

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

A. 510(k) Number:

k112996

B. Purpose for Submission:

New device

C. Measurand:

IgG antibodies to nRNP/Sm, Sm, SS-A (SS-A 60/Ro-52), SS-B, Scl-70, ribosomal P proteins

D. Type of Test:

Qualitative, enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

EUROIMMUN US Inc.

F. Proprietary and Established Names:

EUROIMMUN Anti-ENA Pool ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5100 Antinuclear Antibody Immunological Test System

2. Classification:

Class II

3. Product code:

LLL — Extractable antinuclear antibody, antigen and control

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The EUROIMMUN Anti-ENA Pool ELISA (IgG) is intended for the qualitative determination of IgG class antibodies against nuclear antigens (mixture of nRNA/Sm, Sm, SS-A (SS-A 60/Ro-52), SS-B, Scl-70, and ribosomal P proteins) in human serum. It is used as an aid in the diagnosis of mixed connective tissue disease (MCTD), systemic lupus erythematosus, Sjögren's syndrome and progressive systemic sclerosis, in conjunction with other laboratory and clinical findings.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Dual (450 nm and 620-650 nm reference) wavelength spectrophotometer
(microwell plate reader)

I. Device Description:

The EUROIMMUN Anti-ENA Pool ELISA (IgG) contains the following components: microplate strips coated with a mixture of nuclear antigens (nRNA/Sm, Sm, SS-A (SS-A 60/Ro-52), SS-B, Scl-70, and ribosomal P proteins), calibrator, positive and negative controls, horseradish peroxidase (HRP)-conjugated rabbit anti-human IgG, sample buffer, 10x wash buffer, TMB/H₂O₂ (3,3',5,5'-tetramethylbenzidine/hydrogen peroxide) chromogenic substrate solution, and 0.5 M sulphuric acid stop solution.

J. Substantial Equivalence Information:

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

AESKULISA[®] ANA HEp-2, k081104

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Detection of IgG class antibodies against nuclear antigens	Same
Indications for Use	Aids in the diagnosis of mixed connective tissue disease (MCTD), systemic lupus erythematosus, Sjögren's syndrome and progressive systemic sclerosis, in conjunction with other laboratory and clinical findings	Aids in the diagnosis of certain systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.
Assay Measurement	Qualitative	Same
Methodology	ELISA	Same
Assay platform	96-well microtiter plate	Same
IgG Enzyme conjugate	HRP-labeled anti-human IgG	Same
Substrate	TMB	Same
Matrix	Serum	Same
Reported results	Optical density (OD) ratio	Same
Cut-off	OD ratio 1.0	Same

Differences		
Item	Device	Predicate
Antigen Mixture	nRNA/Sm, Sm, SS-A (SS-A 60/Ro-52), SS-B, Scl-70, and ribosomal P proteins	dsDNA, histones, SS-A (Ro), SS-B (La), Sm, snRNP/Sm, Scl-70, Jo-1 and centromeric antigens and lysed HEp-2 cells
Calibrators and Controls	1 calibrator 2 controls: 1 positive, 1 negative	3 controls: 1 positive, 1 cut-off, 1 negative
Sample buffer	Ready to use	5x concentrate
Wash buffer	10x concentrate	50x concentrate
Stop solution	0.5 M sulphuric acid	1 M hydrochloric acid
Sample dilution	1:201	1:101

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)

DIN EN 13640: 2002 Stability testing of *in vitro* diagnostic reagent; German version
EN 13640: 2002 German and English texts

L. Test Principle:

Antibodies present in patient samples bind to the antigen mixture-coated microtiter wells during an incubation step. The unbound sample is washed away and anti-human IgG enzyme conjugate is added. After washing away unbound enzyme conjugate, the remaining conjugate is incubated with chromogenic substrate solution. The reaction is stopped by adding “stop solution” and color intensity is measured as OD units by spectrophotometer. The ODs are proportional to the amount of bound conjugate, which in turn is proportional to the amount of antibodies bound to the antigen mixture in microtiter wells. Results are expressed as ratios which is a ratio of the OD of the control or patient sample to the OD value of the calibrator.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra- and inter-assay reproducibility of the device was evaluated using 16 and 14 serum samples with different concentrations, respectively. Intra-assay reproducibility was assessed from 16 or 20 determinations on one day. Inter-assay reproducibility was based on 26-30 results from 10 runs performed on 5 days with 2 runs per day. Each sample was assayed in triplicate for each run. For all samples, 100% of the results corresponded to the expected results.

