510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k052133

B. Purpose for Submission:
New Device

C. Measurand:
Anti- CCP antibodies

D. Type of Test:
Qualitative and Semi-quantitative ELISA

E. Applicant:
Eurodiagnostica

F. Proprietary and Established Names:
Immunoscan RA anti-CCP Test Kit

G. Regulatory Information:
1. Regulation section:
   21 CFR 866.5775, Rheumatoid factor immunological test system
2. Classification:
   II
3. Product codes:
   NHX, Antibodies, Anti-Cyclic Citrullinated Peptide (CCP)
4. Panel:
   Immunology 82

H. Intended Use:
1. Intended use(s):
   The Immunoscan RA anti-CCP test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to Cyclic Citrullinated (CCP) in human sera. The assay is used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of Rheumatoid Arthritis (RA), in conjunction with other laboratory and clinical findings. The analysis should be performed by trained laboratory professionals.
   For In Vitro Diagnostic Use.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   The device is for prescription use only.
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm.
   Microplate washer (300μL volume).

I. Device Description:
Each device contains the following: microplate wells coated with citrullinated synthetic peptides; five levels calibrators (25, 50, 200, 800, 1600 units/mL); reference, positive and negative controls (human serum in diluent); wash buffer 20X concentrate; diluent buffer; rabbit anti-human IgG horseradish peroxidase conjugate; TMB (3,3', 5'-tetramethylbenzidin) substrate.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Axis Shield Diastat™ Anti-CCP test Kit
2. Predicate 510(k) number(s):
   k021516
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended use	To aid in the diagnosis of Rheumatoid Arthritis (RA)	Same
Technology	ELISA	Same
Assay Format	Qualitative and semi-quantitative	Same
Reference control	Ready to use	Same
Sample volume required	10 µL	Same
Incubation times	30-30-60 minutes	Same
Platform	96 well microtiter plates	Same

Differences		
Item	Device	Predicate
Antigen	Purified citrullinated synthetic peptides	Purified synthetic cyclic peptide containing modified arginine residues
Sample type and dilution	Serum at 1:50	Serum at 1:100
Positive and Negative controls	Ready to use	Dilute 1:100 with diluted sample diluent
Calibrator	Five Levels (25, 50, 200, 800, 1600 units/mL); ready to use	Five levels (0, 2, 8, 30, 100 U/mL); ready to use
Enzyme-Conjugate	Horseradish peroxidase	Alkaline Phosphatase
Substrate	TMB	PMP (Phenolphthalein monophosphate, Mg^{2+})
Wash buffer	20x Concentrate	16x Concentrate
Stop solution	0.5M sulphuric acid	Sodium hydroxide
OD reading	450 nm	550 nm (540-565nm is acceptable)
Anti-CCP Antibody Results Interpretation	Negative: < 25 units/mL Positive: ≥ 25 units/mL	Negative: ≤ 5U/mL Positive: > 5U/mL

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

L. Test Principle:
   The Immunoscan RA anti-CCP test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to Rheumatoid Arthritis in human serum. The wells of the microtiter plate wells are coated with purified citrullinated synthetic peptides antigen. Diluted serum is applied to the wells and incubated. If specific antibodies are present, they will bind to the antigen in the wells. Unbound material is washed away and any bound antibody is detected

by adding horse radish peroxidase (HRP) labeled anti-human IgG, followed by a second washing step, and an incubation with substrate. The presence of reacting antibodies will result in the development of colour which is proportional to the quantity of bound antibody, and this is determined photometrically.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility*:
   The intra-assay reproducibility was determined by testing six samples eight times. Four samples with high anti-CCP concentrations (88.1-1007.4 U/mL) had a CV of 4.3-12.8% and two samples close to the assay cut-off (33.6-51.7U/mL) had a CV of 8.1-8.4%.

   The inter-assay reproducibility was determined by testing six samples eight times. Four samples with high anti-CCP concentrations (93.1-1105.9 U/mL) had a CV of 6.0-17.7%. Two samples close to the assay cut-off (33.3-52.5 U/mL) had a CV of 7.8-14.5%.

   b. *Linearity/assay reportable range:*
   Three positive sera were diluted serially from neat, 1:2, 1:4, 1:8, and 1:16 dilutions. The values were compared to log 2 of dilution by standard regression. The values indicate that the assay has a linear relationship with serum dilutions.
   The assay reportable range is from 25 units/mL to 1600 units/mL.

