

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

A. 510(k) Number:

k090760

B. Purpose for Submission:

New assay

C. Measurand:

Anti-thyroid peroxidase IgG antibodies and anti-thyroglobulin IgG antibodies

D. Type of Test:

Qualitative and quantitative

E. Applicant:

TheraTest Laboratories

F. Proprietary and Established Names:

TheraTest EL - Anti-TPO

TheraTest EL – Anti-Thyroglobulin

G. Regulatory Information:

1. Regulation section:

21 CFR§ 866.5870 – Thyroid Autoantibody Immunological Test System

2. Classification:

Class II

3. Product code:

JZO - System, Test, Thyroid Autoantibodies

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

See Indications for Use below.

2. Indication(s) for use:

The TheraTest EL-Anti-TPO™ is an enzyme immunoassay for the qualitative detection or quantitative determination of IgG class antibodies against thyroid peroxidase (TPO) in human serum. The TheraTest EL-Anti-TPO™ assay is intended for use as an aid in the diagnosis of autoimmune thyroid disorders, in conjunction with other clinical and laboratory findings.

The TheraTest EL-Anti-Thyroglobulin™ is an enzyme immunoassay for the qualitative detection or quantitative determination of IgG class antibodies against thyroglobulin in human serum. The TheraTest EL-Anti-Thyroglobulin™ assay is intended for use as an aid in the diagnosis of autoimmune thyroid disorders, in conjunction with other clinical and laboratory findings.

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 native human thyroid peroxidase (TPO) or native human thyroglobulin (Tg); a plate frame; assay controls (human serum with or without IgG antibodies against TPO or Tg; 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):

Architect Anti-Tg

Architect Anti-TPO

2. Predicate K number(s):

k052308

k052407

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	aid in detection of autoimmune thyroid disease	same
Assay Range	TPO: LoD – 1000 IU/mL Tg: LoD – 2000 IU/mL	same
Analyte	anti-TPO antibodies anti-Tg antibodies	same
Calibration	MRC 65/093 for Tg Ab, MRC 66/387 for TPO Ab	same

Differences		
Item	Device	Predicate
Conjugate	goat anti-human IgG (Fcγ specific) horseradish peroxidase	Anti-human IgG (mouse monoclonal) acridinium labeled conjugate in MES buffer with protein (bovine)
Capture Antigen	human thyroglobulin- or TPO- coated microwells	Human thyroglobulin- or TPO-coated microparticles in MES buffer with protein (goat) stabilizer.
Detection Method	colorimetric	chemiluminescence
Sample Matrix	serum only	serum and plasma
Assay Format	quantitative and qualitative manual	quantitative automated

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

None referenced.

L. Test Principle:

The TheraTest EL-TPO and the TheraTest EL-Thyroglobulin are solid phase enzyme immunoassays in a 96-well plate format for the measurement of antibodies against tissue TPO or thyroglobulin. Wells are incubated with diluted samples, calibrators, and positive and negative controls. During the incubation, anti-TPO or anti-TG antibodies, if present, 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 TPO or Tg 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 quantitative mode, the absorbance of the sample is converted to a relative value based on the standard curve generated by the calibrators. In the qualitative mode, the result is based on a ratio of the sample to the cut-off calibrator. If the specimen's measured absorbance value exceeds that of the highest Calibrator, the result is reported as >IU/mL (of the highest Calibrator). If exact determination is required, the specimen should be pre-diluted in the provided Specimen Diluent before assaying, and the results should be calculated by taking the dilution factor into account.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision: For both assays, two specimens were tested 20 times within the same assay (within-run precision) and 20 different times in one or two runs per day (between-run precision). Three additional samples were tested in triplicate in 10 runs, one or two runs per day. The results are presented in the following tables:

Imprecision of the EL-Anti-TPO Assay

Mean (IU/mL)	n	Within-run		Between-run	
		Std Dev	% CV	Std Dev	% CV
4.0	30	0.3	7.3 %	0.45	11.3 %
12.5	20	0.6	4.9 %	0.9	8.1 %
22.1	30	0.5	2.7 %	1.7	7.8 %
243.9	20	9.6	3.9 %	22.2	10.9 %
697.9	30	51.7	7.4 %	89.9	12.9 %

Imprecision of the EL-Anti-Thyroglobulin Assay

Mean (IU/mL)	n	Within-run		Between-run	
		Std Dev	% CV	Std Dev	% CV
19.0	30	2.1	10.7 %	2.2	11.5 %
41.5	20	1.0	2.4 %	4.3	10.0 %
67.4	30	1.7	2.5 %	6.8	10.1 %
607.3	20	18.9	3.1 %	51.6	9.6 %
1257	30	67.1	5.3 %	83.0	6.6 %

