510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k131185

B. Purpose for Submission:

New device

C. Measurand:

Anti-Nuclear Antibodies

D. Type of Test:

Qualitative enzyme immunoassay

E. Applicant:

EUROIMMUN US INC.

F. Proprietary and Established Names:

EUROIMMUN ANA Screen ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.5100 – Anti-Nuclear Antibody immunological test system

2. Classification:

   Class II

3. Product code:

   LJM, Antinuclear antibody (enzyme-labeled), antigen, controls

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The EUROIMMUN ANA Screen ELISA (IgG) is intended for the qualitative determination of IgG class antibodies against nuclear antigens (mixture of dsDNA, histones, ribosomal P-proteins, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1 and centromeres) in human serum and plasma (EDTA, Li-heparin, Citrate). It is used as an aid in the diagnosis of mixed connective tissue diseases (MCTD), systemic lupus erythematosus, Sjögren's syndrome, progressive systemic sclerosis and polymyositis, and dermatomyositis, 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:

   Microwell plate reader capable of measuring OD at 450 nm and at 620 nm for dual wavelength readings.

I. Device Description:

The EUROIMMUN ANA Screen ELISA (IgG) consists of a microwell ELISA plate coated with a mixture of dsDNA, histones, ribosomal P proteins, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1 and centromeres antigens. Also included are Calibrator, positive and negative controls, Peroxidase-labeled anti-human IgG conjugate, sample buffer, wash buffer concentrate, TMB chromogen/substrate solution, and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Aesku Aeskulisa ANA Hep-2

2. Predicate 510(k) number(s):
   k081104

3. Comparison with predicate:

Similarities		
Item	Device EUROIMMUN ANA Screen ELISA (IgG)	Predicate Aeskulisa ANA Hep-2
Intended Use	Detection of IgG antibodies to nuclear antigens	Same
Assay Format	Qualitative	Same
Technology	ELISA	Same
Assay Platform	96-well microtiter plates	Same
Calibration	Relative evaluation	Same
Conjugate	Anti-human IgG labeled with horseradish peroxidase	Same
Substrate	TMB	Same
Reported Results	OD Ratio	Same
Cut-Off Level	Ratio 1.0	Same

Differences		
Item	Device EUROIMMUN ANA Screen ELISA (IgG)	Predicate Aeskulisa ANA Hep-2
Antigen Mixture	dsDNA, histones, ribosomal P proteins, nRNP/Sm, Sm, SS-A, SS-B, Scl-70, Jo-1, centromeres	dsDNA, histones, SS-A (Ro), SS-B (La), Sm, snRNP/Sm, Scl-70, Jo-1 and centromeric antigens and lysed HEp-2 cells
Calibrators & Controls	1 calibrator 2 controls: 1 positive, 1 negative	3 controls: 1 positive, 1 cut-off (used for calculation of results), 1 negative
Sample Buffer	Ready for use	5x concentrate
Wash Buffer	10x concentrate	50x concentrate
Stop Solution	0.5 M sulphuric acid	1 M hydrochloric acid
Sample Types	Serum or plasma (EDTA, Li-heparin, Citrate)	Serum
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)

L. Test Principle:

Patient samples are diluted 1:20 in sample buffer, 100 µL of each diluted patient sample and pre-diluted controls and calibrator are added to the antigen mixture coated microtiter wells and incubated for 30 minutes at room temperature. After incubation the microtiter well strips

are washed with wash buffer to remove unbound antibodies and 100 µL of the anti-human IgG enzyme conjugate reagent is added to each microtiter well. After an additional 30-minutes incubation at room temperature, the microtiter wells are again washed 3 times with 300 µl of wash buffer to remove any unbound enzyme conjugate and 100 µL of the chromogen substrate is added. The strips are incubated for 15 minutes at room temperature and 100 µL stop solution is added. The microtiter plates are placed in an ELISA reader and read at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes.

