

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

A. 510(k) Number:

k090753

B. Purpose for Submission:

New assay

C. Measurand:

Anti-CCP IgG antibodies

D. Type of Test:

Semi-quantitative and qualitative immunoassay

E. Applicant:

TheraTest Laboratories

F. Proprietary and Established Names:

TheraTest EL-anti-CCP/2™

G. Regulatory Information:

1. Regulation section:
21 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 TheraTest EL-anti-CCP/2™ test is intended for use in clinical laboratories as an in vitro diagnostic test for the detection and measurement of the IgG class of autoantibodies specific to cyclic citrullinated peptide (CCP) in human serum by enzyme-linked immunosorbent assay (ELISA). It is intended to aid in the diagnosis of Rheumatoid arthritis (RA) in conjunction with other clinical findings and laboratory tests.
2. Indication(s) for use:
Same as Intended use.
3. Special conditions for use statement(s):
For prescription use only.
4. Special instrument requirements:
Spectrophotometric microplate reader with single (450 nm) or dual (450 nm, 620-690 nm reference) wavelength and ELISA plate washer (optional).

I. Device Description:

Each device contains the following: two 96-well ELISA plates with breakaway strips coated with purified synthetic cyclic peptide containing modified arginine residues ; a plate frame; assay controls (human serum with or without IgG antibodies against CCP2); ready to use calibrators; goat anti-human IgG (Fcγ specific) horseradish peroxidase conjugate; TMB chromogen; wash buffer (10X) and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
DIASTAT™ Anti-CCP assay
2. Predicate K number(s):
k023285
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	aid in the diagnosis of rheumatoid arthritis	same
Analyte	anti-CCP antibodies	same
Capture Antigen	synthetic citrullinated peptide	same
Detection Method	colorimetric	same
Assay Format	semi-quantitative and qualitative manual ELISA	same

Differences		
Item	Device	Predicate
Conjugate	goat anti-human IgG (Fcγ specific) horseradish peroxidase	Anti-human IgG (mouse monoclonal) alkaline phosphatase labeled conjugate in Tris buffer
Assay Range	0.135 – 128 U/mL	0.05 - 100 U/mL
Sample Matrix	serum only	serum and plasma

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

CLSI EP17-A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.”

L. Test Principle:

The TheraTest EL anti-CCP2 assay is a solid phase enzyme immunoassays in a 96-well plate format for the measurement of antibodies against CCP. Wells are incubated with diluted samples, calibrators, and positive and negative controls. During the incubation, anti-CCP antibodies, if present in the test sample, bind to the solid phase antigen. The wells are washed, and isotype-specific horseradish peroxidase labeled anti-human immunoglobulin antibody (enzyme conjugate) is added. After incubating the wells with the enzyme conjugate, unbound labeled antibody is removed by washing. A chromogenic substrate solution is added to the wells, and the presence of antibodies to CCP is detected by a color change. The intensity of the color is proportional to the amount of the bound antibody and is read by an ELISA reader. The absorbance value in the blank well (incubated with specimen diluent) is subtracted from the values obtained with samples, calibrators and controls.

In the semi-quantitative mode, the absorbance of the sample is converted to a relative

value based on the standard curve generated by the calibrators. If the specimen's measured absorbance value exceeds that of the highest Calibrator the result is reported as > 128U/mL. In the qualitative mode, the result is based on a ratio of the sample to the cut-off calibrator.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The between-run (total) precision and within-run precision across the assay range was measured by testing 8 specimens in triplicates in 20 separate assays, one or two assays per day. Precision was calculated by the Analyze-it for Microsoft Excel software, and results are presented in the following table:

Sample	Mean (U/mL)	Within-run CV%	Between-run CV%
1	3.2	6.3	9.0
2	4.1	4.2	6.2
3	7.6	4.5	6.5
4	15.5	3.6	5.5
5	21.3	4.1	5.7
6	35.4	4.8	8.4
7	56.4	4.3	6.6
8	100.6	4.1	8.9

Raw data results from the same specimens were analyzed according to the qualitative calculation described in the package insert are presented in the following table:

Sample	Quantitative Result (U/mL)	Target Qualitative Result	Obtained Qualitative Result (n=60)		
			+	equivocal	-
1	3.2	-	0	0	60
2	4.1	-	0	0	60
3	7.6	+	19	41	0
4	15.5	+	60	0	0
5	21.3	+	60	0	0
6	35.4	+	60	0	0
7	56.4	+	60	0	0
8	100.6	+	60	0	0

The sponsor recommends that equivocal samples be reported as equivocal and the patient be re-tested with a fresh sample.

b. *Linearity/assay reportable range:*

The sponsor presented two studies to support the claimed range of Limit of Detection to 128 U/mL for the EL-anti-CCP/2 assay.

In the first study, the highest calibrator (Calibrator 5) and two other specimens positive for anti-CCP (at two levels) were each diluted with Zero Calibrator in five dilutions, from 1:1 to 1:20 and tested in duplicate using the semi-quantitative method. Results from these three specimens were pooled together and regression analysis using data points above the Limit of Detection (LoD) was performed to yield: $y = 1.001x + 1.499$, $R^2 = 0.988$. Samples ranged from 2.6 - 123.2 Units/ml.

An additional three specimens positive for anti-CCP were each diluted with Zero Calibrator in ten dilutions, from 1:1 to 1:10 and tested in duplicate using the semi-quantitative method. Results from these three specimens were pooled together and regression analysis using data points above the LoD was performed to yield: $y = 1.021x + 2.014$, $R^2 = 0.985$. Samples ranged from 3.7 - 109.5 Units/mL and sample recovery ranged from 97 - 126%.

