

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

A. 510(k) Number:

k062045

B. Purpose for Submission:

New device

C. Measurand:

Anti-cyclic citrullinated peptide (CCP)

D. Type of Test:

Enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

Euro-Diagnostica AB

F. Proprietary and Established Names:

EDIA™ Anti-CCP

G. Regulatory Information:

1. Regulation section:
CFR 866.5775 Rheumatoid Factor Immunological Test System
2. Classification:
Class II
3. Product code:
NHX Antibodies, anti-cyclic citrullinated peptide (CCP)
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The EDIA™ anti-CCP test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human sera and plasma. The assay is used to detect antibodies in a single 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:
The EDIA™ anti-CCP test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and semi-quantitation of IgG antibodies to Cyclic Citrullinated Peptides (CCP) in human sera and plasma. The assay is used to detect antibodies in a single 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.
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
A microplate reader capable of reading 540-565nm.

I. Device Description:

The EDIA™ anti-CCP test kit consists of CCP-coated microtiter wells (12strips x 8 wells), 6 levels of anti-CCP calibrators (human plasma in buffer with protein stabilizer and sodium azide), anti-CCP reference control (human plasma in buffer with sodium azide), positive and negative controls (human plasma in buffer and sodium azide, alkaline phosphatase-labeled goat polyclonal antibody to human IgG in Tris buffer with protein stabilizer and sodium azide, enzyme substrate PMP (phenolphthalein monophosphate) in buffer, stop solution (sodium hydroxide) in EDTA and carbonate buffer, 16x wash buffer concentrate (borate buffer with sodium azide) and 5x sample diluent concentrate (phosphate buffer with protein stabilizer and sodium azide). All reagents except wash buffer and sample diluent are ready-to-use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Euro-Diagnostica Immunoscan RA anti-CCP
2. Predicate 510(k) number(s):
k052133
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	EDIA anti-CCP	Immunoscan anti-CCP
Indications for Use	Aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory and clinical findings.	Same
Method	ELISA	Same
Capture antigen	Citrullinated synthetic peptides	Same
Qualitative cut-off: absorbance ratio	<0.95=negative, ≤0.95 to ≥1.0=borderline, >1.0=positive	Same
Controls	Reference, positive and negative	Same

Differences		
Item	Device	Predicate
	EDIA anti-CCP	Immunoscan anti-CCP
Microtiter plate configuration	12 strips x 8 individual wells	12 strips x 8 wells
Conjugate/label	Goat anti-human IgG labeled with alkaline phosphatase	Rabbit anti-human IgG labeled with horseradish peroxidase
Substrate	PMP	TMB
Calibrators (arbitrary units)	0, 2, 8, 30, 100 and 300 U/mL	25, 50, 200, 800, and 1600 U/mL

Differences		
Item	Device	Predicate
Semi-quantitative cut-off: arbitrary units/mL	≤ 5 U/mL= negative, >5 U/mL= positive	≤ 25 U/mL= negative, >25 U/mL= positive
Sample dilution	1:101	1:50

K. Standard/Guidance Document Referenced (if applicable):

None referenced.

L. Test Principle:

The assay principle is ELISA. The wells are coated with cyclic citrullinated peptides. During the first incubation, specific antibodies in diluted serum, will bind to the antigen coating. The wells are then washed to remove unbound antibodies and other components. A conjugate of alkaline phosphatase-labeled goat antibodies to human IgG binds to the antibodies in the wells in this second incubation. After a further washing step, detection of specific antibodies is obtained by incubation with PMP substrate solution followed by the addition of a stop solution. The amount of bound antibodies correlates to the color intensity and is measured in terms of absorbance (optical density (OD)). The absorbance is then calculated against a calibrator curve and the results are given in arbitrary units.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay precision was determined by testing six different samples eight times each.

	High (U/mL)	Medium (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)
Mean	173.9	34.0	9.9	11.8	7.8	9.7
S.D.	13.8	0.6	0.2	0.5	0.1	0.4
% C.V.	7.9	1.9	2.1	4.0	1.9	4.4

Inter-assay precision was determined by testing six different samples eight times each. Results were obtained for three different runs.

	High (U/mL)	Medium (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)
Mean.	183.8	36.6	9.3	11.9	7.8	10.6
S.D.	19.5	3.0	0.9	0.8	0.7	0.9
% C.V.	10.6	8.2	9.8	6.3	9.5	8.9

Batch to batch variation was determined by testing six different samples eight times each. Results were obtained for three different batches.

	High (U/mL)	Medium (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)	Low (U/mL)
Mean	232.5	41.6	11.5	14.1	9.8	13.0
S.D.	30.9	4.5	1.3	1.2	1.0	1.2
% C.V.	13.3	10.8	11.2	8.3	10.4	9.3

b. Linearity/assay reportable range:

The assay measuring range is 0-300 U/mL, based on the calibrator concentrations. Dilution recovery was determined by testing five serial dilutions of three different patient samples.

