510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. **510(k) Number:**
   k070789
B. **Purpose for Submission:**
   New device
C. **Measurand:**
   Anti- cyclic citrullinated peptide (CCP) IgG
D. **Type of Test:**
   Qualitative and semi-quantitative ELISA
E. **Applicant:**
   EUROIMMUN US, LLC.
F. **Proprietary and Established Names:**
   Anti-CCP ELISA (IgG)
G. **Regulatory Information:**

Product Code	Classification	Regulation Section	Panel
NHX, Antibodies, anti-cyclic citrullinated peptide (CCP)	Class II	21 CFR 866.5775 Rheumatoid factor immunological test system	Immunology 82

H. **Intended Use:**
   1. Intended use(s):
      The EUROIMMUN Anti-CCP ELISA (IgG) test kit is intended for the qualitative or semi-quantitative determination of IgG class antibodies against cyclic citrullinated peptides (CCP) in human serum and plasma. It is used as an aid in the diagnosis of rheumatoid arthritis, in conjunction with other laboratory and clinical findings.
   2. Indication(s) for use:
      Same as the intended use
   3. Special conditions for use statement(s):
      Prescription use only
   4. Special instrument requirements:
      ELISA plate reader, automated plate washer (recommended)
I. **Device Description:**
   The device consists of: 12 microtiter strips each with 8 break-off reagent wells coated with synthetic cyclic citrullinated peptides, perioxidase-labelled rabbit anti-human IgG (ready-to-use), chromogen/substrate solution (TMB/H_2O_2), positive and negative human IgG controls (ready-to-use), calibrators (5 levels, human IgG ready-to-use), sample buffer (ready-to-use), wash buffer (10x) and 0.5 M sulphuric acid (stop solution, ready-to-use).
J. **Substantial Equivalence Information:**
   1. Predicate device name(s):

Euro-Diagnostica IMMUNOSCAN RA Anti-CCP Test Kit
2. Predicate 510(k) number(s):
   k052133
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EUROIMMUNE Anti-CCP ELISA (IgG)	IMMUNOSCAN RA Anti-CCP Test Kit
Intended Use	Detection of anti-CCP IgG	Same
Indications for Use	Aid in the diagnosis of rheumatoid arthritis	Same
Technology	ELISA	Same
Enzyme conjugate	HPR anti-human IgG	Same
Substrate	TMB	Same
Controls	Positive and negative	Same

Differences		
Item	Device	Predicate
Sample type	Serum and plasma	Serum
Cut-off	5 RU/mL	25 U/mL
Calibrators	5 levels (1, 5, 20, 100, 200 RU/mL)	5 levels (25, 50, 200, 800, 1600 units/mL)
Wash buffer	10x	20x
Anti-CCP Antibody Results Interpretation	Semi-quantitative: Negative = ≤ 5 RU/mL Positive = >5 RU/mL Qualitative: Negative = OD ratio ≤ 1.0 Positive = OD ratio >1.0	Qualtitative: Negative = < 25 U/mL Positive = ≥ 25 U/mL

K. Standard/Guidance Document Referenced (if applicable):
   None referenced.
L. Test Principle:
   Diluted patient samples, calibrators and controls are incubated in the wells coated with synthetic cyclic citrullinated peptides. Anti-CCP antibodies if present will bind to the antigens in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. Subsequent to washing, rabbit anti-human IgG HRP enzyme conjugates are added and will bind to anti-CCP antibodies in the wells. Unbound HRP enzyme conjugates are removed by washing prior to addition of the enzyme substrate (TMB). If anti-CCP antibodies are present, a blue color will develop. After color development is complete, a stop solution is added which turns the blue color to yellow. The absorbance in each well is read in a microplate reader and is directly proportional to the amount of anti-CCP antibody present.
M. Performance Characteristics (if/when applicable):

1. Analytical performance:
    a. *Precision/Reproducibility:*
        Intra-assay
        To assess intra-assay precision, 7 serum samples containing anti-CCP concentrations ranging from 4.2 to 52 RU/mL were assayed in 20 replicates. The mean %CV was 4.9%. Two-way ANOVA statistics were also calculated.

