

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

A. 510(k) Number:

k051066

B. Purpose for Submission:

Modifications to a cleared device. The applicant made modifications to the capture antigens for four of the test parameters by replacing the biological source (bovine) antigens with human recombinant antigens or by adding a human recombinant component to the biological source antigen. The modified antigens included Sm, SS-A, SS-B and Jo-1. They also modified three calibrators (anti-centromere, anti-ribosomal protein P and anti-Jo-1) by manufacturing them as ready to use. This memo will only address parameters of the device affected by the modifications.

C. Measurands:

Single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), Sm (recombinant), RNP/Sm, SSA (Ro) (recombinant), SSB (La) (recombinant), histones, Scl-70, Jo-1 (recombinant), ribosomal protein P, centromere (human recombinant antigen), chromatin.

D. Type of Test:

Semi-quantitative enzyme-linked immunosorbent assay (ELISA)

E. Applicant:

TheraTest Laboratories, Inc.

F. Proprietary and Established Names:

TheraTest EL-ANA Profiles

G. Regulatory Information:

1. Regulation section:
21 CFR 866.5100 Antinuclear Antibody Immunological Test System
2. Classification:
Class II
3. Product code:
LKP Anti-Sm Antibodies, Antigen and Control
LJM Antinuclear antibody, (Enzyme Labeled), Antigen, Controls
MQA Anti-Ribosomal P Antibodies
LLL Extractable Antinuclear Antibody, Antigen and Control
4. Panel:
Immunology 82

H. Intended Use:

1. Intended use(s):
The EL-ANA Profiles is an in vitro diagnostic test for the detection and measurement of autoantibodies directed against the following autoantigens: single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), Sm^{HR}, RNP/Sm, SSA (Ro)^{HR}, SSB (La)^{HR}, histones, Scl-70, Jo-1^{HR}, ribosomal protein P, centromere and chromatin (nucleosomes). The test system is intended as an aid in diagnosis of systemic lupus erythematosus, Sjögren's syndrome, progressive systemic sclerosis (scleroderma), drug induced lupus, and polymyositis.

2. Indication(s) for use:
Same as Intended use.
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA reader) for 96 well microtiter plates.

I. Device Description:

The assay components consist of: several configurations of microtiter plates coated with antigens: SS-A 60kDa + 52kDa (calf thymus plus recombinant); and Sm (BB'), Jo-1 and SS-B (all human recombinant); IgG enzyme conjugate: goat anti-human IgG (Fcγ specific) conjugated with horseradish peroxidase (HRP); chromogen: containing both the peroxide substrate HRP and 3,3',5,5' tetramethylbenzidine (TMB) as chromogenic indicator; positive and negative controls: human serum; calibrators: antigen specific single calibrators for all antibodies, combined calibrators for ANA/6 and ANA/8; specimen diluent; wash buffer; and stop reagent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
TheraTest EL-ANA Profiles
2. Predicate 510(k) number(s):
k040291
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Assay components:		
Capture antigens: ssDNA, dsDNA, RNP/Sm, histones, Scl-70, ribosomal protein P, centromere and chromatin	Antigens are from thymus (calf or rabbit) or human recombinant (centromere)	Same
IgG enzyme conjugate	Anti-human IgG (Fγ specific conjugated with HRP	Same
Specimen diluent	Phosphate buffer with stabilizers	Same
Wash buffer	10X phosphate buffer	Same
Chromogen	Peroxidase substrate of HRP and TMB	Same
Stop reagent	2M phosphoric acid	Same
Positive control	Human sera containing antibodies	Same
Negative control	Normal human sera	Same

Differences		
Item	Device	Predicate
Capture antigens:		
Sm	Human recombinant BB'	Calf thymus
SS-A (Ro)	Calf thymus (SS-A 60) plus human recombinant SS-A 52kDa	Calf thymus
SS-B (La)	Human recombinant	Calf thymus
Jo-1	Human recombinant	Calf thymus
Calibrators:		
Centromere	Ready to use	Dilute prior to use
Ribosomal protein P	Ready to use	Dilute prior to use
Jo-1	Ready to use	Dilute prior to use

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

None referenced

L. Test Principle:

The device is an enzyme immunoassay with a 96 well plate format. Microplate wells, pre-coated with antigen, are incubated with calibrators, controls and patient samples. During the incubation, antibodies present in the test sample bind to the coated wells. The wells are washed to remove unbound antibodies and labeled goat anti-human IgG is incubated in the wells. The wells are washed again to remove unbound conjugate. As a final step, chromogen is added and autoantibodies are measured using a spectrophotometric plate reader.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Coefficients of variation (CV) for both the intra- and inter-assay reproducibility were all <10% for the modified antigen assays as they were for the predicate device assays.

b. *Linearity/assay reportable range:*

Semi-quantitative autoimmune antibody assays such as these are not expected to yield linear results in dilution studies as is evident in the results shown below.

Sm BB'

Serial dilutions of the Sm BB' calibrator were tested on the BB' antigen. Plotting absorbance versus the dilution factor yielded $y = 1.139x + 0.034$, $r = 0.997$.