Results of this assay are given in arbitrary values (ratio of sample optical density (OD) to OD of cutoff control). The arbitrary values are reported as positive or negative. A ratio of ≥1.0 is a positive result. A ratio of <1.0 is a negative result.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The within-run imprecision was investigated using 6 serum samples with different concentrations covering the measurement range, including 2 samples near the cutoff. Tests were performed according to the package insert with 20 replicates for each sample. The % coefficient of variation (%CV) of the 20 replicates within the run was calculated. The following results were obtained:

Within-run imprecision

	ANA Screen ELISA (IgG) *Ratio*					
Sample	**1**	**2**	**3**	**4**	**5**	**6**
Mean Ratio:	0.3	0.9	1.1	2.5	6.1	7.4
Std. Deviation (SD)	0.031	0.050	0.059	0.066	0.119	0.141
%CV	10.6%	5.8%	5.4%	2.7%	2.0%	1.9%
Range of Ratios:	0.2 – 0.3	0.8 – 0.9	1.0 – 1.2	2.3 – 2.6	5.8 – 6.3	7.1 – 7.7
Expected Result:	Negative	Negative	Positive	Positive	Positive	Positive
% positive:	0%	0%	100%	100%	100%	100%
% negative:	100%	100%	0%	0%	0%	0%

The between-run imprecision was investigated using 10 serum samples with different concentrations covering the measurement range, including 2 samples near the cutoff. Tests were performed according to the package insert with 3 replicates of each sample performed in 10 different runs on 5 days with 2 runs per day. The %CV was calculated by assessing between-run analysis of variance. The square root of the mean square of between-run variation was utilized to calculate the SD of this variance component. The following results were obtained (Ratio):

	ANA Screen ELISA (IgG) *Ratio*					
Sample	**1**	**2**	**3**	**4**	**5**	**6**
Mean Ratio:	0.59	0.85	1.06	2.71	5.99	7.58
SD	0.086	0.095	0.117	0.298	0.366	0.330
%CV	14.6%	11.1%	11.0%	11.0%	6.1%	4.4%
Range of Ratios:	0.5 – 0.7	0.7 – 1.0	1.0 – 1.2	2.5 – 3.0	5.4 – 6.4	7.2 – 8.0
Expected Result:	Negative	Negative	Positive	Positive	Positive	Positive
% positive:	0%	0%	100%	100%	100%	100%
% negative:	100%	100%	0%	0%	0%	0%
Sample	**7**	**8**	**9**	**10**		
Mean Ratio	2.44	4.13	1.02	2.80		
SD	0.303	0.517	0.134	0.488		
%CV	12.4%	12.5%	13.2%	17.4%		
Range of Ratios:	1.6 – 3.1	3.3 – 4.9	0.8 – 1.2	2.0 – 3.5		
Expected Result:	Positive	Positive	Positive	Positive		
% positive:	100%	100%	100%	100%		
% negative:	0%	0%	0%	0%		

The Lot -to- Lot imprecision was investigated using 9 serum samples with different concentrations distributed over the measurement range including 1 sample near cutoff. The samples were tested using 3 different kit lots in 2 different runs with a single replicate of each sample according to the package insert. The mean and SD of each sample in each lot was calculated. The %CV across the three lots was calculated from the SD of the three values in each lot for each sample concentration. The following results were obtained (Ratio):

Lot-to-lot imprecision sorted by increasing Screen ELISA (IgG) Ratios

	ANA Screen ELISA (IgG) *Ratio*								
Sample	**1**	**4**	**6**	**9**	**2**	**8**	**5**	**3**	**7**
Determinations:	6	6	6	6	6	6	6	6	6
Mean Ratio:	0.167	1.100	1.967	1.967	2.083	2.267	3.083	3.667	4.817
SD	0.058	0.087	0.407	0.407	0.202	0.375	0.058	0.462	0.722
%CV	34.6%	7.9%	20.7%	20.7%	9.7%	16.6%	1.9%	12.6%	15.0%
Range of Ratios:	0.1 – 0.2	1.0 – 1.2	1.5 – 2.3	1.5 – 2.2	1.9 – 2.4	1.9 – 2.7	3.0 – 3.2	3.4 – 4.3	4.4- 5.7
Expected Result:	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
% positive:	0%	100%	100%	100%	100%	100%	100%	100%	100%
% negative:	100%	0%	0%	0%	0%	0%	0%	0%	0%

For samples with IgG ratios between 1.0 and 5.0, the lot-to-lot %CV ranged from 2% to 20%.

b. Linearity/assay reportable range:

Not applicable.

