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

A. 510(k) Number:
   k083381
B. Purpose for Submission:
   New assay
C. Measurand:
   Anti-dsDNA antibodies
D. Type of Test:
   Quantitative and semi-quantitative
E. Applicant:
   Euroimmun US Inc.
F. Proprietary and Established Names:
   EUROIMMUN Anti-dsDNA-NcX ELISA (IgG)
G. Regulatory Information:
   1. Regulation section:
      21 CFR §866.5100, Antinuclear Antibody Immunological Test System
   2. Classification:
      Class II
   3. Product code:
      LRM, Anti-DNA antibody (enzyme-labeled), antigen, control
   4. Panel:
      Immunology (82)
H. Intended Use:
   1. Intended use(s):
      See Indications for Use below
   2. Indication(s) for use:
      The EUROIMMUN Anti-dsDNA-NcX ELISA (IgG) test kit is designed for the
      quantitative or semi-quantitative determination of IgG class autoantibodies
      against double-stranded genomic DNA (dsDNA) in human serum and plasma. It
      is used as an aid in the diagnosis of systemic lupus erythematosus, in conjunction
      with other laboratory and clinical findings.
   3. Special conditions for use statement(s):
      For prescription use only
   4. Special instrument requirements:
      Microplate reader capable of measuring OD at 450 nm and 620 to 650 nm.
I. Device Description:
The device consists of strips microplate wells coated with antigen, 3 levels of
calibrator, a positive and a negative control, peroxidase-labeled anti-human IgG
coupled enzyme conjugate, sample buffer, wash buffer concentrate, TMB, and stop
solution.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   FARRZYME Human High Avidity anti-dsDNA Enzyme Immunoassay Kit
2. Predicate K number(s):
   k062183
3. Comparison with predicate:

Similarities		
Item	**New Device**	**Predicate Device**
Intended use	Detection of IgG antibodies to double-stranded DNA as an aid in diagnosis of systemic lupus erythematosus	Same
Technology	ELISA	Same
Assay platform	96-well microtiter plates	Same
Calibration	NIBSC Wo/80 standard	Same
Conjugate	Rabbit anti-human IgG labeled with horseradish peroxidase	Same
Substrate	TMB	Same
Procedure	Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with an anti-human IgG enzyme conjugate; wash step, incubation with substrate; then the addition of a stop solution and reading at 450nm.	Same

Differences		
Item	**New Device**	**Predicate Device**
Assay format	Quantitative or semi-quantitative (using either the 3 calibrators or 1 calibrator only)	Quantitative
Antigen	dsDNA which is complexed with nucleosomes (NcX) and coupled to the solid phase	dsDNA (calf thymus)
Calibrators	3 calibrators 10, 100 and 800 IU/mL	5 calibrators 12.3, 37, 111, 333 and 1000 IU/mL
Controls	2 controls 1 positive, 1 negative	3 controls 1 positive, 1 negative, 1 ssDNA
Samples	Serum or plasma	Serum
Cut Off level	100 IU/mL	30 IU/mL
Assay range	10 - 800 IU/mL	12.3 - 1000 IU/mL
Detection limit	2.6 IU/mL	12.3 IU/mL

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
Calibrators, controls, and diluted patient samples are added to the wells and autoantibodies recognizing the dsDNA antigen bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured human autoantibody. Excess unbound conjugate is removed by another wash step. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate. The intensity of color development is proportional to the concentration of autoantibody in the sample. Microtiter plates are read at 450nm and a reference wavelength of 620 to 650 nm. The controls and patient results are interpreted by comparing them as a ratio of one of the calibrators or to a 3 point calibration curve.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*
      The intra- and inter-assay imprecision of the assay was measured using 13 samples covering the assay range. Intra-assay precision was measured using 20 replicates in one assay while inter-assay precision was measured by testing samples in quadruplicate in 4 or 6 runs (one run per day):

Intra-assay Precision:

Sample	Mean (IU/mL)	SD (IU/mL)	%CV	Sample	Mean (IU/mL)	SD (IU/mL)	%CV
1	31	1.4	4.6	8	110	12.8	11.6
2	38	1.4	3.7	9	119	10.7	9.0
3	41	2.8	6.7	10	157	7.3	4.6
4	60	4.6	7.7	11	318	9.0	2.8
5	81	2.3	2.9	12	543	16.1	3.0
6	85	2.9	3.5	13	713	25.5	3.6
7	95	7.4	7.8				

Inter-assay Precision:

