

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

A. 510(k) Number:

k061376

B. Purpose for Submission:

This is a new device.

C. Measurand:

Antineutrophil Cytoplasmic Antibodies (ANCA)

Anti-MPO (myeloperoxidase)

Anti-PR3 (proteinase3)

D. Type of Test:

ELISA (Semi-quantitative)

E. Applicant:

TheraTest Laboratories Inc.

F. Proprietary and Established Names:

TheraTest EL-ANCA™: anti-MPO

TheraTest EL-ANCA™: anti-PR3

TheraTest EL-ANCA™: anti-MPO and anti-PR3

G. Regulatory Information:

1. Regulation section:

21 CFR§ 866.5660 Multiple Antibodies Immunological Test System

2. Classification:

Class II

3. Product code:

MOB, Anti-neutrophil cytoplasmic antibodies (ANCA)

4. Panel:

(82) Immunology

H. Intended Use:

1. Intended use(s):

TheraTest EL-ANCA™: anti-MPO and EL-ANCA™: anti-PR3 are intended for use in clinical laboratories as an *in vitro* diagnostic tests for the detection and measurement of autoantibodies in human serum directed against human neutrophil myeloperoxidase and proteinase3, respectively.

Measurement of anti-MPO aids in the diagnosis of microscopic polyangitis.

Measurement of anti-PR3 aids in the diagnosis of Wegener's granulomatosis.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

The device is for prescription use only.

4. Special instrument requirements:

- Microplate reader capable of reading absorbance values at 450 nm. If dual wavelength is available, the reference filter should be set at 620-690 nm.
- Automatic microplate washer

I. Device Description:

The device consists of the following: a foil package containing 12 (1 X 8) microwell strips with holder, calibrator, a negative control, a positive control, ANCA Specimen diluent, Wash Buffer concentrate, HRP-labeled goat anti-human IgG conjugate (fc γ specific) and conjugate diluent. All reagents supplied in the kit are ready for use. The kits contain all necessary reagents to perform one or both of the following tests:

1. TheraTest EL-ANCA™: anti-MPO
2. TheraTest EL-ANCA™: anti-PR3
3. TheraTest EL-ANCA™: anti-MPO and anti-PR3

J. Substantial Equivalence Information:

1. Predicate device name(s):
Wielisa™ MPO ANCA
Wielisa™ PR3 ANCA
2. Predicate 510(k) number(s):
k974166
k974167
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	TheraTest EL-ANCA™: anti-MPO and anti-PR3	Wielisa™ MPO ANCA Wielisa™ PR3 ANCA
Intended Use	Detection of anti-MPO and anti-PR3 autoantibodies	Same
Sample type	Serum	Same
Type of test	Semi-quantitative	Same
Assay type	Elisa	Same
Detection Method	Colorimetric	Same
Solid phase capture	Microwells	Same

Differences		
Item	Device	Predicate
Calibrator	One level	5 levels
Conjugate	HRP-goat anti-human IgG	Alkaline phosphatase-goat anti-human IgG
Substrate	TMB	pNPP
Absorbance	450 nm/620 or 690 nm	405 nm

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

None referenced.

L. Test Principle:

The TheraTest EL-ANCA™: anti-MPO and anti-PR3 ELISA test system are solid phase enzyme immunoassay tests. The wells of a polystyrene plate have been coated

with autoantigens. The wells are incubated with specimens, controls and calibrators. During the incubation, the antibody present in the test sample binds to the solid phase. The wells are washed and pre-diluted horseradish peroxidase labeled goat anti-human (Fc gamma specific) is incubated in the wells. Unbound antibody is removed by aspiration and washing. A specific substrate is added and the autoantibody binding is detected by a color change, which is analyzed using a spectrophotometric enzyme immunoassay reader.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
 - a. *Precision/Reproducibility:*

Intra-assay reproducibility was determined by assaying 3 samples 20 times on one plate. Results are shown on the table below.

Test	Mean conc.	Mean %CV
Anti-MPO	54	8.5%
Anti-MPO	101	4.4%
Anti-MPO	201	2.9%
Anti-PR3	25	8.3%
Anti-PR3	52	4.9%
Anti-PR3	75	3.5%

Inter-assay reproducibility was determined by assaying 3 samples in 20 different runs. Results are shown on the table below.

Test	Mean conc.	Mean %CV
Anti-MPO	50	11.1%
Anti-MPO	107	9.7%
Anti-MPO	218	11.2%
Anti-PR3	31	6.8%
Anti-PR3	59	6.0%
Anti-PR3	78	6.0%

- b. *Linearity/assay reportable range:*
Linearity is not claimed for this assay.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
An international reference material for anti-MPO and anti-PR3 antibodies is not available. The assays are calibrated in relative arbitrary units (U/mL).