Reproducibility: The reproducibility of both assays was evaluated by testing clinical samples across the claimed assay range 20 or 30 times each:

Reproducibility of the EL Anti-Thyroglobulin Assay

Sample Value* (IU/mL)	Expected Qualitative Result:	Test result:			% Expected Result
		Neg	Equi-vocal	Pos	
19.0	negative	30	0	0	100%
43.4	equivocal	0	18	2	90%
67.4	positive	0	0	30	100%
535.1	positive	0	0	20	100%
1257	positive	0	0	30	100%

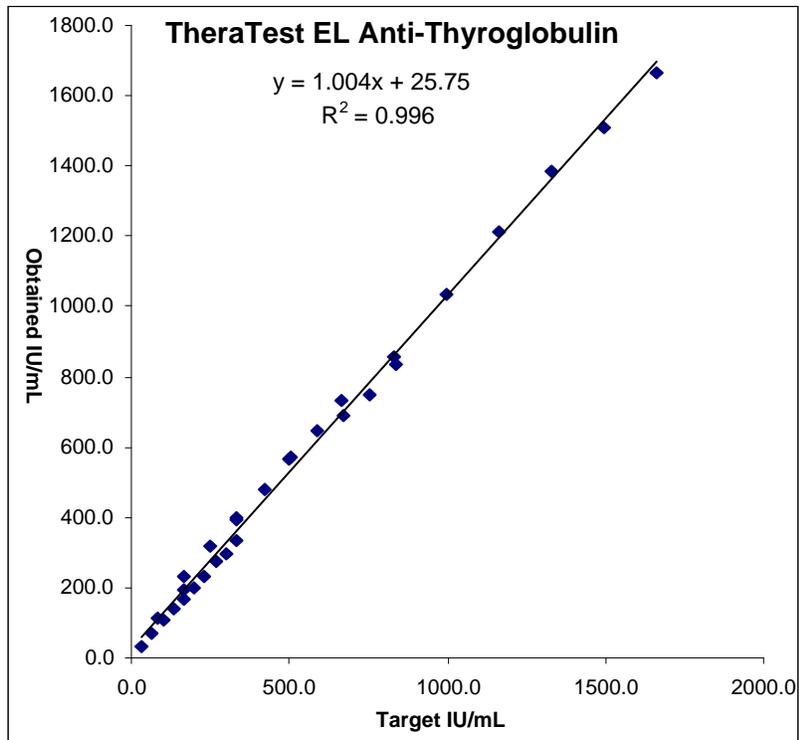
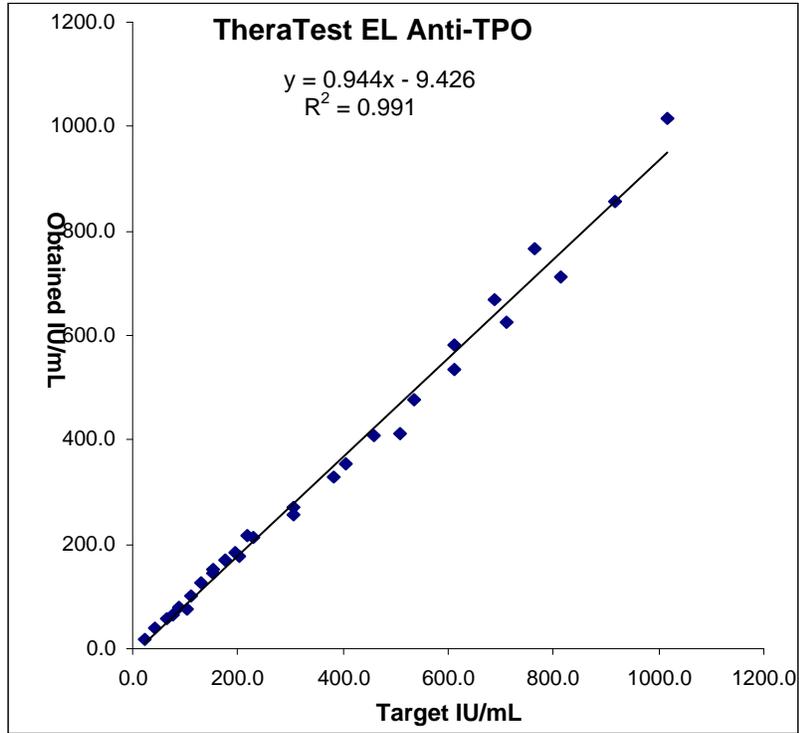
Reproducibility of the EL Anti-TPO Assay

