjunction with other laboratory and clinical findings. The EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG) test kit is intended to be used in a clinical, reference or hospital laboratory. This kit is not designed for point-of-care testing.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For Prescription use only.

4. Special instrument requirements:

CanoScan LiDE Series flatbed scanner using ScanGear and EUROLineScan softwares for automated read (refer to k113439).

**I. Device Description:**

The EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG) consists of antigen coated line blot strips, a positive control (human IgG; 100x concentrate), alkaline phosphatase-labelled goat anti-human IgG conjugate (10X concentrate), sample buffer (ready-to-use), wash buffer (10X concentrate), Nitro blue tetrazolium

chloride/5-Bromo-4-chloro-3-indolylphosphate (NBT/BCIP) substrate solution (ready-to-use), incubation tray and test instruction. Evaluation protocol, reaction control card as well as further accessories for use with EUROLineScan are available separately.

**J. Substantial Equivalence Information:**

1. Predicate device name(s) and 510(k) number(s):

EUROIMMUN Anti-nRNP/Sm ELISA (IgG)	k123261
EUROIMMUN Anti-Sm ELISA (IgG)	k123261
EUROIMMUN Anti-SS-A ELISA (IgG)	k123261
INOVA Quanta Lite™ SS-A 52 ELISA	k063565
EUROIMMUN Anti-SS-B ELISA (IgG)	k123261
EUROIMMUN Anti-Scl-70 ELISA (IgG)	k123261
EUROIMMUN Anti-Jo-1 ELISA (IgG)	k123261
EUROIMMUN Anti-Centromeres ELISA (IgG)	k123261
EUROIMMUN Anti-Ribosomal P-Proteins ELISA (IgG)	k123261

2. Comparison with predicate(s):

Similarities			
Item	Device	Predicate(s)	
Intended Use	Qualitative detection of IgG antibodies against 9 different antigens on the same strip: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B and ribosomal P-proteins in human serum to aid in the diagnosis of autoimmune diseases, systemic sclerosis, poly-/dermatomyositis, mixed connective tissue disease and Sjögren’s syndrome, in conjunction with other laboratory and clinical findings..	Same	
Capture antigens	nRNP/Sm	Purified U1-nRNP complex;U1-nRNP contains RNP as well as Sm reactive proteins	Same
	Sm	Purified Sm antigen	Same
	SS-A	Purified SS-A antigen	Same
	Ro-52	Recombinant Ro-52 antigen	Same
	SS-B	Purified SS-B antigen	Same
	Scl-70	Purified Scl-70 antigen	Same
	Jo-1	Purified Jo-1 antigen	Same
	CENP B	Recombinant centromeres protein B	Same
	Ribosomal P-proteins	Purified ribosomal P antigen	Same
Assay format	Qualitative, positive/negative	Same	

Differences		
Item	Device	Predicate(s)
Assay configuration	All analytes on the same membrane test strip and tested concurrently	Each analyte is coated on polystyrene microwells and tested individually
Result readout method	Manual visual readout or automated instrument readout	Instrument readout
Instrument	A flatbed scanner with EUROLInScan software	Spectrophotometer
Sample Type	Serum	EUROIMMUN: Serum and plasma (EDTA, Li-heparin, Citrate) INOVA: Same
Sample dilution	1:101	EUROIMMUN: 1:201 INOVA: 1:101
Detection antibody	Alkaline phosphatase-labeled anti-human IgG	Horseradish peroxidase-labeled anti-human IgG
Controls	One positive control	EUROIMMUN: Two controls (positive, negative) INOVA: Three controls (high positive, low positive, negative)
Substrate	Nitro blue tetrazolium chloride/ 5-Bromo-4-chloro-3-indolylphosphate	3, 5', 5', 5' Tetramethylbenzidine
Interpretation of results	Positive/negative compared to reaction control card (visual readout) or based on grey scale unit (automated readout)	EUROIMMUN: RU/mL (dsDNA: IU/mL) or ratios INOVA: U/mL

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

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

EN 13640:2002: Stability Testing of In Vitro Diagnostic Reagents

**L. Test Principle:**

The principle of the EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG) is that of an enzyme linked immunosorbent assay (ELISA), using a membrane as the solid phase instead of microtiter wells. Different purified antigens have been coated and applied in easy to read lines (bands) onto a membrane strip.

