510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K040291

B. Analyte:
Anti-Chromatin Antibodies

C. Type of Test:
ELISA, Semi-quantitative

D. Applicant:
TheraTest Laboratories, Inc.

E. Proprietary and Established Names:
EL-ANA Profiles: anti-chromatin

F. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5100 Antinuclear antibody immunological test system
2. Classification:
   Class II
3. Product Code:
   LJM Antinuclear Antibody (Enzyme-Labeled), Antigen, Controls
4. Panel:
   Immunology(82)

G. Intended Use:

1. Intended use(s):
   FOR IN VITRO DIAGNOSTIC USE
   The EL-ANA Profiles™ is intended to measure autoantibodies directed against the following autoantigens: single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), Sm, RNP/Sm, SSA (Ro), SBB (La), Scl-70, Histones, Jo-1, Ribosomal Protein P, Centromere, and **Chromatin (nucleosomes).**
2. Indication(s) for use:
   To aid in the diagnosis of systemic lupus erythematosus and related conditions
3. Special condition for use statement(s):
   For Prescription Use Only
4. Special instrument Requirements:
   Single (450nm) or dual (450nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA reader) for 96 well microtiter plates

H. Device Description:

The TheraTest, Inc. EL-ANA Profiles™ : Chromatin is an antinuclear antibody test system which consists of reagents that measure by ELISA the autoantibodies in serum that react with chromatin, the histone-DNA complex found in the nucleus of eukaryotic cells. Anti-chromatin antibodies are also known as anti-nucleosome, nucleosome-restricted, and anti-(h2A-H2B)-DNA antibodies. The EL-ANA Profiles™ test system already includes tests for autoantibodies against: single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), Sm, RNP/Sm, SSA (Ro), SBB (La), Scl-70, Histones, Jo-1, Ribosomal Protein P, and Centromere. This test is an addition to the same system. It

includes chromatin antigen coated microwells (single antigen plate formats) plus respective Positive and Negative Controls and Calibrator.

This device includes a new component (calibrator). The new calibrator is named EL-ANA Profiles Calibrator (ANA ProCal). The new device takes advantage of the fact that the microtiter plates can be broken into individual wells. Special wells, which are identifiable by having red rim, are created. These wells are coated with goat IgG anti-human $F(ab')_2$ antibodies. A special calibrator is created which contains human IgG in a buffer. When the diluted ANA Profiles Calibrator is added, the anti-$F(ab')_2$ antibody captures the human IgG and displays it on the solid phase similar to that of an antigen-coated well. This calibrator is assigned different number of units for each one of the autoantibodies.
The device is marketed as a panel and there is common labeling for all analytes together. An option of using either a specimen blank or a diluent blank in the procedure is available. An explanation and a special caution note can be found in the Guide to Interpretation section for the user's information.
The only new facet for the EL-ANA Profiles system of devices is the measurement of anti-chromatin antibody; data focused on the performance of the chromatin kit.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   INOVA QUANTA Lite™ Chromatin
2. Predicate K number(s):
   K982603
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	EL-ANA Profiles™ :Chromatin	INOVA QUANTA LITE™ Chromatin
Indications for Use	An aid in diagnosis of systemic lupus erythematosus and related rheumatic diseases	An aid in the diagnosis of drug induced SLE, SLE and related connective tissue diseases.
Methodology	ELISA with purified chromatin from calf thymus	ELISA with purified chromatin from calf thymus
Source of conjugate	Anti-human (goat) conjugated with HRP	Anti-human (goat) conjugated with HRP
Differences		
Item	**Device**	**Predicate**
Calibrator	Unique calibrator to be used with calibration wells (red-rimmed)	Use of Low Positive control as basis of calculation

J. Standard/Guidance Document Referenced (if applicable):
Not Referenced

K. Test Principle:

The TheraTest, Inc. EL-ANA Profiles™ is a solid phase enzyme immunoassay test system. 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 IgG (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. Results are reported as units based on the units in the calibrators provided.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The intra-assay precision was calculated with four specimens (three levels abnormal and one normal tested at 20 times within the same assay. The CV was calculated on the basis of abnormal specimens. All normals remained normal whether using specimen blanks or diluent blanks.