The sponsor presented a study that supported the claim that there is no hook effect up to approximately 1700 U/mL.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no accepted reference standard for anti-CCP. Each lot of assay calibrators is traceable to an internal master calibrator and is validated to determine if they fall within a pre-determined acceptable range. A high positive clinical sample is selected as a control. It is validated against the standard curve and against the curve generated with the new calibrators and must fall within a pre-determined acceptable range. The sponsor has performed real-time stability testing on unopened kits that supports a claim of 12 months at 4°C.
- d. *Detection limit:*
The studies of the assay detection limits were based on CLSI EP17-A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.” The Limit of Blank (LoB) was determined from 60 readings of one blank sample. The measurements were organized according to their value to determine the LoB as the 95th percentile of the blank distribution. The LoB was determined to be 0.0465 Units/mL. The Limit of Detection (LoD) was determined by calculating the SD_s from 60 results obtained from repeated readings of 5 low concentration samples. The pooled estimate from repeated measurements was SD_s = 0.054. The LoD was calculated as 0.135 Units/mL.
- e. *Analytical specificity:*
The analytical specificity of this assay was determined in k023285.

Potential interference from anti-Rheumatoid Factor IgM and anti-dsDNA antibodies was determined by spiking normal and anti-CCP2 positive clinical specimens with the other autoantibodies. No significant ($\pm 10\%$) interference was shown by anti-dsDNA levels of 630 IU/mL and anti-RF IgM concentration of 256 IU/mL. The sponsor did not evaluate the effect of other

endogenous or exogenous substances on the performance of the assays. The package insert contains a caution not to use hemolyzed, lipemic, or icteric samples.

f. *Assay cut-off:*

A cut-off of 5 U/mL was validated by determining the anti-CCP value of 100 blood bank donors (51 female, 49 males). The values were ranked in descending order; only one donor was ≥ 5 U/mL. Therefore the sponsor determined that the upper level of normal was 5 units and set this as the cut-off. Based on the results of the imprecision studies of samples around the cut-off (see above), the sponsor established an equivocal zone at 6 U/mL.

Test	Negative	Equivocal	Positive
Semi-quantitative (U/mL)	≤ 5	$> 5 - < 7$	≥ 7
Qualitative (ratio)	≤ 1.0	$> 1.0 - < 1.4$	≥ 1.4

2. Comparison studies:

a. *Method comparison with predicate device:*

The TheraTest EL-anti-CCP/2 assay was compared to another commercially available ELISA anti-CCP test using a total of 319 specimens: 100 Blood Bank Donors, 119 patients with rheumatoid arthritis (RA) as diagnosed by American College of Rheumatology (ACR) criteria, and 100 patient samples referred by a rheumatologist to a reference lab for anti-CCP testing. Samples below the LoD and above the upper limit of claimed linearity were excluded from method comparison analysis leaving 177 samples for comparison:

		Predicate Device		
		Positive	Negative	Total
EL anti-CCP2	Positive	46	3	49
	Equivocal	2	1	3
	Negative	0	125	125
	Total	48	129	177

Equivocal results are treated as positive:

Positive Percent Agreement = 100% (48/48) 95% CI: 93 - 100
 Negative Percent Agreement = 97% (125/129) 95% CI: 92 - 99
 Total Percent Agreement = 98% (173/177) 95% CI: 94 - 99

Equivocal results are treated as negative:

Positive Percent Agreement = 96% (46/48) 95% CI: 86 - 99
 Negative Percent Agreement = 98% (126/129) 95% CI: 93 - 100
 Total Percent Agreement = 97% (172/177) 95% CI: 94 - 99

b. *Matrix comparison:*

Serum is the only sample type claimed. The user is so advised in the package insert.

3. Clinical studies:

- a. *Clinical Sensitivity:*
The sponsor makes no claim of clinical sensitivity. Claims of clinical sensitivity should be supported by comparing test performance to known clinical diagnoses.
- b. *Clinical specificity:*
The sponsor makes no claim of clinical specificity. Claims of clinical specificity should be supported by comparing test performance to known clinical diagnoses.
- c. Other clinical supportive data (when a. and b. are not applicable):
We estimated the clinical sensitivity of the assay using the results of the assay from the original 119 RA patients and the 100 blood bank donors. The 100 referred for testing samples were not included because there was no information about their clinical diagnosis:

		Rheumatoid Arthritis		
		Positive	Negative	Total
EL anti-CCP2	Positive	83	1	84
	Equivocal	2	0	2
	Negative	34	99	133
	Total	119	100	219

If equivocal samples are considered positive:

Sensitivity: 71.43% 95% CI (62.74%- 78.78%)
 Specificity: 99.0% 95% CI (94.55%- 99.82%)
 Overall Agreement: 84.02% 95% CI (78.59 - 88.28)

If equivocal samples are considered negative:

Sensitivity: 69.75% 95% CI (60.98%- 77.28%)
 Specificity: 99.0% 95% CI (94.55%- 99.82%)
 Overall Agreement: 83.11% 95% CI (77.58 – 87.49%)

- 4. Clinical cut-off:
Not applicable.
- 5. Expected values/Reference range:
The expected value in the normal population is negative. However, the presence of autoantibodies related to RA increases with age. Using the cut-offs established above, 99% of the samples tested were negative for the presence of anti-CCP2 autoantibodies. The sponsor suggests in the labeling that each laboratory establish its own normal range.

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.