High Dose Hook Effect

Not applicable

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

There is no recognized standard or reference material for anti-nuclear antibodies is available. Results of this assay are given in arbitrary values (ratio of sample OD to cutoff control OD).

Calibrators & Controls

The calibrator and controls are derived from human serum, purchased from commercial sources. The serum is tested and found negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. The calibrator is manufactured by dilution of the human serum with stabilizing buffer and adjusted to match the required performance criteria in use with the corresponding microtiter strip lot and the corresponding kit controls. The new lot of calibrator is assayed using a reference panel of at least 8 positive and 2 negative reference sera (distributed over the measurement range). By further dilution or spiking with the original serum, the calibrators are adjusted until the following acceptance criteria are met: At least 6 of the 8 positive sera must be found within the established acceptable ranges. The OD of the negative sera must be below the OD of the calibrator. After the adjustment is completed, the components of the new ELISA kit lot are tested together.

Stability

Three production lots of all kit reagents were tested and determined to be over 12 months for all components. The reconstituted Wash Buffer is stable for up to 28 days. The opened reagents are stable for 6 months.

d. Detection limit:

Not applicable.

e. Analytical specificity:

Cross-reactivity: Cross reactivity was investigated using a total of 82 clinically and serologically characterized samples (10 celiac disease for antibodies against gliadin and tissue transglutaminase, 17 Wegener's granulomatosis for ANCA, 39 rheumatoid

arthritis for antibodies against CCP and 16 infectious diseases antibody positive samples). All samples (except 1 sample with ANCA reactivity from a subject with Wegener's granulomatosis and 1 sample with antibodies against CCP from a subject with rheumatoid arthritis) were negative in the ANA Screen ELISA (IgG), so minimal cross reactivity is expected.

Interference: To investigate the influence from hemoglobin, triglycerides and bilirubin, four different specimens at different ANA concentrations (ratio 0.5 – 7.3) were spiked with potential interfering substances and were incubated with the test system. The recovery in relation to the un-spiked sample without interferent was calculated. The individual recovery of the positive or borderline samples was within the range of 92 – 107%. No significant interference was observed for concentrations of up to 1000 mg/dL for hemoglobin, 2000 mg/dL for triglyceride and 40 mg/dL for bilirubin. Furthermore, the influence from rheumatoid factor was investigated by spiking of 6 different specimens with a rheumatoid factor positive material (characterized nephelometrically). The recovery in relation to the original sample (not spiked) was calculated. The recoveries were found within 100 - 110%. No interference (≤ ± 10%) was observed with rheumatoid factor up to 500 IU/mL.

f. Assay cut-off:

The assay cutoff is a ratio of 1.0.

2. Comparison studies:

a. Method comparison with predicate device:

A comparison study was performed using 158 clinically characterized samples from patients (49 MCTD, 37 systemic lupus erythematosus, 37 Sjögren's syndrome, 19 systemic sclerosis, 16 myositis) and 132 from control groups (10 celiac disease, 17 Wegener's granulomatosis, 39 rheumatoid arthritis, 16 infectious disease and 50 healthy), obtained from different sources. The panel consisted of 101 men and 174 women (and 14 unknown). Age ranged from 7 to 87 years with an average age of 46 years (15 unknown). The samples were tested with the EUROIMMUN ANA Screen ELISA (IgG) and with the Aesku Aeskulisa ANA Hep-2 as the predicate device. The results are shown in the table below. The discrepant samples were from controls and one MCTD sample in the cut-off range.

n = 290		Predicate ELISA	
		positive	**negative**
EUROIMMUN ANA Screen ELISA (IgG)	**positive**	137	3
	negative	5	145

Negative Agreement 145/148 = 98.0% 95% C.I.: 94.2%-99.6%
Positive Agreement 137/142 = 96.5% 95% C.I.: 92.0%-98.8%
Overall Agreement 282/290 = 97.2% 95% C.I.: 94.6%-98.8%

The reactivity of the ANA Screen ELISA (IgG) was verified using the CDC ANA reference panel. All samples were positive with the ANA Screen ELISA (IgG), except for those characterized as nucleolar/U3 RNP (Fibrillarin) and PM-Scl positive. These two target antigens are not included in the antigen spectrum of the ANA Screen ELISA (IgG).