Intra-assay CV

	Spec.blank	Dil blank
High	2.4%	2.4%
Moderate	5.8%	5.3%
Low	3.9%	3.7%

The inter-assay precision was done with same four specimens tested at 20 different times two to four runs per day. The CV was calculated on the basis of abnormal specimens. All normals remained normal whether using specimen blanks or diluent blanks.

Inter-assay CV

	Spec.blank	Dil blank
High	9.1%	9.5%
Moderate	11.8%	9.4%
Low	12.0%	11.4%

b. Linearity/assay reportable range:

The calibrator for anti-chromatin test was tested at two-fold dilutions in triplicate. A linear relationship exists between levels of antibody detected (absorbance) and unit values. Graph shows linearity up to 800 units/mL.

$y = 0.002x^{1.000}$ $r = 1.000$

c. Traceability (controls, calibrators, or method):

A serum sample was selected as a calibrator. This serum was diluted with buffer to provide a specific absorbance when it is reacted with an antigen coated well. An arbitrary unit was pre-assigned to the calibrator since neither CDC nor WHO carry reference standard for this autoantibody. The calibrator is offered as pre-diluted in buffer.
Sera from Blood Donors are used as positive and negative controls.
Selected serum is diluted and subjected to stability experiments. Sample was determined to be stable at 37^{o}C for 7 days and 60 days at 4^{o}C.

d. Detection limit:
Not Applicable

e. Analytical specificity:
Antigen specificity
A serum with known antibodies against the 12 autoantigens were mixed with single autoantigen and incubated in wells coated with the corresponding autoantigen. The percent inhibition of binding by the sera is 95% for chromatin.

Interference
The use of specimen blank or diluent blank does not have any effect on lipemia and hemolysis. However, **diluent blank should not be used with heavily hemolyzed serum**. When a hemolyzed sample was mixed 1:1 with a "clean" negative specimen and tested by the diluent blank method, the results were in slightly abnormal range (<40% above normal limit). An explanation and a special caution note can be found in the Assay Procedure section for the user's information.

f. Assay cut-off:
The cut-off was determined by percentile ranking of 100 blood bank donors. The population was represented by 51 females and 49 males with a median age of 28 years and a range from 16 to 70. The race distribution was 14% Hispanic, 8% Black, 4% Asian and 74% other. Donors that failed criteria for blood donation (based on tests for infectious agents, anemia, etc.) were excluded. It was assumed these donors lack this rare autoantibody. The values obtained from testing the 100 blood bank donors were ranked in descending order, and the cut-off value was obtained by percentile ranking of test values. Cut-off value was 100 units/mL.

2. Comparison studies:

a. Method comparison with predicate device:
The positive agreement and negative agreement of EL-ANA Profiles Anti-chromatin kit compared to the predicate device INOVA Quanta-Lite Chromatin is shown below. Some values are very small and others exceed 100% due to the propensity of the predicate device to yield a slightly higher percentage of false positive results.

Group Tested	Number	Pos. Agreement	Neg. Agreement
Blood bank donors	100	0%	100%
SLE patients	50	92.1%	91.6%
Drug induced lupus patients	46	95.8%	95.4%
Rheumatoid arthritis	50	37.5%	107.1%
All subjects	246	82.4%	108%

Positive and Negative agreement of the device performed with each individual calibrator (true value) and with the EL_ANA ProCal is shown below.

Test Name	No. of spec.	Pos Agreement	Neg Agreement
ssDNA	63	102%	93%
dsDNA	63	96%	102%
Sm	83	111.5%	91.5%
RNP/Sm	63	103.5%	97.1%
SSA	63	100%	100%
SSB	83	85%	103.4%
Histone	63	100%	100%
Scl-70	63	110.5%	94.5%
Jo-1	41	93.3%	103.8%
Ribo-P	41	100%	100%
Centromere	41	100%	100%
Chromatin	41	104.1%	94.1%

Matrix comparison:
Not applicable

3. Clinical studies:
   a. *Clinical sensitivity:*
      Not applicable
   b. *Clinical specificity:*
      Not applicable
   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:
   Refer to Assay Cut-off

M. Conclusion:

Based on the review of the information provided in this 510(k), the TheraTest EL-ANA Profiles™:Anti-Chromatin and the ANA ProCal is **Substantially Equivalent** to the predicate device regulated under 21 CFR 866.5100 Antinuclear antibody immunological test system (Product Code LJM, Antinuclear Antibody (Enzyme-Labeled), Antigen, Controls) Immunology Panel (82)