Autoantibodies in patient samples bind to the bands and are detected via a secondary antibody linked to an enzyme. The strips can be evaluated visually by comparison of the band intensity with the reaction control card or they can be digitized by use of a flatbed scanner and evaluated with the computer software EUROLiScan.

The control band on the strips contains (non-specific) anti-human IgG, which reacts with the sample IgG to show a color reaction if the incubation was performed correctly and so represents a function test on each single strip.

The positive control contains a mixture of the targeted antibodies which bind to the antigen coated on the blot strips. A strip incubated with the positive control shows a positive result. If either the control band or the strip incubated with the positive control is negative, test results are invalid and should be repeated.

The qualitative results are reported for each individual antibody separately as positive or negative. The intensity of the reaction is not reported but only as a means to distinguish between a positive and negative reaction and not as an indication of disease status. The interpretation of the test results does not include a combined score or diagnosis.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

Test results were evaluated by visual or automated reading of the test strips.

For visual evaluation, the reaction intensity of each antigen band on the test strip was compared to the two color bars on the reaction control card that correspond to the intensities just below (negative) and just above (positive) the cut-off.

For automated evaluation, the test strips are scanned using a flatbed scanner and evaluated with EUROLiScan with a cut-off of 11 grey scale units.

*a. Precision/Reproducibility:*

Assay reproducibility was determined by testing 6 or 7 samples that cover the complete range of results (negative, positive and near to cut-off).

The intra-assay reproducibility is based on 20 replicates tested in one day using the same kit lot.

Intra-assay reproducibility:

Antigen band	Sample characterization	Sample number (n)	Visual evaluation result	
			% positive	% negative
<b>nRNP/Sm</b>	pos	2	100	0
	pos (near to cut-off)	2	100	0
	neg (near to cut-off)	2	0	100
	neg	1	0	100
<b>Sm</b>	pos	2	100	0
	pos (near to cut-off)	2	100	0
	neg	2	0	100
<b>SS-A</b>	pos	2	100	0
	pos (near to cut-off)	2	100	0
	neg (near to cut-off)	1	0	100
	neg	2	0	100
<b>Ro-52</b>	pos	2	100	0
	pos (near to cut-off)	1	100	0
	pos (near to cut-off)	1	60	40
	neg	2	0	100
<b>SS-B</b>	pos	2	100	0
	pos (near to cut-off)	1	100	0
	neg (near to cut-off)	1	30	70
	neg	2	0	100
<b>Scl-70</b>	pos	2	100	0
	pos (near to cut-off)	1	50	50
	neg (near to cut-off)	1	0	100
	neg (near to cut-off)	1	45	55
	neg	2	0	100
<b>Jo-1</b>	pos	2	100	0
	pos (near to cut-off)	1	100	0
	pos (near to cut-off)	1	85	15
	neg	2	0	100
<b>CENP B</b>	pos	2	100	0
	pos (near to cut-off)	1	80	20
	pos (near to cut-off)	1	60	40
	neg	2	0	100
<b>Ribosomal P-proteins</b>	pos	2	100	0
	pos (near to cut-off)	2	100	0
	neg	2	0	100

The inter-assay reproducibility is based on 5 different runs using the same kit lot, each run on a different day with 3 replicates per run performed by the same technician. Evaluation was performed both visually using the reaction control card and automatically using the EUROLIneScan software with a cut-off of 11 grey scale units. The results are shown in the table below.