Sample	n	Mean (IU/mL)	SD (IU/mL)	%CV	Sample	n	Mean (IU/mL)	SD (IU/mL)	%CV
1	24	32	1.8	5.6	8	16	110	7.5	6.9
2	24	40	2.4	5.8	9	16	135	11.3	8.4
3	24	42	2.9	7.0	10	16	173	8.3	4.8
4	16	66	4.6	6.9	11	16	338	9.8	2.9
5	24	80	3.1	3.9	12	16	544	35.0	6.4
6	24	88	3.7	4.2	13	16	700	63.1	9.0
7	16	105	8.5	8.1					

   b. *Linearity/assay reportable range:*
      Serial dilutions (8) of 4 serum samples showed the assay was linear across the

claimed measurement range of 10 – 800 IU/mL:

Sample	Concentration range of dilutions (IU/mL)	Regression equation*	(R2)*
1	<2 – 596	y = 0.95x – 3.86	0.9831
2	<2 – 600	y = 0.96x – 6.13	0.9860
3	<2 – 565	y = 0.94x – 0.91	0.9804
4	<2 – 750	y = 0.94x – 6.91	0.9773

*Values above and below the measurement range were not included in the calculation

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   The calibrators are standardized against the WHO reference material Wo-80.

   Opened and unopened kit components were stable for 12 months when stored at 2 – 8°C. Reconstituted wash buffer was stable for up to 28 days. Sample stability claims are based on the recommendations in CLSI H18-A3.

d. *Detection limit:*
   The Limit of Detection (LoD) and Limit of Quantitation (LoQ) were calculated according to the recommendations of ICH Q2B. Both LoD (1.83 IU/mL) and LoQ (5.56 IU/mL) were well below the lower limit of the measurement range.

e. *Analytical specificity:*
   Cross reactivity of the assay was investigated using the CDC ANA Reference Sera panel (n = 12) and the 2002 AMLI Consensus Reference Panel (n = 10). These reference samples were tested on the Anti-dsDNA-NcX ELISA (IgG) according to the package insert. Results were in agreement with the CDC and AMLI characterization. Cross reactivity against Scl-70 and CCP was tested using sera known to test positive for antibodies against these proteins; all were negative when tested in the Anti-dsDNA-NcX ELISA (IgG).

f. *Assay cut-off:*
   See Expected Values section below.

2. Comparison studies:
   a. *Method comparison with predicate device:*
      Two hundred fourteen samples (93 from SLE patients, 54 from rheumatoid arthritis patients, 26 from patients with various ANA-positive connective tissue diseases and 41 from healthy donors) were tested with the EUROIMMUN Anti-dsDNA-NcX ELISA and a predicate assay according to the package inserts. The samples were from 67 men and 154 women; patient age ranged from 19 to 79 years with an average of 47 years:

		PREDICATE	
		positive	negative
EUROIMMUN	positive	25	35
Anti-dsDNA-NcX	negative	3	151

Negative agreement: 81.2% (151/186)   95% CI: 74.8% - 86.5%
Positive agreement: 89.3% (25/28)   95% CI: 71.8% - 97.7%
Overall agreement: 82.2% (176/214)   95% CI: 76.5% - 87.1%

b. *Matrix comparison:*
The suitability of EDTA and citrate plasma was determined by assaying 35 serum samples covering the assay range; corresponding EDTA and citrate plasma samples were drawn simultaneously. Passing-Bablok regression was calculated for each type of plasma. The results of the Passing-Bablok regression analysis were following:

Serum vs.	EDTA plasma	Citrate plasma
Regression	y = 0.99x +1.08	y = 1.00x
95% C.I. intercept	-0.72 – 1.76	-1.98 – 1.39
95% C.I. slope	0.96 – 1.02	0.97 – 1.05
r2	0.994	0.990

Heparin plasma samples were found not suitable for this assay.

3. Clinical studies:
a. *Clinical Sensitivity:*
The clinical sensitivity and specificity of a set of 573 clinically characterized frozen sera was evaluated with the assay. The sensitivity of the EUROIMMUN Anti-dsDNA-NcX ELISA in this cohort was 59.6% (95% C.I.: 52.7 – 66.3%) for SLE while the overall specificity of the device for disease controls was 97.2% (95% C.I.: 95.0 – 98.7%):

Panel	Anti-dsDNA-NcX ELISA (IgG)	
	n	positive (%)
Systemic lupus erythematosus	213	127 (59.6%)
Rheumatoid arthritis	165	7 (4.2%)
Sjögren's syndrome	88	1 (1.1%)
Progressive systemic sclerosis	81	2 (2.5%)
Myositis	26	0 (0.0%)
Total non-SLE	360	10 (2.8%)

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

4. Clinical cut-off**:**
   Not applicable.
5. Expected values/Reference range:
   The purpose of the normal sera studies was to evaluate expected values in the normal population and to confirm the defined cut-off, 100 IU/mL. Samples from 400 apparently healthy blood donors (224 men and 176 women) were measured. Values ranged from 0.6 IU/mL to 72 IU/mL with a mean value of 6.8 IU/mL (standard deviation 8.2 IU/mL).

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.