Intra-assay reproducibility

Sample	Mean Ratio	Ratio Range	Expected Result	% Correct Result
1	0.4	0.3 - 0.4	Negative	100%
2	0.8	0.7 - 0.8	Negative	100%
3	1.4	1.3 - 1.4	Positive	100%
4	2.4	2.2 - 2.6	Positive	100%
5	5.7	5.4 - 6.0	Positive	100%
6	8.3	7.8 - 9.0	Positive	100%
7	0.1	0.1	Negative	100%
8	7.4	6.8 - 7.6	Positive	100%
9	2.8	2.5 - 3.0	Positive	100%
10	4.9	4.7 - 5.2	Positive	100%
11	2.5	2.1 - 2.7	Positive	100%
12	8.3	8.0 - 8.6	Positive	100%
13	10.3	9.5 - 11.1	Positive	100%
14	2.1	2.0 - 2.3	Positive	100%
15	5.6	4.9 - 6.2	Positive	100%
16	2.8	2.5 - 3.0	Positive	100%

Inter-assay reproducibility

Sample	Mean Ratio	Ratio Range	Expected Result	% Correct Result
1	0.4	0.3 - 0.5	Negative	100%
2	0.8	0.7 - 0.9	Negative	100%
3	1.3	1.2 - 1.4	Positive	100%
4	2.7	2.5 - 3.0	Positive	100%
5	5.6	4.9 - 6.1	Positive	100%
6	8.4	7.6 - 9.1	Positive	100%
7	0.2	0.2 - 0.3	Negative	100%
8	6.5	5.3 - 7.3	Positive	100%
9	2.0	1.5 - 2.8	Positive	100%
10	3.5	2.6 - 4.9	Positive	100%
11	1.8	1.1 - 2.1	Positive	100%
12	6.8	5.4 - 8.9	Positive	100%
13	7.1	4.5 - 9.8	Positive	100%
14	2.0	1.6 - 2.4	Positive	100%

Lot-to-lot reproducibility was investigated using 15 sera (3 negative and 12 positive) with a range of ratio values. Each sample/lot combination was tested between 6 and 11 times. The expected result was determined from the sample mean. All of the results correctly matched the expected results.

Sample ID	Mean Ratio	Ratio Range	Lots	Runs/Lot	Total Replicates	Expected Result	Correct Result
1	1.1	1.1 - 1.2	3	2	6	Positive	100%
2	0.9	0.8 - 0.9	3	2	6	Negative	100%
3	0.1	0.1 - 0.2	11	1	11	Negative	100%
5	2.7	2.5 - 3.3	9	1	9	Positive	100%

Sample ID	Mean Ratio	Ratio Range	Lots	Runs/Lot	Total Replicates	Expected Result	Correct Result
6	3.6	3.0 - 4.2	11	1	11	Positive	100%
7	5.0	4.1 - 5.7	11	1	11	Positive	100%
8	8.5	7.0 - 9.8	9	1	9	Positive	100%
9	0.2	0.1 - 0.3	3	2	6	Negative	100%
10	6.3	5.5 - 7.6	3	2	6	Positive	100%
11	2.4	1.9 - 3.0	3	2	6	Positive	100%
12	3.9	2.9 - 5.2	3	2	6	Positive	100%
13	2.2	1.9 - 2.7	3	2	6	Positive	100%
14	6.8	5.9 - 8.2	3	2	6	Positive	100%
15	8.2	7.0 - 9.7	3	2	6	Positive	100%
16	2.0	1.8 - 2.3	3	2	6	Positive	100%

b. *Linearity/assay reportable range:*

Not applicable

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

- i. Traceability: A recognized international standard or reference material for anti-nuclear antibodies is not available. Results of this assay are based on OD reading expressed as in ratios.
- ii. Calibrator and Controls: The OD values of the calibrator and the ratios of the positive and negative controls must lie within the limits stated in the quality control certificate provided with the relevant test kit lot.
- iii. Stability:

Three lots of all kit reagents were tested in real time stability studies. Original sealed products were demonstrated to be stable for 12 months when stored at 2-8°C. Opened products were demonstrated to be stable for 12 months when stored at 2-8°C. The reconstituted wash buffer is stable for up to 28 days (4 weeks).

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

- i. Interference: Four samples with different ENA concentrations (ratios from 0.7 to 8.4) were spiked with varying concentrations of hemoglobin up to 1,000 mg/dL, triglycerides up to 2,000 mg/dL, or bilirubin up to 40 mg/dL. Recoveries of all spiked sample/interferent combinations compared to the measurements for the unspiked samples were calculated. The individual recovery of all samples was within the range of 91% to 105%.

Six samples with ENA concentration ratios from 1.1 to 11.7 were spiked with rheumatoid factor at 500 IU/mL. Recovery in relation to the original unspiked sample was calculated and found to be between 96% and 106%.

ii. Cross Reactivity: The reactivity of the EUROIMMUN Anti-ENA Pool ELISA (IgG) was verified using twelve human reference sera from the CDC ANA reference panel. Samples characterized as homogenous/rim/nDNA (No. 1), nucleolar/U3 RNP (Fibrillarin) (No. 6), centromere (No. 8), and PM-Scl (No. 11) tested negative. All samples with target antigens (Nos. 2, 3, 4, 5, 7, 9, and 12) included in the kit tested positive. CDC sample characterized as anti-Jo-1 positive (No. 10) was also positive. Further testing was conducted to determine whether anti-Jo-1 reactivity was due to the presence of Ro-52 antibodies in the sample. Five samples positive for both anti-Jo-1 and anti-Ro-52 (but negative for nRNA/Sm, Sm, SS-A 60, SS-B, Scl70, and ribosomal P proteins) were positive with the EUROIMMUN Anti-ENA Pool ELISA (IgG). Two anti-Jo-1 positive samples that were negative for anti-Ro-52 antibodies tested negative with the EUROIMMUN Anti-ENA Pool ELISA (IgG). No cross reactivity to Jo-1 is expected.

Cross reactivity was investigated in other disease conditions using 82 clinically and serologically characterized samples. The samples were as follows: 10 celiac disease with antibodies against gliadin and tissue transglutaminase, 17 Wegener’s granulomatosis with anti-neutrophil cytoplasmic antibodies (ANCA), 39 rheumatoid arthritis with anti-CCP antibodies, and 16 infectious diseases antibody positive. All samples except 2 Wegener’s granulomatosis samples were negative with the EUROIMMUN Anti-ENA Pool ELISA (IgG).

f. Assay cut-off:

The assay cut-off is OD ratio of 1.0. Results ≥ 1.0 are positive, and results < 1.0 are negative.

2. Comparison studies:

a. Method comparison with predicate device:

Two hundred seventy-eight (278) clinically characterized samples from patients and control groups were tested with the predicate device and the EUROIMMUN Anti-ENA Pool ELISA (IgG). The samples were from 49 mixed connective tissue disease (MCTD), 26 systemic lupus erythematosus (SLE), 29 Sjögren’s syndrome, 22 systemic sclerosis, 20 polymyositis/dermatomyositis, 10 celiac disease, 17 Wegener’s granulomatosis, 39 rheumatoid arthritis, and 16 infectious disease patients. Fifty healthy individuals were also included.