Inter-assay reproducibility:

Antigen	Sample Characterization	n	Visual evaluation result		EUROLineScan result	
			% pos	% neg	% pos	% neg
<b>nRNP /Sm</b>	pos	4	100	0	100	0
	neg (near to cut-off)	1	0	100	0	100
	Neg	2	0	100	0	100
<b>Sm</b>	pos	3	100	0	100	0
	pos (near to cut-off)	1	100	0	100	0
	neg (near to cut-off)	1	0	100	0	100
	Neg	1	0	100	0	100
<b>SS-A</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg (near to cut-off)	1	0	100	0	100
	Neg	2	0	100	0	100
<b>Ro-52</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	Neg	2	0	100	0	100
<b>SS-B</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg	2	0	100	0	100
<b>Scl-70</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg (near to cut-off)	1	0	100	0	100
	neg	2	0	100	0	100
<b>Jo-1</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg	2	0	100	0	100
<b>CENP B</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg	2	0	100	0	100
<b>Ribosomal P-proteins</b>	pos	2	100	0	100	0
	pos (near to cut-off)	2	100	0	100	0
	neg	2	0	100	0	100

The lot-to-lot reproducibility was tested in 6 different runs (each run in duplicates) using 3 different kit lots. The reproducibility data showed no positive sample was found negative and vice versa.

Inter-lot reproducibility:

Antigen band	Sample Characterization	Sample number (n)	Visual evaluation result	
			% positive	% negative
<b>nRNP/Sm</b>	pos	1	100	0
	neg (near to cut-off)	1	8.3	91.7
	Neg	1	0	100
<b>Sm</b>	pos (near to cut-off)	1	91.7	8.3
	neg (near to cut-off)	1	25	75
	Neg	1	0	100
<b>SS-A</b>	pos	1	100	0
	pos (near to cut-off)	1	83.3	16.7
	Neg	1	0	100
<b>Ro-52</b>	pos	1	100	0
	pos (near to cut-off)	1	91.7	8.3
	Neg	1	0	100
<b>SS-B</b>	pos	1	100	0
	pos (near to cut-off)	1	100	0
	Neg	1	0	100
<b>Scl-70</b>	pos	1	100	0
	pos (near to cut-off)	1	100	0
	Neg	1	0	100
<b>Jo-1</b>	pos	1	100	0
	pos (near to cut-off)	1	100	0
	Neg	1	0	100
<b>CENP B</b>	pos	1	100	0
	pos (near to cut-off)	1	75	25
	Neg	1	0	100
<b>Ribosomal P-proteins</b>	pos	1	100	0
	neg (near to cut-off)	1	25	75
	Neg	1	0	100

The inter-reader reproducibility of 3 samples that cover the complete range of results (negative, positive and near to cut-off) for each antigen was evaluated in 6 runs, each run performed in duplicates by 2 different technicians. No deviation was observed between the individual readings.

b. *Linearity/assay reportable range:*

Not applicable

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

Traceability: There are no recognized standards or reference materials for these analytes.

Controls: Controls are derived from human serum and match pre-specified performance criteria.

Stability:

i. *Real-time and accelerated stability*

The stability studies were performed in accordance with EN 13640:2002 (Stability Testing of *In Vitro* Diagnostic Reagents) to demonstrate unopened kit shelf-life stability (from the date of manufacture when stored at recommended temperature 2-8°C) and opened kit shelf-life stability. The acceptance criteria are that results do not differ more than one result level [pos, pos (near the cutoff), neg (near the cutoff) and neg] from the reference run (real-time stability study: day 0; accelerated stability study and transportation simulation: 4°C stored sample).

For real-time stability study, three lots of all kit components stored at recommended storage temperature 2-8°C were evaluated at different occasions with three samples per antigen band. Original sealed kits and opened kits were demonstrated to be stable for up to 18 and 12 months, respectively, when stored at 2-8°C.