	Samples (RU/mL)						
	1	2	3	4	5	6	7
Mean	52	18	20	26	4.2	4.9	6.3
SD	1.7	1.1	0.8	0.9	0.3	0.2	0.4
%CV	3.4	5.9	4.0	3.6	6.3	4.5	6.6
Overall %CV	4.9						

Inter-assay
To assess inter-assay precision (between-run and between-day), 7 serum samples containing a range of anti-CCP levels (3.9 – 61 RU/mL) were assayed in 4 replicates for 4 runs on 3 different days. The mean values of the 4 runs were used in the calculations of the mean, SD and %CV. The overall %CV was 7.1%. Two-way ANOVA statistics were also provided.

	Samples (RU/mL)						
	1	2	3	4	5	6	7
Mean	61	19	23	34	3.9	4.4	6.2
SD	2.9	1.1	1.4	2.3	0.4	0.4	0.5
%CV	4.7	5.8	6.1	6.6	9.5	9.4	7.8
Overall % CV	7.1						

Lot-to-lot reproducibility
Six different serum samples with anti-CCP levels ranging from 4.0 RU to 55 RU/mL were assayed with 3 different lots of reagents. For each lot, the samples with higher concentrations were tested in duplicate and the samples with lower concentration were tested in 12 replicates. The mean values of the 3 lots were used for calculation. The mean %CV of all samples was 7.6%. Two-way ANOVA statistics were also provided.

	N = 3 lots x duplicates			N = 3 lots x 12 replicates		
	1	2	3	4	5	6
Mean (RU/mL)	55	14	20	4.0	4.3	6.8
SD	2.0	1.3	0.9	0.5	0.3	0.7
%CV	3.7	8.8	4.3	11.2	6.4	11.0
Overall % CV	7.6					

    b. *Linearity/assay reportable range:*
        Linearity
        Six serum samples with high anti-CCP concentrations were used in this study.

Depending on the anti-CCP concentration, four of the samples were diluted at 1:200 and two at 1:100 before serial two-fold dilutions were made. For each sample, there were 6 serially diluted samples to cover the assay measuring range. The device was shown to be linear for the evaluated concentration range of 3.0 to 196 RU/mL.

c.   *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no reference standard for anti-CCP antibodies. Values are arbitrary assigned to the calibrators and controls.
Shelf-life claim for the test kit is 12 months. Reconstituted Wash Buffer is stable up to 28 days and opened reagents are stable for 3-6 months.

d.   *Detection limit:*
The limit of blank was determined by 58 repeated measurements of a negative anti-CCP sample (1 run with 22 determinations and 3 runs with different lot of reagents and 4 determinations). The LoB is 0.4 RU/mL. The limit of detection (LoD) was determined by assaying 3 low positive samples with anti-CCP concentrations ranging from 4.1 to 6.6 RU/mL for 4 runs on 3 days using 3 different lots (174 measurements). The LoD is 2.5 RU/mL.

e.   *Analytical specificity:*
Three serum samples with different anti-CCP concentrations were spiked with potential interfering substances (hemoglobin, triglycerides, bilirubin) in 3 different concentrations were tested. The acceptance criterion for the mean % recovery was 85-115% of the unspiked sample. The mean % recovery was 99% for hemoglobin (101-114%), 100% for triglycerides (91-105%) and 103% for bilirubin (94-106%). Results showed no interference for concentrations of up to 1000 mg/dL for hemoglobin, 2,000 mg/dL for triglyceride and 40 mg/dL for bilirubin.