		Predicate		
		Positive	Negative	Total
EUROIMMUN Anti-ENA Pool ELISA (IgG)	Positive	135	1	136
	Negative	3	139	142
	Total	138	140	278

Positive agreement: $135/138 = 97.8\%$ (95% CI: 93.8% – 99.5%)

Negative agreement: $139/140 = 99.3\%$ (95% CI: 96.1% – 100%)

Overall agreement: $274/278 = 98.6\%$ (95% CI: 96.4% – 99.6%)

Method comparison with individual ENA ELISAs

A comparison study was performed with six monospecific autoantibody ELISAs to ensure that all targets are recognized by the EUROIMMUN Anti-ENA Pool ELISA (IgG). Samples from MCTD, SLE, Sjögren’s syndrome, systemic sclerosis, rheumatoid arthritis, fibromyalgia, infectious diseases and healthy individuals. The combined positive percent agreement was 100% (95%CI: 94.7%-100%), negative percent agreement was 91.7% (95% CI: 80.0%-97.7%) and overall agreement of 96.6% (95%CI: 91.4%-99.1%). There were two false positive samples each for the SSA/SSB and Scl-70 comparison.

b. Matrix comparison:

Serum is the only sample specimen.

3. Clinical studies:

a. Clinical Sensitivity:

Clinical sensitivity was determined from 261 clinically characterized samples from patients with MCTD (n=44), SLE (n=85), systemic sclerosis (n=66), and Sjögren’s syndrome (n=66). The EUROIMMUN Anti-ENA Pool ELISA (IgG) showed an overall sensitivity of 60.9% (95% CI: 54.7 – 66.9%). The results are in the table below.

Condition	Total Samples	Anti-ENA Pool ELISA (IgG)		
		Positive	% Positive	95% CI
MCTD	44	44	100.0	92.0 – 100.0
SLE	85	47	55.3	44.1 – 66.1
Systemic sclerosis	66	30	45.5	33.1 – 58.2
Sjögren’s syndrome	66	48	72.7	60.4 – 83.0
Total	261	159	60.9	54.7 – 66.9

b. Clinical specificity:

Clinical specificity was determined from 171 clinically characterized samples from the following control groups: polymyositis/dermatomyositis (n=26), celiac disease (n=21), Wegener’s granulomatosis (n=17), rheumatoid arthritis (n=39), other autoimmune diseases (n=52), and bacterial/viral infections (n=16). The EUROIMMUN Anti-ENA Pool ELISA (IgG) showed an overall specificity of 96.5% (95% CI: 92.5 – 98.7%). The results are listed below.

Control Group	Total Samples	Anti-ENA Pool ELISA (IgG)		
		Negative	%	95% CI
Polymyositis/dermatomyositis	26	22	84.6	65.1 – 95.6
Celiac disease	21	21	100.0	83.9 – 100.0
Wegener’s granulomatosis	17	15	88.2	63.6 – 98.5

Control Group	Total Samples	Anti-ENA Pool ELISA (IgG)		
		Negative	%	95% CI
Rheumatoid arthritis	39	39	100.0	91.0 – 100.0
Other autoimmune diseases*	52	52	100.0	93.2 – 100.0
Bacterial/viral infections	16	16	100.0	79.4 – 100.0
Total	171	165	96.5	92.5 – 98.7

*Other autoimmune diseases includes autoimmune hepatitis (n=8), primary biliary cirrhosis (n=9), Grave's disease (n=12), Hashimoto's disease (n=11), and type I diabetes (n=12).

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

Not applicable

4. Clinical cut-off:

See Assay cut-off

5. Expected values/Reference range:

The levels of anti-ENA antibodies (IgG) were analyzed in a panel of 200 samples from apparently healthy blood donors. With a cut-off ratio of 1.0, a prevalence of 1.5% was obtained. The mean ratio was 0.2 (SD = 0.37) and the values ranged from 0.0 to 5.0. Users of the kit should generate their own ranges, as stated in the product insert.

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.