Sample Value* (IU/mL)	Expected Qualitative Result:	Test result:			% Expected Result
		Neg	Equi-vocal	Pos	
4.0	negative	30	0	30	100%
11.3	equivocal	0	17	3	85%
22.1	positive	0	0	30	100%
203.4	positive	0	0	20	100%
697.9	positive	0	0	30	100%

* Sample Value determined with the quantitative protocol.

b. Linearity/assay reportable range:

The claimed range of the EL Anti-TPO Assay is 0.4 – 1000 IU/mL; the claimed range of the EL Anti-Thyroglobulin Assay is 3.0 – 2000 IU/mL. To test whether the assays were linear over their claimed range, six samples from different parts of the assay range were diluted between 6 and 10 times. The dilutions were tested with the appropriate TheraTest assay. The measured values were pooled and plotted against the expected values and linear regression analysis was performed:



The sponsor presented a study that supported the claim that there is no hook effect up to 17,000 IU/mL in the EL-Anti-TPO assay, and up to 16,000 IU/mL in the EL-Anti-Thyroglobulin™ assay.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
 Assay calibrators are human serum specimens containing anti-Tg or anti-TPO antibodies. The concentration is determined for every new lot; every new lot of calibrators is assigned IU/mL values by validating them against the International Reference Preparations, MRC 65/093 for Tg Ab, MRC 66/387 for TPO Ab.

An accelerated stability study supports a claim of unopened kit stability of one year; real-time stability studies are underway. Opened kit stability is one year. Studies of serum sample stability show that the stability of the antibodies in serum for one week at 4 – 8 °C.

- d. *Detection limit:*
 The Limit of Blank (LoB) was calculated from reading the zero calibrator 20 times. The Limit of Detection (LoD) was calculated as the LoB+ 1.65*SD (low concentration specimen). Here the low concentration specimen was the negative control. The functional sensitivity of the assays was defined as the lowest concentration that could be quantitatively determined with acceptable precision (< 20%). Two samples with low levels of analyte were tested in triplicates in six runs and the coefficient of variation was calculated. The functional sensitivity claims in the table below are supported by the provided data:

	EL-Anti-TPO assay (IU/mL)	EL-Anti-Thyroglobulin assay (IU/mL)
LoB	0.05	0.44
LoD	0.40	3.0
Functional Sensitivity	1.3	8.2

- e. *Analytical specificity:*
 Serum samples containing known levels of other autoantibodies [anti-dsDNA, anti-myeloperoxidase (MPO) and anti-cyclic citrullinated peptide (CCP) antibodies] were added to anti-TPO and anti-thyroglobulin positive serum specimens and the recovery (%) of the anti-TPO and anti-thyroglobulin antibody concentrations were calculated. There was no significant decrease in recovery. Samples positive for anti-dsDNA, anti- MPO and anti-CCP antibodies were tested with the anti-TPO and anti-thyroglobulin assays but showed no activity.

Cross-reactivity between anti-thyroglobulin and anti-TPO antibodies was examined by studying the inhibition of two serum specimens containing both anti-TPO and anti-thyroglobulin antibodies. These samples were incubated with 10 µg TPO antigen, or 10 µg thyroglobulin antigen, or the same volume of Specimen Diluent, and then assayed in duplicate with the EL-Anti-TPO™

and EL-Anti-Thyroglobulin™ kits. In sample 1, incubation with TPO antigen resulted in 67.5 % inhibition of autoantibody binding in the anti-TPO test but minimal inhibition (15.7 %) of the anti-thyroglobulin test. Incubation with thyroglobulin antigen resulted in 88.7% inhibition of autoantibody binding in the anti-thyroglobulin test, but no inhibition (1.5 %) in the anti-TPO test. In sample 2, incubation with TPO antigen resulted in 97.3 % inhibition of autoantibody binding in the anti-TPO test but no inhibition (0.6 %) in the anti-thyroglobulin test. Incubation with thyroglobulin antigen resulted in 80.8 % inhibition of autoantibody binding in the anti-thyroglobulin test, but no inhibition (-3.7 %) in the anti-TPO test.

The sponsor did not evaluate the effect of 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:*

See Expected Values/Reference Range section below.