CDC panel results

No.	CDC characterization	ANA-Screen	
		Ratio	Result
1	homogenous/rim/nD	3.0	Positive
2	speckled/SS-B	6.8	Positive
3	Speckled	7.7	Positive
4	U1 RNP	7.3	Positive
5	Sm	5.2	Positive
6	nucleolar/U3 RNP (Fibrillarin)	0.0	Negative
7	SS-A/Ro	5.1	Positive
8	Centromere	4.4	Positive
9	Scl-70	6.1	Positive
10	Jo-1	4.9	Positive
11	PM-Scl	0.0	Negative
12	ribosomal P-proteins	2.0	Positive

b. Matrix comparison:

The usability of plasma was investigated using sample pairs each of serum and corresponding plasma (EDTA, Li-heparin, Citrate). Passing-Bablok regression was calculated for the comparison of serum to plasma. Results of the regression analysis and mean % recovery are shown below.

	EDTA plasma	**Li-heparin plasma**	**Citrate plasma**
N	12	12	12
Regression Equation: (y = plasma, x = serum) 95% C.I. of intercept 95% C.I. of slope	y = 0.04 + 0.99 x -0.02 – 0.13 0.96 – 1.02	y = -0.04 + 1.00 x -0.10 – 0.01 0.98 – 1.03	y = -0.00 + 0.99 x -0.02 – 0.05 0.95 – 1.00
Coefficient of determination R^2	0.9988	0.9992	0.9990
Mean %recovery Range of %recovery	103 % 98 – 112 %	99 % 90 – 109 %	98 % 95 – 104 %

3. Clinical studies:

a. Clinical Sensitivity and Clinical Specificity:

Clinical studies were performed in cooperation with different sites. In total 738 clinically characterized samples were investigated for anti-nuclear antibodies (IgG). The EUROIMMUN ANA Screen ELISA (IgG) showed an overall sensitivity of 72.5% (95% C.I.: 68.0 – 76.7%) and a specificity of 95.8% (95% C.I.: 92.9 – 97.7%). 95% C.I. are calculated by the exact method. The results are shown in the table below.

No.	**Panel**	**n**	ANA Screen ELISA (IgG)		
			positive	**%**	**95% C.I.**
1	Mixed connective tissue diseases	21	20	95.2%	76.2 – 99.9%
2	Systemic lupus erythematosus	213	156	73.2%	66.8 – 79.1%
3	Polymyositis/dermatomyositis	26	4	15.4%	4.4 – 34.9%
4	Systemic sclerosis	81	59	72.8%	61.8 – 82.1%
5	Sjögren's syndrome	88	72	81.8%	72.2 – 89.2%
	Total	429	311	72.5%	68.0 – 76.7%

No.	**Panel**	**n**	ANA Screen ELISA (IgG)		
			negative	**%**	**95% C.I.**
6	Celiac disease	10	10	100.0%	69.2 – 100.0%
7	Wegener's granulomatosis	17	16	94.1%	71.3 – 99.9%
8	Rheumatoid arthritis	203	191	94.1%	89.9 – 96.9%
9	Other autoimmune diseases*	63	63	100.0%	94.3 – 100.0%
10	Bacterial/viral infections	16	16	100.0%	79.4 – 100.0%
	Total	309	296	95.8%	92.9 – 97.7%

*from the following groups: AIH (n = 8), PBC (n = 9), Grave's disease (n = 12), Hashimoto (n = 11), celiac disease (n = 11), Diabetes Type I (n = 12)

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 ANA (IgG) were analyzed in a panel of 200 samples from apparently healthy blood donors (120 men and 80 women with an average age of 40 years; age range: 19 – 68 years). The results are shown in the table below.

n	200
Positives	6
Negatives	194
Prevalence	3.0%
Ratio Mean±SD	**0.2 (0.4)**
Ratio Range	0.1-4.5

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.