Crossreactivity was determined using 26 sera from patients with other autoimmune diseases (6 SLE, 5 scleroderma, 5 Sjogren's Syndrome and 10 RA patients with positive IgM RF). No positive results were observed.

f.   *Assay cut-off:*
Four hundred and nineteen RA patient samples and 1144 control samples (400 asymptomatic blood donors, 28 psoriatic arthritis, 35 other arthritides, 108 SLE, 106 Sjogren's Syndrome, 98 Scleroderma, 159 autoimmune thyroiditis, 25 Wegener's granulomatosis, 126 positive anti-parvovirus B19, 54 viral heptitides and 5 positive anti-HIV) were assayed. The cut-off value was determined to be 5 RU/mL by ROC analysis and at the sensitivity and specificity of 78.5% and 98.2% respectively.

2.   Comparison studies:
a.   *Method comparison with predicate device:*
Two hundred and fifty-nine samples from patients clinically characterized positive for RA according to ACR criteria were tested with the EUROIMMUN Anti-CCP ELISA and the predicate device Eurodiagnostica Immunoscan RA Anti-CCP test. The patients consisted of 74 males and 185 females with a mean age of 55y (18y-83y). Results are summarized below.

		Eurodiagnostica Immunoscan		
		+	-	Total
EUROIMMUN	+	213	4	217
	-	3	39	42
	Total	216	43	259

Positive Percent Agreement: (213/216) = 98.6%
Negative Percent Agreement: (39/43) = 90.7%
Overall Agreement: (252/259) = 97.3

b. *Matrix comparison:*
Besides serum, EDTA, heparin and citrate plasma can be used in the EUROIMMUN anti-CCP test. Matrix comparison was performed using four paired serum and plasma samples with different concentrations of anti-CCP antibodies. Four quadruples of each paired samples were serially diluted to cover all concentrations of the calibration curve and the dilutions were tested. Results of the Passing-Bablok regression analyses are summarized below.

	Regression Equation	Slope (95% CI)	Intercept (95% CI)
Serum/EDTA	y = 1.06x – 0.57	1.0 to 1.14	-1.93 to 0.0
Serum/heparin	y = 1.0x + 0.0	0.96 to 1.05	-0.87 to 1.21
Serum/citrate	y = 1.0x + 0.0	0.94 to 1.19	-2.35 to 0.65

3. Clinical studies:
a. *Clinical Sensitivity and Specificity:*
One thousand five hundred and sixty-three clinically characterized retrospective sera were assayed for this study. The sample composition was detailed under Assay Cut-off. Using a cut-off of 5 RU/mL, 329 of the RA patient samples were tested positive and of the 1144 non-RA samples, 1123 were tested negative. The sensitivity and specificity of the EUROIMMUN anti-CCP assay are 78.5% (95% CI 74.3%-82.4%) and 98.2% (95% CI 87.7%-100%). Results are summarized below.

Sample Types	N	Negative	Positive	Positive (%)	Negative (%)	95%CI (%)
RA	419	90	329	78.5		74.3%-82.4
Non-RA						
Blood donors	400	398	2		99.5	98.2-99.9
Psoriatic arthritis	28	28	0		100	87.7-100
Other arthritides	35	32	3		91.4	76.9-98.2
SLE	108	105	3		97.2	92.1-99.4
Sjogren's	106	104	2		98.1	93.4-99.8
Sclerderma	98	95	3		96.9	91.3-99.4
Autoimmune thyroiditis	159	155	4		97.5	93.7-99.3
Wegener's granulomatosis	25	24	1		96.0	79.6-99.9
Anti-Parvovirus B19	126	123	3		97.6	93.2-99.5
Viral hepatitides	54	54	0		100	93.4-100
Anti-HIV	5	5	0		100	98.2-99.9

b. Other clinical supportive data (when a. and b. are not applicable):
      Not applicable.
   4. <u>Clinical cut-off</u>**:**
      See assay cut-off
   5. <u>Expected values/Reference range:</u>
      The reference range was determined by analyzing 400 apparently healthy blood donors consisted of 149 females and 251 males with a mean age of 39 years (18y-68y). The mean concentration of anti-CCP antibodies was 1.2 RU/mL (±0.8 RU/mL) ranging from 0.2 to 8.0 RU/mL. With a cut-off value of 5 RU/mL, two (0.5%) samples were positive.

N. Proposed Labeling:
The labeling is sufficient and 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.