Accelerated and real time stability studies were performed for the kit and its components. Results were acceptable.

- d. *Detection limit:*
Not applicable
- e. *Analytical specificity:*
Cross-reactivity

To assess cross-reactivity of other autoantibodies, 200 serum samples representing 125 blood bank donor samples and 75 disease controls were tested. The disease control group consisted of 25 patients with rheumatoid

arthritis, 25 with scleroderma, and 25 with SLE. Cross-reactivity was observed in 4 SLE patients for anti-MPO and 1 SLE patient for anti-PR3. One scleroderma patient tested positive for anti-MPO.

Interfering substance

The ranges for potentially interfering substances have not been determined. A statement that specimens that are lipemic, hemolyzed or icteric should not be used was added to the instruction manual.

Possible interference of rheumatoid factor in the test system was tested. Three different RF samples were added (10% by volume) to anti-MPO and anti-PR3 positive samples. These mixtures were tested using the EL-ANCA assays.

Data showed rheumatoid factor does not appear to affect the reactivity in the EL-ANCA™ test system.

f. Assay cut-off:

The cut-off value was determined by percentile ranking of 100 blood bank donors. The population was represented by 51 females, 49 males, with a median age of 29 years and a range from 16y to 70y. The race distribution was 14% Hispanic, 8% black, 4% Asian and 74% other. Since both anti-MPO and anti-PR3 are rarely found in normal healthy individuals, 99-100 percentiles were used to establish the upper limit of normal.

Test	Percentile	Normal	Equivocal	Abnormal
Anti-MPO	99-100	≤ 20 U/mL	21-25 U/mL	>25 U/mL
Anti-PR3	99-100	≤ 10 U/mL	11-20 U/mL	>20 U/mL

2. Comparison studies:

a. Method comparison with predicate device:

The TheraTest EL-ANCA™: anti-MPO and anti-PR3 was compared to the predicate devices Wielisa MPO-ANCA and Wielisa PR3-ANCA for a total of 280 specimens: 125 blood bank donors, 37 expected positive for anti-MPO antibodies, 43 expected positive for anti-PR3 antibodies, 25 with SLE, 25 with scleroderma, and 25 with rheumatoid arthritis (RA). Equivocal samples were considered negative in the agreement calculations. There were four samples that were discrepant for anti-MPO in the SLE group. No discrepancy was observed for anti-PR3. Results are summarized in the following two tables.

		Wielisa MPO-ANCA			
		Positive	Equivocal	Negative	Total
TheraTest EL-ANCA™: anti-MPO	Positive	40	0	4	44
	Equivocal	0	1	0	1
	Negative	0	0	135	135
	Total	40	1	139	180

Positive Agreement = 100.0% (40/40)

Negative Agreement = 98.3% (236/240)

Total Agreement = 98.6% (276/280)

		Wielisa PR3-ANCA			
		Positive	Equivocal	Negative	Total
TheraTest EL-ANCA™: anti-PR3	Positive	46	0	0	46
	Equivocal	0	1	5	6
	Negative	0	0	128	128
	Total	46	1	133	180

Positive Agreement = 100% (46/46)
 Negative Agreement = 100% (234/234)
 Total Agreement = 100% (280/280)

b. *Matrix comparison:*
 Serum is the only recommended matrix.

3. Clinical studies:

a. *Clinical Sensitivity and Clinical specificity:*

Clinical sensitivity for the TheraTest EL-ANCA™: anti-MPO and anti-PR3 was determined by testing sera (N=37) from patients expected positive for anti-MPO and (N=43) from patients expected positive for anti-PR3. Anti-MPO and anti-PR3 are rarely found in the same patient. Two PR3 patient samples were found anti-MPO positive and 4 MPO patient samples were found equivocal for anti-PR3. If the equivocal samples were considered negative clinical sensitivity of the TheraTest EL-ANCA™: anti-MPO test and anti-PR3 was 100%.

Clinical specificity was determined by evaluating samples from 125 healthy blood donors, 25 RA, 25 SLE and 25 scleroderma patients. None of the samples from the healthy blood donors were tested positive for the anti-MPO and anti-PR3. One patient with scleroderma and 4 patients in the SLE group were found positive for anti-MPO. One patient in the SLE group was found positive for anti-PR3. If the equivocal samples were considered negative, the clinical specificity of the TheraTest EL-ANCA™: anti-MPO test and anti-PR3 in the disease groups was 94.1% (111/118) and 99.1% (111/112) respectively (see table below).

Group tested	N	MPO Pos.	MPO Equiv	PR3 Pos.	PR3 Equiv.
Expected MPO Pos	37	37	0	0	4
Expected PR3 Pos	43	2	0	43	0
Seropositive RA	25	0	0	0	0
SLE	25	4	1	1	2
Scleroderma	25	1	0	0	0

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 expected value in the normal population is 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.