For accelerated study, three lots of all kits components were stored for 7 days at 37°C and evaluated with 3 samples per antigen band. The same lots stored at 4°C were used as reference. Reagents were shown to be stable for 7 days at 37°C.

ii. *Transport stability*

To simulate transportation of kits from Europe to US, one lot of an example EUROLINE test kit with equal composition and technology as the EUROLINE ENA Profile 9 Ag (IgG) was stored for 7 days at 4°C and -20°C and evaluated with 4 different samples. This simulation showed that no deviation was obtained between the results of the differently stored kits for the different antigen bands.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

- i) Endogenous Interference: Interference testing was performed for each antigen using at least 6 samples that cover the complete range of results (negative, positive and near to cut-off) for each antigen. Each sample was spiked with two different levels of endogenous interfering substances, namely hemoglobin, triglycerides and bilirubin. Evaluation of test results

was performed visually and no significant interference was observed for concentrations up to 500 mg/dL for hemoglobin, 2000 mg/dL for triglycerides and 40 mg/dL for bilirubin.

ii) Cross-reactivity: Analytical cross-reactivity was investigated using the ANA reference panel (n=12) of the Center for Disease Control and Prevention (CDC, Atlanta, USA). The ANA reference panel includes: native DNA, SS-B, RNP/SS-B/SS-A, RNP, Sm, U3-RNP, SS-A, Centromere, Scl-70, Jo-1, PM-Scl, and ribosomal P-proteins. Each reference sample showed the expected reactivity against the EUROLINE ENA antigen bands.

f. *Assay cut-off*:

The cut-off is predefined by the visual evaluation. A sample is positive if the respective band is clearly visible. The cut-off for each antigen on the EUROLINE was set to the lowest limit of a clearly visible band.

To confirm the assay cut-off, 1118 clinically characterized samples sent in to a clinical laboratory for determination of ANA (338 men and 780 women with an average age of 57 years and age range between 18 and 99 years), as well as 150 control samples (79 men and 71 women with an average age of 37 years and age range: 18 – 67 years) were tested. The results showed that negative and positive results can clearly be discriminated by the assay.

2. Comparison studies:

a. *Method comparison with predicate device*:

A comparison study was performed with clinically characterized samples obtained from different sources in Europe and North America. The samples were tested with the EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG) and with the ELISA kits (k123261 or k063565) as the predicate devices. In addition, 5 to 12 artificial samples with antibody concentrations close to the cut-off were created for each antigen by mixing positive samples with negative sample as diluent of the same matrix. The total numbers of samples tested for each antigen differ as shown in the table below.

<b>Antigen</b>	<b>Total test</b>	<b>Gender</b>	<b>Mean age and range</b>	<b>Patient samples</b>
<b>nRNP/Sm</b>	936	738 female; 133 male; 65 unknown	50 (2-91) years; 69 unknown	225 Systemic lupus erythematosus, 53 Mixed connective tissue diseases and 658 control samples*
* 211 Systemic sclerosis , 88 Sjögren's syndrome , 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer and 69 infectious diseases				
<b>Sm</b>	917	715 female; 137 male; 65 unknown	50 (2-91) years; 69 unknown	259 Systemic lupus erythematosus and 658 control samples*
* 211 Systemic sclerosis , 88 Sjögren's syndrome , 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer and 69 infectious diseases				
<b>SS-A</b>	1036	806 female; 165 male; 65 unknown	51 (2-91) years; 69 unknown	229 Systemic lupus erythematosus, 88 Sjögren's syndrome and 658 control samples*
* 211 Systemic sclerosis , 88 Sjögren's syndrome , 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer and 69 infectious diseases				
<b>Ro-52</b>	276	158 female; 53 male; 65 unknown	44 (2-91) years; 69 unknown	81 Systemic lupus erythematosus and 195 control samples*
* 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases				
<b>SS-B</b>	1036	806 female; 165 male; 65 unknown	51 (2-91) years; 74 unknown	229 Systemic lupus erythematosus, 88 Sjögren's syndrome and 719 control samples*
* 211 Systemic sclerosis, 149 polymyositis/dermatomyositis, 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases.				
<b>Scl-70</b>	663	482 female; 111 male; 70 unknown; 5 artificial	54 (2-91) years; 74 unknown	211 Systemic sclerosis and 452 control samples*
* 88 Sjögren's syndrome, 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases, 5 artificial samples mixed to cut-off range				