2. Comparison studies:

a. *Method comparison with predicate device:*

One hundred nine serum samples that had been sent to a reference laboratory for thyroid testing were acquired, stored frozen, and then tested by the sponsor. The samples were not individually identifiable and were not accompanied with clinical or demographic data. Agreement between the proposed assays and the predicate assays is described below:

Agreement between Predicate and EL-Anti-TPO Assays:

		Predicate Assay		
		Positive	Negative	Total
EL-Anti-TPO	Positive	62	0	62
	Negative	4	41	45
	Equivocal*	2	0	2
	Total	68	41	109

Percent Agreement with equivocal results considered *negative* in calculations:

Positive Agreement: $(62/68)*100 = 91.2\%$ (95% CI: 81.8% to 96.7%)

Negative Agreement: $(41/41)*100 = 100.0\%$ (95% CI: 91.4% to 100%)

Total Agreement: $(103/109)*100 = 94.5\%$ (95% CI: 88.4% to 98.0%)

Percent Agreement with equivocal results considered *positive* in calculations:

Positive Agreement: $(64/68)*100 = 94.1\%$ (95% CI: 85.6% to 98.4%)

Negative Agreement: $(41/41)*100 = 100.0\%$ (95% CI: 91.4% to 100%)

Total Agreement: $(105/109)*100 = 96.3\%$ (95% CI: 90.9% to 99.0%)

Agreement between Predicate and EL-Anti-Thyroglobulin Assays:

		Predicate Assay		
		Positive	Negative	Total
EL-Anti-Thyroglobulin	Positive	77	1	78
	Negative	1	29	30
	Equivocal	1	0	1
	Total	79	30	109

Percent Agreement with equivocal results considered *negative* in calculations:

Positive Agreement: $(77/79)*100 = 97.5\%$ (95% CI: 91.2% to 99.7%)

Negative Agreement: $(29/30)*100 = 96.7\%$ (95% CI: 82.8% to 99.9%)

Total Agreement: $(106/10)*100 = 97.2\%$ (95% CI: 92.2% to 99.4%)

Percent Agreement with equivocal results considered *positive* in calculations:

Positive Agreement: $(78/79)*100 = 98.7\%$ (95% CI: 93.1% to 100%)

Negative Agreement: $(29/30)*100 = 96.7\%$ (95% CI: 82.8% to 99.9%)

Total Agreement: $(107/10)*100 = 98.2\%$ (95% CI: 93.5% to 99.8%)

b. Matrix comparison:

This assay is only indicated for serum samples.

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):

4. Clinical cut-off:

Not applicable to these assays.

5. Expected values/Reference range:

The National Academy of Clinical Biochemistry ¹ has suggested reference intervals for thyroid antibody tests should be established on controls selected by a process that would be least likely to include subjects with a predisposition to autoimmune thyroid disease. Based on this recommendation, 108 male healthy blood bank donors with no history of thyroid disease or non-thyroid autoimmune disease were selected as a control population. The median age was of 36 years (range was 16 - 72 years) and predominately Caucasian (70%). Pediatric controls were not included. The TPO antibody and thyroglobulin antibody concentrations in the serum samples of the control subjects were measured with the EL-Anti-

¹ Demers LM, Spencer CA. Laboratory Medicine Practice Guidelines; Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. Eds. LM Demers and CA Spencer. *Thyroid* Vol. 13:3 2003.

TPO and the EL-Anti-Thyroglobulin assays. The results were ranked in descending order, and the cut-off values were obtained by percentile ranking of the values. The results were calculated according to both the quantitative and the qualitative methods.

Quantitative method:

According to the quantitative method, the 97.5th percentile concentration was 13.1 IU/mL for the anti-TPO assay and 40.8 IU/mL for the anti-thyroglobulin assay. To reflect the fact that the healthy and the diseased populations have overlapping autoantibody values, the sponsor introduced equivocal zones around these concentrations. The resulting reference ranges are:

	Negative (IU/mL)	Equivocal range (IU/mL)	Positive range (IU/mL)
Anti-TPO	≤ 10 IU/mL	11-15	≥ 16
Anti-Tg	≤ 35 IU/mL	36-50	≥ 51

For the TPO assay, 96.1% of the control population values were in the negative reference range while 2.9% were positive and 0.9% was in the equivocal range. For the thyroglobulin assay, 96.1% of the control population values were in the reference range, 1.9% were positive and 1.9% were in the equivocal range.

Qualitative method:

Results were also calculated according to the qualitative protocol and were expressed in U/mL. Reference ranges and equivocal zones were determined the same way as described above for the quantitative method. The following reference ranges and equivocal zones were established:

	Negative (U/mL)	Equivocal range (U/mL)	Positive range (U/mL)
Anti-TPO	≤ 5	6 - 8	≥ 9
Anti-Thyroglobulin	≤ 20	21 -30	≥ 31

Using the qualitative method determination, 96.1% of the control population had TPO values in the negative reference range, 2.9% were positive and 0.9% was in the equivocal range. For the thyroglobulin assay, 96.1% of the control population had values in the reference range, 1.9% were positive and 1.9% were in the equivocal 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.