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
   There is no reference standard for anti-CCP. The calibrators and controls (positive and negative) are prepared in-house and arbitrary units are assigned during the development process.

   d. *Detection limit:*
   The detection limit was determined by running the zero standard fourteen times on three different lots. The detection limit of 1.6 Units/mL was calculated by finding the mean plus two standard deviations.

   e. *Analytical specificity:*
   Interference study: Three low positive samples were spiked with bilirubin at 0.2 mg/mL, haemoglobin at 400 mg/dL, lipid at 15 mg/mL and rheumatoid factor at 200 IU/mL. The data indicates that these interferences at the assayed concentrations do not interfere with the anti-CCP results.

   f. *Assay cut-off:*
   The cut-off value of > 25 U/mL was established based on testing of 63 normal blood donor sera, 167 non-RA sera and 219 RA sera. With this cut-off value, 62 normal donor sera (98.4%), 165 non-RA sera (98.8%) and 17 RA sera (7.8%) were negative.

2. Comparison studies:

   a. *Method comparison with predicate device:*
   Testing was performed on 320 samples which included 175 samples from RA patients and 145 samples from normal blood donors. The positive percent agreement was 99.3% (135/136); the negative percent agreement was 96.7% (178/184) and the Overall Agreement was 97.8% (313/320).

		Axis Shield Diastat™ Anti-CCP		
		Positive	Negative	Total
Immunoscan	Positive	135	6	141
RA CCP Kit	Negative	1	178	179
	Total	136	184	320

    b. *Matrix comparison:*
       Not applicable.

3. Clinical studies:

    a. *Clinical sensitivity and specificity:*

       The clinical sensitivity and specificity study were evaluated on 1052 frozen retrospective sera with clinically characterized sera from patients with the following diagnosis: 338 RA, 20 WG; 20 microscopic polyangiitis (MP); 70 SLE; 17 Sjogrën's; 100 IBD; 21 Osteoarthritis; 20 Thyroiditis; 5 EBV; 5 Parvovirus; 9 Mycoplasma;5 Tuberculosis; 8 Yersinia; 3 Salmonella; 5 Chlamydia; 3 Malaria; 9 Borrelia; 5 Syphilis; 3 Infectious endocarditis; 4 Legionella; 3 AST; Schistomiasis; 5 Rubella; 3 Chaga's syndrome; 17 Scleroderma; 20 Multiple sclerosis; 20 IDDM; 20 PM/DM; 20 MCTD; and routine samples. Sensitivity for RA was 75.1%. The overall specificity of the new device for healthy blood donors and disease controls was 98.7%.

N= 1052		Anti-CCP results	
Patient Group	n=	negative	positive
RA	338	84	254
WG	20	20	0
MP	20	20	0
Healthy controls	184	182	2
Disease controls			
SLE	70	68	2
Sjogrën's	17	16	1
IBD	100	97	3
Osteoarthritis	21	21	0
Thyroiditis	20	20	0
EBV	5	5	0
Parvovirus	5	5	0
Mycoplasma	9	9	0
Toxoplasma	5	5	0
Tuberculosis	5	5	0
Yersinia	8	8	0
Salmonella	3	3	0
Chlamydia	5	4	0
Malaria	4	4	0
Borrelia	9	9	0
Syphilis	5	5	0
Infectious endocarditis	3	3	0

Legionella	4	4	0
AST	3	3	0
Schistomiasis	4	4	0
Rubella	5	5	0
Chaga's syndrome	3	3	0
Scleroderma	17	16	1
Multiple sclerosis	21	21	0
IDDM	20	20	0
PM/DM	20	20	0
MCTD	20	19	1
Routine samples	80	78	2

Sensitivity:

    RA: 75.1% (254/338)   95% CI: 70.5% to 79.8%

Specificity:

Healthy controls:	98.9% (182/184)	95% CI: 96.1% to 99.9%
WG:	100% (20/20)	95% CI: 83.2% to 100%
MP:	100% (20/20)	95% CI: 83.2% to 100%
SLE:	97.1% (68/70)	95% CI: 90.1% to 99.7%
Sjogrën's:	94.1% (16/17)	95% CI: 71.3% to 99.8%)
IBD:	97.0% (97/100)	95% CI: 91.5% to 99.4%)
Osteoarthritis:	100% (21/21)	95% CI: 83.9% to 100%
Thyroiditis:	100% (20/20)	95% CI: 83.2% to 100%
Infectious Disease:	98.8% (84/85)	95% CI: 93.6% to 100%
Scleroderma:	94.1% (16/17)	95% CI: 71.3% to 99.8%
Multiple sclerosis:	100% (20/20)	95% CI: 83.2% to 100%
IDDM:	100% (20/20)	95% CI: 83.2% to 100%
PM/DM:	100% (20/20)	95% CI: 83.2% to 100%
MCTD:	95.0% (19/20)	95% CI: 75.1% to 99.9%
Routine samples:	97.5% (78/80)	95% CI: 91.3% to 99.7%

  b. Other clinical supportive data (when a. is not applicable):
    Not Applicable.
4. <u>Clinical cut-off</u>**:**
    Same as assay cut-off.
5. <u>Expected values/Reference range:</u>
    Expected values in the normal population should be negative.

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.