Antigen	Total test	Gender	Mean age and range	Patient samples
<b>Jo-1</b>	626	421 female; 130 male; 75 unknown; 10 artificial	52 (2-91) years; 79 unknown	169 polymyositis/ dermatomyositis and 457 control samples*
* 88 Sjögren's syndrome, 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases, 10 artificial samples mixed to cut-off range.				
<b>CENP B</b>	670	482 female; 111 male; 77 unknown; 12 artificial	54 (2-91) years; 81 unknown	211 Systemic sclerosis and 459 control samples*
* 88 Sjögren's syndrome, 164 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases, 12 artificial samples mixed to cut-off range.				
<b>Ribosomal P-proteins</b>	615	434 female; 89 male; 92 unknown; 2 artificial	45 (2-91) years; 97 unknown	273 Systemic lupus erythematosus and 342 control samples*
* 55 Sjögren's syndrome, 90 rheumatoid arthritis, 15 fibromyalgia, 22 gastrointestinal diseases, 10 liver diseases, 23 thyroid diseases, 10 muscle diseases, 11 skin diseases, 10 renal, 25 cancer, 69 infectious diseases, 2 artificial samples mixed to cut-off range.				

The results and the positive and negative agreement between the device and the predicates are shown in the tables below. 95% C.I.'s were calculated by the exact method.

		Predicate ELISA		Positive Agreement Negative Agreement Overall Agreement % (95% C.I.)
		Positive	Negative	
n=936				88.5% (81.5% - 93.6%)
<b>nRNP/Sm</b>	<b>positive</b>	108	0	100% (99.2% - 100%)
	<b>negative</b>	14	814	98.5% (97.5% - 99.2%)
n = 917				82.9% (66.4% - 93.4%)
<b>Sm</b>	<b>positive</b>	29	2	99.8% (99.2% - 100%)
	<b>negative</b>	6	880	99.1% (98.3% - 99.6%)
n = 1036				99.5% (97.1% - 100%)
<b>SS-A</b>	<b>positive</b>	189	20	97.6% (96.4% - 98.6%)
	<b>negative</b>	1	826	98.1% (97.0% - 98.8%)

		Predicate ELISA		Positive Agreement Negative Agreement Overall Agreement % (95% C.I.)
		Positive	Negative	
n = 276				68.4% (54.8% - 80.1%)
<b>Ro-52</b>	<b>positive</b>	39	1	99.5% (97.5% - 100%)
	<b>negative</b>	18	218	93.1% (89.5% - 95.8%)
n = 1036				100% (94.9% - 100%)
<b>SS-B</b>	<b>positive</b>	71	32	96.7% (95.4% - 97.7%)
	<b>negative</b>	0	933	96.9% (95.7% - 97.9%)
n = 663				96.5% (91.3% - 99.0%)
<b>Scl-70</b>	<b>positive</b>	111	8	98.5% (97.1% - 99.4%)
	<b>negative</b>	4	540	98.2% (96.9% - 99.1%)
n = 626				100% (92.6% - 100%)
<b>Jo-1</b>	<b>positive</b>	48	14	97.6% (96.0% - 98.7%)
	<b>negative</b>	0	564	97.8% (96.3% - 98.8%)
n = 670				96.2% (87.0% - 99.5%)
<b>CENP B</b>	<b>positive</b>	51	5	99.2% (98.1% - 99.7%)
	<b>negative</b>	2	612	99.0% (97.9% - 99.6%)
n = 615				84.6% (71.9% - 93.1%)
<b>Ribosomal P-proteins</b>	<b>positive</b>	44	2	99.6% (98.7% - 100%)
	<b>negative</b>	8	561	98.4% (97.0% - 99.2%)

*b. Matrix comparison:*

Serum is the only test matrix.

1. Clinical studies:

*a. Clinical Sensitivity/Clinical Specificity:*

In total 1149 clinically characterized samples obtained from different study sites were analyzed by the EUROIMMUN EUROLINE ENA Profile 9 Ag (IgG).