SS-A 60 kDa + 52kDa

The in-house standard for SS-A was tested at two-fold dilutions. Plotting absorbance versus the dilution factor yielded $y = 2.575x + 0.130$, $r = 0.993$.

SS-B

The SS-B calibrator was tested at two-fold dilutions. Plotting absorbance

versus the dilution factor yielded $y = 1.345x + 0.008$, $r = 0.998$.

Jo-1

The in-house standard for anti-Jo-1 was tested at two-fold dilutions. Plotting absorbance versus the dilution factor yielded $y = 1.083x + 0.048$, $r = 0.997$.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Not applicable
- d. *Detection limit:*
Not determined or relevant for these assays.
- e. *Analytical specificity:*

Sm BB'

Specificity was demonstrated by running a QC panel of 9 sera with various known specificities. Only the Sm antibody sample was positive. Also, a panel of 25 samples with high levels of Sm antibodies, along with an internal Sm standard and the Sm positive CDC serum specimen were tested. All samples showed a strong reaction with the modified Sm antigen.

SS-A 60 kDa + 52 kDa

Specificity was demonstrated by running a QC panel of 11 sera with various known specificities. Only the SS-A antibody sample was positive. Also, a panel of 10 samples with high levels of SS-A antibodies, along with the SS-A positive CDC serum specimen were tested. All samples showed a strong reaction with the modified mixed SS-A antigen.

SS-B

Specificity was demonstrated by running a QC panel of 8 sera with various known specificities. Only the SS-B antibody sample was positive. Also, a panel of 15 samples with high levels of SS-B antibodies, along with an internal SS-B standard and the SS-B positive CDC serum specimen were tested. All samples showed a strong reaction with the modified SS-B antigen.

Jo-1

Specificity was demonstrated by running a QC panel of 12 sera with various known specificities. Only the Jo-1 antibody sample was positive. Also, a panel of 12 samples with high levels of Jo-1 antibodies, along with an internal Jo-1 standard and the Jo-1 positive CDC serum specimen were tested. All samples showed a strong reaction with the modified Jo-1 antigen.

- f. *Assay cut-off:*

Assay cut-offs for the 4 assays with modified antigens did not change from that originally established with the predicate assays. The cut-offs were validated by running 100 normal sera.

2. Comparison studies:

- a. *Method comparison with predicate device:*

Sm

A comparison between the new assay and the predicate assay for 222 positive and negative samples was presented. The samples included 27 internal QC

known positive samples, 100 normal blood bank donor sera and samples from patients with rheumatoid arthritis (n=20), scleroderma (n=20), and SLE (n=55).

Sm BB'		Native Sm		
		+	-	Total
	+	54	14	68
	-	0	154	154
Total		54	168	222

Positive percent agreement 100% (54/54)
 Negative percent agreement 92% (154/168)
 Overall percent agreement 94% (208/222)

SS-A 60 kDa + 52 kDa

A comparison between the new assay and the predicate assay for 102 positive and negative samples was presented. The samples included 50 blood bank donor sera and 52 samples from patients with SLE.

SS-A 60 kDa + 52 kDa		SS-A 60 kDa		
		+	-	Total
	+	39	3	42
	-	0	60	60
Total		39	63	102

Positive percent agreement 100% (39/39)
 Negative percent agreement 95% (60/63)
 Overall percent agreement 97% (99/102)

SS-B

A comparison between the new assay and the predicate assay for 192 positive and negative samples was presented. The samples included 17 internal QC known positive samples, 100 normal blood bank donor sera and samples from patients with rheumatoid arthritis (n=20), scleroderma (n=20), and 35 samples identified as sera from “abnormal patients”.

SS-B recombinant		Native SS-B		
		+	-	Total
	+	38	2	40
	-	4	148	152
Total		42	150	192

Positive percent agreement 90% (38/42)
 Negative percent agreement 99% (148/150)
 Overall percent agreement 97% (186/192)

Jo-1

A comparison between the new assay and the predicate assay for 192 positive and negative samples was presented. The samples included 17 internal QC known positive samples, 100 normal blood bank donor sera and samples from patients with rheumatoid arthritis (n=20), scleroderma (n=20), and 35 samples identified as sera from “abnormal patients”.

Jo-1 recombinant		Native Jo-1		
		+	-	Total
	+	52	0	52
	-	0	140	140
Total		52	140	192

Positive percent agreement	100% (52/52)
Negative percent agreement	100% (140/140)
Overall percent agreement	100% (192/192)

b. Matrix comparison:

The new and the predicate devices use serum as matrix.

3. Clinical studies:

a. Clinical Sensitivity:

Not done.

b. Clinical specificity:

Not done.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

See assay cut-off.

5. Expected values/Reference range:

A small percentage of the normal population will be positive for these antibodies. Based on testing 100 normal donors and using the established cut-offs, the percentage of negative results with the modified assays was: Sm BB' 99%, SS-A 98%, SS-B, 99% and Jo-1 99%.

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.