Sample	Dilution	Mean Measured Concentration (U/mL)	Calculated Concentration (U/mL)	Dilution Corrected % Recovery
1	1/100	205.0	205.0	100
	1/200	110.5	102.5	108
	1/400	47.3	51.3	92
	1/800	24.8	25.6	97
	1/1600	10.8	12.8	84
2	1/100	138.9	138.9	100
	1/200	70.3	69.5	101
	1/400	40.4	34.7	116
	1/800	18.3	17.4	105
	1/1600	8.7	8.7	100
3	1/100	47.3	47.3	100
	1/200	26.7	23.6	113
	1/400	13.0	11.8	110
	1/800	6.3	5.9	107
	1/1600	3.0	3.0	103

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

There is no recognized standard for quantitatively expressing levels of anti-CCP antibodies. The new assay is calibrated in arbitrary units based on a positive patient serum pool.

The recommended stability of 12 months is based on accelerated stability studies for plates, conjugate, calibrators, controls and substrate. Real time studies are ongoing.

d. Detection limit:

The detection limit of the assay was determined by running the zero calibrator 12 times on three different lots. The detection limit of 0.5 U/mL was calculated by finding the mean plus two standard deviations.

e. Analytical specificity:

Three low positive samples were spiked to the following concentrations in diluted serum samples; bilirubin F at 0.188 mg/dL, bilirubin C at 0.2 mg/dL, hemoglobin at 453 mg/dL, chyle at 0.24 U/dL and rheumatoid factor at 200 IU/mL. The data indicate that the assayed concentrations do not interfere with the anti-CCP results.

f. Assay cut-off:

The assay cut-off level was determined by testing sera from 416 RA patients, 531 non-RA disease patients, and 262 healthy controls from adult subjects. A ROC analysis was performed. The analysis revealed a point with the lowest possible sum of false positives and false negative samples and the cut-off was set at 5 U/mL. Using this cut-off, 99.2% of the normal sera tested were negative, 98.3% of the non-RA disease sera were negative and 76.2% of the RA patient sera were positive.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 678 frozen, retrospective sera were tested to determine the agreement between the new assay and the predicate device. The sera included 416 samples from patients with RA and 262 from apparently healthy blood donors. The study showed the following:

		Immunoscan RA anti-CCP		
		+	-	Total
EDIA anti-CCP	+	317	2	319
	-	5	354	359
	Total	322	356	678

Positive percent agreement	317/322 = 98.4%	95%CI: 96.4 - 99.5%
Negative percent agreement	354/356 = 99.4%	95%CI: 98.0 - 99.9%
Overall agreement	672/678 = 99.1%	95%CI: 97.9 - 99.6%

c. Matrix comparison:

Plasma	N	Range tested (U/mL)	Regression analysis
Heparin	18	0 – 133	$y = 0.9859x + 3.6405, r^2 = 0.9032$
EDTA	15	1 – 84	$y = 1.0369x + 0.2723, r^2 = 0.9899$

3. Clinical studies:

a. Clinical sensitivity:

Patients with clinically defined RA	n	negative	positive	Clinical Sensitivity (95% CI)
	416	99	317	76% (72.1%-80.3%)

b. Clinical specificity:

Clinical specificity for the new assay was determined by testing 793 samples

from 531 non-RA disease patients and 262 asymptomatic healthy blood donors.

	n	negative	positive	Clinical Specificity
Healthy blood donors	262	260	2	99%
Crohn's disease	10	10	0	100%
Colitis ulcerosa	10	10	0	100%
Systemic lupus erythematosus (SLE)	30	30	0	100%
Sjögren's syndrome	17	16	1	94%
EBV	5	5	0	100%
Parvovirus	5	5	0	100%
Mycoplasma	9	9	0	100%
Toxoplasma	6	6	0	100%
Yersinia	8	8	0	100%
Chlamydia	5	4	1	80%
Malaria	4	4	0	100%
Borrelia	9	9	0	100%
Lues	5	5	0	100%
Rubella	5	5	0	100%
Anti-thyroid peroxidase (anti-TPO)	20	20	0	100%
Osteoarthritis	21	21	0	100%
Endocarditis	3	3	0	100%
Tuberculosis	5	5	0	100%
Legionella	4	4	0	100%
Salmonella	3	3	0	100%
AST/ASH (anti-streptococcal antibody)	3	3	0	100%
Schistosomiasis	4	4	0	100%
Chaga's disease	3	3	0	100%
Scleroderma	17	16	1	94%
Multiple sclerosis	20	20	0	100%
Insulin dependent diabetes mellitus	20	20	0	100%
Polymyositis / Dermatomyositis	20	20	0	100%
Mixed connective tissue disease	20	19	1	95%
MPO-ANCA positive	20	20	0	100%
Microscopic polyangiitis				
PR3-ANCA positive	20	20	0	100%
Wegener's granulomatosis				
Ds-DNA positive	40	38	2	95%
Inflammatory bowel disease	80	79	1	99%
Non-RA autoimmune patients	80	78	2	98%
TOTAL	793	782	11	98.6%

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The expected value in the normal population is negative. See assay cut-off.

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.