Panel	Source*	n (men, women)	Mean age and range in years
Systemic lupus erythematosus	1	210 (14,196)	41 (16-80 )
Systemic sclerosis (SSc)	1,2	209 (23,188)	59 (22 – 88 )
Diffuse SSc	1	96	
Limited SSc	1	113	
Polymyositis/ Dermatomyositis	2	149 (38,111)	56 (14 – 85 )
Mixed connective tissue diseases	1,3	53 (7,46)	44 (20 – 69 )
Sjögren's syndrome	1	88 (4,84)	52 (19 –78 )
Rheumatoid arthritis	1	164 (43,121)	54 (19 – 80 )
Fibromyalgia	4	15 (0, 15)	58 (42 –76 )
Gastrointestinal diseases <sup>a</sup>	4	22 (8,7, 7 unknown)	36 (2 –74); 8 unknown
Liver diseases <sup>b</sup>	5	10	
Thyroid diseases <sup>c</sup>	6	23 (3,20)	50 (19 – 73)
Muscle diseases <sup>d</sup>	4	10 (3, 6, 1 unknown)	62 (31– 91); 1 unknown
Skin diseases <sup>e</sup>	4	11 (2,7, 2 unknown)	61 (40 – 84); 4 unknown
Renal diseases <sup>f</sup>	4	10 (5, 1, 4 unknown)	54 (34 – 80); 4 unknown
Cancers <sup>g</sup>	7	25	
Infectious diseases <sup>h</sup>	8	69 (20, 33, 16 unknown)	37 (16 – 62); 17 unknown

\* **1.** Prof. Hiepe, Dr. Egerer, University Clinic Charité, Berlin, Germany; **2.** Prof. Vencovsky, Institute of Rheumatology, Charles University in Prague, Czech Republic; **3.** Dr. Mc Hugh, Royal National Hospital for Rheumatic Diseases, Bath, UK; **4.** Rheumatological practice, Germany; **5.** Prof. D. Bogdanos, Institute of Liver Studies, School of Medicine, Kings's College Hospital, London, UK; **6.** Nuclear medical practice, Germany; **7.** University Clinic Charité, Berlin, Germany; **8.** Dr. Donglai Ma, EUROIMMUN Canada; Medical Center Laboratory, Germany; University of Lübeck, Germany.

<sup>a</sup> Crohn's disease, (n = 5), Ulcerative colitis (n = 5), Celiac disease (n = 5), Diabetes Type I (n = 5), Reflux (n = 2); <sup>b</sup> Autoimmune hepatitis (n = 5), Primary biliary liver cirrhosis (n = 5); <sup>c</sup> Grave's disease (n = 12), Hashimoto's disease (n = 11); <sup>d</sup> Multiple sclerosis (n = 5), Myasthenia gravis (n = 5); <sup>e</sup> Autoimmune dermatoses (n = 6), Psoriasis (n = 5); <sup>f</sup> Renal insufficiency (n = 5), Goodpasture syndrome (n = 5); <sup>g</sup> Prostate carcinoma (n = 5), Ovarian carcinoma (n = 5), Mammary carcinoma (n = 5), Bronchial carcinoma (n = 5), Cervical carcinoma (n = 5); <sup>h</sup> Borreliosis (n = 30), Hepatitis C virus (n = 15), Hepatitis B virus (n = 2), Herpes simplex virus (n = 4), Rubella (n = 15), Measles (n = 1), Parvovirus (n = 2)

The results of the clinical studies are shown in the following tables:

<b>nRNP/Sm</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	19.5% (41/210)	14.4 – 25.5%
Mixed connective tissue diseases	96.2% (51/53)	87.0 – 99.5%
<b>Panel</b>	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic sclerosis	98.6% (208/211)	95.9 – 99.7%
Polymyositis/dermatomyositis	95.3% (142/149)	90.6 – 98.1%
Sjögren's syndrome	98.9% (87/88)	93.8 – 100.0%
Rheumatoid arthritis	100% (164/164)	97.8 – 100%
Fibromyalgia	93.3% (14/15)	68.1 – 99.8%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>98.5% (795/807)</b>	<b>97.4 – 99.2%</b>

<b>Sm</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	8.36% (18/210)	5.2 – 13.2%
<b>Panel</b>	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic sclerosis	100% (211/211)	98.3 – 100%
Polymyositis/dermatomyositis	99.3% (148/149)	96.3 – 98.8%
Sjögren's syndrome	98.8% (87/88)	93.8 – 100%
Rheumatoid arthritis	100% (164/164)	97.8 – 100%
Fibromyalgia	100% (15/15)	97.8 – 100%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>99.4% (855/860)</b>	<b>98.6 – 99.8%</b>

SS-A		
Panel	Sensitivity (%)	95% CI
Systemic lupus erythematosus	43.3% (91/210)	36.5 – 50.3%
Sjögren's syndrome	76.1% (67/88)	65.9 – 84.6%
Panel	Specificity (%)	95% CI
Systemic sclerosis	89.6% (189/211)	84.6 – 93.3%
Polymyositis/dermatomyositis	89.3% (133/149)	83.1 – 93.7%
Mixed connective tissue diseases	92.5% (49/53)	81.8 – 97.9%
Rheumatoid arthritis	95.7% (157/164)	91.4 – 98.3%
Fibromyalgia	93.3% (14/15)	68.1 – 99.8%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	98.6% (68/69)*	92.2 – 100%
<b>Total</b>	<b>93.4% (721/772)</b>	<b>91.4 – 95.0%</b>

\*Borreliosis

Ro-52		
Panel	Sensitivity (%)	95% CI
Systemic lupus erythematosus	35.2% (74/210)	28.8 – 42.1%
Sjögren's syndrome	77.3% (68/88)	67.1 – 85.5%
Panel	Specificity (%)	95% CI
Systemic sclerosis	78.2% (165/211)	72.0 – 83.6%
Polymyositis/dermatomyositis	55.0% (82/149)	46.7 – 63.2%
Mixed connective tissue diseases	81.8% (43/53)	68.0 – 90.6%
Rheumatoid arthritis	94.5% (155/164)	89.8 – 97.5%
Fibromyalgia	93.3% (14/15)	68.1 – 99.8%
Gastrointestinal diseases	95.5% (21/22)*	77.2 – 99.9%
Liver diseases	60% (6/10)**	26.2 – 87.8%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	96.0% (24/25)^	79.6 – 99.9%
Infectious diseases	98.6% (68/69)^^	92.2 – 100%
<b>Total</b>	<b>81.9% (632/772)</b>	<b>79.0 – 84.5%</b>

\* Crohn's disease; \*\* 4 Autoimmune hepatitis; ^ Ovarian carcinoma; ^^ Hepatitis C virus

<b>SS-B</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	20.0% (42/210)	14.8 – 26.1%
Sjögren's syndrome	47.7% (42/88)	37.0 – 58.6%
<b>Panel</b>		
	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic sclerosis	95.3% (201/211)	91.5 – 97.7%
Polymyositis/dermatomyositis	97.3% (145/149)	93.3 – 99.3%
Mixed connective tissue diseases	100% (53/53)	93.3 – 100%
Rheumatoid arthritis	98.2% (161/164)	94.7 – 99.6%
Fibromyalgia	100% (15/15)	78.2 – 100%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>97.7% (754/772)</b>	<b>96.3 – 98.6%</b>

<b>Scl-70</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic sclerosis (SSc)	30.6% (64/209)	24.4 – 37.4%
Diffuse SSc	60.4% (58/96)	49.9 – 70.3
Limited SSc	5.3% (6/113)	2.0 – 11.2
<b>Panel</b>		
	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	100% (210/210)	98.3 – 100%
Polymyositis/dermatomyositis	99.3% (148/149)	96.3 – 100%
Mixed connective tissue diseases	100% (53/53)	93.3 – 100%
Sjögren's syndrome	100% (88/88)	95.9 – 100%
Rheumatoid arthritis	99.4% (163/164)	96.6 – 100%
Fibromyalgia	100% (15/15)	78.2 – 100%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	90.9% (10/11)*	58.7 – 99.8%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>99.7% (856/859)</b>	<b>99.0 – 99.9%</b>

\* Psoriasis

<b>Jo-1</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Polymyositis/dermatomyositis	24.2% (36/149)	17.5 – 31.8%
<b>Panel</b>	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	100% (210/210)	98.3 – 100%
Systemic sclerosis	99.1% (209/211)	96.6 – 99.9%
Mixed connective tissue diseases	100% (53/53)	93.3 – 100%
Sjögren's syndrome	100% (88/88)	95.9 – 100%
Rheumatoid arthritis	99.4% (163/164)	96.6 – 100%
Fibromyalgia	100% (15/15)	78.2 – 100%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	96% (24/25)*	79.6 – 99.9%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>99.6% (917/921)</b>	<b>98.9 – 99.9%</b>

\* Ovarian carcinoma

<b>CENP B</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic sclerosis (SSc)	18.0% (90/209)	36.3 – 50.1%
Limited SSc	74.3% (84/113)	65.3 – 82.1%
Diffuse SSc	6.3% (6/96)	2.3 – 13.1%
<b>Panel</b>	<b>Specificity (%)</b>	<b>95% CI</b>
Diffuse SSc	93.7% (90/96)	86.9 – 97.7%
Systemic lupus erythematosus	98.6% (207/210)	95.9 – 99.7%
Polymyositis/dermatomyositis	97.3% (145/149)	93.3 – 99.3%
Mixed connective tissue diseases	98.1% (52/53)	89.9 – 100%
Sjögren's syndrome	96.6% (85/88)	90.4 – 99.3%
Rheumatoid arthritis	98.8% (162/164)	95.7 – 99.9%
Fibromyalgia	93.3% (14/15)	68.1 – 99.8%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>98.3% (844/859)</b>	<b>97.1 – 99.0%</b>

<b>Ribosomal P Proteins</b>		
<b>Panel</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>
Systemic lupus erythematosus	4.8% (10/210)	2.8 – 8.6%
<b>Panel</b>	<b>Specificity (%)</b>	<b>95% CI</b>
Systemic sclerosis	100% (211/211)	98.3 – 100%
Polymyositis/dermatomyositis	100% (149/149)	97.6 – 100%
Mixed connective tissue diseases	98.1% (52/53)	89.9 – 100%
Sjögren’s syndrome	100% (88/88)	95.9 – 100%
Rheumatoid arthritis	100% (164/164)	97.8 – 100%
Fibromyalgia	100% (15/15)	78.2 – 100%
Gastrointestinal diseases	100% (22/22)	84.6 – 100%
Liver diseases	100% (10/10)	69.2 – 100%
Thyroid diseases	100% (23/23)	85.2 – 100%
Muscle diseases	100% (10/10)	69.2 – 100%
Skin diseases	100% (11/11)	71.5 – 100%
Renal diseases	100% (10/10)	69.2 – 100%
Cancer	100% (25/25)	86.3 – 100%
Infectious diseases	100% (69/69)	94.8 – 100%
<b>Total</b>	<b>99.8% (858/860)</b>	<b>99.2 – 100%</b>

b. Other clinical supportive data (when a. is not applicable):

Not applicable.

4. Clinical cut-off:

See Assay cut-off.

5. Expected values/Reference range:

The levels of autoantibodies against nRMP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B and ribosomal P-proteins were analyzed in a panel of 173 samples from US asymptomatic blood donors of mixed age and sex (79 men, 94 women with a mean age of 38.3 years and age range between 19 and 50 years). The result provided in the table below showed that the % samples found positive for each autoantibody ranges from 0 to 3.5. It is recommended that each laboratory determine its own normal range based on the population and equipment used.

n=173	EUROLINE ENA Profile 9 Ag (IgG)	
	Positive	%
nRNP/Sm	0	0
Sm	5	2.9
SS-A	6	3.5
Ro-52	6	3.5
SS-B	1	0.6
Scl-70	1	0.6
Jo-1	0	0
CENP B	1	0.6
Ribosomal P-proteins	0	0

**N. Proposed Labeling:**

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

**O. Conclusion:**

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